POLLINIS A NONPROFIT AND INDEPENDENT NGO FUNDED EXCLUSIVELY BY DONATIONS FROM INDIVIDUALS TO PROTECT WILD AND HONEY BEES AND PROMOTE SUSTAINABLE AGRICULTURE IN ORDER TO HELP PRESERVE POLLINATORS.

Comments to the draft EFSA Bee Guidance Document (2022) POLLINIS

GENERAL COMMENTS

As a general comment, we remark a switch of approach in the draft GD compared to the 2013 version. The 2013 GD was clearly committed to the general objective of protection, by adopting a precautionary and pragmatic approach to address, as much as possible, potential risks. For instance, when facing the lack of OECD guidelines to test crucial aspects of toxicity, the approach was, whenever possible, to propose feasible experimental protocols (study designs) to address the problem and produce relevant data. Similarly, in presence of inconclusive or insufficient data to conclude about the relevance of a potential source of risk, the attitude of the 2013 version was to adopt a precautionary approach by including as much as possible that potential risk in the assessment.

Regretfully, this approach is not always present in the draft GD. Concerning effect assessment, a robust scheme to address effects, in particular combined and sublethal, is still lacking. This lack is of particular concern when one considers the quantity of pesticide classes found in real world samples (Tosi et al., 2018; Traynor et al., 2021). In the absence of OECD validated test guidelines to assess an effect, the approach in the updated version is to ignore this effect, despite the availability of draft guidelines and robust non-guidelines studies and tests, whose utilisation would be crucial to better characterize the risk and thus better protect bees. In certain cases, even existing OECD validated guidelines are not mandatorily requested (this is the case, for instance, of the acute toxicity tests for bumble bees, OECD 246 and 247).

Similarly, concerning exposure estimation, in the absence of sufficient data to decide about the relevance of a source of risk, the approach is just to ignore that potential source of risk. While we acknowledge the important effort of refinement of exposure which has been conducted, with the introduction of several new factors (pre-flowering factor, landscape factor, half-life in plant matrices, etc.), we regret that such an effort does not lead to a more robust protection, because it mainly targets the risk of **overestimating** exposure, while the risk of **underestimating** exposure has not been sufficiently dealt with.

Not only important routes of exposure have not been included in the draft GD (e.g., honey dew, extrafloral nectaries, resin, as well as soil and contaminated nesting materials for solitary bees) but also some of those that were part of the 2013 GD have been removed from the new assessment (e.g. contaminated water). This is regretful, and a regression compared to the 2013 guidance document. The reason often evoked not to cover certain routes of exposure is the lack of sufficient data to understand their relevance.

In our view, the burden of proof should be reversed in these cases: instead of ignoring potential sources of risk because of lack of data confirming their relevance, a potential

route of exposure should be part of the risk assessment till there are sufficient data to exclude its relevance. Indeed, scientific uncertainty should not prevent prudent actions: scientific information does not need to demonstrate definitively the cause-and-effect relationship between risk and harm or the precise relevance of a risk, once it is acknowledged that such a risk may reasonably exist. Therefore, in case of uncertainty concerning a potential source of risk, the correct approach must be to apply the precautionary principle, i.e. to assume that we cannot exclude this risk (at least for the time being) and therefore consider it into the assessment. This approach is important not only to remain on the safe side, but also to collect data that could help better define the relevance of a certain route of exposure or the need of a specific test in the future. Unnecessary routes of exposure / tests could always be eliminated from the risk assessment at a later stage, once proved they are redundant.

Finally, the risk assessment for wild bees has been weakened: the first tier (laboratory tests) for wild bees is not mandatory in the updated version. Such tests are crucial, not only to better characterize the risk for bumble bees and solitary bees, but also to generate further research and data. This need is actually well recognized by both risk assessors and risk managers, who estimated that higher tiers studies should be in certain cases conducted for bumble bees and solitary bees « in order to gain more robust data on the effects of pesticides on those bees » (Draft GD, lines 31-2). We consider that the same principle applies to laboratory studies, and therefore these studies must be conducted: even in the absence of a defined effect threshold, first tier studies will provide important data to better characterize the risk. Increasing the current level of knowledge would certainly improve the accuracy of the risk assessment in future.

In conclusion, in order to avoid an insufficient risk assessment, the draft GD should include more exposure routes and a more robust scheme to address combined and sublethal effects, while also introducing a mandatory battery of laboratory tests for bumble bees and solitary bees. It is true that only few OECD guidelines are available to address these effects/species, but, at the pace of pollinators' decline we witness today (Seibold et al., 2019; Wagner, 2020), of which EFSA experts are well aware, we need a more proactive and pragmatic approach toward protection to prevent further decline, one which relies also on draft guidelines and robust non-guidelines studies and tests, even if the latter have not been validated yet. Indeed, extinction is running faster than protocol validation.

More specifically, in order to avoid an insufficient risk assessment, we suggest the following (see specific comments on the corresponding chapters of the draft GD for more details):

Exposure

1) For the dietary, only exposure via nectar and pollen for both adult bees and larvae is addressed. We recommend to include other routes of exposure, namely contaminated matrices (e.g., honey dew, extrafloral nectaries, resin etc.) that could lead to oral residue intake. This is in line with the precautionary principle and also a means of collecting more data to understand the precise relevance of these routes of exposure.

2) Risk assessment for ground-nesting bees should include exposure impacts from pesticides in soil and nesting materials.

3) Exposure through contaminated water should be reintroduced in the risk assessment as a precautionary measure, despite data uncertainty.

Effects

1) Tests at the last stages of their OECD publication, as well as some non-OECD robust protocols, need to be included in the draft GD. We are confident that their inclusion in the risk assessment would also propel their standardization and harmonization.

2) Despite the recognition of the central role of sublethal effects, the proposed risk assessment scheme is still overwhelmingly focused on lethal effects. We advocate for a more refined and holistic assessment that does not only focus on lethality but uses harmonised methods to test sublethal and relevant combinations. Therefore, assessment of sublethal effects should be reinforced, by including other tests (e.g. HPG, PER) besides the homing flight. As per the latter, the homing flight test should be mandatory at least for all neurotoxic insecticides (see ~~ANSES 2018~~). Considering the large and troubling lack of information on the sublethal toxicity of pesticides to bees, multiplying tests during the risk assessment, and combining their results, will have an important impact on protocol standardization and data availability.

3) It is important to stress that sublethal effects should be directly assessed also for bumble bees and solitary bees, and not only for honey bees as presently planned in the draft GD. Robust protocols exist and must be used. Similarly, the assessment of potential time-reinforced toxicity should not be limited to honey bees.

4) The proposed risk assessment still relies too heavily on honey bees. First tier assessment of bumble bees and solitary bees should be mandatory, at least for the acute toxicity, given that validated or draft OECD Guidelines for these tests are available. We also strongly recommend to include chronic and larval toxicity tests for these species, based on existing (ring-tested or other) available protocols. The fact that, regretfully, risk managers have decided not to establish a determined threshold for mortality, which consequently prevents the setting of trigger values for laboratory tests, shouldn't be a reason not to perform these tests, which could nevertheless provide precious information and data. In a situation where the absence of SPGs prevents to ascertain a potential unacceptable impact at tier 1, and decisions over an unacceptable impact need to be taken at higher tiers, the line of evidence provided by tier 1 tests is all the more important to help weighting and integrating the evidence.

5) **The guidance does not address the risk assessment of combinations of more than one PPP.** This is a major weakness of the risk assessment, preventing to evaluate the real risk: the combined toxicity of insecticides, fungicides and herbicides on bees needs to be considered. We welcome the future inclusion of a system-based approach to assess multiple stressors, and recognize the limits of the current scientific tools to address the impact of all non-intentional mixtures. Nevertheless we deem important for the time being to include at least an assessment of the most common intentional binary combinations and mixtures (tank mixtures), with special attention to synergic effects between certain classes of fungicides and insecticides.

6) Indirect effects should be taken into consideration, at least through assessment factors. It should be noted that the interrelations between species and ecosystems are included in the definition of environment established by regulation (EC) 1107/2009 (art. 3.13): "'environment' means waters (including ground, surface, transitional, coastal and marine), sediment, soil, air, land, wild species of fauna and flora, and any interrelationship between them, and any relationship with other living organisms".

SPECIFIC COMMENTS

Pathways of PPP exposure for bees (Ch 1.4, p. 13)

We acknowledge the important effort of refinement of exposure which has been conducted, with the introduction of several new factors (pre-flowering factor, landscape factor, half-life in plant matrices, etc.). Unfortunately, such an effort does not lead to a more robust protection, because it mainly targets the risk of **overestimating** exposure, while the risk of **underestimating** exposure has not been sufficiently dealt with.

Not only important routes of exposure have not been included in the draft GD (e.g., honey dew, extrafloral nectaries, resin, as well as soil and contaminated nesting materials for solitary bees) but also some of those that were part of the 2013 GD have been removed from the new assessment (e.g. exposure via contaminated water). This is regretful, and a regression compared to the 2013 guidance document. The reason often evoked not to cover certain routes of exposure is the lack of sufficient data to understand their relevance. In our view, the burden of proof should be reversed in these cases: instead of ignoring potential sources of risk because of lack of data confirming their relevance, a potential route of exposure should be part of the risk assessment till there are sufficient data to exclude its relevance.

1)For the dietary, only exposure via nectar and pollen for both adult bees and larvae is addressed (p. 14). We recommend to **include other routes of exposure**, namely contaminated matrices (e.g., **honey dew, extrafloral nectaries, resin** etc.) that could lead to oral residue intake. This is in line with the precautionary principle and also a means of collecting more data to understand the precise relevance of these route of exposure.

2)**Risk assessment for ground-nesting bees should include exposure impacts from pesticides in soil and nesting materials**: while the draft GD acknowledges that for most species of bumble bees and solitary bees nesting in the soil, exposure by contact with contaminated soil/mud/leaves may be relevant, as already reported in the EFSA PPR Panel (2012), this route of exposure is not addressed, because of insufficient information (lines 460-63, p. 14). However, recent research has again confirmed the importance of this route of exposure, and an efficient ecotoxicology model to assess this risk to ground-nesting bees exists (see Willis Chan et al. 2019; Anderson and Harmon Threatt 2019). Therefore, **exposure via contaminated soil must be included in the risk assessment**.

3)**Exposure through contaminated water should be reintroduced in the risk assessment** as a precautionary measure, despite data uncertainty. The potential overestimation of the amount of water an individual bee consumes (value W of the equation for calculating the exposure toxicity ratio - ETR) based on the study of Free and Spencer-Booth (1958), is compensated by the absence of estimation of potential contamination by contact or inhalation, being the ETR calculated exclusively for a dietary scenario. As per the implausible assumptions concerning the water scenario for larval honey bees in the 2013 GD observed by the working group (Annex E, p. 6), they don't represent an obstacle to reintroduce the water

scenario, given that, as remarked by the working group, an assessment for the effects of water in larvae is unnecessary, as the issue of PPPs in larval food is already directly addressed within the risk assessment framework.

4) Also, exclusion of guttation water is arguable. According to Annex E, the exclusion of guttation water was mainly based on one review (Zumkier et al., 2019) of industry studies (Bayer, Syngenta and BASF). However, results from these studies are not probatory, because, as the EFSA working group itself recognizes, « Each individual report concluded that guttation water from seed-treated crops does not reduce colony size by more than 10% or induce any detectable adverse effects on honey bee colonies. However, none of the studies met the criteria for a valid assessment based on EFSA (2013) and most (21/25) of the studies failed to meet our criteria for a valid assessment, largely based on their failure to demonstrate that the colony had been exposed to PPP via foraging from guttation fluid » (Annex E, p. 22). Therefore « … (we cannot determine if) if absence of effects is due to a real absence or to a study design unsuitable for detecting these effects » (Annex E, p. 7).

5) We acknowledge that lack of information entails several challenges for the assessment of contamination via water. However, we consider that this route of exposure should nevertheless be included in the risk assessment while in parallel addressing, through specific research programmes, the main research needs identified by the working group, namely: 1) fundamental information on how much water individual bees consume; 2) evidence that exposure to PPPs via water is a realistic and important exposure route; and 3) the practical difficulties of assessing effects of PPPs in foraged guttation water on honey bee colonies in a field scenario.

6) Exposure through inhalation for very volatile substances should be compulsory.

7) 7) The exclusion of each of the above-mentioned routes of exposure will result in an underestimation of real exposure, which should be compensated through robust **assessment factors**.

Scope of the Guidance document (Ch. 2, p. 15)

a) **Indirect effects are ignored (p. 15)**. Indirect effects should be taken into consideration, at least through assessment factors. Especially for oligolectic wild bees, these effects can be very important. It should be noted that the interrelations between species and ecosystems are included in the definition of environment established by regulation (EC) 1107/2009 (art. 3.13): "'environment' means waters (including ground, surface, transitional, coastal and marine), sediment, soil, air, land, wild species of fauna and flora, and any interrelationship between them, and any relationship with other living organisms".

b) **The guidance does not address the risk assessment of combinations of more than one PPP.** This is a major weakness of the risk assessment, preventing to evaluate the real risk. We welcome the future inclusion of a system-based approach to assess multiple stressors, and recognize the limits of

the current scientific tools to address the impact of all non-intentional mixtures. Nevertheless we deem important for the time being to include at least an assessment of the most common intentional binary combinations and mixtures (tank mixtures), with special attention to synergic effects between certain classes of fungicides and insecticides.

Specific Protection Goals (Ch. 3.1, p. 16)

=> While acknowledging that the definition of the SPGs is outside the EFSA mandate, we reiterate our concerns about the SPG for honey bees: while very strong hives may be able to withstand 10% mortality, there will be a cost to compensate for the losses (more larvae to rear and therefore the need for extra food, to the detriment of the colony, and eventually of the beekeepers). Furthermore, if the hives have an average or low population, this will obviously be more serious and the consequence may be the more or less rapid death of the colonies. Similarly, pathogens can weaken weak colonies even more.

=> We consider that the decision to rely on the undefined threshold option for the protection goals for bumble bees and solitary bees, will be highly detrimental to the objective of protection of wild bees. We believe that, based on available data, these SPGs could have been determined. Together with the other associations representing civil society in the ad-hoc stakeholder group (PAN, Beelife and Apimondia), POLLINIS has already expressed its concerns to the risk managers (see our letter to the EC and SCoPAFF representatives dated 06/07/2022).

Spatial scale => We suggest a **reconsideration of the definition of the spatial scale** (now limited to the edge of the field) **including the treated field for solitary bees**. As the ecological entity of SB is defined as the « population » (e.g. group of females and males of the same species nesting in the same place spatially defined by the maximum foraging range) (cf. lines 535-6, p. 17), how can we assume that the treated field is not part of that space? As recognized by the WG, « bumble bee and solitary bee nests located in the field may be exposed to pesticides, e.g. via direct exposure of larvae and adults to soil residues, contaminated nest materials. ». Limiting the spatial scale to the edge of the field will result in an insufficient risk assessment for wild bees. Furthermore, we stress the fact that often osmiae (and bumble bees as well) are placed directly into the treated field for pollination purposes. Therefore, not considering the spatial scale of the treated field will also result in an insufficient pollination service.

Landscape factor (LF $_{po}$, LFne) (Ch. 5.3.7, p. 47)

=> **Landscape factor weakens the chronic toxicity test**. How is it possible to verify that the collected pollen origin is not from another contaminated area? Considering that non-treated areas are often contaminated by multiple pesticide residues, and that even insects in nature conservation areas were found to be contaminated with on average of 16.7 pesticides (Bruhl et al. 2019) we suggest a LF of 1 also for chronic and larvae tests in the Tier 1 exposure assessment.

Residue Unit Dose (RUD) (Ch. 5.3.8, p. 48)

=> The new data set used by the WG is composed exclusively of non-published studies submitted for regulatory purposes (industry studies). The data in Appendix F of EFSA (2013) were not included in this analysis, because they also included open literature studies and were compiled according to several worst-case criteria (Supplementary Document, lines 2107-2114, p. 67). The Residue Unit Dose (RUD) is an important parameter and its calculation should include the largest available amount of data, despite the additional challenges for comparison that a more heterogenous data-set (including also open literature studies) implies.

Half-life in pollen and nectar (DT50po, DT50ne) (Ch. 5.3.9, p. 50)

=> The database used to calculate half-life in pollen and nectar included 39 DT50 values for **10 active substances** (for pollen), and 22 DT50 values for **7 active substances** (for nectar) only (Supplementary Document, fig. 21, p. 76), of which several DT50 values (31% and 41% of the whole dataset for pollen and nectar, respectively) were derived from trials conducted with the active substance sulfoxaflor, that degrades faster than other substances, especially for pollen. Considering that environmental conditions, pesticide application patterns, chemical properties, and plant varieties cause considerable variations in residue levels in nectar and pollen (Li, 2022), we stress that that spatiotemporal, chemical, and plant-related factors must be carefully considered for the widest possible range of substances, in order to refine pesticide exposure assessment for honeybees. Therefore, the database used to calculate half-life in pollen and nectar may be not representative of the whole range of substances and related half-life in pollen and nectar. Further investigation and a larger database would be necessary to establish these values.

Half-life in plant matrixes (DT50 $_{pnt}$) (Ch. 5.3.10, p. 50)

=> To confirm the default plant DT50 value of 10 days established in the updated GD, further investigations are necessary, including a derivation of a plant DT50 in a quantitative way.

Effect assessment in lower tiers (Ch. 6, p. 63)

=> The WG proposes defining the effect endpoints as function of both LD50,j and slopej , determined by a log-logistic dose-response function.

=> The a priori selection of a specific dose-response model may lead to overlook non-monotonic dose-response data; model averaging should be recommended.

Toxicity studies (Ch. 6.1.2 , p. 64)

=> Toxicity studies (tier 1) should be mandatory also for bumble bees and solitary bees. For acute toxicity validated OECD tests or draft guidelines are available.

=> Chronic toxicity tests for bumblebees can be performed according to the latest ring test protocol (2019) based on OECD guidelines No. 245 (2017) and No. 247 (2017)1. We strongly encourage the WG to include this protocol into the risk assessment.

=> It would be crucial to integrate a larval toxicity test for solitary bees: larvae of solitary bees of the genus Osmia feed on unprocessed pollen during development, which make them extremely vulnerable to pesticides. This risk should be addressed, by integrating the oral toxicity protocol for solitary bee larvae developed by Claus et al., 2021 (see also Feraerts et al., 2020) into the risk assessment.

Active substances and Plant Protection Products (Ch. 6.1.3, p. 66)

=> The growing evidence of adverse effects caused by « inactive » ingredients (e.g., ingredients such as co-formulants, adjuvants, solvents, carriers), highlights the need to carefully assess the risk of both these ingredients and pesticide formulations to bees (Rinkevich et al., 2015; Straw & Brown, 2022). To this end, in order to guarantee that the toxicity of a plant protection product is either the same or lower than the active substance tested, the ratio of 3 indicated in the 2022 GD to be used for the investigation of potential higher toxicity of the PPP based on the acute toxicity endpoints should be lowered to 1.5.

=> In case of indication of higher toxicity of the PPP, not only chronic and brood data should be provided, but also testing of sublethal effects for the PPP, as clearly established by Regulation (EU) 284/2013 (10.3.1): « (when) the the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the active substance tested, … testing by both acute (oral and contact) and chronic toxicity, **including sub-lethal effects**, shall be conducted. »

Extrapolation between species (Ch. 6.5, p. 70)

=>**Extrapolation factors for bumble bees and solitary bees are exclusively derived from acute (mostly contact) tests, but applied to all types of tests with adult bees, despite being uniquely derived from the acute (mainly contact) tests.** (2246-51, p. 70). Especially for larvae and chronic assessments, this may not be sufficient. Testing of BB and SB with available tests GD, draft guidelines or other available protocols would allow a more precise qualification of toxicity for these species as well as gathering more data.

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[1]A protocol for chronic toxicity for bumble bees (bumble bee adult chronic oral test (ICPPR) has ben developed and a validation report is in preparation to initiate the process to develop it into an OECD Guideline document. It could already be integrated in the new GD.

=> A **Tef = 1 is proposed from honey bee larvae to bumble bees and solitary bee species** (lines 2268-9). However, the relative sensitivity of solitary bees to honey bees for chronic and developmental endpoints remains unknown (Claus et al., 2021). The larval stage is of utmost importance for the fitness of the solitary bee: it is therefore necessary that larval toxicity tests are conducted for solitary bees, without extrapolating the risk assessment on a Tef=1 from honey bee larvae.

=> Slopej from tests with bumble bees and/or solitary bees should be used.

=> More research is needed on the issue of inter-species sensitivity. A recent study (Kueh Tai et al., 2022) with oral and contact bioassays for Leioproctus paahaumaa, a solitary ~~ground-nesting~~ bee, and A. mellifera, using imidacloprid and dimethoate, showed that bees responded inconsistently; L. paahaumaa were 36 and 194 times more susceptible to oral and topically applied imidacloprid than A. mellifera, but showed comparable sensitivity to dimethoate. This study stresses the **urgent need for more comparative inter-species toxicity studies.**

=> The extrapolation factors presented in this section are estimated from the relationship between LD50 and bee weight. Vulnerability of solitary bees compared to honey bees does not depend only on weight and sensitivity, because dissimilarities in life history traits between solitary and social bee species: univoltinism with flight period during time of pesticide application, underground nesting cavities in soil close to the treated crop, contaminated nest-building material such as mud from treated fields, short foraging range combined with a nest location close to treated crop, absence of trophallaxis, oligolectic larval diet on pollen origina~~ting from treated crops~~ and low body weight are all linked with a possible increased risk (Brittain and Potts, 2011). How are these specific risks taken into consideration?

Summary of the selection of hazard parameters for the risk assessment (Ch. 6.6, p. 72)

=> The GD should clearly state that tests with bumble bees (OECD Test Guideline No. 247 and 246) and solitary bees (Osmia spp. Roessink I, 2019 and Roessink I, 2017) are compulsory and must be used as a reference to derive hazard parameters for these groups of bees.

=> It would be crucial to conduct also a larval development test protocol for solitary bees, considering oral exposure of unprocessed pollen is mainly during this life stage (Eeraerts et al., 2020, Sgolastra et al., 2015). A larval test protocol is suitable to assess, apart from lethal effects, sublethal effects, for example, on development and longevity. The oral toxicity protocol for solitary bee larvae developed by Claus et al., 2021 could be used in the context of screening and first tier evaluation (see also Eeraerts et al., 2020).

Hazard parameters for the risk assessment of bumble bees (Ch. 6.6.2, p.74)

=> The new GD should clearly state that for bumble bees, OECD TG 246 and 247 acute tests MUST be provided in the dossier (and not « may be provided » as presently stated in the draft GD, lines 2355-56) with both active substance and representative PPP.

=> HB SlopeJ without assessment factors may not be enough protective.

Hazard parameters for the risk assessment of solitary bees (Ch. 6.6.3, p. 74)

« For solitary bees, since standard tests are not yet available, the risk assessment should generally be based on hazard parameters previously selected for honey bees, with an extrapolated LD50,j obtained after applying the appropriate Tef to the honey bee LD50,j, as explained in Section 6.5. The honey bee slopej can be used 'as is' for solitary bee risk assessment as well. However, when studies based on publicly available test protocols or draft OECD TG e.g. on O. rufa are available (likely for acute exposure only), they can be used to derive the hazard parameters for the solitary bee risk assessment. When this is the case, the LD50 (or surrogate LD50) from those studies could be used to obtain the extrapolated LD50,j after applying the appropriate Tef. » (lines 2377-85)

=> **The new GD should clearly state that for solitary bees, hazard parameters must be derived from studies based on draft OECD TG (Roessink I, 2019 and Roessink I, 2017) for acute toxicity, and that experimental protocols for larval toxicity test (e.g. the protocol in** Claus et al., 2021**) should be employed. The LD50 (or surrogate LD50) from those studies should be used to obtain the extrapolated LD50,j after applying the appropriate Tef.**

Options for refinement (Ch. 6.7, p. 74)

The draft GD states that if studies are available with multiple species belonging to the same bee group, the geomean approach could in principle be used to combine LD50 values after having applied the appropriate Tef to each of them.

=> Any merging of data (either geometric mean or SSD) between studies available with multiple species belonging to the same bee group is not protective enough when considering the great variably existing among bee species. To ensure a more conservative approach, The appropriate TEF should be instead applied on the the lowest LD50. (The Lowest LD50 should be selected and then the TEF applied, or at least grouping by major groups.

Interpretation of the result (Ch. 7.3.1, p. 83)

=> The draft GD acknowledges the lower resilience and higher vulnerability to stressors of bumble bees and especially solitary bees compared to honey bees (lines 2688-90, p. 83) and therefore suggests « a conservative approach to interpret the result » (lines 2693-4, p. 83). How does this work in practice? How these differences and potential greater vulnerability are going to be taken into consideration in the RA results?

Time-reinforced toxicity (Ch. 8, p. 83)

=> The draft GD proposes an assessment strategy for TRT based on the results of the honey bee chronic toxicity study according to OECD guideline 245 (OECD, 2017c), as an analysis performed by the WG has shown that data from a ten-day study, in in combination with a modified GUTS-RED-SD model, can be used to reliably predict the toxicity for a longer exposure period (Section 5 of Annex G to the Supplementary Document. If sufficient mortality is observed in this study, the results also form a good basis for assessing whether the substance shows time-reinforced toxicity » (Annex G - lines 788-99)(Annex G - lines 788-99).

=> The **model-based approach (analysis from the** toxicity data from the first 10 days of the study, combined with **a modified GUTS-RED-SD model) suggested by the** GD is a promising tool to predict TRT (the extrapolation of the time to 50% effect is predicted with an uncertainty of about +/- 30%); however, for the time-being, we consider that real time-to- effect studies, providing the advantage of a prolonged observation, should be favored. This will allow also the detection of long-term toxicity without TRT. To this end, the methodology used in Tosi et al., 2019 (a time-to-death approach in which individuals were monitored until at least 50% of controls died) should be applied. This study revealed long-term lethal and sublethal effects of flupyradifurone, which impaired bee survival and behaviour at field-realistic doses (down to 11 ng/bee/day, corresponding to 400 µg/kg) that were up to 101-fold lower than those reported by risk assessments (1110 ng/bee/day), despite an absence of time-reinforced toxicity. This study also demonstrates that current laboratory risk assessments of pesticides most likely underestimate their impact because the 10-day observation period is too short and too focused on lethal impacts. Mandatory real time-to- effect studies will also allow the production of more data for the refinement of the model-based approach. Therefore, we suggest that the TRT test (at least in the form of time-to-death test as in Tosi et al., 2019) is included as a mandatory and systematic test.

=> T**o be coherent with the regulatory mandate of wild bee protection, such tests should be conducted also for bumblebees and solitary bees**. TRT test for bumblebees can be conducted according to the latest ring test protocol of the Chronic Toxicity Test for bumblebees (2019) based on OECD guidelines No. 245 (2017) and No. 247 (2017). For solitary bees, see the protocol in Azpiatzu et al., 2022.

Sublethal effects on honey bees in risk assessment (Ch. 9, p. 91)

=> **the WG has decided to focus primarily on a subset of sublethal effects, in particular those that very obviously alter bee behaviour**. **The focus on bee behavior for sublethal effets, while important, is not sufficient**. Other important sublethal effects, especially those related to reproduction **(HB sperm quality for instance)** and physiology and immune system of bees (Di Prisco et al., 2013) must be included. The sublethal and long-term impacts of pesticides may be severely underestimated by relying on short-term and lethal impacts. We also suggest a more accurate and thorough assessment of abnormal behaviours, possibly made more feasible for mass assessments by publicly available behavioural descriptions and video analyses adapted for standard risk assessments (Tosi et al. 2019).

=> HPG tests and PER tests need to be included in tier 1. Even if standardized protocols for these tests do not exist yet, well established protocols have been performed for many years and represent an efficient and cost-effective additional source of data. We also feel confident that their inclusion in the regulatory risk assessment will facilitate their rapid standardization.

=> Hypopharyngeal glands (HPGs) are essential for the colony's development because they produce, among other, royal jelly to feed both larvae and the queen. They may serve as an indication of sublethal toxicity (Hatjina et al., 2013). The WG agreed to no longer include a risk assessment for HPG development in the revised Guidance Document mostly based on the lack of scientific data and standardized testing methods available. While the WG indicates that there might be a lack of studies demonstrating a direct link between HPG and the colony size reduction, the lack of information (and standardized tests) should not be a reason for the HPG assessment removal. Especially as authors of recent studies suggest that the HPG toxicity might be indeed linked to the development and productivity decline of the bee (and bumblebee) colonies which can be directly linked to colony fitness, and size (Karedla et Al. 2022; Minnameyer et Al. 2021).

Additionally, the WG examined studies from industry dossiers submitted to EFSA in which the effect on HPG development was measured to investigate the likelihood of the situation that the NOED for HPG is at least a factor of 33 lower compared to the LDD50 for the chronic oral exposure (i.e., HPG risk assessment failing when the chronic risk for adults is acceptable). However, the whole data set shown by the WG is composed of only 7 examples of active substances. The number of results of HPG development measurements (7) the WG uses as an argument, is insufficient for a thorough evaluation whether the HPG assessment is of added value compared to the chronic risk assessment for mortality. More data sets should be assessed before such a conclusion. If more data sets are not available, as the WG indicates, additional studies should be performed to fill the gaps. Therefore, we suggest maintaining the HPG assessment, while performing more studies assessing the link between HPG and colony size and establishing standardized methods (size of the acini and protein content in the gland) to assess the development of the HPGs.

=> The PER test was not included or mentioned in the 2022 revised version. However, the PER has been evaluated as an "efficient complementary test for an overall assessment of the negative effects of pesticides on bee functionality" in recent scientific production (Ferreira et al., 2022). Up to date, this test is frequently used in scientific studies investigating pesticide-mediated behavioural changes, proving its continuous relevance (Riveros et al. 2022; Vaughan et al. 2022; Cartereau et al., 2022). Moreover, this test can be easily conducted for bumblebees as well (Siviter et al. 2022). Given the lack of sufficient variety of tests for sublethal effects included in the draft document and the fact that well established protocols for PER exist (Giurfa et al., 2012; Smith et al., 2014; Takeda, 1961), we suggest the inclusion of PER test in the revised version of GD.

=> In the draft GD is stated that « if a reliable and complete higher tier risk assessment is available (see Chapter 10), there is no need to specifically consider sublethal effects and an assessment does not need to be carried out. » (lines 3002-04, p. 91). In our opinion, an assessment of sublethal effects (lower tier RA) should always be included in the dossier. It could be also useful to introduce the Sublethal Toxicity Ratio (SubTR) as an additional endpoint (the ratio between a pesticide's LOAEL and LD50) as suggested by Tosi et al., 2022.

=> **Sublethal effects should be assessed also for BB & SB**. Sublethal effects on activity, locomotion, homing flights, reproduction perturbations, such as reduced fertility of queens or reduced lifespan of queens and drones of Bombus terrestris (Whitehorn et al., 2012) have been described in wild bee species. For solitary bees, the protocol in Claus et al., 2021 allows to simultaneously assess larvae toxicity and sublethal effects could be employed.

Using pattern of sublethal effects seen in the laboratory tests (Ch. 9.2.2, p. 93)

=> The WG propose using the regular observations required in OECD 213, 214 and 245 ((OECD, 2017c, OECD, 1998b, OECD, 1998a)) to determine if exposure to a PPP influences the behaviour of bees in laboratory experiments. (lines 3055-7). We suggest that observations of sublethal effects based on standard OECD guidelines, should be further refined using recently published behavioural protocols and videos (Tosi et al. 2019).

Homing flight study (Ch. 9.4, p. 97)

The draft GD states that the homing flight study can only be used as a refinement of a concern indicated from acute exposure (i.e. at the second screen, if the NOEDhoming test is higher than the PEQ for acute exposure, there is no concern from acute sublethal effects on foraging behaviour).

=> Homing flight study should be performed systematically (without passing through the screening steps, i.e. even if the NOEDhoming test is lower than the PEQ for acute exposure), for all the neurotoxic pesticides, as recommended by ANSES (2018).

Metabolite (Ch. 11, p. 114)

=> In the EFSA 2013 document, risk assessment for metabolites was triggered when “the metabolite is exceeding 10% (total radioactive residues or TRRs) **or** 0.01 mg/kg is identified in the plant metabolism study”.

However, in the new revised document EFSA 2022 the rule changed and the RA of metabolites is now triggered only when “residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **and** 0.01 mg eq/kg in residue studies in pollen and nectar or metabolism studies in primary and rotation crops” **OR** “residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **or** 0.01 mg eq/kg in residue studies in pollen and nectar or metabolism studies in primary and rotation crops, **and** their parent substance is of acute toxicity to bees.”

=> A metabolite can be more toxic than the parent compound in bees (for example in the case of triclopyr or acetochlor (Lewis et al., 2016)), i.e. a metabolite can be toxic to bees even if the parent substance is not of acute toxicity. Therefore we suggest that the rule of

when the RA of metabolites is triggered remains the same as it was described in EFSA 2013.

Mixtures (Ch. 12, p. 121)

=> Tank mixtures are not within the scope of the draft guidance, but still they may result in enhanced toxicity. **The combined toxicity of insecticides, fungicides and herbicides on bees needs to be considered**: while acknowledging that is a present impossible to have an exhaustive approach, prioritizing the testing of the more common intentional mixtures (tank mixtures) and the testing of the binary combinations that occur most frequently in the field could be a good start to assess exposure (and related risks) in real world, as well as to fill critical data gaps quickly. Experimental evaluation of mixtures should be done. Testing for potential synergies of pesticides that have a greater probability to cause harmful interaction effects based on their chemical characteristics (i.e., mode of action) (Tosi et al. 2019) and their likelihood of co-exposure in the real world should be mandatory.

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