1.3 New industry research and approaches that could help to improve the risk assessment on bees

Mark Miles[1], Anne Alix, Roland Becker, Natalie Ruddle, Axel Dinter, Laurent Oger, Ed Pilling, Amanda Sharples, Gabe Weyman

[1]Bayer CropScience, Monheim am Rhein, 40789 Germany,
E-mail contact:mark.miles@bayer.com

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Abstract

The crop protection industry recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013 is not a realistically feasible way forward. It is based on extremely conservative assumptions, its study requirements lack clarity and are not workable and guidelines for a number of studies are unavailable or not validated. Industry therefore believes that a revision of the assessment scheme for use by regulatory authorities is needed. Building on an analysis of the proposed developments in the EFSA Bee Guidance Document, we suggest proactive and practical approaches.

We believe our approaches provide comparable levels of protection to the EFSA approach and are based on the current scientific state of the art for bee pollinator risk assessment. Key features are the focus on honey bees as a representative species, the definition of core data packages, concentration on main exposure routes and the proposal of more realistic assumptions for the risk assessment process.

Industry believes that this practical approach is both a realistic and protective way forward for bee risk assessment and would welcome the opportunity to engage in a technical discussion with Member States experts and EFSA on this topic in order to help establish a workable and protective solution as soon as possible.

1.4 Honey bee nectar foragers feeding themselves and the colony: a review in support of dietary exposure assessment

Sara Rodney[1], John Purdy[2]

[1] Intrinsik Corp, 1125 Colonel By Drive, Carleton Campus, CTTC-3600 Ottawa, ON, Canada, K1S 5R1
[2] Abacus Consulting Services Ltd, 8725 Twiss Road, P.O. Box 323, Campbellville, ON, Canada, L0P 1B0

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Abstract

Quantitative knowledge regarding the foods collected and ingested by nectar foraging honey bees (Apis mellifera) is essential for accurately assessing risk associated with pesticide residues in their diet. Although a very large and diverse body of research is available covering many years of research in the literature, much of this research was designed for purposes other than risk assessment and the accumulated knowledge has not been comprehensively reviewed and consolidated from the viewpoint of pesticide risk assessment. Accordingly, in the interest of advancing all tiers of pollinator risk assessment, and identifying data gaps, we strove to gather, assess, and summarize quantitative data relating to nectar forager collection, consumption and sharing of nectar within the colony. Data pertaining to nectar forager provisioning before foraging flights, quantities of nectar brought back to the hive, frequency and duration of foraging trips and energetics was reviewed. Recommendations for future research in support of refined honey bee risk assessment will be discussed.

Keywords: honey bee, forager, nutrition, diet, pesticide exposure, risk assessment, Monte Carlo

Background – The objective of this review was to compile quantitative information regarding nectar forager ingestion of nectar to support pesticide risk assessment. We also identified data gaps in information needed to support honey bee dietary risk assessment. The current pollinator risk assessment guidance published in 20141, by the U.S. Environmental Protection Agency, Canadian Pest Management Regulatory Agency, and California Department of Pesticide Regulations (the Agencies) follows the typical tiered approach. The Tier 1 assessment involves a deterministic calculation in which laboratory toxicity data and conservative exposure assumptions

of contact and ingestion are compared to obtain a risk quotient (RQ). The RQ is then compared to specified levels of concern. The dietary portion of the exposure assessment estimates pesticide ingestion rates based on food intake and residues in pollen and nectar. The Tier I Nectar Ingestion Rate Equation is used for the nectar component2:

$$D_{nectar} = S_F \times \sum_{i=0}^{T}\left(\frac{D_i \times F_i}{P_i}\right) + S_R \times \left(\frac{24 - \left(\sum_{i=0}^{T} D_i \times F_i\right)}{P_{ave}}\right)$$

Where: Dnectar = Nectar ingestion rate (mg/bee/day)

SF = Amount of sugar required for flight (mg/hr)

T = Number of trips per day

Di = Duration of foraging trip i (hr)

Fi = Fraction of time spent flying during trip i

Pi = The proportion of sugar in nectar collected during trip i

SR = The amount of sugar required to meet resting metabolic rate (mg/hr)

Pave = The average proportion of sugar in nectar (30%)

The Tier I assessment assumes no dissipation of pesticide in nectar or honey, and that the proportion of residues relative to the amount of sugar in nectar and honey are constant. When not flying, it was assumed based on a review by Winston3 that nectar foragers consume 0.7 mg sugar/hr . To estimate distribution statistics of nectar ingestion rates, the Agencies conducted Monte Carlo simulations for 10,000 individual nectar foragers, for which the input factors were varied according to Table 1. These factors were apparently treated as independent variables in the simulation.

Table 1 Variables, Input Values and Distributions for Forager Dietary Nectar Exposure Estimation

Variable	Distribution Assumption	Mean	SD	Min	Max	Source(s)
Number of trips/day	Lognormal	10	3	1	150	Winston, 1987[3]
Sugar requirement during flight (mg/hr)	Uniform	NA	NA	7	12	Balderrama *et al*., 1992[4]; Gmeinbauer and Crailsheim, 1993[5]
Duration of each foraging trip (hr)	Uniform	NA	NA	0.5	1.33	Winston, 1987
Fraction of trip spent flying	Uniform	NA	NA	0.5	0.9	Based on Rortais *et al*. (2005)[6]
Sugar content of nectar (proportion)	Lognormal	0.3	0.1	0.1	0.6	2012 White Paper[2]

The resulting median of the distribution was 292 mg nectar/bee/day. This estimate was incorporated into the Tier 1 dietary assessment for nectar foragers, and the BeeREX (v1) risk assessment model.

Worker honey bee characteristics relevant to risk assessment –Worker bees develop through a series of task groups roughly in sequence but with both variability and plasticity7. Newly emerged workers clean hive cells; at 3 days, they begin feeding larvae; at 10 days, they receive, process, and distribute food in the colony; and around 22 days of age, they begin defending the hive and foraging8. Foraging has the highest risk of mortality. In a 36-day study with 47 radio-tracked free foraging honey bees conducted in Meilin, China, the median lifespan was 26 days and nearly all the bees were dead within 36 days9.

The diet of a worker bee also changes with its age. Workers from 1-9 days old consume on average 3-8 mg pollen/bee/day under both natural foraging and caged feeding conditions. The protein and amino acids in pollen are needed for both gland development and brood feeding. After this,

foragers consume very little pollen, e.g. 0.04 mg pollen/bee/day 10-11. Nectar ingestion increases during brood feeding and comb building activities, and remains significant to support the flight of foragers 6, 12-13.

Nectar and water are carried in the crop, which in honey bees, is enlarged and expandable to form the "honey stomach." After consumption, both liquid and solid foods are initially held in the honey stomach. There is no significant uptake of nutrients through the walls of the honey stomach14-19. The next section of the alimentary canal is the ventriculus or stomach where primary digestion and nutrient absorption occurs. It is connected to the honey stomach by a valve-like structure, the proventriculus, which controls the actual entry of food into the ventriculus. The inlet of the proventriculus, which has been called the "stomach mouth", effectively isolates the material carried in the crop from the material that is to be consumed by the bee5, 19.

The cycle of activity and energy use by worker bees reflects the changes in their activities as they progress through different task groups with age. Young bees spend most of their time in the hive and are sporadic in their activities throughout the day and night. Older workers spend more time defending the hive and foraging, becoming distinctly diurnal. Bees of foraging age may actually forage sporadically during daylight hours and also may take days off, even if the foraging conditions are good. These inactive foragers form an essential pool of workers available under normal conditions to be recruited to various tasks as needed20. Foraging and flight activity can also be limited to the time of day consistent with nectar availability of a particular food source21.

Honey bee workers are ectothermic when at rest in the hive and while executing many of the tasks they perform inside the hive. They become endothermic when disturbed, when fanning to cool the hive, when the colony requires heat to maintain an optimal temperature, or in flight and foraging activities 22-23.

Nectar Collection And Distribution In The Hive: Foragers collecting pollen or nectar take in enough nectar by trophallaxis for the round trip before they leave the hive24-26. Some nectar may be taken from in-coming foragers as part of the recruitment process, but most provisioning comes from hive bees (In artificial circumstances forager bees may consume the nectar they collect directly4). Both the concentration and the volume of sugar taken in are regulated according to multiple factors, including the anticipated total energy required for the round trip and the number of previous trips taken to the same source. Crop loads in departing foragers ranged from 0.7 to 3.57 µL/bee 26-28. The target sugar concentration provided to the foragers is achieved by mixing nectar solutions at different stages of evaporation, from honey to freshly collected nectar26.

Nectar Collection – distance and time travelled: Reported foraging distances vary widely, but with adequate resources around the hive, measures of centrality generally fall within 2 km of the hive. The maximum reported nectar foraging distance was 13 km for nectar foragers 29. It has been observed that the number of bees found foraging decreases exponentially with distance from the hive 30-32. Reported values for time per nectar foraging trip range from 21 minutes (lowest average) to 2.5 hr (maximum individual). The time per trip depends on multiple factors including the distance travelled, the number of flowers required to collect a load, the time taken per flower and the overall pollinator population in the area 3, 33-34.

Volume and Concentration of Nectar Collected: In studies with artificial sugar solutions, crop loads in returning foragers at the hive ranged from 5-60 µL/bee, and have been shown to increase with increasing source flow rate, temperature, sugar concentration, and distance from the hive. With natural nectar, crop loads did not exceed 48 µL/bee; with means ranging from 13.6 to 25.6 µL/bee35-36. Nectar foragers do not always fill their crops due to the metabolic cost of transport, and a possible drive to maximize energetic efficiency.

Nectar collected and held in the crop by foragers is transferred back to the hive. Receiver bees take incoming nectar from incoming foragers 37. They transfer some of it to storage cells for honey production, but also provide portions to multiple recipients in the hive so that the nectar distributed in in the colony by sequential trophallaxis. Multiple exchanges of partial crop loads

lead to such extensive mixing that the total food resource in the crops of the bees in the colony has been referred to as the "communal crop." The speed of this process is remarkably fast; individual trophallactic transfers take only 8-14 seconds. Tracer studies show that both sugars and other materials contained in nectar spread throughout the adult bees in the colony within hours and into larvae within 2 days37-40.

Number of Trips/Day: The number of trips per day is influenced by the quality of a source in terms of sugar concentration, constancy, and the experience of the bee with previous flights. Foraging rates at artificial feeders placed near the hive can be much higher and are not representative of free foraging honey bees. An extreme value of 150 trips per bee per day was included in Winston's review3 and this was used as the maximum in the Monte Carlo analysis to support the risk assessment guideline. However, this value was obtained with data from artificial feeder experiments. The highest reported average from naturally foraging was more than an order of magnitude lower, at 10 trips per day33, 41-43.

Several studies report the time spent outside the hive by individual bees (Table 2). Consistent with the research done on resting bees, on average, bees of foraging age spend only a few hours or less per day outside the hive 9

Table 2 Time Outside the Hive

Time (hr/day)	Bees	Method	Remark	Reference
≤4.5 for 70% of observed bees	A subset of 300 bees	Marked bees	Identified nectar foragers	Thom *et al*., 2000[42]
0 - 6.25 (range)	47	Radio tagged	All foragers, Meilin Town China	He *et al*., 2013[9]
1.38 ± 4.32 to 3.06 ± 12.8	9 samples of 212-536	Radio tagged	All foragers, oilseed within 1 km, UK; includes treated fields	Thompson *et al*., 2016[41]

Nectar or honey ingestion: Estimates of nectar or honey ingestion by individual forager bees are available from tracer experiments, weight differentials or respirometer studies of metabolism. Respirometer experiments in which either oxygen use or carbon dioxide production are measured are more common. These values can be converted to energy burned, and equivalent mass of sugar consumed. The average energy consumption reported for resting bees between 15 and 35 °C ranged from 0.10 to 2.10 mg of sugar/hr [22, 44-45]. Only the results of Stabentheiner et al[22] apply specifically to workers of foraging age. These reports should be reviewed with caution, as honey bees are easily roused to a sustained endothermic state of higher energy use even when they appear to be at rest, leading to overestimation of the resting metabolic rate17,18.

From the results of 7 studies of metabolic rates or sugar consumption of untethered active bees, the low, moderate and high estimate of the metabolic rate of forager bees was 5.56, 10.8 mg and 15.7 mg/hr respectively [4, 46-51] .

Estimation of Nectar Ingestion Rate – Considering the weight of evidence in the literature, the ingestion rate of 292 mg/bee/day is likely too high to represent a median value because it is based on averages of 10 trips per day of 55 minutes each, which equates to 550 minutes or 9 hr outside the hive. Although this duration outside the hive foraging may be possible, it is not an expected or central value. The nectar ingestion rate was recalculated as follows: An array of all 27 possible permutations of the low, moderate and high values of foraging time, foraging metabolic rate and non-foraging metabolic rate was set up as in Table 3. The total time outside the hive per day based on RFID tracking data was considered more robust than estimates of the number and duration of trips, and it accounts for foraging time in general.

Table 3 Input Values for Estimation of Honey Bee Forager Nectar Ingestion Rate

Variable	Assumption	Value
Time outside the hive (minutes)	Low	60
	Moderate	185
	High	375
Foraging metabolism/Assumed metabolism outside the hive (mg sugar/hr)	Low	5.56
	Moderate	10.8
	High	15.7
Non-foraging metabolism/Assumed metabolism inside the hive (mg sugar/hr)	Low	0.1
	Moderate	1.04
	High	4.4

Results and Conclusion: The recalculated sugar requirements for honey bee nectar foragers ranged from 8 to 176 mg sugar/bee/day, illustrating the expected high variability. Using the moderate values gave a central estimate of 55 mg sugar/bee/day. This corresponds to 183 mg nectar/bee/day (or approximately 162 µL), which is approximately 1.6-fold lower than the median estimate of 292 mg nectar/bee/day in the guidance1. For context, it may take between 6 and 12 trips to collect this amount of 30% sugar nectar. For 3 hr outside the hive, this corresponds to between 15 to 30 minutes per trip. These estimates are comparable to literature values reported for trip frequency and duration52. Estimates are strongly influenced by the sugar content assumption, which will be affected by preference and availability. Also, crop loads on arrival at the hive are likely to be more concentrated than at collection due to absorption of water through the crop. Further refinements of these estimates are in progress.

Data Gaps/Recommendations - Much of this exercise and that of the White Paper relies on limited data, and extreme simplification of complex and highly variable processes. There is a need to improve understanding of time spent outside the hive for nectar foragers in the US agroecosystems, and determine proportions of time spent at various levels of energy expenditure during nectar foraging (e.g., flying, hovering, resting, and endothermal versus ectothermal states), the distribution of sugar concentrations of nectars collected by nectar foragers, giving special consideration to treated crops53.

For more refined assessment of exposure we should account for the fate and behavior of the pesticide, and in this regard, it would be useful to determine the relative amounts of fresh nectar, aged nectar, ripened honey and water ingested.

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