1 **DRAFT GUIDANCE OF EFSA**

2 EFSA Draft Guidance Document on the Risk Assessment of Plant 3 Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)[1]

4 **European Food Safety Authority**[2, 3]

5 European Food Safety Authority (EFSA), Parma, Italy

6 **ABSTRACT**

7 The Guidance Document is intended to provide guidance for notifiers and authorities in the context of 8 the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 9 1107/2009. The scientific Opinion on the science behind the development of a risk assessment of Plant 10 Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided 11 the scientific basis for the development of the Guidance Document. Specific Protection Goals were 12 agreed in consultation with the Standing Committee on the Food Chain and Animal Health. The 13 Guidance Document suggests a tiered risk assessment scheme with a simple and cost effective First 14 Tier to more complex Higher Tier studies under semi-field and field conditions. Each of the tiers will 15 have to ensure that the appropriate level of protection is achieved.

16 In the current document only the chapters which were not included in the first round of public 17 consultation (20 Sep. – 12 Nov. 2012) are presented. The other chapters are currently under revision 18 taking into account the comments received in the first round of public consultation.

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23 **KEY WORDS**

24 Honey bees, risk assessment, Guidance Document, Pesticides, *Apis mellifera*, *Bombus*, Solitary bees

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SUMMARY

EFSA was asked by the European Commission to develop a Guidance Document on the risk assessment of Plant Protection Products on bees. The Guidance Document is intended to provide guidance for notifiers and authorities in the context of the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009. The scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided the scientific basis for the development of the Guidance Document.

The process of the development of the Guidance Document follows the methodology of definition of Specific Protection Goals (SPG) as outlined in the Scientific Opinion of EFSA's PPR Panel (EFSA, 2010). The Standing Committee on the Food Chain and Animal Health was consulted for the appropriate levels of protection (e.g. to make choices on the magnitude of effects, duration of effects and exposure percentiles).

The Guidance Document suggests proposed the implementation of a tiered risk assessment scheme with a simple and cost effective First Tier to more complex Higher Tier studies under semi-field and field conditions. Each of the tiers will have to ensure that the appropriate level of protection is achieved.

More detailed guidance on specific aspects of laboratory studies and Higher Tier risk assessments are given in the Appendices. A need was identified for test protocols for bumble bees and solitary bees. Potential protocols are available in the published literature and first proposals are made in the Appendices. It is important that fully validated test protocols are developed in future.

In the current document only the chapters which were not included in the first round of public consultation (20 Sep. – 12 Nov. 2012) are presented. The other chapters are currently under revision taking into account the comments received in the first round of public consultation.

Note: If there is no abstract then the summary will begin on the first page and the key words section will appear after the summary.

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80

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is currently revising the European Guidance Document on terrestrial ecotoxicology elaborated by the Commission and experts from Member States. In the context of this revision, the bees risk assessment will also be addressed.

Members of the European Parliament and beekeepers' associations have expressed their concerns to the Commission as to the appropriateness of the current risk assessment scheme, and in particular on the EPPO[4] "Environmental risk assessment scheme for Plant Protection Products – Chapter 10: honeybees" revised in September 2010 with ICPBR[5] recommendations.

Considering the importance and the sensitiveness of this issue, and in line with the aim of the Commission Communication on Honeybee Health (COM (2010) 714 final)[6] adopted on 6 December 2010, the Commission considers that the revised EPPO assessment scheme would need further consideration by EFSA in an Opinion on the science behind the risk assessment for bees and that a Guidance Document on the risk assessment of Plant Protection Products on bees should be developed.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

A scientific Opinion of the PPR Panel on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus spp*. and solitary bees) will be prepared.

In particular the following issues will be addressed:

- The assessment of the acute and chronic effects of Plant Protection Products on bees, including the colony survival and development.
- The estimation of the long-term effects due to exposure to low concentrations
- The development of a methodology to take into account cumulative and synergistic effects.
- The evaluation of the existing validated test protocols and the possible need to develop new protocols, especially to take into account the exposure of bees to pesticides through nectar and pollen.

In order to have the possibility for stakeholders and the interested public to comment on the draft Guidance Document, we propose to include a round of public consultations on the draft Guidance Document. An Opinion on the science behind the Guidance Document could be delivered by April 2012 and a final Guidance Document in December 2012.

CONTEXT OF THE SCIENTIFIC OUTPUT

The Guidance Document is intended to provide guidance for notifiers and authorities in the context of the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009.

[4] European and Mediterranean Plant Protection Organization
[5] International Commission for Plant-Bee Relationships Statutes
[6] Communication from the Commission to the European Parliament and the Council on Honeybee Health, COM(2010) 714 final, adopted on 06/12/2010

116 The scientific Opinion on the science behind the development of a risk assessment of Plant Protection
117 Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided the
118 scientific basis for the development of the Guidance Document.

119 A public consultation is foreseen in order to give stakeholders and the interested public the
120 opportunity to comment on the draft Guidance Document.

121

122 ## 1. Introduction

123 The draft Guidance document which was sent out for public consultation in September 2012 was
124 intended to address the risk to bees from exposure of bees from direct contact and from oral uptake of
125 residues in pollen and nectar. In the meantime new information on the exposure to guttation became
126 available from the ongoing peer-review on neonicotinoids. This information helped to develop an
127 approach to address guttation exposure. In parallel the working group updating the aquatic Guidance
128 Document has developed a method on how to integrate the assessment of metabolites. This enabled
129 the working group for the bee risk assessment to make recommendations for harmonised assessment
130 of metabolites also in the risk assessment for bees. In addition the chapter on uncertainty analysis was
131 finalised. In order to give stakeholders the opportunity to comment on these new approaches a second
132 round of public consultation is launched.
133
134 A draft guidance is presented in the current document for assessing the risk to honeybees resulting
135 from exposure via contaminated water from (i) guttation water, (ii) surface water and (iii) water from
136 puddles in the field. The relationship between the risk assessments resulting from these different
137 exposure routes is as follows: all the risks have to be assessed and if one of them leads to breaching of
138 the specific protection goal, the overall conclusion is that the risk is unacceptable if there are no
139 suitable risk mitigation measures. As follows from the remainder of this document, the risk
140 assessments proposed here are less complicated than the risk assessment from consumption of nectar
141 and pollen. E.g. if the substance is applied after the guttation period, there is no risk resulting from
142 guttation. See following sections for the details.
143

144 ## 2. Assessment of risk from exposure to contaminated water

145 ### 2.1. Assessment of risk from exposure to guttation water

146

Outlined below is a theoretical risk assessment scheme aimed at assessing the risk to honey bees from the consumption of guttation fluid. The lower tiers of the scheme simply assumes that guttation fluid contains the active substance at a proportion of the water solubility and that honey bees take and consume it as water. The scheme also assumes that foragers collect guttation fluid and take it to the colony where it is incorporated in to brood food (e.g. royal jelly) and then fed to larvae.

The first part of the scheme assumes that crops produce guttation fluid, forager honey bees collect and consume guttation fluid and that guttation fluid is fed indirectly via brood food to larvae. Whilst these assumptions are true, the extent to which they occur is unknown and hence this leads to uncertainties in the scheme.

The uncertainties include, but are not limited to, the following:

1. the degree to which guttation occurs - the scheme, as presented, assumes that guttation occurs in every crop albeit within the guttation period. The scheme does not currently specifically consider the likely occurrence of guttation, for example does it occur in all crops all of the time that are treated or only a percentage of treated crops? (Please note that this issue is covered in the exposure flow chart (Box 2), however it is a generic issue and hence appropriate to all uses etc.)

2. the degree to which honey bees forage guttation fluid - the scheme assumes that honey bees will forage on and collect/consume guttation fluid. The scheme does not consider that honey bees may not forage on guttation fluid and may collect/consume water from other sources in preference. Only in the highest tier (field study) this issue is covered.

3. the use of guttation fluid in royal jelly and other brood food - the scheme assumes that

guttation fluid is used in brood food. It is unknown whether this is likely or not or the extent to which this may occur.

All of the above points mean that the initial tiers of the scheme are precautionary and hence are likely to result in many failures and the need for higher tier studies. Guidance is provided regarding how to carry out higher tier exposure and effect studies, however it is uncertain as to how practical these are, for example there is a lack of experience to indicate the precise environmental conditions required to ensure that guttation occurs and that the concentration in the fluid is appropriate (i.e. equivalent to a 90th percentile). This issue is addressed by requesting five studies for seed treatments whereas for spray applications two studies are recommended.

The above points indicate that further information is required to make the following scheme more robust. Further information is required on the following:

1. likely occurrence of guttation in terms of crop/calendar year combinations (see Box 2 of the flow charts)
2. likely use of guttation fluid by honey bees, including the likelihood that it will be fed via brood food to larvae

In addition to the above, feedback on the design of higher tier studies is welcomed.

147
148
149 All bees need water for their metabolism (Nicolson 2009), however, at the moment it is not possible to
150 quantify the level of exposure for non-*Apis* bees. Moreover, the very high level of water fluxes in
151 honey bees at the colony level should be sufficiently protective for bumblebees and solitary bees. For
152 these reasons, it is proposed to focus the risk assessment for guttation water on honey bees only.
153
154 From the available literature and regulatory studies (see also EFSA 2012a) effects on bees were
155 observed from exposure to guttation droplets under the following conditions:
156
157 1. residues of a highly bee toxic substance in guttation droplets
158 2. high water demand of the bee colony
159 3. bee colony close to the field where guttation occurs
160 4. no alternative sources of water
161
162 Guttation tends to occur more frequently under high soil moisture and high air humidity. In some
163 crops such as onions, carrots and sugar beet guttation (information JKI[7]) is rarely observed while in
164 others (e.g. maize) guttation occurs frequently. It is not possible on the basis of the available
165 information to rule out exposure to guttation droplets from certain crops or under certain conditions
166 and therefore this, along with potentially high residues, means that the assessment has to currently be
167 conducted for all crops and uses.
168
169 Guttation water occurring in treated crops may contain very high pesticide concentrations (EFSA
170 2012a, Appendix H). Therefore, the following risk assessment is proposed. It is possible that guttation
171 water of plants other than the treated crop may contain the applied substance (e.g. weeds in the treated
172 field, plants in field margins, adjacent crops, succeeding crops). These other plants are not covered in
173 the scheme below as the risk for the treated crop will pose a greater risk than these other plants.
174
175 The screening is based on several worst case assumptions such as the highest water consumption rate
176 observed in literature at 35°C (Free & Spencer-Booth, 1958) and maximum water solubility as the
177 concentration in guttation droplets. It is considered not necessary to include contact exposure in the
178 screening because the screening step for oral uptake is based on worst case assumptions and will
179 identify highly bee toxic substances for higher tier assessments. In higher tier studies bees will be

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180 exposed by oral uptake and contact exposure. Potential effects on other life stages (larvae) will also be
181 assessed in the higher tier studies.
182
183
184 **The sequence for the risk assessment is the following:**
185 (please see text below “Exposure assessment and risk assessment flow chart” for further details on
186 each of the points)
187
188 **1. Check whether exposure is negligible.**
189 If exposure is concluded to be negligible then a low risk to bees from guttation can be concluded.
190
191 **2. Check whether guttation occurs for <10% of crop/calendar-year combinations.**
192 If it is less than 10% then the exposure is considered as negligible otherwise go to point 3. If no data
193 are available then also go to point 3.
194
195 **3. Calculate the ETR for adult and larvae consuming guttation water based on conservative**
196 **assumptions**.
197
198 **The ETR values for adult bees are calculated as follows:**
199
200 Acute adult
201
202 $$ETR_{acute} = W * PEC / LD50 \quad (1)$$
203
204 where W = 11.4 µL/bee and is the uptake of adult bees. Where the PEC is the concentration in the
205 guttation water in µg/µL and is assumed to be 40% of the water solubility for the acute risk assessment
206 in the first tier. The LD50 is the oral LD50 in µg per adult bee.
207
208 Chronic adult
209
210 $$ETR_{chronic} = W * PEC / LC50 \quad (2)$$
211
212 where W = 11.4 µL/bee and is the uptake of adult bees. Where the PEC is the concentration in the
213 guttation water in µg/ µL and is assumed to be 22% of the water solubility for the chronic risk
214 assessment in first tier. The LC50 is the LC50 (in µg per bee) based on an exposure period of 10 days.
215
216 **The ETR for larvae is calculated as follows:**
217
218 $$ETR_{chronic} = W * PEC / NOEC \quad (3)$$
219
220 where W is 111 µL for larvae (consumed over 5 days). The PEC is the time weighted average
221 concentration in the guttation water in µg/µL over 5 days and the initial concentration is based on 29%
222 of the water solubility. The NOEC (in µg per bee) is based on an exposure period of 5 days.
223
224
225

In the above scheme the initial PEC is based on using 40% of water solubility for the acute assessment, 22% for the chronic PEC for adults and 29% for the chronic PEC for larvae, both for seed treatments, spray applications and granules (see Appendix A).

The above ETR should be compared to the acute ETR to the trigger of 0.106 and the chronic ETR to the trigger of 0.03 and the larval ETR trigger of 0.2 (for details on the trigger value see Appendix A and D).

If the ETR value is below the triggers then the protection goal is met otherwise proceed in the risk assessment. Before conducting higher tier studies it is an option to refine the exposure estimate as outlined under point 4 (see also Risk assessment and exposure flow chart below).

4. Refinement of the exposure calculation.

The exposure estimate can be refined with residues measured in the crop of concern (see figure 1). The PEC guttation needs to cover the 90th percentile in guttation fluid for the crop of concern. The location, growth stage and environmental conditions need to be considered.

For the chronic assessment of adult bees the peak concentration should be used unless there is information which could justify the use of a 10d-twa PEC.

For spray and granular applications it is proposed to use the PEC pore water scenarios as a first refined approximation of the concentration in guttation fluid (90th percentile scenarios for the three regulatory zones are available, see EFSA 2012a).

For seed treatments it is proposed to refine the exposure estimate by conducting field studies and to measure the concentrations in guttation water.

Using these exposure data, the above ETR should be recalculated.

5. The above ETR should be compared to the acute ETR to the trigger of 0.106 and the chronic ETR to the trigger of 0.03 and the larval ETR trigger of 0.2 (for details on the trigger value see Appendix A and D).

The protection goal is met if the ETR value is below the trigger values if not proceed with semi-field studies.
In the semi-field study, it needs to be demonstrated that the protection goals are met. See recommendations on the design of semi-field studies below. If the protection goals are not met in the semi-field study then proceed with field studies (see specific recommendations for semi-field and field studies below).

Exposure assessment and risk assessment flow chart:

The first step in the flow chart is to check whether the substance is applied after the guttation period (**box 1**). If this is the case, there is no exposure.

In **box 2** it is checked whether guttation water occurs for less than 10% of the crop/calender-year combinations. If so, there is unlikely to be exposure for the 90th percentile case. It may be possible to include information on the daily temperature in determining whether exposure to guttation water may occur as it is well known that bees forage for nectar and pollen usually only above 12°C. However, this threshold does not apply to water foraging, i.e. collection for water occurs at temperatures less than 12°C. Therefore, it is probably not feasible to refine the risk assessment based on air temperature.

At this moment, there is no detailed guidance for box 2. So it usually will be necessary to proceed with the next step (**box 3**) and calculate the acute and chronic ETR.

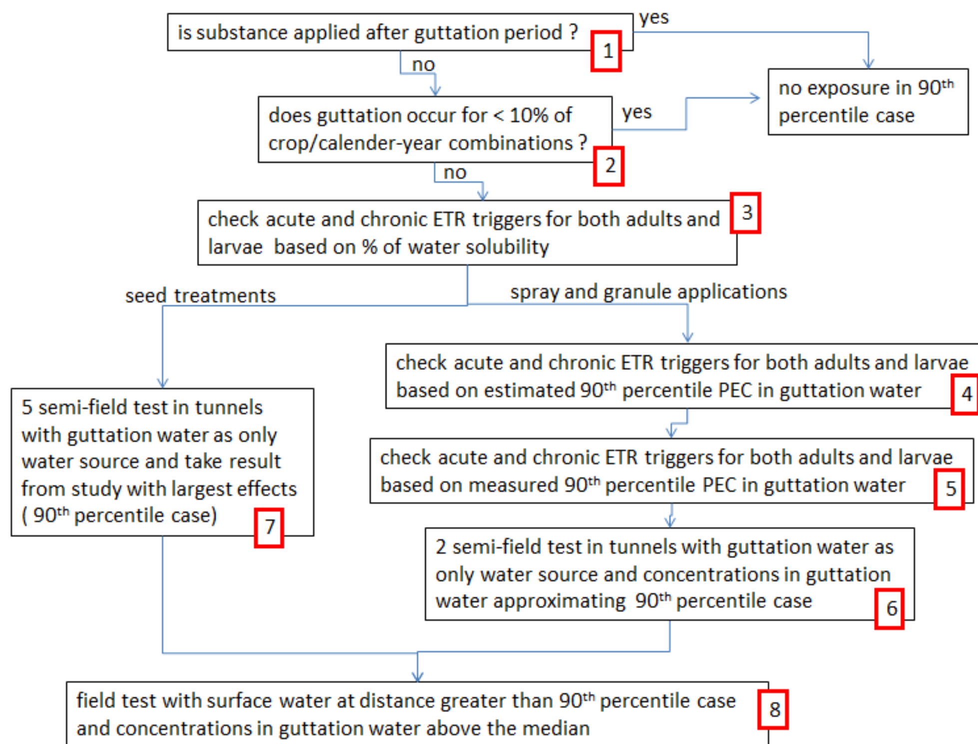

Figure 1: Flow chart for the assessment of the risk resulting from guttation water. The numbers of the boxes are used in the text for their identification.

After **box 3**, the flow chart has two branches: one for the seed treatments (left) and one for the spray and granule applications. For the spray and granules applications, the estimation can be based on estimating the concentration of substance in the transpiration stream of the plants with models describing pesticide fate in the soil-plant system. However, for the seed treatments this is not possible as easy-to-use models for the behaviour of pesticides in a plant growing from a seedling that was coated with the pesticide are not readily available.
So for seed treatments it is not yet possible to perform scenario calculations with models describing pesticide fate in the soil-plant system as described before. It is also less clear which factors will lead to high or low concentrations. Therefore, it is proposed to perform for the seed treatments five semi-field experiments in the area of use of the seed treatment (**box 7**) and to measure in these studies also the concentrations in the guttation water to characterise the exposure.

The assessment for the spray and granule applications continues in **box 4** where the % of water solubility is replaced with an estimated 90th percentile concentration in guttation water. EFSA (2012c) developed a tiered approach for assessing 90th percentile pore water concentrations in the top layer of soil for annual crops under conventional and reduced tillage (assuming ploughing over 20 cm every year). Scenarios were selected for the three regulatory zones (South-Centre-North) for simulations with numerical models. These models calculate uptake of substances by the crop assuming passive uptake based on the concept of the transpiration stream concentration factor (TSCF). This concept assumes that the concentration of substance in the transpiration stream of the plant is a constant fraction (i.e. this TSCF) of the concentration in the water that is taken up by the plants. It is proposed

313 to use these scenarios in combination with a TSCF of 1 and to assume that the concentration in the
314 guttation water is equal to the concentration in the transpiration stream of the plant. Because this
315 approach has so far not been tested, it is proposed to multiply simulated peak concentrations in the
316 transpiration stream with a model uncertainty factor. Uncertainties are also related to the concentration
317 of the compound in the guttation droplet compared to the transpiration stream. As a starting point an
318 uncertainty factor of five is suggested. Once such tests for a range of conditions and substances have
319 become available and have shown that the approach is conservative enough, this model uncertainty
320 factor may be lowered. For other systems than annual crops under conventional or reduced tillage no
321 pore water scenarios are available. So these cannot be dealt with in this tier and have to be referred to
322 higher tiers.
323
324 The above is recommended only for granules that are broadcasted or incorporated into the soil. It is
325 not applicable for granules that are buried with the seed (e.g. in-furrow and band treatments) as the
326 simulations mentioned above are based on the assumption that the granules are distributed
327 homogeneously in an horizontal plane in the soil. Granules buried with the seed are likely to lead to
328 exposure that is more similar to that of the seed treatments. Therefore, it is proposed to use the same
329 approach as for the seed treatments (see above).
330
331 If the simulations with the numerical models do not result in acceptable risk, the next step is to
332 perform field experiments to assess the 90th percentile concentration in the guttation water (**box 5**). For
333 spray and granule applications these have to be targeted to the 90th percentile combination of soil and
334 weather conditions based on the EFSA pore water scenarios used in the simulations. This is likely to
335 lead to the requirement that the field study has to be carried out in a soil with low organic matter
336 content and at a location with a relatively low temperature (see EFSA, 2012c). As described before
337 EFSA (2012c) only considered annual crops under conventional or reduced tillage. For the other
338 systems (e.g. permanent crops) it is proposed to base the 90th percentile conditions on the assumption
339 that these occur under conditions of a combination of a low organic matter content and a relatively low
340 temperature in the area of use of the substance. It is proposed to perform at least two experiments in
341 the area of use of the substance targeted to measure concentrations for 90th percentile cases.
342
343 From these experiments both the peak concentration and the 5- and10-day TWA concentrations could
344 be derived (note that this has the consequence that the 90th percentile peak and the 90th percentile
345 TWA concentration may be based on different experiments). The 10-d TWA can be used to refine the
346 exposure assessment for the adults provided that the use of a TWA is justified.
347
348 The next step is to perform semi-field studies in tunnels in which the guttation water is the only water
349 source (**box 6**) and in which both exposure concentrations and effects on the bees are measured. For
350 the spray and granule applications it is proposed to perform two semi-field studies for soils and
351 meteorological conditions that are expected to generate 90th percentile concentration levels (same
352 procedure as in box 5).
353 If all these steps have not demonstrated that the specific protection goals are achieved, the conclusion
354 has to be that guttation water, if used as the only water source, is likely to lead to unacceptable effects.
355 However, if given the choice bees prefer permanent water sources (streams, ditches, ponds, rivers)
356 over temporary water sources like guttating plants. So in the presence of such permanent water sources
357 high concentrations in the guttation water are unlikely to lead to adverse effects in the hive. Therefore,
358 in **box 8** field studies are proposed under 90th percentile worst-case conditions with respect to the
359 presence of permanent water sources both for seed treatments and spray and granule applications. This
360 means that the assessment moves to the landscape level and the main driver for the effect assessment
361 then becomes the distance of the hive to the nearest water source. Therefore, it is proposed to conduct
362 field studies in which the distance to the nearest permanent water source is equal or larger than the 90th
363 percentile case in the area of use of the substance.
364
365 These distances can be assessed via GIS procedures. The concentrations in the guttation water are
366 expected to play only a minor role at this level of the risk assessment. Therefore, it suffices if the
367 concentrations in the guttation water are above the median case for the area of use of the substance. In

these field studies both the concentrations in the guttation water and the effects on the bee hive have to be assessed. The selection of the soil and meteorological conditions for these field studies can for the spray and granule applications be selected based on the EFSA pore water scenarios used in box 4. For the seed treatments the selection can be based on the field experiments performed in box 7. For the number of fields/replicates to achieve a sufficient power to detect effects please see chapter 4.

Risk mitigation for exposure to guttation

From the available information it is evident that effects on bees from exposure to guttation water were only observed when no alternative sources of water were in the vicinity of the hive. The provision of water could mitigate the risk.

The distance of the colony to the field where guttation occurs is also of importance. Guttation was observed very frequently in grasses and in the vegetation outside of the field. Such vegetation could be more attractive for bees to collect guttation water then the crop plants. Furthermore the available data suggest that bees prefer permanent water sources to guttation droplets. Therefore a vegetated buffer strip and permanent water bodies in the vicinity of the field could mitigate the risk from guttation water. It could be an option to restrict uses (planting of seed treated crops) to fields where permanent water bodies such as ponds or streams are in the close vicinity. However, the available information is not sufficient to give an exact recommendation on the minimum distance to the next permanent water body that is needed to avoid that bees use guttation droplets from treated fields. Research would be needed to investigate the distance at which permanent sources of water are preferred over guttation droplets collected in the field.
In principle it would also be possible to develop a tier based on a landscape-level approach for guttation water considering all the other guttating plants in the foraging area, e.g. based on a criterion that less than a specified percentage of the water foragers will collect contaminated guttation water. However, current knowledge seems insufficient to develop such an approach.

Another option is to provide the bee colonies with an alternative water source. This should be considered at MSs level. At this moment it is not yet clear whether this is acceptable across the EU.
Overall it is concluded that more information is needed to decide on the efficiency of different risk mitigation options.

2.2. Assessment of risk from exposure to surface water

As bees will drink from surface water present in the agricultural environment, it is proposed to consider the possible effects of consumption of surface water by bees. In the first instance, it is proposed to base this on checking whether the triggers for the acute and chronic adult ETR and larvae ETR are met as calculated with Eqns 1, 2 and 3 using again a daily water consumption W of 11.4 µL for adult bees and 111 µL (5 days) for larvae.

As regards the PEC, the regulatory acceptable concentration (RAC) from the aquatic risk assessment should be used. It should be noted that the highest RAC from the aquatic risk assessment should be used as the PEC because this is most conservative

It is expected that the RAC from the aquatic risk assessment is low enough in order not to lead to any effects on bees drinking from surface water. Only in case of substances which are particularly toxic to bees compared to aquatic arthropods (crustaceans and insects) there could be a risk to bees. In such cases a potential risk would be indicated by the first tier calculation above.

If the triggers are not met, the exposure in surface water can be mitigated following the procedures described by FOCUS (2001; 2007a,b). Please note that this does not imply acceptance of these

421 procedures by EFSA because EFSA never reviewed FOCUS (2001) which formed the basis for
422 FOCUS (2007a,b).
423

424 ## 2.3. Assessment of risk from exposure to water in puddles

425 Bees may also consume water from puddles in fact there is some evidence to indicate that they even
426 seem to prefer puddle water over water from streams and ditches. EFSA (EFSA 2012a, p. 218)
427 reviewed the assessment of the concentrations in puddle water by EFSA (EFSA 2008a) and concluded
428 that it may not be sufficiently conservative. EFSA (2012b) recommended that the concentrations in the
429 puddle water are estimated from the concentrations in the runoff water from the FOCUS runoff
430 scenarios (R1-R2-R3-R4, see FOCUS, 2001) relevant for the use.
431
432 It is proposed to check as a first tier whether the triggers for the acute and chronic ETR for adult and
433 the ETR for larvae are met as calculated with Eqns 1 and 2 and x using again a daily water
434 consumption *W* of 11.4 µL/bee and 111 µL/larvae (5days larvae) using the concentrations in the
435 runoff water from the four FOCUS runoff scenarios. The peak concentration of each of the relevant
436 R1-R4 scenarios should be calculated and the highest value should be taken. The justification for this
437 conservative approach is that EFSA has not yet evaluated the appropriateness of these FOCUS
438 scenarios. Please note that FOCUS (2001) provided guidance only for running these scenarios for
439 spray applications; guidance for running them for seed treatments and granules can be found in EFSA
440 (2004).
441
442 The concentrations in the runoff water of the R1-R4 scenarios may be considerably higher than the
443 concentrations in the surface water of these scenarios. This is due to FOCUS (2001) assuming that
444 only 20% of the upstream catchment of the stream is treated with the substance and that concentrations
445 from runoff events generate small water volumes that may then be strongly diluted in the streams.
446 Moreover, the normal risk mitigation measure used for surface water (runoff reduction by buffer
447 strips) is not relevant for the consumption of puddle water by the bees. It is therefore desirable to
448 develop a probabilistic higher-tier approach for the concentration in puddle water that is targeted at the
449 90th percentile worst-case exposure for the hives at edges of treated fields in the area of use of the
450 substance. This approach has to combine the likelihood of occurrence of puddles in the treated fields
451 in the first months after application of the substance with the concentrations in the puddle water.
452 However, the development of such an approach was not possible within the time frame of the writing
453 of this guidance document as it would require a considerable amount of work and expertise in the field
454 of soil physics which was not available to the workgroup.
455

A decision needs to be taken whether the first conservative tier for the exposure to puddle water should be implemented in the risk assessment procedure or whether this should wait until also the higher-tier approach has been developed.

456
457
458

459 # 3. Risk assessment scheme for metabolites

460 Sinclair (2009) investigated the toxicity of metabolites in relation to the parent compound of several
461 PPPs (60 a.s. and 485 transformation products) to aquatic organisms and demonstrated that the
462 majority (70%) of transformation products had either a similar toxicity to the parent compound or are
463 less toxic. However, a significant proportion (30%) were more toxic than their parent compound and
464 4.2% of transformation products were more than an order of magnitude more toxic. Over 90% of the
465 observed increases in toxicity of the metabolite could be explained by the presence of a toxophore,
466 differences in accumulation (i.e. hydrophobicity) or differences in mode of action (for example active
467 components of pro-PPPs or highly reactive metabolites). Furthermore, the investigation showed that a
468 transformation product that is more hydrophobic than its parent compound and does not have
469 pesticidal activity is unlikely to be more toxic than its parent to sensitive species that have a receptor

site relevant to the parent mode of action. This information is integrated in the risk assessment scheme below.

The proposed risk assessment scheme for metabolites covers only metabolites that might occur in the pollen and nectar. The scheme does not cover metabolites that may be present in guttation fluid, honey dew, surface water and puddles. Depending upon the design of the plant metabolism study may mean that metabolites present in the soil and subsequently taken up by the plant *may* not be covered. If the plant metabolism study includes exposure of the soil then this route *may* be covered. Similarly, if the study is designed to assess metabolism in following crops, then soil metabolites *may* be addressed. Further work is required to develop a scheme that covers all potential metabolites.

As a starting point the information from plant metabolism studies is used. These studies are designed to identify metabolites at usually one point in time. Each metabolite exceeding 10% (total radioactive residues or TRR) or 0.01 mg/kg is identified in the plant metabolism study. These studies do not necessarily cover the flowering of the crop. In the following scheme, the metabolism in the crop is extrapolated to other plants e.g. adjacent crops or weeds. This leads to uncertainties in the assessment but in the absence of other data it is proposed to use the plant metabolism studies in the first tier.

If a well designed field study is conducted and the presence of metabolites was confirmed then the risk to metabolites is considered to be covered and no separate assessment for the metabolites needs to be performed.

1. Identify plant metabolites from plant metabolism studies in which the parent substance is applied in the same way as for the intended use. For following crops this should include application to bare soil. Are there any identified metabolites formed in amounts of >10% (TRR) or 0.01 mg/kg ?

   Yes: Go to 2
   No: No further assessment is required.

2. Is it clear that the toxophore relevant for the toxicity to bees has been lost from the molecule (see Note1)?

   Yes: No further assessment is required
   No or unclear: Go to 3

3. Calculate the acute and chronic ETR values based on 10 times higher toxicity than the parent compound. Multiply the RUD value for the parent compound with the maximum percentage of the metabolite (TRR) observed in plant metabolism studies in any matrix analysed (except roots) to estimate the exposure. The following equations should be used:

$$PECmet = Ftrr \times M(met)/M(par) \times RUDpar \times AP \text{ (Application rate)}$$

PECmet = PEC metabolite
Ftrr = Fraction of metabolite formed (% of total radioactive residues)
M(met) = Molar mass of the metabolite
M(par) = Molar mass of the parent molecule
RUD(par) = Residue per unit dose of the parent molecule
AP = Application rate

The ETR values need to be calculated for adult (acute and long-term) and larvae. First tier ETR trigger values breached?

Yes: Go to 4
No: No further assessment is required.

4. Determine the acute and chronic toxicity to adult bees and larvae specific for the metabolite (e.g. experimentally derived or QSAR) and calculate the first-tier ETRs (the same assessment as for the parent compound). First tier ETR trigger values breached?

Yes: Consider higher tier refinement
No: No further assessment is required.

Note1: Identification of toxophore

Substances that have a specific mode of action, like pesticides, contain a structural feature or moiety that gives the toxic property. This structural feature is referred as the toxophore, or toxophoric moiety. The substance causes toxicity through the interaction of its toxophore with a biomolecular site (e.g., receptor). Substances that are structurally similar could contain the same toxophore (or may yield a common toxophore upon metabolism) and may therefore have a common toxic effect.

For the assessment of the metabolite it may be possible for the applicant to provide a reasoned case as to if the molecule contains a toxophore or if it has been lost following transformation. Toxophores for each of the major classes of PPP have been identified by looking for sub-structural similarities within a pesticidal class by Sinclair *et al*. (2009), which can be used to support argumentation. A number of ways have been identified to define domain of applicability, which may be used to decide if toxophores are present or not (Nikolova and Jaworska 2003; Dimitrov *et al*. 2005; Jaworska *et al*. 2005; Netzeva *et al*. 2005). In case it cannot be clearly shown that the toxophore is not present in the molecule it should be assumed that the toxophore remains and that the molecule has a specific mode of action.

3.1. Alternative information replacing experimental studies

The principles for assessing metabolites should in essence be the same as those for active substances. However, in contrast to the active substance, data requirements for metabolites do not always have to be addressed by experimental studies. Applicants are invited to address the open questions by any other available information in support of a scientific and rational assessment. If chemical analysis confirm that the metabolite was present in the pollen and nectar of the original test (e.g. field study) then it can be concluded that the risk from the metabolite is addressed by this study providing that exposure of foragers to the required concentration has been achieved. Furthermore, for this extrapolation to be valid it is also important that the time period after the measured metabolite concentration was of sufficient length for observation of effects.

563 Toxicity testing with metabolites

564 For metabolites which require experimental studies, the same testing scheme as for active substances
565 is generally required. As regards the issue of accumulative toxicity, if the active substance is
566 considered to fail the Haber's Law test, then it is assumed that the metabolite(s) will as well. This is
567 accepted as being worst case. In this situation when the risk from the active substance is refined, it is
568 important to consider the risk from the metabolite(s) as well.

569 **3.2. Risk Assessment for Metabolites**

570 In principle, the risk assessment process for metabolites will be similar to that for active substances,
571 albeit recognizing that risk assessment cases will not always require specific study data for certain
572 metabolites. If preliminary risk assessments indicate potential concerns then, as for parent molecules,
573 risk refinement is possible either by refining effect concentrations or by refinement of the exposure
574 concentration.

575 If higher-tier studies have been conducted with the active substance, or a relevant formulation, these
576 studies may have also assessed the risk from the metabolites. It is advised that if a higher-tier study,
577 e.g. field study, is being carried out then appropriate analysis should be conducted so that an
578 assessment of both the exposure and effects of any metabolites can be made.

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583 **4. Uncertainty analysis**

584 **4.1. Approaches for characterising uncertainty in higher-tier assessments[8]**

585 Regulation (EC) No 1107/2009 lists under Annex II criteria for approval of active substances, safeners
586 and synergists under 3.8 Ecotoxicology, point 3.8.1 "…*The assessment must take into account the*
587 *severity of effects, **the uncertainty of the data**, and the number of organisms groups which the active*
588 *substance, safener or synergist is expected to affect adversely by the intended use."* This implies that
589 uncertainties in the data should be considered.

590 Regulation (EC) No 1107/2009 refers for decision making to Annex VI of Directive 91/414.

591

592 Point 2.5.2.1 in Annex VI to Directive 91/414/EEC states that no authorisation shall be granted unless
593 it is "clearly established" that no unacceptable impact occurs. The term 'clearly establish' implies a
594 requirement for some degree of certainty. First-tier assessments use standardised scenarios and
595 decision rules which are designed to provide an appropriate degree of certainty. Higher tier
596 assessments are not standardised, and so the degree of certainty they provide has to be evaluated case
597 by case. The need for risk assessments to include characterisation of uncertainty has also been
598 emphasised at senior policy levels in the EU[9] (see also Sterling 2010).

599 Methods for characterising uncertainty can be grouped into three main types:

600 • Qualitative methods: using words to describe the certainty of an outcome, or to describe how
601 different the true outcome might be compared to an estimate.

[8] After paragraph 6.8 and 6.9 of Bird and mammal guidance document (EFSA 2009).
[9] E.g. "Even though it is not a subject that lends itself easily to quantification, I would urge you to take account of the risk manager's need to understand the level of uncertainty in your advice and to work towards a systematic approach to this problem." (Madelin, 2004).

602 • Deterministic methods: generating deterministic quantitative estimates of impact for a range of
603 possible scenarios. This shows the range of possible outcomes (e.g. a range of ETRs) and can be
604 accompanied by qualitative descriptions of their relative probabilities (traditional 'worst-case'
605 assessments are an example of this).

606 • Probabilistic methods: these give numeric estimates of the probabilities of different outcomes
607 (Luttik *et al.* 2011). These probabilities may be estimated statistically (e.g. when quantifying
608 measurement or sampling uncertainty, or as outputs from probabilistic modelling). However, they
609 may also be estimated subjectively, by expert judgement.

610 All uncertainties affecting an assessment should be considered at least qualitatively. To reduce the risk
611 of overlooking important uncertainties, it is recommended to systematically consider each part of the
612 assessment (e.g. different lines of evidence, different inputs to calculations, etc.) and list all of the
613 sources of uncertainty together with a description of the magnitude and direction of their potential
614 influence on the expected level of impact. As well as evaluating each individual source of uncertainty,
615 it is also essential to give an indication of their combined effect. It is recommended to use a tabular
616 approach to facilitate and document this process, as illustrated in Tables 1 and 2. This is based on an
617 approach used in some EFSA opinions (EFSA, 2005; 2007a; 2007b; 2008b), but adapted to increase
618 clarity by introducing separate columns to describe uncertainties that act in different directions.

619 Research in social science has shown that there is a general tendency for experts to underestimate
620 uncertainties. It is therefore important that risk assessors should be aware of the potential magnitude of
621 common uncertainties in the assessment of risks to organisms. For example, assessors should be aware
622 of the potential magnitude of measurement uncertainties (e.g. methods used for determining the
623 number of dead bees (i.e. forager mortality) and of the potential magnitude of sampling uncertainty
624 associated with small and moderate sized datasets).

625 In some cases, a qualitative evaluation of uncertainties may be sufficient to establish clearly (i.e. with
626 sufficient certainty) that unacceptable levels of impact will not occur, as it is required by the 'unless'
627 clause in Annex VI. In other cases, a purely qualitative evaluation of uncertainty may not give a
628 sufficiently clear picture of the range of possible outcomes. In such cases, one option is to obtain
629 additional data to reduce uncertainty. This may usefully be targeted on the uncertainties that appeared
630 largest in the qualitative evaluation. However, an alternative option is to refine the characterisation of
631 the uncertainties progressively, by evaluating some of them using first deterministic methods and then,
632 if necessary, probabilistic methods. This implies a tiered approach to the treatment of uncertainties,
633 which starts by evaluating all uncertainties qualitatively and progresses either by reducing uncertainty
634 (by obtaining additional data) or by refining the evaluation of selected uncertainties (either
635 deterministically or probabilistically), until the point where it can be 'clearly established' whether an
636 unacceptable impact will occur (as required by the 'unless clause in Annex VI).

637 **Table 1:** Tabular approach recommended for qualitative evaluation of uncertainties in refined
638 assessments. The +/- symbols indicate whether each source of uncertainty has the potential to make
639 the true risk higher (+) or lower (-) than the outcome of the refined assessment. The number of
640 symbols provides a subjective relative evaluation of the magnitude of the effect (e.g. +++ indicates an
641 uncertainty that could make the true risk much higher). If the effect could vary over a range, lower and
642 upper evaluations are given (e.g. + / ++). If possible, the user should indicate the meaning of different
643 numbers of symbols (e.g. two symbols might be used to represent a factor of 5, and three symbols a
644 factor of 10). See Appendix C for some practical examples.

Source of uncertainty	Potential to make true risk lower	Explanation	Potential to make true risk higher	Explanation
Concise description of first source of uncertainty	Degree of negative effect	Short narrative text explaining how this factor could make true		

	(e.g. - - -)	risk lower		
Second source of uncertainty			Degree of positive effect (e.g. +++)	Short narrative text explaining how this factor could make true risk lower
Add extra rows as required for additional sources of uncertainty	-	Note: many uncertainties may act in both positive and negative directions	+	
Overall assessment	Narrative text describing the assessor's subjective evaluation of the overall degree of uncertainty affecting the assessment outcome, taking account of all the uncertainties identified above. The overall assessment should be a balanced judgement and not simply a summation of the plus and minus symbols.			

645 It is unlikely that it will ever be practical – or necessary – to quantify all uncertainties, so every
646 deterministic or probabilistic assessment should be accompanied by a qualitative evaluation of the
647 unquantified uncertainties. Also, it should be remembered that deterministic and probabilistic methods
648 often require assumptions (e.g. about distribution shapes) that are themselves uncertain, and these
649 additional uncertainties should be included in the qualitative evaluation. Therefore, every refined
650 assessment should contain at least a qualitative evaluation of uncertainties.

651 The overall magnitude of uncertainty associated with an assessment will often be very large. This
652 should not be regarded as implying a failure of risk assessment; on the contrary, it provides essential
653 information for decision-making (Madelin 2004; Stirling 2010).

654 It should be noted that for pesticides where several different types of refined assessment are used, the
655 uncertainties affecting each one will be different. In such cases it is recommended to evaluate the
656 uncertainties affecting each approach separately. The contribution of the multiple assessment
657 approaches (multiple lines of evidence) in reducing overall uncertainty can then be evaluated by
658 weight-of-evidence in the final risk characterisation (see next section).

659 Appendix C provides some further information on the types of issues that should be considered when
660 determining the uncertainty in higher tier studies. Appendix C also contains a brief worked example.

661 In summary, it is recommended that:

662 • Every refined risk assessment should be accompanied by at least a qualitative evaluation of the
663 uncertainties affecting it, using a systematic tabular approach. In assessments with multiple lines
664 of evidence, the uncertainties affecting each line of evidence should be evaluated separately.

665 • In cases where qualitative evaluation of uncertainty is not sufficient to determine whether it is
666 clearly established that no unacceptable impact will occur, the assessor may either (a) seek further
667 data to reduce the uncertainty, or (b) refine the evaluation of the existing uncertainties using
668 quantitative methods (which can be either deterministic or probabilistic).

669 **4.2. Risk characterisation and weight-of evidence assessment**

670 Risk characterisation is the final step of risk assessment. At this point, all relevant information or
671 evidence that has been gathered is used to produce an overall characterisation or description of the
672 risk, in a form that is suitable for decision-making.

673 To be useful for decision-making, the risk characterisation should focus on evaluating whether the
674 relevant protection goals are satisfied for the pesticide under assessment: the magnitude of effects on
675 colonies should not exceed 7% reduction in colony size and forager mortality should not be increased
676 compared to controls by a factor of 1.5 for 6 days or a factor of 2 for 3 days or a factor of 3 for 2 days.
677 Often, risk characterisation will involve combining several different types of refined assessment, each
678 providing a separate indication of the risk. For example, an applicant might submit a refined exposure

assessment, together with some additional toxicity studies and/or a proposal for mitigation. These need to be integrated in an overall risk characterisation that takes appropriate account of each, so as to provide the best basis for decision-making. This process of combining available 'lines of evidence' to form an integrated conclusion or risk characterisation is frequently referred to as 'weight-of-evidence' assessment (e.g. EC, 2002; Hull and Swanson, 2006). This term reflects the principle that the contribution of each line of evidence should be considered in proportion to its weight.

It is recommended that the following approach is taken regarding a weight-of-evidence assessment:

- Consider all relevant lines of evidence, including the first-tier assessment. Retention of the first-tier assessment is appropriate in all cases, as it is relevant to consider whether it was borderline or failed by a large margin.
- Evaluate the uncertainties associated with each line of evidence. This should be done by applying the approaches described in the preceding section to each line of evidence separately. The characterisation of overall uncertainty for each line of evidence is then used in the weight-of-evidence assessment, as in principle the weight given to each line of evidence should be proportionate to its certainty.
- Form overall conclusions by using expert judgement to combine all lines of evidence, weighted according to their certainty, and give more weight to the most certain, but also take due account of the less certain. High certainty implies high weight. If one line of evidence implies a much narrower range for the risk than another line of evidence (i.e. higher certainty), then the true risk is most likely to fall inside the range of the former.
- Be sure to take full account of the uncertainties and to include a fair description of the range of possible outcomes in the final risk characterisation. Identify the outcome that is considered most likely, but do not give it more emphasis than is justified by the evidence.
- If different lines of evidence conflict (e.g. a high ETR but no effects in a field study), this should be considered a form of uncertainty. No line of evidence should be completely discounted unless it is wholly invalid or irrelevant. Instead, as stated above, each line of evidence should contribute to the overall conclusion in proportion to its certainty.
- If the overall characterisation of risk is expressed qualitatively, choose words very carefully to describe the outcome and its uncertainty as clearly as possible. For example the phrase 'on balance' is often used to focus on one of several possible outcomes, e.g. "on balance, it is concluded there will be no mortality". This type of statement is not appropriate, because it fails to communicate the degree of certainty (e.g. 'on balance' could mean 51 % certainty, or 99 %)[10].
- A weight-of-evidence assessment is inevitably subjective. Different assessors may vary in their weighing of the evidence, especially when uncertainty is high. Therefore, it is essential to document the assessment in detail, including the outcome and uncertainty for each lines of evidence considered, and explaining how they were combined to reach conclusions about the overall outcome and its uncertainty.

It is recommended that a systematic tabular approach to documenting the weight-of-evidence assessment, such as that illustrated in Table 2. The tabular format provides a concise yet clear summary of the lines of evidence considered and how they were combined. It also helps the reader to evaluate whether the assessment was balanced, and aids consistency of approach between pesticides.

It should be noted that Table 2 summarises the major types of uncertainty for each line of evidence, and not just the overall uncertainty. This is recommended because it helps the assessor to take account of some important strengths and weaknesses of different types of refined assessment (see for instance EFSA (2009)).

[10] Note that the standard of evidence required by the 'unless' clause is 'clearly establish', which is much stronger than 'on balance'.

724 The subjectivity of weight-of-evidence assessment can impede the formation of an independent view
725 when this is based on the assessment of another person. Therefore, when a weight-of-evidence
726 assessment is submitted by an applicant, it would be prudent for the regulatory authority to conduct
727 their own weight-of-evidence assessment separately, compare their conclusion with that of the
728 applicant, and consider the reasons for any differences.

729 It is sometimes objected that characterising uncertainty is unhelpful in decision-making. In fact, it is
730 essential for risk assessors to characterise uncertainty, as is clear from Directive 91/414/EEC ('clearly
731 establish') and from policy statements by the European Commission (Madelin, 2004). Furthermore,
732 practical options exist for dealing with uncertainty in decision-making. Two of the principal options
733 are to request more data to reduce uncertainty, or to request more refined evaluation or analysis of the
734 existing uncertainty. A third option is to counter the uncertainty by applying risk mitigation options, so
735 that the chance of adverse impacts is limited to an acceptable level[11]. However, choosing between
736 options for dealing with uncertainty involves risk management considerations outside the scope of this
737 document such as the acceptability of effects, the degree of certainty required and potentially other
738 factors such as the cost and time required for further refinement, the need to respect legal deadlines for
739 authorisations, and the consequences of risk mitigation or non-authorisation (e.g. reduced efficacy,
740 reduced choice of pest control options in agriculture, risk of resistance, etc.).

741 In summary:

742 • Every refined risk assessment should conclude with an overall characterisation of risk, in terms
743 relevant for decision-making. It is recommended to begin with the consideration of whether the
744 evidence makes any mortality or reproductive effects unlikely (the surrogate protection goal).
745 Where this is not satisfied, attention should turn to characterising the levels of mortality and
746 reproductive effects that may occur, and using this to evaluate whether there is a high certainty that
747 the magnitude of effects on colonies should not exceed 7% reduction in colony size and that
748 forager mortality should not be increased compared to controls by a factor of 1.5 for 6 days or a
749 factor of 2 for 3 days or a factor of 3 for 2 days

750 • The overall characterisation of risk should be derived by a qualitative weight-of-evidence
751 assessment considering all relevant lines of evidence and their uncertainties using a systematic
752 tabular approach (e.g. Table 2). If the overall characterisation is expressed qualitatively (in words)
753 rather than quantitatively, great care should be taken to describe the outcome and its uncertainty as
754 clearly as possible.

755 • The first-tier assessment should always be included as one of the lines of evidence, and given
756 appropriate weight (this will be higher for acute risks of sprayed pesticides than for other types of
757 assessment).

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759 **Table 2:** Tabular approach recommended for qualitative weight-of-evidence assessment,
760 summarising the conclusion and uncertainties for several lines of evidence and using them to develop
761 an overall conclusion. See Appendix C, Tables C3 and C4 for practical examples. The +/- symbols
762 indicate whether each source of uncertainty has the potential to make the true risk higher (+) or lower
763 (-) than the indicated outcome. The number of symbols provides a subjective relative evaluation of the
764 magnitude of the effect (e.g. - - - might indicate an uncertainty that could reduce risk by an amount
765 equivalent to reducing a TER by about a factor of 10). If the effect could vary over a range, lower and
766 upper evaluations are given (e.g. - / ++ or + / ++).

	Lines of evidence *(add more columns if appropriate)*		
	First-tier assessment *(should*	Second line of	*Add one column for*

[11] "In cases where both the potential risk and scientific uncertainties are high, the risk manager may conclude that a precautionary approach is appropriate." (Madelin, 2004).

	always be included)	evidence	*each line of evidence*
Main contributions to uncertainty:			
Concise description of first major source of uncertainty	+ and – symbols (see legend)		
Second uncertainty			
Add one row for each major source of uncertainty			
Conclusions for individual lines of evidence	Insert overall assessment for each line of evidence		
Overall conclusion	Insert overall conclusion giving appropriate weight to each line of evidence, taking account of their relative certainty (more uncertainty = less weight). The overall conclusion should be a balanced judgement and not simply a summation of the plus and minus symbols.		

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866 **APPENDICES**

Name	Appendix Title
A	BACKGROUND TO THE EXPOSURE ESTIMATES AND TRIGGER VALUES USED IN THE RISK ASSESSMENT FOR GUTTATION
B	TEST PROTOCOLS TO ASSESS THE EFFECTS OF PESTICIDES IN GUTTATIONS ON HONEY BEES
C	ASSESSMENT OF UNCERTAINTY
D	TRIGGER VALUES

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A. Background to the exposure estimates and trigger values used in the risk assessment for guttation

For seed treatments the estimation of the time weighted average concentrations expressed as a percentage of water solubility is based on available measurements as model simulations are yet available. EFSA (2012a) provided an overview of available measurements in guttation water of plants grown from treated seeds and this will be used in the following estimation.

The vast majority of the measurements were carried out with maize seeds treated with imidacloprid, clothiadin and thiamethoxam at rates ranging from 0.5 to 1.25 mg per seed. The few measurements of concentrations in guttation water available for other crops (winter oil seed rape, winter barley, sugar beet and wheat; see Figure H7 of EFSA, 2012a, and Reetz et al., 2011) show concentrations that are considerably lower than those found for maize. The estimated values have been based on the results for maize as this is expected to result in conservative estimates for all crops.

Most of the measurements for imidacloprid, clothianidin and thiamethoxam in maize guttation water consider the course of time of the concentration after emergence. These measurements usually show a sharp exponential decline in the concentration water in the first few weeks after emergence of the guttation fluid. The highest value found for imidacloprid in field studies was about 250 mg/L (Figure H5 of EFSA, 2012a). The highest value found for clothianidin in field studies was about 100 mg/L (Figure H7 of EFSA, 2012a). The highest value found for thiamethoxam in field studies was 172 mg/L (Table H1 of EFSA, 2012a). However, in a greenhouse study under extremely dry conditions a maximum thiamethoxam concentration as high as 1154 mg/L was found (Tapparo *et al*., 2011). The water solubility of imidacloprid is 610 mg/L, that of clothianidin is 340 mg/L and that of thiamethoxam is 4100 mg/L (FOOTPRINT database). Based on this limited information we propose to assume as a default estimated peak concentration 40% of the water solubility, i.e. the max residues were never more than 40% of water solubility..

Figure H7 of EFSA (2012a) contains also concentrations in maize guttation fluid of methiocarb showing a maximum of about 5 mg/L. The water solubility of methiocarb is 27 mg/L (FOOTPRINT database) which would give a default of about 11 mg/L so indeed above the measured maximum of 5 mg/L. Table H1 of EFSA (2012a) gives fipronil concentrations of 46 and 77 mg/L in maize guttation fluid from a laboratory study. However, the water solubility of fipronil is about 4 mg/L (FOOTPRINT database) so these measurements are unlikely to be reliable. Therefore, on the basis of these data, it is considered that the above proposal to use 40% of the water solubility is sufficiently precautionary.

The available measurements of the course of time of the concentration usually show an exponential decline (Figures H2 to H5 of EFSA, 2012a). As the underlying data were not available, declines were fitted visually by drawing a straight line and the following were obtained:

1. half-lives of 3.3, 3.6 and 4.6 days for clothianidin from Figures H2 and H4,
2. a half-life of 2.3 days for imidacloprid from Figure H5, and
3. a half-life of 3.0 days for thiamethoxam from Figure H2.

Figure H7 showed first an increase of the concentration of clotianidin up to the maximum of about 100 mg/L followed by a sharp decrease. This decrease could be described with a half-life of 1.1 days. Based on this information it is proposed to use a half-life of 5 days to calculate the estimated TWA concentrations in guttation fluid. This is considered to be conservative. In case semi-field studies are available (box 7 of the flow chart in Figure 1), it is preferable to derive the TWA from the measured decline in these studies.

Thompson (2010) showed data from a Swiss field study on decline of clothianidin concentrations in guttation water of maize seedlings: the concentration was initially about 30 mg/L and it declined below 15 mg/L within 5 days. Reetz *et al*. (2011) found initial concentrations of clothianidin of about

928 8 mg/L in a German field study and this concentration decreased to below 1 mg/L within a week.
929 Therefore, on the basis of the above, the proposed time course of the concentration in the guttation
930 fluid is considerably more conservative than these findings.
931
932 In case of exponential decline, the TWA concentration can be calculated with:
933
934 $C/C_0 = (1\text{-}e^{-kt}) / k\, t$
935 (3)
936
937 where C is the concentration as a function of time, C_0 is the concentration at the start, k is the rate
938 coefficient of the decline (equal to ln 2 divided by half-life) and t is the time period for averaging.
939 Using a half-life of 5 days for t = 10 days, gives C/C_0 = 0.54, so the 10-d TWA concentration can be
940 obtained by multiplying the peak concentration with 0.54. So this becomes 0.54 × 40% = 22% of the
941 water solubility. Similarly the 5-d TWA concentration becomes 29% of the water solubility.
942
943 **Larval water consumption** - the assessment of larvae exposure is based on the conservative
944 assumption that all the larvae food is from honey which is diluted with contaminated water.
945
946 It is assumed that a honey bee worker larva needs 59.4 mg sugar and 1.5-2 mg pollen per 5 days
947 (EFSA 2012a, Appendix D). If the lowest pollen value is used, the food consumption is 60.9 mg dry
948 material over 5 days (i.e. 59.4 mg + 1.5 mg = 60.9 mg dry material in their food.
949 The water content of larvae food is 73.51% for young larvae within the first two days and 64.9% for
950 older larva from day 3-5 (Haydak, 1943). The corresponding dry matter percentages are 26.49 % for
951 young larvae and 35.1 % for old larvae. The amount of water over 5 days is calculated as 169 mg
952 (60.9 mg/26.49*73.51) or 112.6 mg (60.9 mg/35.1*64.9) for young and old larva, respectively. In this
953 calculation, the honey is assumed to be uncontaminated and the water content of honey is assumed to
954 be 18% (White, 1976). The consumption of contaminated water was therefore 138.6 mg and 92.3 mg.
955 The average over 5 days from consumption of larvae food with 73.51% water (2 days) and larvae food
956 with 64.9% water (3 days) was 110.82 mg over 5 days. For the following calculations this has been
957 rounded to 111 mg (assumed equal to 111 µL) over 5 days.
958 The water consumption was also calculated with other methods resulting in slightly lower water
959 consumption rates.
960 The use of a 5 d time weighted average PEC is proposed since the half life for the decline of residues
961 in guttation is assumed to be very short (see above). It is acknowledged that the use of a TWA
962 concentration may underestimate the exposure of the first larval stages which consume more water in
963 relation to their body weight than the older larval stages. However the loss of early larval stages from
964 a peak exposure would not have such a high energetic cost for the colony than loosing later larval
965 stages. The exposure of later larval stages is covered by the time weighted average approach and
966 hence considered to be protective enough.
967
968 **Worker bee water consumption** –the assessment of adult worker bee exposure is based on a water
969 consumption of 11.4 µL/bee. This water consumption is based on Free & Spencer-Booth (1958) who
970 measured water consumptions ranging from 5.8 to 11.4 µL/d at 35°C. At 30°C they found much lower
971 water consumption than at 35C (at most 0.8 µL /d). In the hive, adult workers keep the brood
972 temperature between 32 °C and 36 °C with a mean of 34.5 °C (Himmer, 1927; Kronenberg and Heller,
973 1982; Seeley and Heinrich, 1981). However, Becher *et al.* (2010) showed that the in-hive temperature
974 linearly decreased from the core of the brood nest to the periphery with a slope of 0.45 °C/cm. Thus,
975 11.4 µL/bee is considered to be a conservative value.
976
977 **TER trigger for drinking guttation water**
978 The trigger values were calculated according to the methodology outlined in Appendix D.
979 M = mortality (background mortality)
980 E = exposure (=dose)
981 M = E x 50/LD50
982 E = M x LD50/50

983
984 The ETR trigger is calculated as 0.106, 0.156 and 0.26 for background mortalities of 5.3, 7.8 and 13.
985 Given the limited dataset on background mortality it is proposed to use the most conservative value of
986 0.106.
987
988

B. Test Protocols to assess the effects of pesticides in guttation on honey bees

The residues in guttation droplets represent a potential exposure route for bees. Specific test protocols in semi-field and field conditions are required to assess the effects of guttation to honey bees if the risk is not acceptable in the first tier. In this section the recommendations on how to carry out these tests are provided.

SEMI-FIELD TEST

In general the test should be designed as proposed in appendix O of the final Guidance Document but specific recommendations listed below have to be considered:

1. Test crop: the study should be carried out in the crop where the plant protection product will be registered. The study should be performed at the emergence of the crop or when the plants are in very young stages because at this moment the residues concentrations in guttation droplets are higher. It is important to irrigate the soil in order to maintain good humidity conditions for the production of guttation, but puddles must be avoided.
2. Duration of the study: it is recommended that the bees are exposed to the guttation fluid for two weeks. This exposure period is considered a good compromise between the duration of guttation in the field and the maximum period of confinement of the colonies in the tunnel (or cage, or tent) before a colony decline. However, after exposure in the tent, the observations should last for another 28 days in open-field studies. A pre-exposure period (5 days) is required before introducing the colonies in the tunnel. During this period bee mortality should be recorded and should demonstrate stable background mortality.
3. Treatments: tunnels (or cages or tents) with treated and untreated crops (control) have to be used.
4. Assessments: the occurrence of the guttation fluid and the number of dead bees (in the dead bee traps and on linen sheets) should be recorded every day during the study. The residue analyses must be performed on the guttation fluid (at emergence and at several successive assessments during the study) and dead bees (only in case of abnormal mortalities). Colony development should be assessed as proposed in appendix O of the final GD.
5. Feeding: honey bees must be fed with sugar paste and pollen (free of pesticide contaminations) during the study. The pollen quality must be the same in the control and treated tunnels.
6. No water source must be supplied during the exposure period.
7. The tunnels should be covered in order to avoid the dilution of the active ingredient by rain and that the bees can take the water from the rain and not from the guttation.

FIELD TEST

In general the test should be designed as proposed in appendix O of the final Guidance Document but specific recommendations listed below have to be considered:

1. The test crop: the study should be carried out in the crop where the plant protection product will be registered. The study should be performed at the emergence of the crop or when the plants are in very young stages because in this moment the residues concentrations in guttation droplets are higher.
2. Location of the colonies in the field: colonies should be placed at the edge of the treated fields in order to maximize the exposure to guttation.
3. Duration of the study: from emergence of the crop up to 6 weeks after emergence. The colony survival after wintering should be recorded.
4. The test is considered valid if at least one guttation event occurs
5. Assessments: the occurrence of the guttation and the number of dead bees (in the dead bee traps and on linen sheets) should be recorded every day during the study period. The residue analyses must be performed on the guttation (at emergence and at several successive assessments during the study) and dead bees (only in case of abnormal mortalities). Colony development should be assessed as proposed in appendix O.

1042 6. Feeding: food should be provided via additional honey combs in the hives or sugar paste in case
1043 no forage is available during the test (e.g. in autumn).
1044 7. Permanent water sources should be located as far away as possible from the hives and test fields
1045 (a minimum distance of 200 m was chosen arbitrarily because considered applicable).
1046

1047 **C. ASSESSMENT OF UNCERTAINTY**

1048
1049 As outlined in the chapter on uncertainty every refined assessment should contain at least a qualitative
1050 evaluation of uncertainties. Outlined below is some guidance aimed at aiding the determination of
1051 uncertainty in higher tier studies. The guidance falls into two separate sections. The first is aimed at
1052 providing an indication of the type of questions or issues that should be considered by a risk assessor
1053 when they are assessing higher tier studies. It should be noted that this list is not exhaustive and will
1054 vary from study to study. The second section is a brief illustration of the assessment of uncertainty for
1055 two fictitious datasets. It should be noted that is only a brief example and is aimed at highlighting the
1056 way in which such an assessment could be presented.
1057
1058 **Uncertainty analysis for individual higher tier studies (residues studies and effects studies)**
1059 Outlined below is a proposal for a checklist to characterize the uncertainty in the higher tier studies.
1060 The points listed are not definitive or exhaustive and will change from study to study. The outcome of
1061 the analysis of this assessment can feed in the overall assessment of uncertainties (as in the tables
1062 below).

1063 The example below is for an application via a spray and covers both the exposure and effects part. It is
1064 provided for illustrative purposes only. It is provided to highlight the types of questions that should be
1065 considered by the risk assessor when they are evaluating higher tier studies.

1066 This type of assessment should be repeated for all exposure scenarios and accompanying assessments
1067 (e.g. adjacent crops or following crops)

1068

1069 **Exposure studies**
1070
1071 **Table C1:** Uncertainty matrix for the exposure refinement with measurements of residues in fields
1072 (according to Appendix G in the Guidance Document)
1073

Source of uncertainty	Detailed description	Assessment of its level (low, medium, high)	Justification for the assessment
Measurement in nectar, pollen and dust: sampling			
Location of test sites (fields) and strategy for choosing them			
Measurement of the applied amount			
Sampling method (e.g., random, etc.)			
Residue analysis			
Number of samples			
Location of samples and strategy for choosing them			
Sampling timing (peak concentration covered?)			
Measurement in nectar, pollen and dust: analytical method			
Quantification and detection limits			
Analytical method used (and if other methods exist, with their comparative performance, handling of samples after collection in field)			
Statistics			
Preparations of raw data (e.g., pooling) before statistical analysis			
Statistical method used for identifying the average of one treated field (at the peak concentration) (this has to be			

Source of uncertainty	Detailed description	Assessment of its level (low, medium, high)	Justification for the assessment
repeated for the other fields)			
Confidence interval			
Potential confounders			
Influence of the temperature and weather conditions of the year (e.g., no extreme weather conditions, prolonged rain period, etc.)			

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1077 **EFFECTS Studies**

1078 **Table C2:** Uncertainty matrix for the effects in a field study (Appendix on effects studies)

Source of uncertainty	Description	Assessment of its magnitude (low, medium, high)	Justification for the assessment
Precision of the effects measurement			
For the assessment of the colony strength were details provided on the methodologies used. For visial assessment: e.g. competence of the observer and provide pictures of the evaluation of the bee population)			
Measurement of mortality (techniques used)			
Measurement of foraging activity including behavioural effects (techniques used)			
Correct experimental conditions and parameters			
Location of test sites (fields) and strategy for choosing them			
Duration of observation during the flowering period			
Total duration of the flowering period (in days) versus duration during which the hives were exposed			
Quantitative level of the diseases mentioned in the guidance, at the beginning and in the end of the experimentation			
Choice of the crop used			
Extrapolation from one crop to another			
Population size (in number of bees) at the beginning and the end of the experiment			
Area of alternative foraging sources available			
Method for measuring the area of alternative foraging sources (e.g., questionnaires with farmers)			

Source of uncertainty	Description	Assessment of its magnitude (low, medium, high)	Justification for the assessment
Area of each study site			
Genetic origin of the colonies			
The queen – age and sisterhood with queens of other hives			
Origin of the colonies (where were they before the experiment)			
Distance between the control and test sites			
Frequency of hive observation			
Time for hive observation (how many minutes, at which time of the day, what happens if the weather does not allow observation)			
Potential confounders			
Area of attractive crops present in the stocking zone, after the exposure period			
Hives nourishment during the stocking period (quantity, frequency, content – e.g., sugar syrup),			
Estimated surface covered by other plants in an area of 3 km (radius) around the hive and if these plants are attractive to bees, split in the following categories: - other crops, - weeds in the treated field - adjacent crops - plants on field margins			
Farmers' practices of application and dosing in the foraging area of the test and control colonies			
Exposure assessment in the effects study			
Maximum in time of concentration of residues in nectar and pollen entering the hive adequately assessed?			
Statistics			
Studies designed to detect required effect thresholds (no. of hives and study sites were sufficient)			
Statistical method used			
Confidence interval			
Statistical power			
Statistical unit used			
Further preparations of raw data (e.g., pooling) before statistical analysis			

1079

1080 **Qualitative assessment of uncertainty**

1081 Outlined below are two examples of an assessment of the uncertainty of a dataset and accompanying
1082 risk assessment. It should be noted that these are very brief, however they aim to illustrate the manner
1083 in which the information could be presented.

1084

1085 **Example 1**

1086 Background
1087 The product is to be used on oilseed rape as a spray before and during flowering. The following
1088 assessment only covers the risk from the consumption of nectar and pollen from the treated crop. The
1089 assessment of uncertainty should be repeated for all other routes of exposure, for example adjacent
1090 crops, field margins etc.
1091 First tier: All HQ and ETR fail the relevant trigger values, however the compound doesn't pose a risk
1092 via accumulation. The use of risk mitigation measures have been considered, however they would
1093 remove the usefulness of the product and therefore higher tier data and associated assessment is
1094 required.
1095 Higher tier study submitted:
1096 Studies on the residues in pollen and nectar were conducted according to the Guidance
1097 Document, i.e. a range of sites representative of where the product will be grown within the
1098 zone (i.e. sites represent a range of soil and climate conditions). The number of sites selected
1099 is in line with the Guidance Document. Data have also been submitted to indicate the 'dilution
1100 factor', i.e. a factor that takes in to account the difference between residues in pollen and
1101 nectar from the treated plants and those in the colony.
1102 Field studies on oilseed rape – residues in all studies/hives have been determined to be at least
1103 equivalent to the 90th percentile exposure estimate. Sufficient studies submitted to detect
1104 required effect.
1105 Effects on colony strength were <7%; mortality <1.5 times the control over 3 days.
1106
1107 **Table C3:** Worked example of a qualitative assessment of the uncertainty in a field study

Source of uncertainty	Potential to make true risk lower	Explanation	Potential to make true risk higher	Explanation
Exposure studies	+++	All studies conducted according to the Guidance Document, i.e. an appropriate range of soil/climate conditions. Studies submitted to determine dilution factor are acceptable.	-	True exposure is unlikely to be worst.
Exposure in field studies	+++	Exposure in field studies were in line with that determined to occur as a result of residue studies. In-field measurements of foraging and pollen identification indicate adequate exposure as well.		True risk is unlikely to be worst than this as in reality dilution due to adjacent crops and flowering weeds will occur.
Effects in field studies	+++	Demonstration that bees were exposed to at least a 90th percentile, colonies were healthy and monitored throughout.	-	Only potential issue is that different strains of bees may react differently from those selected.
Overall assessment	Underlying studies are in line with those recommended and as a result uncertainties minimal and would indicate that the true risk is lower than that assessed.			

1108
1109
1110 **Example 2**
1111 Use on oilseed rape as a spray before and during flowering.

1112 First tier: All HQ and ETR fail the relevant trigger values, however the compound doesn't pose a risk
1113 via accumulation. The use of risk mitigation measures have been considered, however they would
1114 remove the usefulness of the product and therefore higher tier data and associated assessment is
1115 required.
1116 Higher tier study submitted:
1117 Studies on the residues in pollen and nectar were conducted according to the Guidance
1118 Document, however only one study was carried out. No work has been carried out to
1119 determine potential dilution factor.
1120 Field studies on oilseed rape – residues in pollen and nectar are in line with the above study.
1121 Monitoring of bee activity indicated that bees were foraging the crop in line with the control
1122 (i.e. both in terms of bees/m^2 and pollen analysis).
1123 Effects on colony strength were <7%; mortality <1.5 times the control over 3 days.
1124
1125 **Table C4:** Worked example of a qualitative assessment of the uncertainty in a field study

Source of uncertainty	Potential to make true risk lower	Explanation	Potential to make true risk higher	Explanation
Exposure studies	+	Only one exposure study was submitted. No other information regarding the potential exposure were available.	--	Uncertainty as to the likely exposure levels of bees.
Exposure in field studies	+	Exposure in field study was potentially in line with that determined in one study. No other information available. The true residue could be much higher.	--	Uncertainty as to what the exposure has been in the field studies.
Effects in field studies	+	Lack of demonstration that bees were exposed to at least a 90th percentile in-hive, although evidence that the bees foraged the treated crop.	--	Exposure could be less than the 90th percentile, hence the effects could be greater.
Overall assessment	Much uncertainty regarding the exposure, therefore there is a lack of certainty as to whether the SPG will be met.			

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D. TRIGGER VALUES

Use of HQ approach for solid formulations

EFSA (2012a) propose that it is possible to use the HQ approach, along with the associated trigger value as part of the seed treatment/granule, or solid formulation scheme. In particular EFSA (2012a) propose using it in the assessment of risk from dust drift.

The original concept behind the HQ approach and the associated trigger value was developed for spray applications. To read across to solid formulations, there needs to be an assessment of whether a solid formulation poses an equivalent (or lower) risk to sprays. In order to do this there should be a consideration of the toxicity of a spray formulation versus the toxicity of dust from a solid formulation, as well as a consideration of exposure

As regards toxicity, it is likely that in terms of toxicity, that when expressed in equivalent terms (i.e. µg a.s./bee), that a spray formulation is *potentially* more toxic than the active substance and that a solid formulation is probably of similar toxicity to the active substance.

Exposure from spray formulations will mainly consist of oral and contact. Exposure via the oral route may occur when the bees consume contaminated pollen or nectar, water, guttation fluid which has either been contaminated directly by spray deposit or via systemic action of the active substance. As regards contact exposure, this is possible if the bee is sprayed directly or comes in to contact with spray deposits. It should be noted that when a bee cleans itself, it may then consume what is deposited on it.

As for exposure from dust from solid formulations, it is considered that the routes will be similar as for sprays above. In addition, it is feasible that if dust is present in or on the flower then a bee may come in to contact with this when working flowers. This may then be taken up orally when the bee cleans or is cleaned by others in the hive; it is feasible that this route could be greater compared to the similar route for spray applications.

According to the above, the toxicity of the formulation of a solid formulation is likely to be less than that for a spray formulation, as regards exposure, this is likely to be similar, although there is a possibility that the may be greater exposure compared to the spray from deposition of the dust in flowers. Taking all this together it is feasible that using a HQ approach may be appropriate and hence would mean the same as for a spray treatment – see earlier.

The HQ is calculated with the in-field dose. Soil treatments and sowing of seeds are usually performed on bare soil, which means that bees are not expected to be exposed in the field. The off-field dose will always be (much) lower than the in-field dose (*refer to dust drift values elsewhere*). This means that the calculated HQ is much higher than the HQ relevant for the off-field. This may possibly cover the uncertainties regarding the extrapolation of the LD50 determined for liquid formulation to dust.

Risk quotients and First Tier trigger values

The Toxicity Exposure Ratio, or TER, is a risk quotient that is calculated for each particular combination of a non-target organism and a PPP. Conventionally, the quotient is calculated as the ratio of the intake of the PPP that is lethal to half the subjects exposed, or the LD_{50}, and the level of environmental exposure, denoted E. Here we generalize the principle to any response variable, lethal or sublethal. Therefore, the dose required to reduce performance on any variable, including survivorship, is denoted by D_{50}. Thus, the TER is given by:

$$\text{TER} = D_{50}/E \qquad \text{Eqn D1}$$

Higher Tier testing is invoked when the TER is less than the trigger criterion, *T*, i.e.

$$D_{50}/E < T \qquad \text{Eqn D2}$$

Algebraic rearrangement of Eqn D2 shows that Higher Tier testing is invoked when the environmental exposure exceeds 100/*T* % of the D_{50}:

$$E > D_{50}/\ T \qquad \text{Eqn D3}$$

For lethal effects, the trigger criterion typically has been set at ten, so that Higher Tier testing is invoked when the environmental exposure exceeds 10% of the LD_{50}:

$$E > D_{50}/10 \qquad \text{Eqn D4}$$

It is necessary to establish the maximum level of potential threat that can be expected from a PPP that has been eliminated from further consideration by First Tier testing. Specifically, we must establish the effect of a PPP that has just exceeded the trigger value by having a level of environmental exposure of $E = D_{50}/\ T$. The degree of detrimental effect due to a dose of $D_{50}/\ T$ depends on the dose-response relationship, which is typically a sigmoidal function (Figure D1).

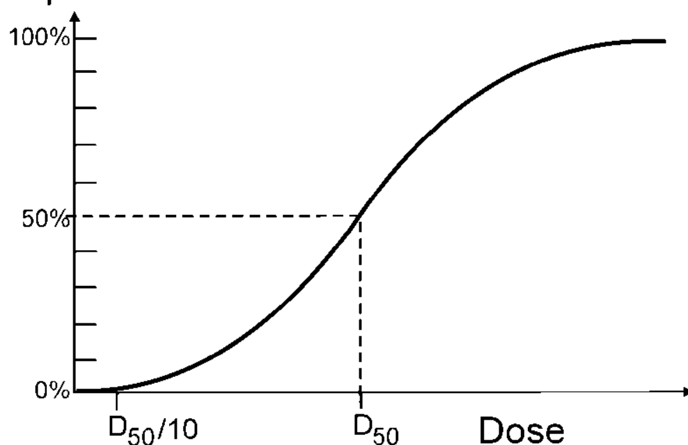

Figure D1: A typical dose-response relationship where 'Dose' (*x*-axis) indicates the environmental exposure of an individual organism and 'Response' (*y*-axis) indicates the percentage of individuals that exhibit the response being measured. D_{50} denotes the dose at which 50% of individuals respond and for the case where the trigger criterion $T = 10$, $D_{50}/10$ denotes one tenth of this exposure.

Provided that the dose-response relationship is sigmoidal and that its gradient accelerates at the lowest doses, the maximum response to a particular dose is given by a linear relationship, $response = dose \times 50/\ D_{50}$ (Figure D2).

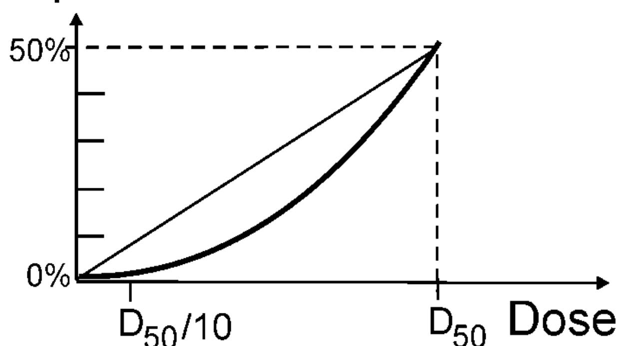

Figure D2: The lower left quadrant of the dose-response relationship from Fig. 1. If the dose-response relationship is sigmoidal, its gradient must accelerate in this quadrant, which implies that the maximum response to $D_{50}/10$ is given by a linear relationship, *response* = *dose* × 50/ D_{50}. The slope of this relationship is obtained because starting from the origin there is a rise of 50% in response across a run of D_{50} and the slope of a linear relationship is given by rise over run.

Given that *response* = *dose* × 50/ D_{50}, the maximum response to an exposure, or dose, of D_{50}/T is obtained by $D_{50}/T \times 50/D_{50}$, or $(50/T)$%. For the case where the trigger criterion $T = 10$, we obtain a maximum response of (50/10)%, or 5%. Consequently, we consider that the use of a trigger criterion of $T = 10$ provides a reasonable safeguard for most protection goals.

Notes

To defend this conclusion, the following must be further justified by evidence: that dose-response relationships for PPPs are linear or sigmoidal. Gathering this evidence is a target for further research.

Note that the dose-response relationships presented here are generic and not necessarily based on mortality. It is an open question as to whether an exposure of $D_{50}/10$ based on mortality testing will safeguard sublethal responses to a level below 5%. Other endpoints may be more sensitive than mortality and so resolving this question requires further research.

There is always statistical uncertainty associated with working from dose-response relationships fitted to experimental data. Our guidelines will need to make reference to necessary levels of statistical power etc. in this context.

Determining a trigger value for an acute oral exposure

Overview:- By assuming that the dose-response relationship is linear in the low-dose range, it is possible to identify the maximum exposure whose impact (imposed mortality) meets a specified protection goal. By definition, it is possible to link this maximum exposure, or uptake, to the HQ.

Principles:- Let A denote the field application rate of a compound (kg a.i. ha^{-1}) and let RUD denote the residue unit dose of the bee's diet (mg a.i per kg diet at A = 1 kg a.i. ha^{-1}). Let c denote the daily consumption rate (kg diet day^{-1}) and let d denote the duration of the exposure in days. If U denotes the uptake of a compound by an individual bee (mg a.i), then

$$U = A \times RUD \times c \times d \qquad \text{Eqn D1}$$

1262 Let LD_{50} (units of mg) denote the 48 h consumption of a.i. that causes mortality in 50% of exposed
1263 bees. Dividing both sides of Eqn D1 by LD_{50} yields:
1264
1265 $$U / LD_{50} = (A \times RUD \times c \times d) / LD_{50} \quad \text{Eqn D2}$$
1266
1267 Since by definition the hazard quotient is given by $HQ = A / LD_{50}$, we replace this quotient in the right
1268 hand side of Eqn D2 and rearrange terms to obtain:
1269
1270 and hence:
1271
1272 $$HQ = U / (RUD \times c \times d \times LD_{50}) \quad \text{Eqn D3}$$
1273
1274 Assuming that the dose-response relationship is linear through the origin (i.e. zero dose-dependent
1275 mortality in the control dose) in the dosage range from zero to LD_{50} (see justification above), the
1276 maximum dietary exposure (mg a.i. kg^{-1}) that meets a protection goal of mortality less than *M*% is
1277 given by $U = M \times LD_{50}/50$, which is explained as follows.
1278
1279 Let *X* denote the exposure that causes the maximum mortality permitted under the Specific Protection
1280 Goals. Assume that the dose-response relationship is a straight line defined by *mortality* =
1281 *exposure**50/LD_{50}. (This assumption is conservative because it produces higher mortality at low
1282 doses than an accelerating sigmoidal curve). Note that this dose-response relationship passes through
1283 the origin (zero dose-dependent mortality above background at zero dose) and that *mortality* = 50% at
1284 *exposure* = LD_{50} as required.
1285
1286 The point (*U*, *M*) lies on the dose-response relationship with coordinates *mortality* = *M, exposure*= *U*,
1287 so we can find *U* given *M*. When *mortality* = *M* and *exposure* = *U*, we use *mortality* =
1288 *exposure**50/LD_{50} to obtain:
1289
1290 $$M = U*50/LD_{50} \quad \text{Eqn D4}$$
1291
1292 and rearrangement yields the required
1293
1294 $$U = M \times LD_{50}/ 50 \quad \text{Eqn D5}$$
1295
1296 We now use this result as follows. Substituting the expression for U given by Eqn D5 into Eqn D3
1297 yields:
1298
1299 $$HQ = (M \times LD_{50}/ 50) / (RUD \times c \times d \times LD_{50}) \quad \text{Eqn D6}$$
1300
1301 and algebraic simplication produces:
1302
1303 $$HQ = M / (50 \times RUD \times c \times d) \quad \text{Eqn D7}$$
1304 *Worked example.*
1305
1306 Assume $RUD = 12.5 \times 10^{-3}$ mg a.i. mg^{-1} (which is 12.5 ppm), $c = 128 \times 10^{-3}$ mg d^{-1}, and $d = 2$.
1307
1308 If the protection goal specifies $M \leq 5.3\%$ then solving Eqn D7 yields
1309
1310 $HQ = 5.3/(50 \times 12.5 \times 10^{-3} \times 128 \times 10^{-3} \times 2) = 5.3/0.16 = 33$
1311
1312 The HQ trigger values are calculated as follows based on daily mortality rates based on life
1313 span/mortality data of foragers retrieved from literature (see Annex T of the final GD on mortality
1314 rates):
1315

1316

	Lowest observed mortality	10th percentile	Median
Daily background mortality	5.3	7.8	13
HQ trigger	33	49	81

1317
1318 The HQ trigger values for bumble bees and solitary bees were recalculated based on daily mortality
1319 rates of 4.4% (bumble bees) and 5% (Osmia) resulting in values of 27.5 and 31.5. An additional
1320 assessment factor of 5 is suggested to account for higher susceptibility of forager losses in bumble
1321 bees and uncertainties related to differences in species sensitivity distribution in solitary bees.
1322
1323
1324 **Determining a trigger value for an acute contact exposure**
1325
1326 This scenario covers direct overspray of bees sitting on a plant or on the ground in field. In the
1327 Opinion of the PPR panel (EFSA, 2012a) it is proposed to assume "as a conservative assumption that
1328 honey bees in the field during or shortly after spray applications are exposed to a mass corresponding
1329 to the mass sprayed to 1 cm^2 of the field". (Note that 1 $cm^2 = 10^{-8}$ ha.)
1330
1331 As above the exposure/dose a bee receives is denoted as U and can be calculated as follows:
1332
1333 $$U = A \times 10^{-8} \quad \text{Eqn D8}$$
1334
1335 Since the application rate is given in kg a.s./ha it needs to be multiplied by 10^6 to express it in mg a.s./
1336 cm^2.
1337
1338 $$U = 10^{-2} \times A \quad \text{Eqn D9}$$
1339
1340 Dividing both sides of the Eqn D9 by LD50 (contact) yields:
1341
1342 $$U / LD_{50} = 10^{-2} \times A / LD_{50} \quad \text{Eqn D10}$$
1343
1344 The hazard quotient is given by HQ = A / LD_{50}. We replace the quotient on the right hand side of Eqn
1345 D10:
1346
1347 $$U / LD_{50} = 10^{-2} \times HQ \quad \text{Eqn D10}$$
1348
1349 The rearranged equation is:
1350
1351 $$100U / LD_{50} = HQ \quad \text{Eqn D11}$$
1352
1353
1354 As above the point (*U,M*) in the dose-response curve can be used to find the dose at a certain
1355 mortality.
1356
1357 When *mortality* = *M* and *exposure* = *U*, we use *mortality* = *exposure**50/LD_{50} to obtain:
1358
1359 $$M = U*50/LD_{50} \quad \text{Eqn D4}$$
1360
1361 and rearrangement yields the required
1362
1363 $$U = M \times LD_{50} / 50 \quad \text{Eqn D5}$$
1364

We now use this result as follows. Substituting the expression for U given by Eqn D5 into Eqn D11 yields:

$$HQ = 100\,(M \times LD_{50} / 50) / LD_{50} \qquad \text{Eqn D12}$$

and algebraic simplication produces:

$$HQ = 2M \qquad \text{Eqn D13}$$

Workedl example.

If the protection goal specifies $M \le 5.3\%$ then solving Eqn D13 yields

$HQ = 5.3 \times 2 = 10.6$

The HQ trigger values are calculated as follows based on daily mortality rates based on life span/mortality data of forager honey bees retrieved from literature (see Annex T of the final GD):

	Lowest observed mortality	10th percentile	Median
Daily background mortality	5.3	7.8	13
HQ trigger	10.6	15.6	26

The HQ trigger values for bumble bees and solitary bees were recalculated based on daily mortality rates of 4.4% (bumble bees) and 5% (*Osmia*) resulting in values of 8.8 and 10. An additional assessment factor of 5 is suggested to account for higher susceptibility of forager losses in bumble bees and uncertainties related to differences in species sensitivity distribution in solitary bees.

Determining a trigger value for an oral 10 day exposure.

Overview:- This procedure finds the maximum dietary exposure of a compound that causes a level of mortality over 10 days that would impose no more than a negligible impact on a honeybee colony, as required by the Specific Protection Goals. The required proportional elevation in mortality is determined from the Khoury model (Khoury et al. 2011) and assuming the standard parameterisation of Henry *et al.* (2012. Science 336: 348-50), which is conservative in assuming that the colony has a relatively low capacity to replenish lost foragers (Cresswell & Thompson 2012. Science, *in press*) and then this is applied to a more conservative estimate of the background rate of mortality under field conditions. The exposure required to cause this elevation is determined from a laboratory dose-response relationship.

1. Find the daily mortality rate in the Khoury model that causes a 7% decrease in colony size over 10 days (see the magnitude of a 'negligible effect' in the Specific Protection Goals). Denote this rate by $m_{7,10}$

2. Find ratio of $m_{7,10}$ to the 'background' rate of daily mortality assumed in the Khoury model* (i.e. 0.154). The maximum relative increase in daily mortality rate that meets the Specific Protection Goal is $I = m_{7,10}/0.154$

3. Assume that the environmentally relevant background rate of daily mortality under field conditions is m_E. Therefore, the maximum rate of mortality that meets the Specific Protection Goals for the

relevant environment is $I \times m_E$. The maximum increment above background level is therefore $max.increment = (I - 1) \times m_E$

4. For the compound in question, consider the dose-response relationship between oral dietary exposure dosage (mg a.i. kg^{-1}) and mortality rate and determine the compound's LC_{50}, where LC_{50} denotes the exposure dosage necessary to produce 50% mortality after 10 days.

Assuming that the dose-response relationship is linear through the origin (i.e. zero dose-dependent mortality in the control dose) in the dosage range zero to LC_{50} (see justification in Appendix A), the maximum dietary exposure (mg a.i. kg^{-1}) that meets the protection goal is given by $max.increment \times LC_{50}/50$, which is explained as follows.

Let X denote the exposure that causes the maximum mortality permitted under the Specific Protection Goals. Assume that the dose-response relationship is a straight line defined by $mortality = exposure*50/LC_{50}$. (This assumption is conservative because it produces higher mortality at low doses than an accelerating sigmoidal curve). Note that this dose-response relationship passes through the origin (zero dose-dependent mortality above background at zero dose) and that $mortality$ = 50% at $exposure = LC_{50}$ as required.

The point ($max.increment, X$) lies on the dose-response relationship with coordinates $mortality = max.increment$, $exposure = X$, so we can find X given $max.increment$. When $mortality = max.increment$ and $exposure = X$, we use $mortality = exposure*50/LC_{50}$ to obtain:

$$max.increment = X*50/LC_{50}$$

and rearrangement yields

$$X = max.increment \times LC_{50}/ 50.$$

5. Let T denote the trigger value for the TER and by definition $T = LC_{50} / exposure$ so substituting $exposure = X = (max.increment \times LC_{50}/ 50)$ yields

$$T = LC_{50} / (max.increment \times LC_{50}/ 50)$$

and algebraic simplification yields $T = 50/ max.increment$.

Worked example (labelled by steps above).

1. The solution to the Khoury model that yields 7% reduction in colony size after 10 days is $m_{7,10} = 0.195$.

2. Therefore $I = 0.195/0.154 = 1.27$

3. If $m_E = 5.3\%$, $max.increment = 0.27 \times 5.3 = 1.43$

5. Trigger value = $50/1.43 = T = 34$

The TER trigger values are calculated as follows based on daily mortality rates based on life span/mortality data of foragers retrieved from literature (see Annex T of the final GD):

	Lowest observed mortality	10th percentile	Median
Daily background mortality	5.3	7.8	13
I	1.27	1.27	1.27
Max. increment	0.27 x 5.3 = 1.43	0.27 x 7.8 = 2.1	0.27 x 13= 3.5
TER Trigger	34	23	14
ETR Trigger	0.03	0.04	0.07

1468
1469 The ETR trigger values for bumble bees and solitary bees were recalculated based on daily mortality
1470 rates of 4.4% (bumble bees) and 5% (*Osmia*) resulting in values of 0.024 and 0.027, respectively.
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1474 **GLOSSARY [AND/OR] ABBREVIATIONS**

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a.i.	active ingredient
a.s.	active substance
BBCH	Growth stage; uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species
CA	Concentration Addition
EA	Exposure Assessment
EC50	Concentration required killing half the members of a tested population after a specified test duration
ECx	Concentration with x% level of effect compared to the control
EPPO	European and Mediterranean Plant Protection Organization
ERC	Ecotoxicologically Relevant type of Concentration
ETR	Exposure toxicity ratio
EU	European Union
FOCUS	FOrum for Co-ordination of pesticide fate models and their Use
Guttation	Appearance of drops of xylem sap on the tips or edges of leaves of some vascular Plants
GD	Guidance Document
HQ	Hazard quotient i.e. the quotient of the application rate and the acute oral or contact toxicity
ICPBR	International Commission Plant Bee Relationship
IGR	Insect growth regulator, group of compounds that affect the ability of insects to grow and mature normally
Lab	Laboratory
LC50	Dose required killing half the members of a tested population after a specified test duration
LOD	Level of Detection
LOQ	Level of Quantification
NOAEC	No Observed Adverse Effect Concentration

NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
OECD	Organization for Economic Co-operation and Development
PEC	Predicted Exposure Concentration
PPP	Plant Protection Product
PUF	Plant Uptake Factor
RAC	Regulatory Acceptable Concentration
RUD	Residue Unit Dose
SCFoCAH	Standing Committee on Food Chain and Animal Health
SPG	Specific Protection Goal
TU	Toxic Unit
TER	Toxicity Exposure Ratio
TSCF	Transpiration Stream Concentration Factor