# Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-Apis bees

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that a risk assessment demonstrates low risks to human health and the environment, among which includes pollinators. Currently risks are evaluated for honey bees and for non-target arthropods (NTA) of cultivated ecosystems. The actual protection of pollinators other than the honey bees, as for example for non-Apis bees, in relation to these risk assessments has recently been questioned and requires further investigation. We present the findings from a comparison of Hazard Quotient (HQ) value calculations to assess the risk to honey bees, non-target arthropods and to non-Apis bees (with the application of an additional safety factor of 10). Calculations were based on publicly available ecotoxicological data.

Results: The risk to NTA, honey bees and non-Apis bees, as depicted by HQ values, indicated a higher fail rate for NTA than for bees, but a similar pass / fail rate for non-Apis bees when compared to the NTA scheme. Outcome of the risk assessment for NTA using extended laboratory tests gave similar pass/fail rates compared to the screening step for honey bees.

Conclusion: A screening step for non-Apis bees could be developed based on data available on honey bees and NTAs.

**Keywords**: risk assessment, non-Apis bees, pollinators, pesticides, non-target arthropods.

## 1. Introduction

Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species.[1] These include pollinators such as the honey bee[2,3] but also other non-target arthropods (NTA), the latter covering species within the agricultural landscape and more specifically, a wide range of groups such as pollinators, herbivores, predators, parasites, fungivores and detritivores.4 In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection and conservatism. Tier I screening risk assessments are thus intended to rapidly exclude those substances which pose a low risk to non-target organisms and to focus resources on those for which a potential risk cannot be excluded and further studies may be undertaken to characterize the conditions and occurrence of risks.

In the case of the honey bee, the Tier I screening risk assessment is based on a Hazard Quotient (HQ) approach[3]. This HQ is calculated by dividing application rate by the LD50 (Lethal Dose for 50% of the organisms exposed in the test). Similarly for NTA, the HQ is calculated on the basis of the LR50 (Lethal Rate 50% of the organisms exposed in the test) of two indicator species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*),4 in the same way as the honey bee calculation using the application rate.

In the risk assessment process as currently used in Europe, both the robustness and suitability of the NTA and honey bee HQ as a screen step for spray applied products has been validated.[4,5] In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed as a trigger for higher tier risk assessment.[6]

The actual protection of pollinators other than honey bees, (e.g. non-Apis bees), that is achieved by these risk assessments has recently been questioned and need further investigation identified. At the Pellston workshop on Pesticide Risk assessment for Pollinators,[7] it was suggested that for risk assessment the honey bee could be a suitable surrogate species for other bee species. However, to account for potential differences in the sensitivity between the honey bee as a test organism and

other non-Apis bees a safety factor of 10 (for interspecies differences) was suggested to be applied to the trigger value used for the HQ calculated for honey bees.

To explore the effectiveness and impact of this approach, HQ values were calculated to assess the risk to honey bees and non-target arthropods. These were compared to a hypothetical HQ based on the honey bee data to cover non-Apis bees which included additional assessment factor of 10. Publically available ecotoxicological data for honey bees and NTA were used. This paper presents the outcome of this analysis and derives some recommendations in the perspective of the development of a screening step in a risk assessment scheme dedicated to non-Apis bees.

## 2. Data analysis

For this initial exploratory analysis Draft Assessment Reports (DAR) and ESFA conclusion reports8 were examined and LR50 values for both NTA indicator species and LD50 values for honey bees were collected into a Microsoft excel spread sheet. The good agricultural practice (GAP) i.e. application rates of active substances, disclosed in the documents was used for exposure component of the the HQ calculations.

### 2.1 Tier I risk assessment for honey bees

For honey bees the HQ calculated was based on the highest single application rate for each representative use (g active substance/hectare). The HQ value is calculated by dividing the LD50 derived from acute oral and contact toxicity tests[9,10] expressed as microgram of active substance/bee, by the application rate.[3]

### 2.2 Tier I and Tier II risk assessment for NTA

For NTA, the exposure rate was calculated using the methods of Escort-24 based on the application rate. In the case of repeated applications, a Multiple Application Factor was applied in the exposure calculation to accounted accumulated residues from preceding applications to exposure due to the final application, thus leading to the highest exposure estimate. The Tier I HQ was calculated using LR50 values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* (as grams active substance/ha) derived from glass-plate tests.[3,4,11]

In the Tier II risk assessment for NTA, a 50% effect trigger is applied which is equivalent to an HQ trigger of 1 (based on LR50 values for *Aphidius rhopalosiphi* and *Typhlodromus pyri* derived from extended laboratory tests).[4,11] These tests differ from the Tier I tests in that arthropods are exposed to the product on a natural substrate (i.e. treated leaves) rather than an inert substrate (i.e. glass-plates).

### 2.3 Tier I risk assessment for non-Apis bees

The tier I risk assessment for non-Apis bees was performed by applying an extra safety factor of 10 to the trigger values used for the Tier I risk assessment for the honey bee.

### 2.4 Interpretation of the HQ values

For honey bees, if a calculated HQ value for a given product use was below the trigger value for the two exposure routes, it was concluded that the use was of low risk.

For NTA, if a calculated HQ value for a given product use was below the trigger value for the for the two species, it was concluded that the use was of low risk.

The following HQ triggers values were employed to define low risk to each ecological entity:

- Honey bees – HQ trigger = 50
- NTA – HQ trigger = 2
- Non–Apis bees – HQ trigger = 5 (i.e. 1/10th of the honey bee trigger to account for inter-species variation).

## 3. Results

A total of 93 product uses (for 74 active substances) were employed in the analysis. Results are presented as pass rate, i.e. the rate of products uses for which the outcome of the Tier I risk assessment calculation is below the trigger value for honey bees, NTA and non-Apis bees (Table 1).

**Tab. 1** A comparison the honey bee, non-target arthropod (NTA) and the proposed non-Apis bee hazard quotient (HQ) as a screening tool for tier I for 93 product uses (for 74 active substances)

| | | Product uses | | |
|---|---|---|---|---|
| **% of compound uses passing at Tier I** | **All** | **Herbicide** | **Fungicide** | **Insecticide/acaricide** |
| Honey bee HQ 50 | 75% | 100% | 91% | 23% |
| Non-Apis HQ 5 | 46% | 64% | 53% | 15% |
| NTA HQ 2 | 42% | 58% | 47% | 15% |
| Total number of uses | 93 | 33 | 34 | 26 |
| % NTA passes at Tier II (i.e. based on LR50 from extended laboratory studies) | 70% | 97% | 82% | 8.7% |

**Tab. 2** List of herbicide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

| **Compound** | **use** | **Honey bees** | **Non *Apis*-bees** | **NTA** |
|---|---|---|---|---|
| acetochlor | h | | > | |
| aclonifen | h | | > | |
| amidosulfuron | h | | | |
| azimsulfuron | h | | | |
| benfluralin | h | | > | |
| bensulfuron-methyl | h | | | |
| bifenox | h | | | |
| chloridazon | h | | > | |
| clodinafop | h | | | |
| clomazone | h | | | |
| clopyralid | h | | | |
| cycloxydim | h | | > | |
| diclofop-methyl | h | | > | |
| diflufenican | h | | | |
| dimethachlor | h | | | |
| fenoxaprop-P | h | | | |
| fluazifop-P | h | | | |
| flurochloridone | h | | > | |
| metazachlor | h | | > | |
| metosulam | h | | | |
| metribuzin | h | | > | |
| napropamide | h | | | |
| nicosulfuron | h | | | |
| oxadiazon | h | | | |
| penoxsulam | h | | | |
| picloram | h | | | |
| prosulfocarb | h | | > | |
| quinmerac | h | | | |
| quizalofop-P-ethyl | h | | | |
| rimsulfuron | h | | | |
| tralkoxydim | h | | > | |
| tribenuron-methyl | h | | | |
| trisulfuron-methyl | h | | | |

Note : > indicates that the honey bee toxicity end point used was the highest dose tested, (typically 100 µg active substance/ bee) rather than a defined LD50 value. In these cases it may be that a higher endpoint could be determined to refine the risk assessment.

A list of active substance names which pass and fail at Tier I for honey bees, NTA and non-Apis bees is presented for herbicides (Table 2), fungicides (Table 3) and insecticides/acaricides (Table 4).

**Tab. 3** List of fungicide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

| Compound | use | Honey bees | Non-*Apis* bees | NTA |
|---|---|---|---|---|
| azoxystrobin | f | | > | |
| carbetamide | f | | | |
| cyflufenamid | f | | | |
| cyprodinil | f | | > | |
| difenoconazole | f | | | |
| dimoxystrobin | f | | | |
| dithianon | f | | > | |
| dodemorph | f | | > | |
| dodine | f | | > | |
| epoxiconazole | f | | | |
| fenpropimorph | f | | > | |
| fludioxonil | f | | | |
| fluopicolide | f | | | |
| fluoxastrobin | f | | | |
| fosetyl-al | f | | > | |
| mandipropamid | f | | | |
| metrafenone | f | | | |
| prochloraz | f | | | |
| proquinazid | f | | | |
| prothioconazole | f | | > | |
| pyrimethanil | f | | > | |
| tebuconazole | f | | | |
| tolylfluanid | f | | > | |

Note : > indicates that the honey bee toxicity end point used was the highest dose tested, (typically 100 µg active substance/ bee) rather than a defined LD50 value. In these cases it may be that a higher endpoint could be determined to refine the risk assessment.

### 3.1 Pass rate for honey bees (HQ < 50)

Out of the total of 93 product uses, 70 (75%) were observed to pass at Tier I using a trigger of 50, indicating that 25% of all product uses required further evaluation, testing and risk assessment. When these uses were summarised by product type (herbicide, fungicide and insecticide / acaricide), it can be seen that all 33 (100%) herbicide uses and 32 out of 34 (91%) fungicide uses pass at Tier I. For insecticide/acaricide uses, 20 out of 26 (77%) results indicated that further evaluation was required.

Of the insecticide/acaricide uses which did not indicate a risk at Tier I (n = 6) these included compounds of known low toxicity to bees, highly selective products and insect growth regulators (IGR) which are not toxic to adult stages (Table 4). Note that IGRs are known to display toxicity towards developmental stages and thus can pose a risk to larvae. Consequently for the three IGR compounds further evaluation would have been automatically triggered for larval and brood effects so overall only three uses with HQ values above 50 were considered to be low risk at Tier I. However, this would not preclude additional testing based on evidence present in other parts of the dossier.

Finally, as it can be observed in Table 4, the screening step for honey bees reliably identified the need for further investigation for all neurotoxic insecticides.

**Tab. 4** List of insecticide/acaricide compounds which pass and fail at Tier I for honey bees, non-target arthropods (NTA) and non-Apis bees. White cells indicate pass at Tier I, grey cells indicated further evaluation is necessary

| Compound | use | Honey bees | Non-*Apis* bees | NTA |
|---|---|---|---|---|
| acequinocyl | i | | | |
| acetamiprid | i | | | |
| alphacypermethrin | i | | | |
| carbaryl | i | | | |
| diazinon | i | | | |
| dimethoate | i | | | |
| etofenprox | i | | | |
| fenoxycarb | i | | | |
| formetanate | i | | | |
| imidaclopid | i | | | |
| indoxacarb | i | | | |
| malathion | i | | | |
| methomyl | i | | | |
| oxydemeton | i | | | |
| phosmet | i | | | |
| pirimicarb | i | | | |
| pyridaben | i | | | |
| spiromesifen | i | | | |
| tau-fluvalinate | i | | | |
| triflumuron | i | | | |

### 3.2 Pass rate for non-target arthropods (HQ < 2)

Tier I risk assessment for NTA indicated an overall pass rate for all uses of 42% (39 out of 93). The pass rate was 58% for herbicide uses (19 out of 33), 47% for fungicide uses (16 out of 34) and 15% for insecticide/acaricide uses (4 out of 26), using an HQ trigger of 2 using both indicator species.

When findings from extended laboratory tests are considered for NTA in a Tier II risk assessment the pass rate was 97%, 82% and 8.7% for herbicides, fungicides and insecticides/acaricides respectively (Table 1).

As observed for honey bees of the four insecticide/acaricide uses which passed the screening step, one was a highly selective compound with a specialised mode of action and the remaining three products IGRs which are not toxic to adult stages. IGRs may pose a potential risk to immature arthropods and on mode of action the IGR compounds would trigger further evaluation using the appropriate tests. Consequently only a single use was considered of low risk at Tier I for NTA.

### 3.3 Pass rate for non-Apis bees (HQ < 5)

For non-Apis bees the pass rates were expectedly lower than for honey bees with 46% for all uses considered altogether (43 out of the total number of uses (n = 93)) (Table 1). This trend for fewer passes in the Tier I risk assessment compared to the honey bee was also seen when analysed by product type. For herbicide uses, 21 out of 33 (64%), for fungicide uses 18 out of 34 (53%) and for insecticide/acaricide uses 4 out of 26 (15%) passed at tier I using an HQ trigger of 5.

The four uses which passed the tier I risk assessment for non-Apis bees were the same as those for the NTA assessment and for the same reasons (i.e. highly selective compound with a specialised mode of action and IGRs). The three IGR compounds would have automatically triggered further evaluation of risk due to their mode of action. For the insecticide/acaricide uses which failed at Tier I for non-Apis bees these were based on actual measured LD50 values not 'greater than' values.

**4. Discussion**

In total 93 product uses (for 74 active substances) were employed in the analysis using publicly available data, conclusions and independent expert opinions. These represented the range of active substances evaluated between 2004 – 2001, and represent the range of product uses as herbicides, fungicides and insecticides/acaricides.

In a review by Mineau et al[12] the Tier I risk assessment for honey bees using an HQ trigger of 50 has been shown to adequately screen for spray applied products for which a risk cannot be excluded and thus warrant further investigation. The capacity of the Tier I to screen out substances of low risk from those which required further assessment was evaluated through a review of the honey bee kill incidents recorded in the United Kingdom Wildlife Incident Investigation Scheme (WIIS).[12] This analysis supported the utility and efficacy of the Tier I screening methodology, provided that special considerations on the mode of action, (as seen above for IGR and specific modes of actions and use patterns) are also considered in the risk assessment process.

The Tier I risk assessment for NTA identified far more uses for further evaluation compared to the honey bee assessment using an HQ of 50 while the Tier II risk assessment for NTA based on extended laboratory studies identified a similar number of product uses to pass to the Tier I honey bee risk assessment. As for honey bees, the Tier I risk assessment for NTA is intended to effective identify spray applied products for which a risk cannot be excluded. This is demonstrated by this analysis and has been validated and reviewed using laboratory and field data and sensitivity analysis.[13,14]

As expected, lowering the trigger value from 50 to 5 to account for non-Apis bees leads to far more product uses failing at Tier I. However, for herbicides all non-Apis bee Tier I failures were due to the endpoint being a 'greater than' value as the study did not determine a LD50 for honey bees. This is the same for all the fungicide uses which fail at Tier I (with the exception of prochloraz). For these uses studies to derive the actual LD50 may be necessary to enable a full evaluation as the real pass rate for herbicides and fungicides could be higher than predicted by the limitations of these data.

In this analysis a 10-fold safety factor to cover non-Apis bees was used only as an example and is likely to be highly conservative or even over conservative. The possible utility of value of 5 was discussed at the SETAC Pellston workshop on Pesticide Risk assessment for Pollinators workshop.[7] The selection of a definite value should however also consider available data on the relative sensitivity of the honey bee compared to other pollinating species. Recent work comparing the toxicity of dimethoate to a range of different bees indicated a very narrow toxicity range for bees, with the honey bee appearing in the middle of the range, thus indicating that the honey bee can be considered a suitable sentinel species (Figure 1).[15] It is hoped that researchers will continue to work on non-Apis bees and the database will continue to be expanded in the future.

The justification for the application of a safety factor needs to be supported by an evaluation of the uncertainties for which it is expected to account. In this analysis, the application of the 10-fold safety factor leads to the identification of a large number of herbicide and fungicide products and uses that would require further evaluation. This is in part due to mathematic biases related to the use of 'greater than' values and high application rates. However, an analysis of the uncertainties in relation to the anticipated effects of herbicides or fungicides to bees should be clearly defined when deciding on the need for a safety factor. In additional, in defining an appropriate screening step, differences in biology between bee species may be more important than differences in sensitivity, as they may affect the exposure profile.

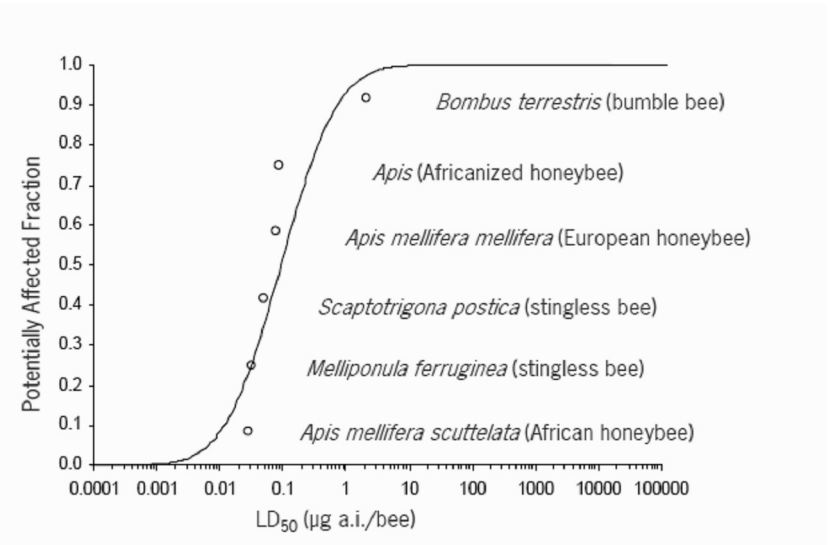


**Fig. 1** Species sensitivity distribution to dimethoate for different bees species (from Roessink et al., 2011).[15]

As discussed during the SETAC Pellston workshop on Pesticide Risk assessment for Pollinators workshop,[7] some NTA species may better represent the exposure routes for non-Apis bees. Any existing data on these species should be considered when analysing the level of risks expected to non-Apis bees. As an example, adult parasitoids such as *Aphidius rhopalosiphi* feed on nectar and are thus a good representative for exposure conditions of pollinating species. Similarly, the ground-dwelling beetle *Aleochara bilineata*, (a standard species used in the European risk assessment to NTA) is tested for sensitivity to plant protection products applied to the soil and as such data may be considered informative for ground nesting bees. In cases where a refined risk assessment has been triggered for NTA, the data set developed in the European process may contain information on several different species in the laboratory and more when semi-field/field testing has been undertaken (Table 5).[4] For field tests, inventories of species identified in the tested crop may also yield useful information in evaluating whether there is a particular concern for non-Apis species which would need to be investigated further.

**Tab. 5** Testing methodologies developed for the risk assessment to non-target arthropods developed in European process of evaluation of pesticides (from Candolfi et al., 2001)[4]

| **Testing scale** | **Species (and stages tested)** |
|---|---|
| Tier I Laboratory: artificial substrate | *Aphidius rhopalosiphi* (adults + life cycle) |
| | *Typhlodromus pyri* (protonymphs + life cycle) |
| Tier II (extended) Laboratory : natural substrate | *Aleochara bilineata* (adults + life cycle) |
| | *Aphidius rhopalosiphi* (adults + life cycle) |
| | *Chrysoperla carnea* (larvae + life cycle) |
| | *Coccinella septempunctata* (larvae + life cycle) |
| | *Orius laevigatus* (nymphs + life cycle) |
| | *Pardosa* sp. (adults) |
| | *Poecilus cupreus* (adults) |
| | *Trichogramma cacoeciae* (adults + life cycle) |
| Semi-field | e.g. *Poecilus cupreus* (adults) |
| | Methods can be adapted for many species |
| Field | Arthropods (populations and communities) |

In comparing the proportion of uses which passed at Tier I using the HQ trigger of 5 for non-Apis bees with the results of the NTA analysis, it was found that the numbers were very similar to the NTA analysis leading to slightly fewer Tier I passes. It has to be noted however, that the NTA analysis was conducted on data generated for the purpose of risk assessment and are not influenced by 'greater than' values. Thus the NTA Tier I risk assessment is far more conservative than the Tier I risk assessment for honey bees and similar to the non-Apis bee assessment presented in this paper. The two standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are indeed considered as the most sensitive of the non-target arthropods tested under the European Escort scheme.4 Also due to the limitations of the non-Apis evaluation discussed above it can be concluded that based on this assessment the NTA Tier I risk assessment is more conservative than the proposed non-Apis risk assessment based on honey bee data and a HQ of 5.

Due to the sensitivity of the tested sentinel species, the range of biological traits they represent and the conservatism of the screening step as observed above, NTA may then be reliable surrogates for non-Apis bees in a screening step of risk assessment. When the outcome of HQ calculation for honey bees indicates possible concerns for pollinating insects, additional information available for NTA as laboratory or extended laboratory tests could be analysed as a screening step. As shown above, the Tier I risk assessment for NTA appeared to be the most restrictive and could then be used as a screening step for non-Apis bees. When a risk cannot be excluded on the basis of Tier I calculations, all available data on NTA including extended laboratory tests and higher tier data could be examined in order to identify any reliable information addressing the possible risks to non-Apis bees, taking into consideration biological traits, plant-insect relationship and exposure routes specific to non-Apis bees.

This initial investigation has demonstrated the potential for existing risk assessment schemes to provide a suitable screen step for non-Apis bees. However, before any such scheme can be developed additional risk assessment and further testing will probably be necessary for a number of products, and more specifically for insecticides/acaracides which may also lead to the necessity for additional testing methods and species. These issues are being dealt with within the OECD where a working group is currently identifying the requirements in terms of testing in light of risk assessment needs.

## 5. Conclusions

The comparison of Tier I risk assessments for honey bees, NTA and non-Apis bees based on a representative sample of 93 product uses indicated that all three are able to rapidly identify compounds and uses for which the potential for adverse effects cannot be excluded. The outcome of screening steps were consistent for insecticides/acaricides for which possible effects are expected to be the highest and those which passed the Tier I assessment would, owing to their mode of action, have triggered a dedicated risk assessment according to current guidance documents.[2,3,4] However, the Tier I screening step for NTA and non-Apis bees presented in this paper had poor discrimination for herbicide and fungicide product uses with many products potentially triggering higher tier investigations (such as extended laboratory tests for NTA). The current honey bee screen was effective at identifying substances of high risk (i.e. known insecticides and acaricides) and allowed for low risk products (most herbicides and fungicides) to pass demonstrating that the scheme was meeting screening requirements.

Taking into account sensitivity, biological and screening aspects, NTA appear promising surrogate species for screening risks to non-Apis bees. The Tier I risk assessment for NTA appeared to be the most restrictive of the three groups on the basis of available data. Thus a screening step for non-Apis bees could be developed based on the Tier I risk assessment for NTAs followed by the use of Tier II (extended laboratory data) to cover non-Apis bee risk assessment without impacting the level of protection. However, when compared to the findings of the honey bee analysis this may be an over conservative screening step. As for the honey bee, further information such as field monitoring is needed to adjust the risk assessment hypothesis. A dedicated ICPPR working group is currently working on monitoring protocols with the aim to address the issue. Then additional testing as

defined by OECD will be needed in order to fill the related gaps of laboratory and higher tier testing in the risk assessment.

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