Assessing homing failure in honeybees exposed to pesticides: Guez’s (2013) criticism illustrates pitfalls and challenges

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I have been involved in a publication showing that sublethal exposures to thiamethoxam, a neonicotinoid pesticide, increase the risk of homing failure in foraging honeybees (Henry et al., 2012a). Along with other recent toxicological studies on free-ranging bees (Gill et al., 2012; Schneider et al., 2012; Whitehorn et al., 2012), those results have motivated the European Food Safety Agency (EFSA, 2012) to reconsider the relevance of risk assessment of plant protection products on bees, currently based on the sole lethality criterion. Among the potential indicators of sublethal hazard, homing failure is a suitable candidate (EFSA, 2013) because (i) it integrates multiple physiological and cognitive functions such as orientation, spatial memory, associative learning and muscular flight activity, and (ii) it may be straightforwardly converted into a mortality rate. Homing failure studies are now being replicated for extending the study of behavioral impairments to other substances (Matsumoto, 2013) and even to pathogens (Li et al., 2013). The forthcoming expansion of homing studies in bees underscores the need to set standards for homing failure measurement. In our original contribution (Henry et al., 2012a), we calculated mortality due to homing failure, \( m_{hf} \), as the proportion of nonreturning treated foragers relative to expectations given by the proportion of returning control foragers. This relative control-treatment homing difference returns a mortality probability equivalent to the statistical effect size of the exposure.

However, Guez (2013a) criticized our calculation of \( m_{hf} \), arguing that dividing the homing difference by control expectations falsely inflates the mortality estimates. Instead, he claimed \( m_{hf} \) should simply read as the absolute control-treatment homing difference. Beside our in-depth reply, alerting on the necessity to properly fix experimental biases (Henry and Decourtye, 2013), Guez (2013b) persisted in his criticism. As a surrogate for our relative homing difference formula \([\text{Equation 1 in Guez (2013b)}]\), he recommends either the use of his absolute homing difference formula \([\text{Equation 2 in Guez (2013b)}]\) or an alternative formula measuring the proportional increase in post-exposure homing failure \([\text{Equation 3 in Guez (2013b)}]\).

I show here that both alternatives are intractable and cannot be properly implemented in a honeybee population dynamics model (Khoury et al., 2011) as in our original study (Henry et al., 2012a). I aim to resolve the disagreement by clarifying several key features of the population models involved. I understand that Guez’s (2013a,b) implicit assumption that homing experiments are based on the same temporal scale than the parameters of the honeybee population dynamics model (Khoury et al., 2011). This, however, is incorrect, as shown below. His tentative Equation 2 underestimates mortality, while Equation 3 may return severely overestimated mortalities. I hope this cautionary note will help risk assessors identifying some important pitfalls and challenges in the assessment of post-exposure homing failure in bees.

A MORTALITY PROBABILITY CAN’T BE MORE THAN 100%

A mortality probability can’t exceed 1 (i.e., 100% of the initially surveyed individuals died). Yet, Guez’s (2013b) Equation 3 returns \( m_{hf} \) values of up to 155%. Readers should be aware that Equation 3 does actually not estimate a mortality rate per se, but a relative difference in homing failure. The resulting values are not bounded within [0,1] and therefore should not be combined with natural mortality as shown in subsequent equations. As control homing failure gets closer to 0, \( m_{hf} \) in Equation 3 (Guez, 2013b) rapidly gets much larger than 1 and eventually tends to ∞. So does the result of the subsequent equation, which is intended to give daily mortality rate for use in the colony population model. Guez’s (2013b) Equation 3 is therefore inappropriate and liable to overestimate mortality. The hypothesis of a low or null control homing failure is plausible and is even a desirable property for any homing experiment. For instance, control homing failure was only 1.5% in experiment 3 of Henry et al. (2012a). In Guez’s (2013b) example, if one substitutes the 17% control homing failure by a 1.5% value, Equation 3 would return \( m_{hf} = \frac{(0.432 - 0.015)}{0.015} = 27.8. \) Subsequently, total daily mortality would reach an unrealistic level of \( m_{total} = 0.154 + 0.154 \times 27.8 = 4.44 \) (foragers have an overall daily mortality probability of 44%).

A MORTALITY PROBABILITY IS DIMENSIONLESS

By definition, probabilities and proportions are dimensionless. Yet, Guez (2013b) recurrently expresses homing failure in \( \text{individuals.day}^{-1} \), and even insists on this property to justify the combination of different mortality parameters (“Importantly, since [Control homing success], [Control
HOMING FAILURE DUE TO EXPERIMENTAL STRESS IN NOT TRIVIAL

Beyond the temporal mismatch, it is critical to properly account for any bias resulting from the experimental stress. Guez's Equation 2 seems to hold true if “…we assume that most of the homing failure observed in the control is due to natural predation” (Guez, 2013b). This assumption is however too far reaching because the experimental stress substantially contributes to homing failure in Control bees. One might tentatively estimate the magnitude of experimental stress in Control bees by comparing homing statistics from the most challenging experiment vs. from the least challenging one [i.e., Experiments 2 and 3, respectively, in Henry et al. (2012a)]. When released from an unfamiliar place 1 km away from their colony, 16.9% of the control bees failed homing. When released from a familiar place and in the vicinity of their colony, only 1.5% of the bees failed homing. The 15.4% difference is therefore mostly attributable to the increased experimental challenge imposed to bees, and is of the same order of magnitude as homing failure attributable to pesticide exposure (though the most challenging experiment might also include a greater natural mortality simply due to increased homing duration). This needs be investigated in further studies.

FUTURE CHALLENGES IN HOMING FAILURE ASSESSMENT

I have highlighted here some pitfalls on the way to assess honeybee homing failure in the context of dietary exposure to pesticides. Most importantly, homing experiments can’t be assigned the same temporal basis as the daily population parameters in colony dynamics models (Khoury et al., 2011), and this must be properly accounted for.

important challenges for future studies are listed below.

(i) Homing failure and (sub-)chronicle exposure. As pointed out by Guez (2013a) and also in earlier discussions (Cresswell and Thompson, 2012; Henry et al., 2012b), mortality with daily natural mortality. Implementing mortality due to homing failure into population dynamics models might be further improved. In earlier studies (Cresswell and Thompson, 2012; Henry et al., 2012a), mortality was combined with daily natural mortality as an additional independent probability \( m_{total} = m_{natural} + m_{hf} \). One might however consider that post-exposure homing failure only occurs at the end of the foraging day, and therefore only applies on the portion of foragers who escaped natural mortality that day. Under this assumption, total mortality would be slightly reduced \( m_{total} = m_{natural} + m_{hf} - [m_{natural} \times m_{hf}] \). I suggest to search into that direction to set a lower bound scenario for \( m_{hf} \).

(ii) Disentangling the contributions of natural mortality and experimental stress in homing experiments. As shown earlier, homing failure due simply to the experimental condition is arguably not trivial. One last challenge is the assessment of the relative contribution of natural mortality and experimental conditions to total homing failure, i.e., the magnitude of the experimental effect that must be controlled for in the calculation of \( m_{hf} \).

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