1.18 Linking protection goals to trigger values using compound specific properties: Chronic risks to bees

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Abstract

In the EFSA guidance document for the assessment of risk of plant protection products for bees the screening and tier I trigger for chronic risk to bees is linked to a trigger value which is intended to meet a certain level of protection. However, the methods used to derive the trigger of 0.03 do not take into account several factors including the shape and nature of the dose-repose used to generate the endpoint. This means that the resultant proposed trigger leads to a large over estimation of risk with a large number of compounds failing the risk assessment and being incorrectly identified as a higher chronic risk to honey bees. We analyzed the methods used in the selection of the trigger of 0.03 and propose simple adaptations to evaluate all active substances to the same level of protection by taking into account the type of endpoint and the dose response relationship. We found that by using the correct dose-response relationships we could accurately ensure that the desired level of protection was met. We checked our proposal using real-life examples of seven substances registered for use within the European Union and discuss how these proposals could be used to inform risk assessors and risk managers as well as potentially reducing the number of false positive and negatives in a risk assessment.

Keywords: Honeybee, risk assessment, protection goals, triggers, pesticide

Introduction

In the EFSA guidance document for the assessment of risk of plant protection products (PPP) to bees a number of new trigger values are proposed (EFSA 2013). One of concern due to its conservative nature is the honey bee chronic oral trigger of 0.03. In effect a substance is considered low risk if the 10 day chronic LDD_{50} is 34x higher than the estimated exposure. An impact analysis indicated that using this trigger almost all substances would not pass the screening or tier I risk assessment leading to higher tier evaluations even for substances of low toxicity (Alix et al 2013, Miles et al 2018). Where a risk assessment is designed to meet a certain level of protection the triggers need to ensure that there is a low possibility of false positives but without generating excessive false negatives. The suggested scheme and trigger (EFSA 2013) is over conservative and leads to an excessive number of false positives.

In this paper we set out some mathematical solutions to ensure that the Specific Protection Goal (SPG < 7% colony reduction) can be met without the generation of excessive false positives in the risk assessment. We show how the information from the dose-response analysis can be used to calculate the trigger needed to meet the SPG as defined by EFSA (2013) and also suggest alternative approaches when the 10 day chronic LDD_{50} endpoint is not available and only a NOEDD could be generated. In addition the problem is considered from the opposite position to indicate the level of protection actually observed from the calculated ETR value by using the concept of the Individual Effect Chance (IEC). We illustrate the utility of these approaches with real life examples of risk assessments.

Material and methods

We analysed the methods and the underlying assumptions used in EFSA 2013 to calculate the actual level of protection afforded by a trigger of 0.03 to a range of plant protection products (PPP). This was done by comparing the differences between the use of the trigger described by a linear model (fixed to 0.03) with triggers linked specifically to hypothetical compounds meeting a Log-Probit dose-response relationship for a range of different slopes which is more like the real-life situation.

The specific protection goal (SPG) linked to the trigger of 0.03 is set to ensure that a maximum of 7% reduction in colony size is not exceeded. The honey bee forager model of Khoury et al (2011)

was used to translate an increase in forager mortality to the SPG as 1.27x in hive background mortality (5.3%) over 10 days. This means the maximum increment in mortality is:

Max increment = 0.27 x 5.3 = 1.43 % mortality

(i.e. equivalent to no more than 1 dead bee in 70).

Using a linear interpolation model the chronic trigger was set as:

10 day Chronic trigger LDD_{50} = 50%/1.43% = 34 (0.03)

As this uses a linear model the trigger overestimates the required level of protection as true dose-response relationships are sigmoidal rather than linear (Finney 1952). The area between the linear and sigmoidal functions (Figure 1) represents the overestimation of meeting the SPG. A linear model also does not take into account the true shape and slope of the dose-response. The use of a linear model like this ensures that the SPG will virtually never be exceeded. However, as already stated the linear model brings with it an over simplification and the identification of many low risk uses as high risk to bees.

For many non-insecticidal substances it has been observed by the authors that the measurement of a $LDD_{50chronic}$ is not technically possible due to low toxicity and/or limited solubility (e.g. many herbicides and fungicides). In these cases a no observable effect daily dose (NOEDD) can be determined. The use of a NOEDD also leads to an exceedance of the level of protection and false positives (i.e. low risk is indicated at $^1/_{34}$ of the NOEDD) as the trigger was calibrated for a $LDD_{50chronic}$.

To make it possible to conduct a meaningful risk assessment where there is no $LDD_{50chronic}$ but a NOEDD is available we calculated a suitable trigger which offers at least the same level of protection. As the NOEDD is at a part of the dose-response where the relationship is rather flat we can use the calculation of EFSA using the linear interpolation model but assuming the NOEDD is equivalent to the $LDD_{10chronic}$ which is now a common place approach in ecotoxicology:

Chronic trigger NOEDD = 10%/1.43% = 6.99 (0.143)

Consequently, where no $LDD_{50chronic}$ endpoint is available the NOEDD or LD_{10} endpoints can be used with a trigger of 0.143 in place of 0.03. This ensures that the protection goal will be met without the need for an over conservative assessment.

To ensure that the required level of protection is met and not exceeded the following approaches are proposed. Where the type of model and slope are known and there is a quantified $LDD_{50chronic}$ endpoint this information is used to calculate the appropriate trigger or use a look up table (see Table 1).

Alternatively, information about the model and slope can be used to calculate the individual effect chance (IEC) indicated by the observed ETR value as this is proportionate to the level of effect expected at the given exposure level. If the trigger is breached or the IEC calculation indicates a mortality rate of higher than 1 in 70 then further investigation is needed as the protection goal is not met. The IEC can be calculated using the following formula assuming a dose-response model based on a probit assumption (i.e. log normal distribution of individual sensitivity)

$$\log LD_k = \log LD_{50} + (z/b)$$

where: z is the standard normal deviate and b equals slope.

To test and illustrate the utility of these approaches we conducted a risk assessment for seven pesticide active substances belonging to Bayer AG (three herbicides, two fungicides and two insecticides) as test cases. For each compound the endpoint ($LDD_{50chronic}$ or NOEDD) from a scientifically valid honey bee 10 day chronic feeding test was taken from the report along with information about the dose-response model and slope where available (Table 2). A worst case typical European Union use pattern was selected and ETR values calculated using the tier 1

method of EFSA (2013) and the shortcut value (SV = 5.8) for a downward directed spray applications.

Results and discussions

In almost every hypothetical case the level of protection achieved greatly exceeded SPG of <7% colony reduction (Table 3) when the difference between linear and sigmoidal dose-response relationships are considered. For example the conditions where, the trigger value of 0.03 meets the SPG for a given $LDD_{50chronic}$ are only met where the slope (b) of a Log-Probit dose-response relationship is 1.43. If the slope is greater than this the level of protection will exceed the SPG which is the case for the majority of compounds and generates a large number of false positives. This will differ for different models (e.g. Log-Logit and Weibull) but always leads to exceeding the desired level of protection.

The resultant ETR values (Table 2) were compared to a trigger of 0.03 and in this case only one active substance was shown to pass (herbicide 3 with an ETR = 0.012). The main reason for this active substance and use to pass the risk assessment was that it is a compound of low bee toxicity with a very low use rate (10 g a.s./ha). As over 85% of our examples failed to meet the chronic risk trigger of 0.03 we looked more closely at the type of data we had. For five of the examples a defined $LDD_{50chronic}$ toxicity endpoint was available with information about the dose-response relationship. Using this information we calculated the actual triggers required to meet the SPG. For the remaining two substances the studies were conducted as limit tests where no mortality was observed so the $NOEDD/LDD_{10chronic}$ trigger of 0.143 was applied (one of these was herbicide 2 which had passed at tier 1 with an ETR less than 0.03). When the trigger was adjusted to account for either the shape of the dose-response (i.e. sigmoidal vs. linear) or for the endpoint (NOEDD in place of the $LDD_{50chronic}$) four of the seven substances passed the risk assessment while retaining the required level of protection demanded by EFSA 2013. This included herbicide 1 and also fungicide 2 and insecticide 1. The latter two are currently registered for use in flowering crops where bees may be present based on no unacceptable effects in higher tier data (semi-field and field studies). Insecticide 2 which is toxic to bees with chronic 10 day LDD_{50} = 0.0137 µg a.s./bee/day unsurprisingly did not pass using the modified trigger of 0.657 (based on a Log-Probit model and a slope of 12), whereas for herbicide 2 the adjust trigger based on the compound specific properties indicated a high margin of safety.

Using the information from the endpoint and dose-response relationship (where available) the calculated ETR values can be used to predict the level of morality that would be expected to occur due to exposure to the estimated exposure level (by using the Individual chance of effect or ICE concept. If the ICE indicates that less than 1 bee out of 70 will die (i.e. 1.43% mortality) then the risk assessment will meet the SPG. It was possible to calculate the IEC for five substances. For herbicide 1 exposure was predicted to lead to the death of one additional bee out of 5038 bees (0.02% mortality). High level of safety was also shown for fungicide 2 and insecticide 1 with very low levels of mortality predicted at the exposure level used in the risk calculation indicating how over conservative the use of a trigger of 0.03 would be. For insecticide 2 the ICE calculation indicated that all bees would die at the exposure level used in the risk calculation. Where the dose-response information was missing we compared the calculated ETR values to the trigger of 0.143. Where the ETR was below this trigger (herbicide 3) we could conclude that less than 1 in 70 bees would die and for herbicide 2, where the ETR was above the trigger that it could not be excluded that more than 1 in 70 bees could die.

The seven examples illustrated how over conservative the trigger of 0.03 is if applied to a chronic risk assessment calculation. In almost all cases a high risk was indicated. By taking information about the endpoints and (where available) about the dose-response relationship it can be seen that in over half the cases the trigger incorrectly identified a risk where none was present. Although we precisely calculated the trigger to meet the SPG for each substance we also present a look up table (Table 1) for trigger which may prove to be useful tool for risk assessors wishing to

apply the correct trigger to meet the SPG of less than 1.43% mortality. Risk managers may find the ICE calculation useful as it puts the actual level of predicted effects into context and such information could be useful in evaluating the effectiveness of risk mitigation.

Conclusions

The use of a trigger of 0.03 in chronic risk assessment for honey bees leads to a large number of substances failing the risk assessment and requiring a higher tier evaluation.

An analysis of the method used to define the trigger and of real-life ETR values for a selection of active substances registered in the E.U. (e.g. use of a linear model) indicated that there was high possibility of incorrectly identifying low risk substances as high risk.

We present a simple method to evaluate all active substances to the same level of protection by taking into account the type of endpoint (i.e. $LDD_{50chronic}$ or NOEDD) and the slope of the dose response relationship which is compound specific.

The actual level of protection afforded by a given exposure toxicity ratio (ETR) as the individual chance of effect (ICE) can be calculated allowing for better informed decision making by risk managers.

The number of false positive and negatives in a risk assessment could be reduced by using specific triggers based on the properties of the test substance.

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Table 1 Look up table for triggers to meet Specific Protection Goal (SPG < 7% colony reduction) for chronic risk assessment

Compound slope (b)	Trigger adjusted for slope to meet SPG of 1 dead bee in 70		
	Log-Probit	Log-Logit	Weibull
1	0.0065	0.014	0.0062
1.43	0.03	0.052	0.029
2	0.080	0.120	0.079
3	0.186	0.244	0.184
4	0.284	0.347	0.281
5	0.365	0.429	0.362
6	0.432	0.494	0.429
7	0.487	0.546	0.484
8	0.532	0.589	0.530
9	0.571	0.625	0.569
10	0.604	0.655	0.602

Table 2 Chronic risk assessment case studies for adult honey bees. Exposure Toxicity Values (ETR) values in bold do not pass the trigger of 0.03, shaded cells indicate that further refinement is needed following consideration of endpoints and compound toxicological properties to meet the Specific Protection Goal (SPG < 7% colony reduction).

Compound code	GAP[1] (kg a.s./ha)	Endpoint (µg a.s./bee/day)	Model	Slope	ETR[2]	Actual trigger required to meet SPG	Individual chance of effect (ICE)
Herbicide 1	0.238	LDD_{50} = 14.5	Probit	3.468	0.0952	0.234	1 in 5038
Herbicide 2	0.48	NOEDD > 4.4	N/A	N/A	0.633	0.143	≥1 in 70
Herbicide 3	0.01	NOEDD > 4.7	N/A	N/A	0.012	0.143	≤1 in 70
Fungicide 1	0.15	LDD_{50} = 2.62	Probit	2.603	0.332	0.144	1 in 9
Fungicide 2	0.25	LDD_{50} = 10.2	Probit	3.529	0.142	0.240	1 in 722
Insecticide 1	0.0075	LDD_{50} = 0.53	Probit	3.080	0.08	0.195	1 in 2744
Insecticide 2	0.06	LDD_{50} = 0.0137	Probit	11.997	25.4	0.657	1 in 1

[1]GAP : Good Agricultural Practice, i.e. use rate of active substance/ha; [2]ETR = SV x use rate / Endpoint, where SV = 5.8 (EFSA 2013).

Table 3 Effect of sigmoidal dose-response relationship and slope on implied level of protection vs. a linear model

Slope (b) Log-probit model	Individual effect chance at (IEC) 0.03 threshold	Mortality (%) using trigger of 0.03	Risk overestimate	Adjusted Trigger to meet SPG
1.43	1 in 70	1.43	None	0.03
2.0	1 in 862	0.12	13x	0.082
3.0	1 in 407,000	0.00025	59850x	0.189
4.0	1 in 1.78×10^7	0.00000006	26,296,399x	0.285
5.0	1 in 7.55×10^{13}	0.0000000000013	1,110,160,000,000x	0.367

Figure 1 Linear (assumed) vs. sigmoidal (actual) dose response functions (modified after EFSA 2013). The area between the linear and sigmoidal lines represents the overestimation caused by using a model which does not accurately represent the dose-response relationship.

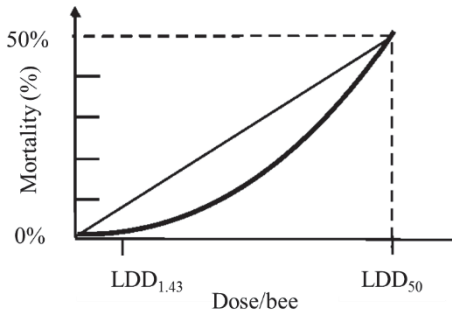

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Foto
Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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