Hydroxymethylfurfural: A Possible Emergent Cause of Honey Bee Mortality?

Lara Zirbes,* Bach Kim Nguyen,† Dirk C. de Graaf,‡ Bruno De Meulenaer,# Wim Reybroeck,§ Eric Haubruge,† and Claude Saegerman⊥

†Unit of Functional and Evolutionary Entomology, University of Liege, Gembloux Agro-Bio Tech, Passage des déportés 2, 5030 Gembloux, Belgium
‡Laboratory of Zoophysiology, Department of Physiology, Ghent University, Krijgslaan 281 S2, 9000 Gent, Belgium
#Department of Food Safety and Food Quality (nutriFOODchem unit, partner in Food2Know), Ghent University, Coupure Links 653, 9000 Gent, Belgium
§Technology and Food Science Unit, Institute for Agricultural and Fisheries Research, Brusselsesteenweg 370, 9090 Melle, Belgium
⊥Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR-ULg), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster 20, B42, 4000 Liège, Belgium

ABSTRACT: Hydroxymethylfurfural (HMF), a common product of hexose degradation occurring during the Maillard reaction and caramelization, has been found toxic for rats and mice. It could cause a potential health risk for humans due to its presence in many foods, sometimes exceeding 1 g/kg (in certain dried fruits and caramel products), although the latter still is controversial. HMF can also be consumed by honey bees through bad production batches of sugar syrups that are offered as winter feeding. In Belgium, abnormal losses of honey bee colonies were observed in colonies that were fed with syrup of inverted beet sugar containing high concentrations of HMF (up to 475 mg/kg). These losses suggest that HMF could be implicated in bee mortality, a topic that so far has received only little attention. This paper reviews the current knowledge of the presence of HMF in honey bee environment and possible consequences on bee mortality. Some lines of inquiry for further toxicological analysis are likewise proposed.

KEYWORDS: hydroxymethylfurfural (HMF), honey bee, mortality, syrup

INTRODUCTION

Global pollinators have declined in abundance and diversity, which can affect natural ecosystems and agriculture.1,2 Specifically, for several years, abnormal mortalities and weakening of honey bee colonies have been often observed in Europe and North America.3 Bee populations in Europe have nonetheless been seriously affected by human activities. Between 1970 and 2007, the number of honey bee colonies in Europe gradually decreased from over 21 million to about 15.5 million.3,4 Moreover, beekeepers in Europe and also in North America have repeatedly been confronted with elevated and sometimes unexplained winter losses.6−8 A multitude of factors that may contribute to increased winter losses have been discussed comprehensively in the recent literature: invasive species, increased pathologies, climate, food resources, and low farmland biodiversity. Most prominently among them were the invasive mite Varroa destructor and pathologies caused by viruses and the microsporidian Nosema spp.6−13 Hydroxymethylfurfural (HMF) present in syrups for bee feeding during winter could be a new factor implicated in bee mortality.

Indeed, in 2009−2010, abnormal losses of bee colonies were observed in Belgium. Later analyses showed that some of these colonies had been fed during winter with syrup of inverted beet sugar, which presented a concentration of HMF up to 475 mg/kg due to a bad production batch.14 Several studies confirm a toxic effect of the HMF on the health of the bee.15−17 However, the absence of toxicological data does not allow establishing a standard to guarantee no toxic effects for honey bees.

The objective of this review is multiple: understanding the mechanisms of formation of HMF, its presence in bee environment, its toxicity for honey bees, and its implication in bee mortality.

HMF MECHANISMS OF FORMATION

5-Hydroxymethyl-2-furaldehyde or, as it is more commonly referred to, 5-hydroxymethylfurfural consists of a furan ring, containing both aldehyde and alcohol functional groups (Figure 1).

HMF is a common Maillard reaction (the nonenzymatic browning) product formed through the reaction between reducing sugars and amino acids during heat treatment of food.18−20 HMF can also be formed through acid-catalyzed

Figure 1. 5-Hydroxymethylfurfural.
dehydration of hexoses, via 1,2-enolization followed by two consecutive dehydration steps followed by a self-condensation and further dehydration.\textsuperscript{19,21,22} Figure 2 presents the main pathways to HMF formation in foods. HMF can be produced from all hexoses and also from those oligo- and polysaccharides that can yield hexoses upon hydrolysis. However, it appears to be more selectively produced from keto-hexose, notably from D-fructose.\textsuperscript{23,24} HMF can also appear in products where water coexists with monosaccharides in acidic medium.\textsuperscript{25} The activation energy for HMF formation is higher than that for HMF degradation, with the result that the maximum obtainable concentration increases with increasing temperature.\textsuperscript{26} Apart from temperature, the rate of HMF formation in foods is dependent on the type of sugar,\textsuperscript{27} pH,\textsuperscript{28} water activity,\textsuperscript{22,29} and concentration of divalent cations in the medium.\textsuperscript{30}

The formation rate of HMF is increased by a higher enolization rate as well by a higher proportion of acyclic and furanose forms of fructose.\textsuperscript{26} Detailed mechanisms of HMF formation were recently reviewed by Morales in 2009.\textsuperscript{19}

### HMF IN THE ENVIRONMENT AND IN BEE ENVIRONMENT

In overview, HMF is used in the synthesis of some fuel additives, organic compounds, and novolak type resins.\textsuperscript{31,32} It is an intermediate in the synthesis of several crown ethers.\textsuperscript{33} HMF is also utilized to produce polymers, surfactants, solvents, pharmaceuticals, and plant protection agents.\textsuperscript{32,34} It is normally formed during thermal decomposition of sugars and carbohydrates. Moreover, glucose infusions are commonly used as vehicles for administering a variety of drugs. During its production, the solutions must be sterilized and HMF formation can occur.\textsuperscript{19} Furthermore, HMF in foodstuffs has received special attention for years. Indeed, HMF is widely recognized as a marker of quality deterioration, resulting from excessive heating or inappropriate storage conditions in a wide range of foods containing carbohydrates.\textsuperscript{19} In fact, the Codex Alimentarius of the World Health Organization and the European Union (EU Directive 110/2001) have defined a maximum HMF quality level in honey (40 mg/kg) and in apple juice (50 mg/kg) as a deterioration and heat treatment indicator. The HMF is also detected in spirits, wine, and other...
alcoholic beverages, coffee, milk, fruit juices, vinegars, adult and baby cereals, and breads.

In bee environment, HMF is naturally present in honey in low quantity. It is produced by the action of the normal honey acidity on reducing sugars and sucrose at ambient temperature and is also considered as a quality indicator for honey. As previously mentioned, to avoid heat treatment or long storage of honey, Directive 2001/110/CE imposed HMF maximal concentrations in honey of 40 mg/kg for temperate regions and 80 mg/kg for tropical climates.

Different methods were applied to analyze HMF content in honey including gas chromatography coupled with mass spectrometry, a sensible method for minor constituents. The International Honey Commission recommends three methods for the analysis of HMF content in honey: two spectrophotometric methods (White and Winter methods) and one RP-HPLC method. These methods were recently compared to show that White and Winter methods are fast but very few specific and sensitive, whereas the RP-HPLC method is slower but offers more precise results. In 1998, Anklam noted in a review that "the suitability of the analytical methods for HMF is unsatisfactory and requires further investigation ....", and it is still true.

Bees can also be exposed to HMF through syrup that is offered as winter feeding. Indeed, it is a common beekeeping practice to replenish the food reserves of a bee colony after honey has been yielded in autumn. When bees feed on sugar syrup, they metabolize saccharose into glucose and fructose using invertase. To facilitate the feeding of the bees, some beekeepers supply them with ready-made food before winter. This ready-made food is generally composed of inverted sugar syrup, in which fructose and glucose are directly available for honey bees. This ready-made food is therefore more susceptible to HMF formation. Moreover, beekeepers give homemade syrup based on sucrose and water and, sometimes, they add some ingredients such as vinegar or citrus juice that could enhance HMF formation. These compounds acidify syrups and increase HMF production. A long-term storage could bring an important evolution of the HMF content in syrups. Moreover, no study has focused on HMF metabolism in the digestive system of honey bees after oral ingestion, whereas pathways for HMF biotransformation were summarized in mammalian systems and makes the bees more susceptible to secondary infections, pesticides, and probably other toxicants such as HMF. Another study has tested acaricides, fungicides, and drug interactions on honey bee mortality and found that approximately half the acaricide–acaricide and acaricide–fungicide combinations tested showed evidence of interactions, nearly all of which were agonistic and resulted in increased acaricide toxicity. On the other hand, two antimicrobial–acaricide combinations tested presented antagonistic interactions with the acaricide Fenpyroximate. Antagonistic interaction is characterized by decreased toxicity of a drug or pesticide combination. Moreover, agonistic interactions between the model enzyme inhibitors piporonyl butoxide (PBO) and three acaricides (r-fluvalinate, coumaphos, and Fenpyroximate) used in hives to eradicate the mite V. destructor were necessary to de

HMF. However, few studies show cytotoxic effect on human blood cells or DNA damages in several human cell lines after 3 h of exposure to 100 mM HMF. Due to this potential risk for human health, some mitigation strategies of HMF in food were proposed, focusing on the most innovative and potentially exploitable at the industrial level. Preventive and removal strategies were proposed at different levels of food processing including formulation, processing, and postprocessing.

As explained previously, there has been an incident of honey bees that were exposed to HMF through syrup offered to bees as winter feeding. HMF seems to be toxic to honey bees: intestinal tract ulceration was suspected, which seemed to be lethal. However, very few studies observed any toxicity of HMF to honey bees. Jachimowicz et al. found that the HMF content of 150 mg/kg in commercially acid-hydrolyzed invert sugar syrup caused a mortality of 50% within 16 days after the start of the feeding. HMF concentration of 30 mg/kg seems harmless to honey bees; therefore, many specialists recommended that its concentration in inverted syrup may not exceed 20 mg/kg as it is in most honeys. It was previously advised to control the HMF content of inverted sugar syrups before they were given to bees for feeding. Several years later, during experiments on the quality of syrups used for bee feeding, Ceksterye and Racy have suggested that HMF content of 48 mg/kg in sugar syrup from maize was harmless for wintering honey bees. Interestingly, they found that the content of HMF present in the initial syrups decreased in the syrups deposited by bees in the comb, suggesting that the bee organism is able to metabolize the HMF to some extent. Later, Le Blanc et al. used caged honey bees to evaluate the HMF dose–response effect on bee mortality with a high-fructose corn syrup (HFCS), a saccharose replacement for honey bees in the United States. They observed 50% of bee mortality after 19 days for HFCS with 150 mg/kg HMF. This is very close to the results of Jachimowicz et al. After 26 days, they compared bee mortality for different HMF doses (57, 100, 150, 200, and 250 mg/kg) and found that only HFCS enriched with 250 mg/kg induced a significantly lower survival.

The toxicity of HMF to honey bees was increased by syrup crystallization. Indeed, during the crystallization process, a part of the syrup becomes solid and the HMF was concentrated in the liquor, being the unique phase accessible to honey bees. These papers seemed to indicate that HMF participates in honey bee mortality, but standardization of experiments is necessary to define a HMF LD₅₀ for bees.

Moreover, influence of HMF on bee mortality has to be associated with other mortality causes. For example, when the mite Varroa destructor parasitizes bees, it weakens their immune systems and makes the bees more susceptible to secondary infections, pesticides, and probably other toxicants such as HMF. Another study has tested acaricides, fungicides, and drug interactions on honey bee mortality and found that approximately half the acaricide–acaricide and acaricide–fungicide combinations tested showed evidence of interactions, nearly all of which were agonistic and resulted in increased acaricide toxicity. On the other hand, two antimicrobial–acaricide combinations tested presented antagonistic interactions with the acaricide Fenpyroximate.
identified. These interactions imply that P450 enzymes play a role in detoxifying these acaricides in honey bees. An agonistic interaction is defined by an elevated toxicity of a drug or pesticide combination. Some beekeepers give syrup to honey bees in combination with a treatment against V. destructor with acaricides, exposing honey bees to possible interactions between HMF and acaricides. Another study investigated the integrative effect of the microsporidian Nosema spp. and an insecticide (imidacloprid). Imidacloprid alone, in a concentration similar to that found in the natural environment, had no effect on honey bee mortality, but a synergistic interaction was demonstrated on honey bee mortality when this pesticide was combined with Nosema spp.

HMF, as some pesticides, could also be an indirect factor in bee mortality by modifying the natural behavior of honeybees. For example, recent research showed that a nonlethal exposure of honey bees to thiamehtoxam (a neonicotinoid systemic pesticide) influences indirectly bee mortality due to homing failure at levels that could annihilate bee colonies. In conclusion, hydroxymethylfurfural is toxic for mice and rats, and some studies also suggest health risks for humans. It is not clear, though, what the impact of HMF and eventually other sugar degradation products is on honey bees, how they are metabolized in the bees, and what the impact is on bee behavior and mortality.

In this review we highlight that HMF can present a toxicological risk for honey bees giving rise directly or indirectly to bee mortality. We also noted the absence of toxicological data making it currently not possible to establish an action limit on the HMF content of sugar syrups used for bee feeding to manage the risk. Further experiments are necessary to evaluate the implication of HMF in honey bee mortality and to determine the maximal concentration in HMF authorized in winter feed syrups for honey bees.

**AUTHOR INFORMATION**

**Corresponding Author**
*L.(Z.)* E-mail: entomologie.gembloux@ulg.ac.be.

**Funding**
This study was funded by the Federal Public Service of Heath, Food Chain Safety and Environment (Contract RF 11/6248).

**Notes**
The authors declare no competing financial interest.

**REFERENCES**


