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Review

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Hydroxymethylfurfural: a possible emergent cause of honey bee mortality?

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Abstract

Hydroxymethylfurfural (HMF), a common product of hexose degradation as 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 1g/kg (in certain dried fruits and caramel product), 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 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 were likewise proposed.

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

Introduction

Global pollinators are 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 [4, 5]. 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, 9-13]. 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-furancarboxaldehyde, or as it is more commonly referred to as 5-hydroxymethylfurfural, consists of a furan ring, containing both aldehyde and alcohol functional groups (Fig.1).

5-hydroxymethylfurfural (HMF) is a common Maillard reaction (the non-enzymatic 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-catalysed dehydration of hexoses, via 1,2-enolisation followed two consecutive dehydratation steps followed by a selfcondensation and further dehydratation [19, 21, 22]. Fig. 2 presents the main pathways to HMF formation in foods. HMF can be produced from all hexoses, and also from those oligo- and polysaccharides which can yield hexoses upon hydrolysis. However, it appears to be more selectively produced from keto-hexose, notably from D-fructose [23, 24]. HMF can also appear in product where water coexists with monosaccharides in acidic medium [25]. The activation energy for HMF formation is higher than for HMF degradation with the result that the maximum obtainable concentration increases with increasing temperature [26]. Apart from temperature, the rate of HMF formation in foods is dependent on the type of sugar [27], on pH [28], on water activity [22, 29] and on the concentration of divalent cations in the media [30].

The formation rate of HMF is increased by a higher enolisation rate as well by a higher proportion of acyclic and furanose forms of fructose [26]. Detailed mechanisms of HMF formation were recently reviewed by Morales in 2009.

HMF in environment and in bee environment

In overview, HMF is used in the synthesis of some fuel additives, organic compounds and of novolak type resins [31, 32]. It is an intermediate in the synthesis of several crown ethers [33]. HMF is also utilized to produce polymers, surfactants, solvents, pharmaceuticals and plant protection agents [32, 34]. It is normally formed during thermal decomposition of sugars and carbohydrates. Moreover, glucose infusions are commonly used as vehicles for administrating a variety of drugs. And during its production, the solutions must be sterilized and HMF formation can occur [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 [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 (40mg/kg) and in apple juice (50mg/kg) as deterioration and heat-treatment indicator. The HMF is also detected in spirits, wine and other alcoholic beverages [35-37], coffee [38], milk [39], fruit juices [40-42], vinegars [43], adult and baby cereals [30, 43, 44], and breads [45].

In bee environment, HMF is naturally present in honey in low quantity. It is produced by action of the normal honey acidity on reducing sugars and sucrose at ambient temperature [19] 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 [46] imposed a HMF maximal concentration in honey of 40 mg/kg for temperate regions and 80 mg/kg for tropical climates.
Different methods were applied to analyse HMF content in honey including gas chromatography coupled with mass spectrometer, a sensible method for minor constituents [47]. The International Honey Commission [48] recommends three methods for the analyse of HMF content in honey: two spectrophotometric methods [49, 50], and one RP-HPLC method [36]. These methods were recently compared [51] to show that White and Winter methods are fast but very few specific and sensitive whereas RP-HPLC method is more slow but offer more precise results. In 1998, Ankalm [52] noted in this review that "... the suitability of the analytical methods for HMF is unsatisfactory and requires further investigation..." and it is always 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 sugar syrup, they metabolise saccharose into glucose and fructose using invertase [53]. 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 where fructose and glucose are directly available for honey bees. This ready-made food is therefore more susceptible for HMF formation. Moreover, beekeepers give home-made syrup based on sucrose and water and sometimes, they add some ingredients like 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 focused on HMF metabolism in the digestive system of honey bees after oral ingestion whereas pathways for HMF biotransformation were summarized in mammalians [54].

All these elements have to be considered to understand how HMF can contaminate honey bees and evaluate its impact on honey bees' mortality.

Toxicity of hydroxymethylfurfural

There is an increased interest for HMF and furan derivates since data became available on the toxicity of these molecules. In fact, various animal experiments showed that HMF has a number of structural alerts that pose possible genotoxic and carcinogenic risks. Some studies revealed that HMF may induce genotoxic and mutagenic effects in bacterial and human cells and promote colon and liver cancer in rats and mice[55-59].

Human exposure to HMF can occur through pharmaceutical preparation, cigarette smoke and consumption of a number of commonly available beverages and foods including breads, honeys, fruit juices or jam. Humans can be exposed to HMF by inhalation, ingestion or skin absorption. Although HMF is not yet considered a harmful substance for humans [60], the subject is still a matter of debate. Some scientists have estimated the daily intake of HMF at 30-150 mg per person [61] but no long-term cancer bioassays have been presented on HMF. However, few studies show cytotoxic effect on human blood cells [62], and DNA damages in several human cell lines after 3h exposure to 100 mM of HMF [63]. 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 industrial level [64]. In this review, preventive and removal strategies were proposed at different level of food process including formulation, processing and post-processing [64].

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 [15]. However, very few studies observed any toxicity of HMF to honey bees [15-17]. Jachimowicz et al. (1975) found that the HMF content of 150 mg/kg in commercially acid hydrolysed 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 the honey bees, therefore many specialists recommended that its concentration in inverted syrup may not exceed 20 mg/kg as it is in most honeys [65]. It was previously advised to control the HMF content of inverted sugar syrups before they were given to bees for feeding [16]. Several years later, during experimentations on the quality of syrups used for bee feeding, Ceksteryte and Racys (2006) 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 which was present in the initial syrups decreased in the syrups deposited by bee in the comb, suggesting that bee organism is able to metabolise the HMF to some extent [66]. Later, Le Blanc et al. [17] 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 bee in the USA. 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[16]. After 26 days, they compared bee mortality for different HMF doses (57, 100, 150, 200 and 250 mg/kg) and they 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 crystallization process, a part of syrup becomes solid and the HMF was concentrated in the liquor being the unique phase accessible to honey bees [67]. These papers seemed to indicate that HMF participates to honey bees' mortality but standardisation 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 [3, 68, 69] and probably to others toxicants as HMF. Another study has tested acaricides, fungicides and drug interactions on honey bees' mortality and they 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 present antagonistic interactions with the acaricide fenpyroximate [70]. 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 (tau-fluvanilate, coumaphos and fenpyroximate) used in hives to eradicate the mite *Varroa destructor* were identified. These interactions imply that P450 enzymes play a role in detoxifying these acaricides in honey bees [70]. 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 *Varroa destructor* with acaricides, exposing honey bees to possible interactions between HMF and acaricides. Another study investigated the integrative effect of the microsporidian *Nosema* and an insecticide (imidacloprid). Imidacloprid alone, in similar concentration as found in natural environment, had no effect on honey bee mortality [71] but a synergistic interaction was demonstrated on honey bees mortality when this pesticide was combined with *Nosema* spp. [72].

HMF, as some pesticides, could also be an indirect factor in bee mortality by modifying the natural behavior of honeybees. For example, a recent research showed that a nonlethal exposure of honey bees to thiamethoxam (neonicotinoid systemic pesticide) influences indirectly bee mortality due to homing failure at levels that could annihilate bee colonies [73].

In conclusion, hydroxymethylfurfural (HMF) is toxic for mice and rats, and some studies also suggest health risks for humans. It is not clear though what is the impact of HMF and eventually other sugar degradation products on honey bees, how they are metabolized in the bees, what is the impact on the bees 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 in order to manage the risk. Further experiments are necessary to evaluate the implication of HMF in honey bees’ mortality and to determine the maximal concentration in HMF authorized in winter feed syrups for honey bees.

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Figure 1: 5-hydroxymethylfurfural

Figure 2: Proposed reaction scheme for the formation of 5-hydroxymethylfurfural in food (adapted from [54]).

Figure 1

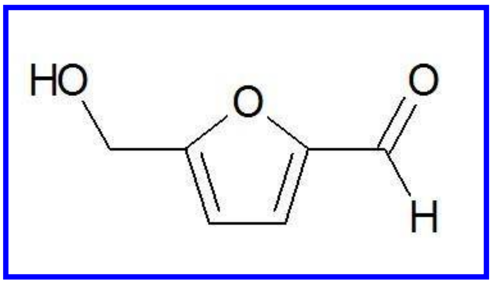
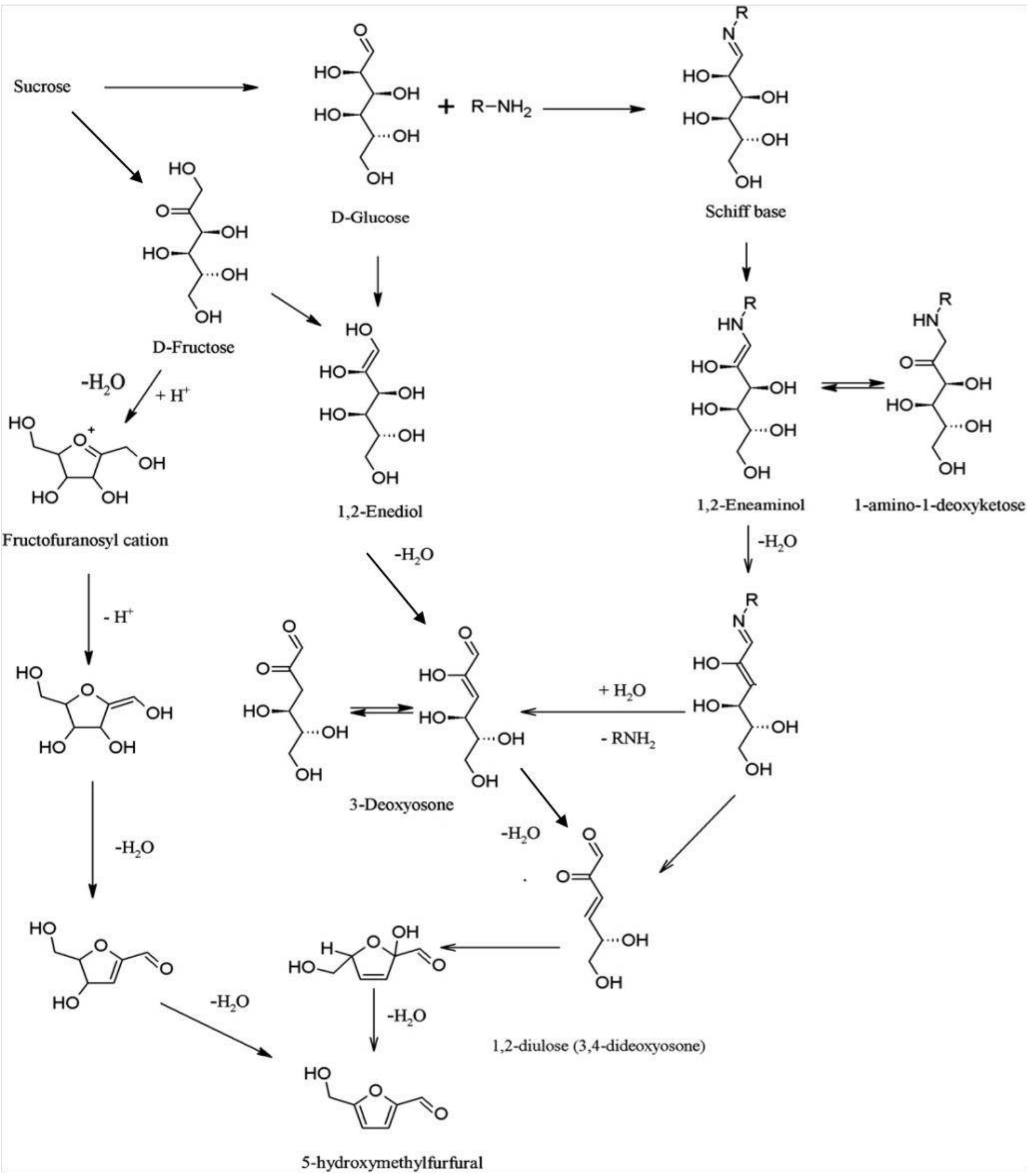
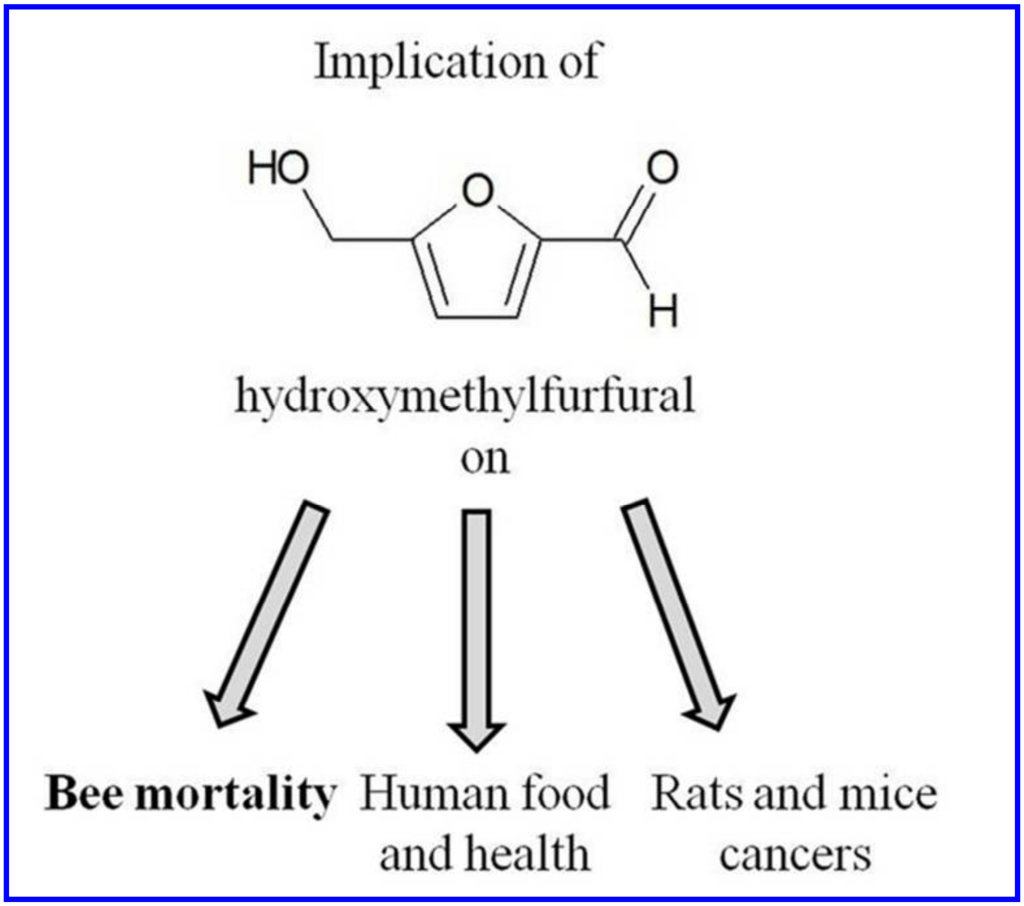


Figure 2


Sucrose
D-Glucose
R−NH2
Schiff base
D-Fructose
1,2-Enediol
1,2-Eneaminol
1-amino-1-deoxyketose
-H2O
+ H+
Fructofuranosyl cation
- H+
3-Deoxyosone
+ H2O
- RNH2
1,2-diulose (3,4-dideoxyosone)
5-hydroxymethylfurfural



53x47mm (300 x 300 DPI)