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Risk assessment of beeswax adulterated with paraffin and/or stearin/stearic acid when used in apiculture and as food (honeycomb)

European Food Safety Authority (EFSA)

Abstract

EFSA has established purity criteria and technical specifications for beeswax used in apiculture. The risks to honey bees and humans that are exposed to beeswax adulterated with paraffin and/or stearin/stearic acid and their possible contaminants was assessed. Exposure of honey bees may occur via contact or oral routes and of humans via consumption of honey contaminated with constituents of adulterated beeswax or honeycomb contained in honey pots. EFSA gathered information from various sources, such as scientific literature and the media (MedISys). From the appraisal and statistical analysis of the classic and advanced methods used for beeswax authentication, it is concluded that purity testing should include at least two physico-chemical parameters complemented with advanced analytical methods for a reliably sensitive detection (limit of detection <5%) and quantification of beeswax adulterants. Four exposure scenarios were defined for bees. In the absence of toxicological endpoints, it was not possible to reach a conclusion on the risk posed to bees. However, stearin/stearic acid at certain levels can induce detrimental effects on bee brood. For humans, the working group considered the exposure to waxes (largely consisting of *n*-alkanes and containing hardly any aromatic compounds with more than two aromatic rings) to be of low concern. The consumption of beeswax adulterated with paraffin would result in an increased exposure to certain contaminants for which a potential concern has already been identified, such us mineral oil saturated hydrocarbons. Exposure to food-grade stearin and its contaminants would not be of concern, although the latter might slightly contribute to the overall exposure to some contaminants such as polycyclic aromatic hydrocarbons, dioxins and dioxin-like polychlorinated biphenyls. Since gaps in knowledge and data were found, recommendations are listed to support future risk assessments on the impact of adulterated beeswax on both humans and bees.

Key words: beeswax, stearin, paraffin, honeycomb, fraud, adulteration, risk

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Summary

Beeswax is a natural product produced by honey bees (*Apis mellifera* L.) for the construction of combs used for food storage (e.g. honey/nectar and pollen/beebread) and brood rearing. Although being a product in contact with honey as food, beeswax used in apiculture as comb foundations for honey production is only subject to regulatory safety requirements when used as food additive E901 or as pharmaceutical-grade beeswax (*cera flava*, *cera alba*). There is no such regulatory framework available for beeswax when used in apiculture (as comb foundations and/or crude beeswax used for their production) nor, therefore, as honeycomb for human consumption.

Following a notification by the Rapid Alert System for Food and Feed on the occurrence of beeswax adulteration in the EU and based on previous studies (both from the scientific literature and official authorities) raising concerns, the European Commission asked EFSA to provide scientific and technical assistance on this issue. In particular, to determine the technical specifications and purity criteria for beeswax when used in apiculture and as food (in the form of honeycomb inside honey pots), and to assess the risks to honey bees and humans when the beeswax is adulterated with paraffin and/or stearin/stearic acid.

Before conducting such assessments, EFSA gathered data and information on beeswax adulteration from various sources (i.e. study reports commissioned by the European Commission; Eurostat data on beeswax trade to and within the EU; a call for studies in EU Member States on adulterated beeswax; and information from the media on MedISys and from the scientific literature). Paraffin and stearin/stearic acid are the most reported adulterants in beeswax, but their prevalence in Europe is not well documented.

Preliminary studies requested by the European Commission and conducted by Belgium, France and Germany confirmed an association between the presence of stearin/stearic acid and health effects on honey bees. As for humans, it is suspected that stearin/stearic acid as well as paraffin and their contaminants contained in adulterated beeswax used in apiculture could also pose harm to humans consuming the honey or honeycomb. In order to verify this risk hypothesis and make the required assessments, detailed information was gathered and reported on hazard identification (i.e. type of wax and origin of the adulterants), hazard characterisation (adverse effects on honey bees and humans and a dose–response assessment) as well as estimates of exposure in bees and humans to these adulterants and their contaminants.

An inventory of the various purity criteria and the related physico-chemical analytical methods and advanced chromatographic and spectroscopic methods for beeswax analysis were collected from the legislation on food additives, the *European Pharmacopoeia* and the scientific literature. A weighting analysis was performed on those methods by several experts and validated by a statistical analysis. This exercise indicated that a routine purity test (authenticity control) involving the quantification of adulterants (paraffin, stearin/stearic acid) with a reliable detection (limit of detection <5%) requires at least two physico-chemical parameters, complemented with advanced analytical methods (gas chromatography–mass spectrometry, high-temperature gas chromatography (HTGC) with flame ionisation detection, HTGC/mass spectrometry or Fourier-transform infraRed spectroscopy coupled with attenuated total reflectance accessory).

Hazards due to dietary exposure to petroleum-based waxes (called hydrocarbon waxes in this report) have been assessed by the EFSA Panel on Contaminants in the Food Chain, in its Opinion on mineral oil hydrocarbons in food (EFSA CONTAM Panel, 2012). The no-observed-adverse-effect level for induction of liver microgranulomas in Fischer 344 rats by the most potent mineral oil saturated hydrocarbons (MOSH; low or medium melting point wax, consisting primarily of *n*-alkanes) of 19 mg/kg bw per day was used as a reference point for calculating margins of exposure. There is a paucity of information regarding the possible presence of contaminants or toxic substances present in hydrocarbon waxes. Mineral oil aromatic hydrocarbons (MOAH), which can also be considered as largely alkylated poly aromatic hydrocarbons, PAH) are known to be present as minor components in different types of hydrocarbon waxes. There is no information on the type of wax used for the adulteration of beeswax, but it is likely that the cheapest, hence least refined waxes would mainly be used. Among the waxes commercially available, slack waxes (CAS# 64742-61-6) are poorly refined and may have higher MOAH content than highly or semi-refined waxes.

Stearin is a product of fats and oils that are consumed in food products and, therefore, it is not expected to raise safety concerns for humans if consumed as such in beeswax. There is no information about the type of stearin used for the adulteration of beeswax, but it is again likely that stearin of the lowest value, such as animal by-product stearin, is being used. Stearin may be of plant (e.g. palm oil and fat) or animal origin (e.g. tallow and lard). To increase the melting point, it is either fractionated (the stearin being the crystallised fraction) or obtained by hydrogenation of the unsaturated fatty acids.

The effects on bees are understudied and still debated. Four exposure scenarios were defined for bees (via contact and oral routes) for larvae and adults (bees producing wax and nurses). The first two scenarios were on (worker and drone) larvae via physical (wetting) contact with contaminated wax (first scenario) or via consumption of pollen/beebread stored in wax and eventually contaminated through the migration of the adulterants from the wax to the beebread (second scenario). The third scenario was on in-hive adult bees (those manipulating and building beeswax combs) via mastication of contaminated wax. The fourth scenario was on nursing bees (those preparing food for larvae containing fresh pollen). The adulterants being lipophilic, only the lipid content in bee matrices was considered (pollen/beebread and royal jelly). The most exposed bees are those producing wax, followed by bees manipulating propolis with beeswax, larvae and nurses. However, toxicological data for different adulteration levels and endpoints comprising the testing of acute, chronic and sublethal toxicity are not available. This information is necessary to comprehensively assess the risks to honey bees from exposure to adulterated beeswax and their contaminants. A few studies tested the effect of hydrocarbon waxes, stearic and palmitic acids on bees. These studies show impacts on brood (mortality rates from 45% up to 80%) beeswax adulterated with 50% of hydrocarbon waxes, 5% of stearic acid, 7.5% of palmitic acid and 10% for mixture of fatty acids.

In humans, the Working Group considers the exposure to waxes (largely consisting of n-alkanes and containing hardly any aromatic compounds with more than two aromatic rings) are of low concern. The consumption of beeswax adulterated by paraffin would result in an increased exposure to certain contaminants for which a potential concern has been already identified. Exposure to food-grade stearin and its contaminants would not be of concern, although the latter might slightly contribute to the overall exposure to some contaminants such as PAHs, dioxins and dioxin like PCBs. Beeswax adulterants and their contaminants are lipophilic, they are not expected to migrate to honey.

Since major gaps in knowledge and data were found, a comprehensive list of recommendations has been made to support future risk assessments on the impact of adulterated beeswax on both humans and bees.

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1. Introduction

1.1. Background and terms of reference as provided by the European Commission

In June 2017, the Commission was informed by the Belgian authorities about contamination of beeswax intended for apiculture use[1]. The adulterated beeswax originated from China and Ukraine.

In July 2017, the case created by Belgium was sent through the EU Food Fraud Network (European Commission, online-a), a dedicated network for cross-border non-compliance for food and feed, with a specific request to Germany and Spain. The Belgian authorities found paraffin (between 1.7% and 5.8% with one sample having an adulteration of 52%) and stearin (adulteration up to 25%) in honeycomb (animal by-product (ABP) Category 3) intended for apiculture use and thus posing a potential risk of adulterated beeswax entering the food chain in the form of honeycomb and/or a risk for bee health. Stearic acid and certain paraffins are actually authorised in plastic food contact materials under Commission Regulation (EU) No 10/2011[2].

The EU Food Fraud Network set up by the Commission is closely following the issue of beeswax adulteration. Member States were alerted through the Rapid Alert System for Food and Feed (RASFF) (European Commission, online-b) by RASFF news 17-844 (Appendix A) which raised awareness among stakeholders to request contractual guarantees from their suppliers on the purity of beeswax (European Commission, online-c).

There is a potential risk of adulterated beeswax entering the food chain in the form of honeycomb. Companies are including honeycomb with honey in pots to demonstrate the authenticity of the product. Contaminated wax sheets in those cases are integrated into the honeycomb and can be eaten by the consumers as indicated on the product label.

In November 2017 and July 2018, the problem was highlighted by the Commission in the Civil Dialogue Groups – Directorate-General for Agriculture and Rural Development. This forum assists the Commission and helps to maintain a regular dialogue on all matters relating to the Common Agricultural Policy, including rural development, and its implementation.

In August 2017, the Commission's Joint Research Centre (JRC) assessed the technical quality of analytical methods which have been used by researchers to detect adulteration of beeswax with stearin and/or paraffin themselves. The methods currently used for this purpose are at the research stage and have not been validated by collaborative studies, which is a requirement for official control purposes. The JRC recommended using gas liquid chromatography with flame-ionisation or mass spectrometric detection to detect and quantify the amount of paraffin and stearin added to beeswax. They also recommended the establishment of purity criteria for beeswax (when used in apiculture and as food when used in honey pots) in the absence of any such standard in the EU legislation (Appendix B).

The Commission has also asked the Member States to provide information on this issue via a questionnaire. In particular, information from three Member States, Belgium, France and Germany, confirmed links between the presence of stearic acid and its health effects on bees, namely brood development disturbance and increase of larva mortality.

The topic of adulterated beeswax was put on the agenda of the Working Party of Chief Veterinary Officers in March 2018, an enquiry into the French processing sector for beeswax for beekeeping applications was presented by France, and beeswax adulteration was highlighted by the Commission.

[1] Unless specifically stated, 'beeswax' does not mean a reference to the food additive 'beeswax, white and yellow (E901)'.

[2] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

1.2. Terms of reference

In accordance with Article 31 of Regulation (EC) No 178/2002[3], the Commission asks EFSA to provide scientific and technical assistance concerning beeswax adulteration with paraffin and stearin.

In particular, the scientific technical assistance should comprise:

1. in the absence of a specific standard in the EU legislation, the establishment of purity criteria and technical specifications for beeswax when used in apiculture and as a food in honey pots;

2. an evaluation of the impact of the migration of stearin and paraffin contained in beeswax intended for apiculture use on bee health, and possible safety concerns for humans due to consumption of honey contaminated with constituents of adulterated beeswax, or direct consumption of honeycomb.

1.3. Interpretation of the terms of reference

To address the mandate from the European Commission, the working group suggested differentiating the assessment made for bee health from the assessment made for human health. Therefore, the questions put by the European Commission are the following:

ToR 1. The establishment of **purity criteria and technical specifications for beeswax** when used in apiculture and as a food in honey pots (honeycomb).

ToR 2. Evaluation of the possible **health concerns for honey bees** due to their exposure to adulterated beeswax and to other bee products contaminated with constituents of adulterated beeswax.

ToR 3. Evaluation of the possible **health concerns for humans** due to the consumption of honey contaminated with constituents of adulterated beeswax or due to consumption of honeycomb contained in honey pots.

Beeswax is produced by different types of bees (all managed bees of the genus *Apis* and some species of the genus *Bombus*, *Melipona* and *Trigona*). For the purpose of this mandate, the focus is on beeswax produced by the western honey bee *Apis mellifera* L.

Different terms are used to refer to beeswax under the European legislation, in scientific literature and in the apiculture sector. The working group provided a description of the terminology used in this report (Table 1).

When assessing the risks to human health, beeswax refers only to beeswax in the form of a honeycomb and its synonyms, i.e. comb, comb wax. Consumers can also be exposed to adulterated beeswax through the consumption of products such as comb honey and chuck honey as defined in Council Directive 2001/110/EC[4].

The EU legislation on ABP[5] defines beeswax (also referred to as honeycomb) as an 'apiculture by-product' not intended for human consumption. For the purpose of this mandate, when assessing the risks to honey bee health, beeswax refers to all the different terms used in apiculture for this product, i.e. crude beeswax (raw beeswax) and beeswax block comb foundation (beeswax foundation, wax foundation, beeswax sheet, wax sheet) which are defined in Table 1 of this report. Further details on ABP legislation are provided in Section 1.4.1.

Apiculture accessories made from synthetic or semi-synthetic beeswax[6], in the form of microcrystalline wax, are not assessed, nor are means of adulteration of beeswax other than by paraffin and/or stearin/stearic acid and their contaminants.

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[4] Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ L 10, 12.1.2002, p. 47–52.

[5] Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. OJ L 273, 10.10.2002, p. 1–95.

[6] ECHA substance infocard on 'Beeswax, synthetic'. https://echa.europa.eu/substance-information/-/substanceinfo/100.068.421

Finally, pesticides and heavy metals have been reported as beeswax contaminants. However, as their origin is mainly due to environmental pollution and probably not related to adulteration, they were not assessed.

Table 1: Terminology used in this technical report

Terms	**Working group interpretation**
Beeswax	Natural wax produced by honey bees (*Apis mellfera* L.) for the construction of combs used for food storage (e.g. honey/nectar and pollen/beebread) and brood rearing. Beeswax is a lipid-based organic compound (natural wax) produced by the worker bees by four pairs of wax glands located on the inner side of the 4th to 7th abdominal sternites. The wax production phase primarily starts on day 9 and peaks at day 12 until day 18. *Adapted from Svečnjak et al. (2019a).*
Comb wax	Internal structure in the honey bee hive composed of a mass of characteristic hexagonal cells made of beeswax. *Adapted from Svečnjak et al. (2019a)*
Comb wax synonyms	Comb, honeycomb
Crude beeswax	Term used in apiculture for beeswax obtained by melting the honeycomb and wax cappings (after removal/extraction of honey) and removing foreign matter by melting it by boiling water (alternatively by steam or solar wax melting procedure). Crude beeswax is described as light yellow to dark yellow (light-brown) solid with a characteristic pleasant hive-like odour (originating from honey, propolis, pollen and honey bees) and granular, non-crystalline fracture when broken, insoluble in water, partially soluble in alcohol/ethanol, very soluble in ether and completely soluble in fatty and essential oils (white beeswax may be obtained by bleaching yellow beeswax). *Adapted from Council of Europe (2020).*
Crude beeswax synonyms	Raw beeswax, beeswax block
Comb foundation	Term used in apiculture for a sheet of beeswax with imprinted bottoms and the beginnings of walls of characteristic hexagonal cells (comb) obtained by melting, sterilising and pressing or casting crude beeswax using specific beeswax-embossing mould machines. Comb foundations are intended for further use in beekeeping (insertion in the bee hives within wired frames) as a comb foundation for further in-hive comb construction. *Adapted from Svečnjak et al. (2019a)*
Comb foundation synonyms	Beeswax foundation, wax foundation, beeswax sheet, wax sheet
Beeswax in apiculture	This term refers to comb foundations and crude beeswax to produce beeswax for its use in apiculture. This also refers indirectly to beeswax used as food in honey pots (honeycomb) as honeycomb is constructed by the bees on the comb foundations.
Paraffin	In chemistry, 'paraffin' is a synonym of 'alkane' (i.e. saturated hydrocarbon). In colloquial language, the term paraffin refers to solid hydrocarbon waxes (CAS No. 8002-74-2), comprised mainly of high molecular weight linear alkanes (Sections 1.4.3. and 3.3.2.1), and in this report it refers to the adulterant. 'Hydrocarbon wax' is used, comprising the 'low and medium melting point wax' (also called 'paraffin wax') and the 'microcrystalline wax' (of higher molecular mass than the low and medium melting point wax).
Stearin	'Stearin' has at least three meanings:

	(i) In chemistry, the term stearin (or tristearin, glyceryl tristearate, CAS No. 555-43-1) corresponds to the tristearic acid triglyceride in which all three glycerol hydroxyls are esterified with stearic acid. (ii) In the professional language of the fat industry, the term stearin refers to the solid fraction of oils and fats, i.e. triglycerides predominantly incorporating saturated fatty acids, as opposed to the olein, which is the liquid fraction. (iii) In colloquial language, 'stearin' refers to any solid fatty acid or mixture of free solid fatty acids, such as stearic and palmitic acid. In this report, 'stearin' corresponds to meaning (ii) and 'stearin/stearic acid' corresponds to meaning (iii). However, since those adulterants come from fraudulent practices, their precise composition is not always known.
Palmitin	'Palmitin' is a white, crystalline, water-insoluble powder, $C_{51}H_{98}O_6$, prepared from glycerol and palmitic acid: used in the manufacture of soap. In some cases, consider palmitin (CAS No. 67701-03-5) as a mixture of palmitic acid and stearic acid.

The current categorisation of beeswax in apiculture as an ABP Category 3 material, i.e. not intended for human consumption[7] does not prevent the presence of contaminants and/or adulterants. Moreover, it allows commercialisation of beeswax used in apiculture without previous quality (authenticity) control. These adulterants mainly include paraffin and stearin/stearic acid as well as their contaminants and can result from fraudulent practices performed during beeswax recycling (i.e. intentional introduction of adulterants to beeswax, referred to as adulteration) or from incidental processes (i.e. unintentional/unaware use and distribution of adulterated beeswax; Section 1.5). An example is described in the mandate and RASFF news 17-844 (Appendix A) concerning adulterated beeswax from China and Ukraine detected on the Belgian apiculture market (see Section 1.1).

These adulterants may pose health concerns to honey bees which might be in contact (i.e. from larvae developing in wax and from adults manipulating the wax when building combs) or consume contaminated food (stored in beeswax) and to humans via the consumption of honey or honeycomb. Preliminary studies requested by the Commission and conducted by Belgium, France and Germany confirmed an association between the presence of stearin/stearic acid and health effects on honey bees, namely brood development disturbance and increase of larva mortality (e.g. FASFC, 2008; Reybroeck, 2017; Reybroeck and Van Nevel, 2018; Aupinel, 2018) or impact on bee colonies (BGD, 2017). Stearin/stearic acid as well as paraffin and their contaminants contained in beeswax used in apiculture may pose harm to honey bees, in particular the brood, which develops inside beeswax cells, nurses which feed larvae with pollen contained in beeswax, workers which produce and masticate beeswax and foragers which consume nectar/honey previously stored in beeswax cells. They could be exposed to adulterants contained in beeswax both via contact and oral exposure.

Stearin/stearic acid as well as paraffin and their contaminants contained in beeswax used in apiculture could endanger the health of humans that consume honey or honeycomb placed in the honey pot. In this scenario, humans are assumed to ingest the adulterants and their contaminants migrated from the adulterated honeycomb to the honey or through the consumption of the adulterated honeycomb.

Detailed information is needed on the hazards (adulterants and their contaminants), hazard characterisation (adverse effects on honey bees and humans; dose–response assessment) as well as estimates on exposure of bees and humans to the adulterants and their contaminants.

For honey bees, experimental studies assessing the impact of known amounts of adulterants contained in waxes (usually expressed as a percentage of adulterated beeswax) can be used to determine the potential effects. Honey bee exposure is derived from worst-case scenarios of contact or ingestion of known amounts of larva food (i.e. royal jelly and beebread) and adult food (nectar/honey, pollen/beebread) stored in comb wax or mastication of known amounts of adulterated beeswax. Hazard

[7] Regulation EC No 1069/2009.

characterisation is based on previous evaluations made on these chemicals in the food area. Human exposure is derived from worst-case scenarios of ingestion of adulterated honey or beeswax.

Finally, if the available information is insufficient for decision-making, a plan will be developed to acquire new data. If this applies, specific knowledge and data gaps to be filled by future research and data collection efforts will be listed by the working group. Such recommendations will be provided to support risk management decisions and evidence-based risk assessments of adulterated beeswax.

1.4. Additional information

1.4.1. Legislation on beeswax

Beeswax in the form of honeycomb is the first natural 'packing material' for honey. Despite contact with honey as food, beeswax used in apiculture (as comb foundations) for honey production is not subject to any obligatory quality controls prior to being placed on the market (Svečnjak et al., 2019a). Quality controls apply to beeswax used as food additive E901 (EFSA, 2007) or as a pharmaceutical product (Council of Europe, 2020).

1.4.1.1. Beeswax intended for use as an animal by-product for apiculture

The European legislation on ABPs defines beeswax (and implicitly honeycomb) as an 'apiculture product' used in beekeeping[8] and categorises beeswax as an ABP not intended for human consumption[9]. It prohibits importation into and transit through the EU of beeswax in the form of honeycomb[10].

Beeswax for apiculture is commercialised in the EU as an ABP Category 3. Regulation (EC) No. 1069/2009[11] defines Category 3 ABPs as products of animal origin or foodstuffs containing products of animal origin that are not intended for human consumption for commercial reasons or due to manufacturing or packaging defects or other defects from which no risk to public or animal health arise.

Purity criteria and technical specifications are not available for beeswax for apiculture.

1.4.1.2. Beeswax intended for human consumption as a food contact material or as a food additive

Regulation (EC) No 178/2002, laying down the general principles and requirements of the food law (General Food Law Regulation), is applicable to beeswax when intended for consumption as food or feed. This includes its use as a food contact material or as a food additive.

Article 5 of Commission Regulation (EU) No. 10/2011 includes the EU list (Annex 1) of substances authorised for the use as additive or polymer production aid in plastic materials and articles without a specific migration limit (though restricted by the overall migration limit of 60 mg/kg food).

Commission Regulation (EU) No 231/2012[12], laying down specifications for food additives, includes beeswax (white or yellow) as an authorised food additive for specific uses (e.g. food coating).

EU legislation on products of animal origin for human consumption[13] does not list beeswax in the form of honeycomb as a product of animal origin for human consumption.

[8] Regulation (EC) No 1774/2002.

[9] Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. OJ L 54, 26.2.2011, p. 1–254.

[10] Chapter VIII, Art 25 of Commission Regulation (EU) No 142/2011.

[11] Annex XIV Table 2 of Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). OJ L 300, 14.11.2009, p. 1–33.

[12] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

[13] Council Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption. OJ L 18, 23.1.2003, p. 11–20.

1.4.1.3. Beeswax intended for use as a pharmaceutical product

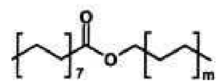
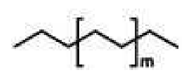
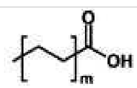
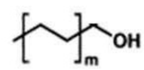
The *European Pharmacopoeia* (Council of Europe, 2020 is a reference for the quality control of medicines. The standards provide a scientific basis for quality control during the entire life cycle of a product.

The *European Pharmacopoeia* 10th edition (Council of Europe, 2020) contains monographs and methods for the analysis of beeswax that is indexed as beeswax yellow (or *cera flava*) and beeswax white (or *cera alba*). They have not been amended since their establishment and first publication in the 5th edition. The 10th edition includes information on an ongoing revision for white beeswax (*cera alba*) with regard to a test for adulteration and the revision of the ester value, saponification value, glycerol and other polyols. The information is available at the knowledge database section, where similar updates are available for yellow beeswax (*Cera flavia*).

1.4.2. Beeswax chemical composition and physico-chemical properties

More than 300 individual components have been reported in beeswax from various species of honey bee (Tulloch, 1980; Giumanini et al. 1995; Jiménez, et al., 2004, 2006, 2007; Maia and Nunes, 2013; Waś et al., 2014a, 2014b; Svečnjak et al., 2019a). Although their concentrations may vary depending on the honey bee species and their geographical origin, only small differences are observed in the concentration of the individual components and substance classes (EFSA, 2007). A typical composition of beeswax from *A. mellifera* L. is shown in Table 2.

Table 2: Beeswax composition from *A. mellifera* L. (adapted from Aichholz and Lorbeer, 1999)

Components	*A. mellifera* L. (%)	General structural formula
Esters total[(a)]	57.4	
monoesters	40.8	
hydroxymonoesters	9.2	
diesters	7.4	
Hydrocarbons total	15.7	
alkanes	12.8	
alkenes	2.9	
Free fatty acids total	18.0	
Free fatty alcohols total	0.6	
Total	91.7	

(a) Only the structural formula of alkylesters of palmitic acid is shown as an example.

Fatty acid monoesters with long chain linear alcohols (mainly C_{30}–C_{32}) are the most abundant compounds in beeswax, with alkyl palmitates (C_{38}–C_{52}) and alkyl esters of oleic acid (C_{46}–C_{54}) constituting the predominant structures (EFSA, 2007).

Hydroxymonoesters are long-chain alcohols and consist mainly of hydroxypalmitic acid esters as palmitic acid diolesters, whereas diesters and hydroxydiesters consist mainly of diesters and acylated hydroxyacid esters (EFSA, 2007).

n-Alkanes with an odd number of carbon atoms (C_{23}–C_{33}) constitute the predominant hydrocarbons in beeswax with heptacosane (C_{27}) being the most abundant in *A. mellifera* beeswax, followed by nonacosane (C_{29}), hentriacontane (C_{31}) and pentacosane (C_{25}), in decreasing order (Jiménez et al., 2004, Serra Bonvehí and Orantes Bermejo, 2012; Maia and Nunes, 2013; Waś et al., 2014a, 2014b).

The most common alkenes in *A. mellifera* beeswax are those with odd-numbered carbon atoms (C_{27}–C_{39}). For some of them, two isomers have been detected (Maia et al., 2013; Waś et al., 2014b). The percentage of unsaturated components increases with the chain length: above C_{33}, only unsaturated species are present (Serra Bonvehí and Orantes Bermejo, 2012). Low levels of dienes (23:2, 25:2, 31:2 and 33:2) have been observed, for which the double bound positions have not been established (Jiménez et al., 2004, 2007; Maia et al., 2013). The alkenes with 31 and 33 carbon atoms, either monounsaturated or diunsaturated, were most abundant (Jiménez et al., 2004; Maia et al., 2013).

Free fatty acids in beeswax are mainly unbranched and saturated, with even carbon numbers from C_{20} to C_{36}. Tetracosanoic acid (C_{24}) has been reported as the most abundant free fatty acid in *A. mellifera* beeswax (6%) (EFSA, 2007).

Free fatty alcohols make up generally less than 1% of beeswax and have a chain length of C_{24}–C_{36} (Jiménez, 2006, 2007; Serra Bonvehí and Orantes Bermejo, 2012).

Some carbohydrates (uncharacterised) and unidentified compounds, making up a few per cent are also present in beeswax.

In honey bees, chemical communication is the primary mode of intra-colony communication. Fatty acids that act as nestmate recognition cues include four unsaturated acids, namely palmitoleic, oleic, linoleic and linolenic acids, and two saturated fatty acids, namely palmitic and lignoceric acids (stearic acid is inactive) (Breed, et al.,1995a, 1995b, 1998, 2004; Fröhlich, et al., 2000; Brockmann et al., 2003; d'Ettorre et al., 2006). Fatty acids are also of great importance for beeswax and honeycomb consistency and firmness (Buchwald et al., 2005, 2006, 2009).

1.4.3. Origin, production and uses of paraffins

The term 'paraffins' may have different meanings according to the context. It can be used as a synonym for alkanes, which are divided into three groups based on their structure: normal alkanes (normal paraffins) branched alkanes (branched paraffins), cycloalkanes (cyclo-paraffins or naphthenes). In some cases, paraffins refer to normal (linear) alkanes, whereas branched or isoalkanes are also called iso-paraffins. Alkanes containing fewer than five carbon atoms are gaseous at room temperature, those having 5 to 17 carbon atoms are liquids, and the *n*-alkanes having more than 17 carbon atoms are solids.

In other cases, the term 'paraffin' refers to paraffin waxes. The long-chain *n*-alkanes largely dominate their composition (the amount of *n*-alkanes usually exceeds 75% and may reach almost 100%). Microcrystalline wax differs from refined paraffin wax in that the molecular structure is more branched and the hydrocarbon chains are longer, resulting in a higher molecular weight). To avoid confusion, in this report the term 'paraffin' was used when it refers to the adulterant and 'hydrocarbon waxes' covers paraffin waxes (low and medium melting point waxes) as well as other types of petroleum-based wax such as microcrystalline waxes or unrefined waxes (slack waxes).

Paraffins are mainly obtained from crude oil refining (see Fig. 1). They are crystallised from base oils, a high boiling fraction obtained by vacuum distillation.

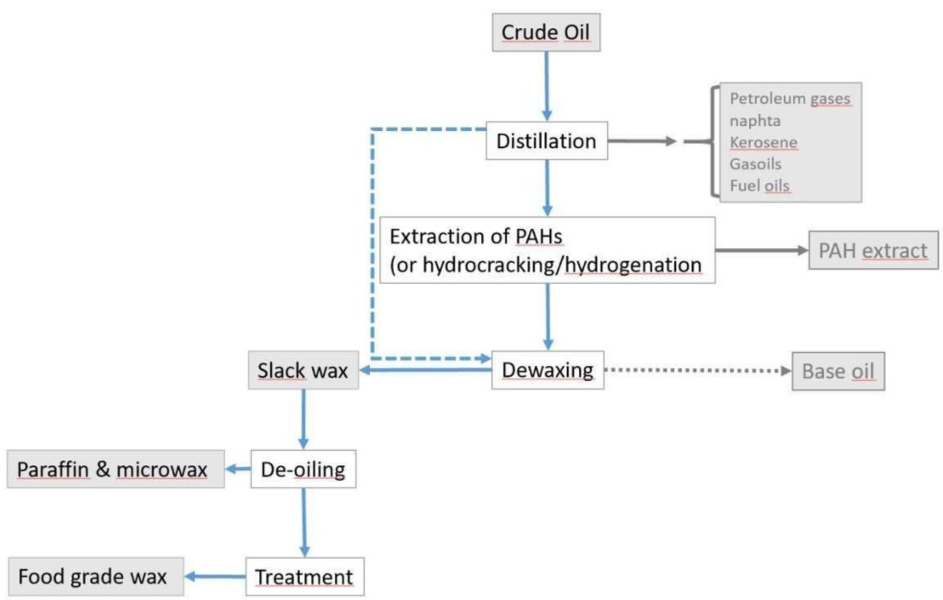


Figure 1: Major steps in crude oil refining for wax production (adapted from Carrillo, 2017). *Blue dashed lines represent a possible minor route in the production process of paraffins. The grey dotted line indicates several undetailed steps.*

Slack waxes are intermediate products in the production of waxes and are obtained by dewaxing refined or unrefined vacuum distillate fractions by a crystallisation process. Depending on the distillate fraction, they contain hydrocarbons of 12–85 carbon atoms, mainly *n*-alkanes. Heavier oil fractions yield slack waxes with increasing proportions of isoalkanes, cycloalkanes (naphthenes) and alkylated aromatic hydrocarbons (MOAH). Slack waxes usually still contain 10–30% oil. Paraffin and microcrystalline waxes are produced by de-oiling them.

Low to intermediate melting point waxes mainly consist of *n*-alkanes ranging from C_{20} to C_{50} (Jafari Behbahani et al., 2015). They are translucent white to yellow and have a well-defined macrocrystalline structure of large needles or plates, with a melting point in the range of 43 to 68°C, typically around 55°C. They may contain a low proportion of MOAH.

Microcrystalline waxes are of a higher molecular mass (predominantly in the range between C_{41} and C_{51}) and contain a higher percentage of iso- and cycloalkanes (EFSA ANS Panel, 2013a). Microcrystalline waxes usually melt at between 60 and 95°C

Depending on purity and melting properties, hydrocarbon waxes are used, for example, for candles, coating paper or clothes, in food contact materials, as food additives, in histology preparations and in cosmetics.

Hydrocarbon waxes are used in pharmaceuticals and cosmetics if compliant with the purity specifications on polycyclic aromatic hydrocarbons (see Chuberre et al. 2019 for review).

Low viscosity waxes derived from petroleum-based or synthetic hydrocarbon feedstocks (FCM 93) and high viscosity waxes (microcrystalline wax; FCM 94) are authorised for use in food contact materials (see below). FCM 93 must comply with the following specifications: average molecular weight not less than 350 Da; viscosity at 100°C not less than 2.5 cSt (2.5×10^{-6} m^2/s); content of hydrocarbons with carbon number less than 25, not more than 40% (w/w). A specific migration limit (SML) of 0.05 mg/kg food is specified and this wax is not to be used for articles in contact with fatty foods. For FCM 94, the specifications are: average molecular weight not less than 500 Da; viscosity at 100°C not less than 11 cSt (11×10^{-6} m^2/s); content of mineral hydrocarbons with carbon number less than 25, not more than 5% (w/w). No SML is specified.

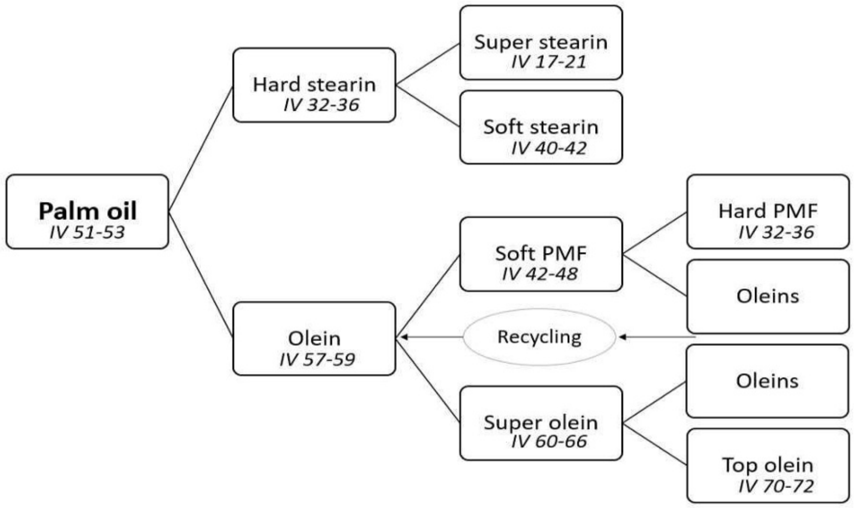
The presence of high amounts of paraffin (> 50%) in beeswax has been reported by several authors (Jiménez et al., 2007; Serra Bonvehí and Orantes Bermejo, 2012; Svečnjak et al., 2015).

The composition of hydrocarbon waxes, including impurities and contaminants, varies based on their source and the refining process used to produce a finished product. During the petroleum refining process (distillation/extraction/hydrocracking/hydrogenation/de-oiling), most of the undesirable substances are eliminated. However, sulfur, oxygen and nitrogen compounds, as well as MOAH, may still be present, in particular in weakly refined products and those of low price (Lijinski, 1960). Lau et al. (1997) found significant amounts of PAH, chlorophenols, chlorobenzenes and polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans in hydrocarbon wax used by candle manufacturers. Using nuclear magnetic resonance spectrometry, Palou et al. (2014) did not find aromatic hydrocarbons in hydrogenated industrial waxes (i.e. intensely refined waxes).

1.4.4. Stearin origin, production and use

In chemistry, the term 'stearin' or 'tristearin' corresponds to glycerol tristearate (CAS No. 555-43-1), i.e. the triglyceride in which all three glycerol hydroxyls are esterified with stearic acid, but can also be the common name for stearic acid or a mixture of stearic acid and palmitic acid.

'Stearin' is also used to refer to the solid fraction obtained by crystallisation from fats or oils of animal or plant origin. During the fractionation of vegetable oils, the solid fraction (stearin) is separated from the remaining liquid fraction (olein) by means of a filtration or a centrifugation process (Fig. 2). For instance, palm stearin is the solid fraction of palm oil produced by partial crystallisation. In palm stearin, trisaturated triacylglycerols and disaturated-monounsaturated triacylglycerols constitute more than 75% of the total, and palmitic acid (not stearic acid) is the major fatty acid. Stearin can also be obtained from animal fats, such as lard and tallow (Kincs, 1985). Free fatty acids can be present as a minor fraction in stearin from plant or animal origin. Today, additional fractionation steps can yield very low iodine value stearins, known as super stearin. Such super stearin contains approximately 90% saturated fatty acids, predominantly palmitic acid, most of them in esterified form. It is very hard below 40–45°C and progressively melts at 65–70°C (Gibon, 2012).



IV = iodine value; PMF = palm mid fraction

Figure 2: Dry multiple fractionation of palm oil (adapted from Siew Wai Lin, 2011)

Food-grade stearin is used to provide increased stability in foods that require solid fat functionality. In particular, stearin of plant origin finds applications in frying fats, confectionary, margarines, shortening, cocoa butter substitute and in the manufacture of components for moulding and coatings.

However, not all fats, oils and their side products are of food grade or intended for food. Stearin of animal origin possibly accruing as a by-product not intended for human consumption, falls under Regulation (EC) No 1069/2009. Large amounts of fats/oils and by-products are used for animal feeds and the chemical industry, e.g. for the production of detergents. Stearin is also a common name for stearic acid, or its mixture with palmitic acid, which is used in the production of candles. In theory, stearin could be produced from edible fats and oils contaminated incidentally (e.g. by leaking lubricating, hydraulic or heating oils) or as a result of fraud (EFSA, 2008b) or discarded (Grob et al., 2001).

1.4.4.1. Evaluations on beeswax and its adulterants when used in apiculture

The risk of contaminated and/or adulterated beeswax to honey bee health was assessed by the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) in the advice 18-2018 (FASFC, 2018). The Scientific Committee proposed action limits for re-melted beeswax placed on the market: (i) the acid value of the wax should be greater than, or equal to, 17 and less than, or equal to, 24; (ii) the ester value (= saponification value – acid value) of the wax should be greater than, or equal to, 63 and less than, or equal to, 87; (iii) contamination of beeswax by heavy metals should be below or equal to 3 mg/kg, 2 mg/kg and 1 mg/kg for arsenic, lead and mercury, respectively; and (iv) pesticide and veterinary drug residues in the wax should be less than 0.6 mg/kg for acrinathrin, 400 mg/kg for amitraz, 0.4 mg/kg for carbofuran, 2 mg/kg for chlorpyrifos(-ethyl), 40 mg/kg for coumaphos, 0.06 mg/kg for cyfluthrin, 0.3 mg/kg for cypermethrin, 40 mg/kg for DDE, 40 mg/kg for DDT, 0.1 mg/kg for deltamethrin, 1.5 mg/kg for flumethrin, 0.03 mg/kg for imidacloprid, 0.09 mg/kg for lindane (γ-HCH), 0.2 mg/kg for mevinphos, 1.5 mg/kg for pyridaben, 20 mg/kg for tau-fluvalinate, 0.04 mg/kg for thiamethoxam, and 2 mg/kg for thymol.

1.4.4.2. Evaluations on beeswax and its adulterants when used as food

EFSA evaluations

Beeswax: In 2007, EFSA adopted a Scientific Opinion on the use of beeswax as a food additive 'Beeswax (E901) as a glazing agent and as carrier for flavours' (EFSA, 2007). The Panel concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not a safety concern.

The following relevant EFSA evaluations were identified on the basis that the substances assessed contain common components with the adulterants identified in beeswax.

Paraffin and stearin components: EFSA has previously assessed the safety of the main stearin components, stearic and palmitic acid, as food additives and nutrients in food and of paraffins in food contact materials. With regard to stearic acid and palmitic acid, the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA Panel) adopted a Scientific Opinion on dietary reference values for fats (NDA Panel, 2010) and the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) a Scientific Opinion on the re-evaluation of fatty acids (E570) as a food additive, which included the evaluation of these two specific fatty acids (EFSA ANS Panel, 2017a). Typical palmitic and stearic acid compositions of common edible fats and oils range between 2 and 44%, while the contribution of fatty acids (E570) as a food additive represented on average only 1% of the overall exposure to saturated fatty acids from all dietary sources (food additive and regular diet). In addition, stearin (considered as a triglyceride of stearic and palmitic acid) is related to the re-evaluation of mono- and di-glycerides of fatty acids (E471) as food additives (EFSA ANS Panel, 2017b), where the presence of potential impurities of safety concern from the raw materials used as a source of fatty acids is addressed. Paraffin is approved for use as a food contact material (FCM 93) in plastics under Commission Regulation 10/2011 for the category 'waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks, low viscosity, with an SML at 0.05 mg/kg food'.

Microcrystalline wax: In 2013, the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) concluded that microcrystalline wax used as a food additive (E905) with the currently authorised uses (as a surface treatment agent on confectionary, decorations and coatings and chewing gum and as a surface treatment agent on melon, papaya, mango and avocado) would not be of safety concern (EFSA ANS Panel, 2013a).

Microcrystalline wax is approved for use in plastic food contact materials (FCM 94) under Commission Regulation 10/2011 (described as waxes, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks, low viscosity) with no restrictions other than the generic overall migration limit of 60 mg/kg food.

Mineral oils: In 2012, the Panel on Contaminants in the Food Chain (CONTAM Panel) published an Opinion on mineral oil hydrocarbons (MOH) in food (EFSA CONTAM Panel, 2012). The mineral oil saturated hydrocarbon (MOSH) fraction of MOH includes the hydrocarbon waxes. It was reported that in the dietary surveys of the general populations across Europe, dietary exposure to MOSH ranged between approximately 0.03 and 0.3 mg/kg/bw per day and was found to be higher in younger consumers than in adults and the elderly. Honey was not identified as a significant source of exposure. The no-observed-adverse-effect level (NOAEL) for induction of liver microgranulomas by the most potent MOSH, 19 mg/kg bw per day, was used as a reference point for calculating margins of exposure (MOEs) for background MOSH exposure. MOEs ranged from 59 to 680. Hence exposure to MOSH via food in the EU was considered to be of potential concern.

In 2013, the ANS Panel assessed medium viscosity white mineral oils with a kinematic viscosity of between 8.5 and 11 mm^2/s at 100°C for the proposed uses as a food additive (EFSA ANS Panel, 2013b). It noted that the potential dietary intake of mineral oils with a kinematic viscosity ≥11 mm^2/s at 100°C as a food additive in high consumers would reach up to approximately 10 mg/kg bw/day for toddlers. The Panel established a group acceptable daily intake (ADI) for both medium and high viscosity mineral oils of 12 mg/kg bw/day based on a two-year study on chronic toxicity and carcinogenicity in rats fed with the two mineral oils. It concluded that, although the exposure is below the ADI, other sources of mineral oil need to be considered.

Other evaluations from the Scientific Committee on Food, JEFCA and FDA

Mineral hydrocarbons, including low to intermediate melting point and microcrystalline waxes (E905), have been evaluated several times for their safe use as food additives by both the Scientific Committee on Food (SCF) in 1990 and 1995 (SCF, 1992, 1997) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), but most recently in 2009 (JECFA, 1992, 1993, 1995, 2002, 2010).

The SCF stated that there are insufficient data to establish the safety of hydrocarbon waxes (SCF, 1997). JECFA noted at its 39th meeting that long-term toxicity studies had indicated that petroleum-derived hydrocarbon waxes and microcrystalline waxes were non-toxic and non-carcinogenic (JECFA, 1992, 1993). The Committee therefore established an ADI not specified 'for these waxes for the following uses: chewing-gum base, protective coating, defoaming agent, and surface finishing agent'. However, the ADI 'not specified' for hydrocarbon waxes was withdrawn at the JECFA's 44th meeting (JECFA, 1995), because in new 90-day studies in rats there were toxicological effects at all dose levels.

At JECFA's 44th meeting (JECFA, 1995), an ADI of 0–20 mg/kg bw was established for hydrotreated, high-melting point microcrystalline wax and clay-treated microcrystalline wax, based on new short-term feeding studies showing no adverse effects up to the highest dose tested of 2% microcrystalline wax in the diet (JECFA, 1995). Based on the same studies, the SCF likewise established an ADI of 0–20 mg/kg bw (SCF, 1997). At the 59th meeting, JECFA (JECFA, 2002) assessed additional studies including long-term ones. The ADI for microcrystalline waxes was confirmed, specified by an average relative molecular mass of ≥500 and a carbon number at the 5% distillation point of ≥25. Although the Committee was not able to establish an ADI for low-melting-point waxes, a temporary group ADI of 0–0.01 mb/kg bw for medium and low viscosity mineral oils was set.

Hydrocarbon waxes approved for use as a food contact additive in plastics under Commission Regulation 10/2011, are described as 'waxes, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks, high viscosity' (FCM 94). The specifications are the same as those used by the JECFA in 2002. Later, the use of hydrocarbon waxes of a lower molecular mass was authorised (FCM 93). They are described as 'waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks, low viscosity, with an SML of 0.05 mg/kg food' and there is a restriction 'not to be used for articles in contact with fatty foods for which simulant D is laid down'. It is specified by 'average molecular weight not less than 350 Da; viscosity at 100°C not less than 2.5 cSt (2.5 × 10^{-6} m^2/s); content of hydrocarbons with carbon number less than 25, not more than 40% (w/w)'. This entry in the list of Regulation (EU) 10/2011 is presently under re-evaluation.

1.5. Beeswax in apiculture: recycling, adulteration and contamination pathways

Beeswax is produced by different types of bees (all managed bees of the genus *Apis* and some species of the genus *Bombus*, *Melipona* and *Trigona*). For the purpose of this mandate, the focus is on beeswax produced by the western honey bee *Apis mellifera* L. Secretion of wax scales may occur in adult honey bees from day 3 to day 21 post-emergence (Hepburn et al., 1991). However, the wax production phase primarily starts on day 9 and peaks at day 12 until day 18 (Hepburn et al., 1984, 2014). Beeswax is a lipid-based organic compound (natural wax) produced by the worker bees using four pairs of wax glands located on the inner side of the 4th to 7th abdominal sternites (Locke, 1961; Cassier and Lensky, 1995). The new wax scales are masticated by the worker bees and used to build honeycomb cells in which the brood is raised and where nectar and pollen are stored (Thompson, 2012; Ravoet et al., 2015). Over the season, when the colony is developing, new comb foundations are needed. In that process, beekeepers need new beeswax, which comes from recycling old wax.

Beeswax is used for various applications, such as candles, cosmetics, medicinal ingredients and food additives, but in the context of this mandate, the focus is on beeswax used for comb foundations. With this application, it eventually re-enters the beekeeping industry and can end up in honey pots as honeycomb (displayed as honeycomb).

While there is an ISO standardisation in preparation, there are currently no guidelines available to beekeepers providing instructions on how to recycle beeswax. Several good beekeeping management practice guidelines were proposed (e.g. Bee Research Institute, 2009; KonVIB/FAB-BBF, 2009; ITSAP, 2018; FAO, 2019; El Agrebi et al., 2019, 2020) with the following recommendations:

1. The use of beeswax of good quality, which could be ensured with the following actions in place:
    i. Registration of official manufacturers subject to official veterinary supervision.
    ii. Plants which manufacture comb foundation and process combs from various sources with a system of critical points in place (hazard analysis and critical control points).
    iii. Inspections of comb foundation performed on a regular basis at all official manufacturers with regard to composition (to avoid adulteration and ensure a limited level of chemical residues) and microbiology (to ensure the absence of pathogens). Remove old waxes and those for which health problems have been noted from the bee circuit or destroy them.

iv. Each beekeeper should solicit an attestation from the manufacturer (or trader) to ensure the chemical and microbiological safety status of the beeswax comb foundation.
v. Each beekeeper should follow guidelines on the beekeeping management practices of its country (e.g. use preferentially natural veterinary active substances that do not remain in beeswax and do not affect the hive products or use veterinary medicinal products under veterinary supervision).

2. The renewal of the body frames: the replacement of the old frames from the brood chamber by low residue beeswax comb foundation in order to ensure a complete frame turnover in the hive after 2 to 3 years is recommended. The beeswax capping (the white covering on sealed honeycomb) processed by the beekeepers themselves are the best source of beeswax for frame replacement.
3. The traceability and management of beeswax: register the new comb foundations introduced into the hives; keep samples of beeswax for future analysis if needed; specify the origin and the source of beeswax (e.g. commercial, organic, recycled, capping or initiation).
4. The restocking of empty built frames: built frames must be protected from pests, mould and rodents.

Beeswax can be contaminated by the residues of plant protection products and veterinary substances through different pathways (FASFC, 2018; Svečnjak et al., 2019a). Beekeepers can use chemical substances (e.g. veterinary substances like acaricides and biocides) to treat beehives, notably to control the *Varroa destructor* mite, a parasite of bees that causes bee varroosis. Applying varroacides in honey bee colonies leaves residues in bee products, especially in beeswax, in which they accumulate with years of treatment, given that they are mostly fat-soluble and non-volatile. Veterinary substances can also be applied to honey bee colonies to control other bee diseases, such as American foulbrood (*Paenibacillus larvae*), European foulbrood (*Melissococcus plutonius*), and nosemosis (*Nosema apis* and *N. ceranae*). Moreover, insect repellents can be used by beekeepers against the wax moths (*Achroia grisella* and *Galleria mellonella*) in stored combs (Wilmart et al., 2016). The European Medicines Agency (EMA) provides the list of active substances and commercial products authorised in the EU for beekeeping, (EMA, 2019).

Foraging bees themselves can bring back environmental contaminants to the hive, e.g. PAH, heavy metals (lead, cadmium, chromium, manganese and zinc), PCBs and residues of the plant protection products used in agriculture. Carriers are contaminated water, nectar, pollen, propolis or/and honeydew (Porrini et al., 2002). These residues can contaminate the beeswax of the existing combs.

Beeswax from non-EU countries may be contaminated with antibiotics used in beekeeping (Reybroeck et al., 2012) and/or in agriculture that are not allowed under European legislation.

Adulteration of beeswax occurs with paraffin and, to a lesser extent, with stearin/stearic acid, palmitin and tallow (Serra Bonvehí and Orantes Bermejo, 2012; FASFC, 2018; Svečnjak et al., 2019a). Paraffin is the most widely used adulterant due to its wide availability, low price, and physico-chemical properties (chemically inert, white or colourless and odourless) (Svečnjak et al., 2015). Paraffin in beeswax may alter the composition of honey by fermentative or oxidation processes (Svečnjak et al., 2019b).

After its use, beeswax is usually re-melted and re-used within the beekeeping sector (Wilmart et al., 2016), which leads to the accumulation of residues (EMA, 2019; Reybroeck and Van Nevel, 2018; El Agrebi et al., 2019).

2. Data and methodologies

2.1. Information provided by the European Commission

The following documentation has been provided by the Commission as background information:

1. Member States have been alerted through the RASFF notification, reference number 17-844 (Appendix A), on cases of adulterated beeswax reported by Belgium and other EU Member States.
2. The JRC has assessed the quality of the analytical methods used by researchers to detect adulteration of beeswax with paraffin and/or stearin/stearic acid and produced a report (Appendix B).
3. The EU Member States submitted information via a questionnaire.
4. Information from three Member States, Belgium, France and Germany (e.g. see FASFC, 2018; Reybroeck, 2017; Reybroeck and Van Nevel, 2018), confirmed links between the presence of stearic acid and health effects on bees, namely brood development disturbance and increase in larva mortality. Later, Slovakia submitted a report on a risk assessment of pesticide residues in beeswax, which highlighted the importance of the quality of beeswax for the health and vitality of the bee colony.

2.2. Call for studies by EFSA

On 27 May 2019, EFSA launched a call to complete the information already provided by the Commission, open until 10 July 2019. The objective was to retrieve the latest studies and data available on adulterated beeswax with paraffin and/or stearin/stearic acid in the EU. Information on the title of the study, the authors, date of publication, doi, URL and abstract, as well as a reference to the area covered by the study, i.e. human health, honey bee health, and the area of expertise (analytics, physico-chemical characterisation, purity criteria or risk assessment) was registered.

The call for studies was sent to the EU Bee Partnership, the US Bee Informed Partnership, COLOSS (Prevention of honey bee colony losses) and EFSA networks: the Emerging Risk Engagement Network (EREN) and the Stakeholder Discussion Group on Emerging Risks (StaDG-ER).

In total, nine replies, including 18 documents, were received from representatives of six Member States (Belgium, Bulgaria, Czechia, Germany, Sweden and Switzerland) as well as from COLOSS. In addition to some studies already provided by the Commission, the targeted groups provided additional studies on wax quality assessments, technical specifications for beeswax and studies related to methodologies for paraffin analysis in beeswax, like the one submitted by the Ministry of Agriculture of the Czech Food Authority on attenuated total reflectance Fourier-transform infrared spectroscopy (ATR–FTIR).

The German Federal Institute for Risk Assessment (BfR) provided information on two studies on residue and authenticity analyses for beeswax as well as the analyses performed on the impact of adulteration of different kinds of beeswax on honey bee health.

The Federation of Swedish Farmers submitted the results on the analysis of 48 samples of beeswax (comb foundation and block). Almost half of the samples were of Swedish origin and the others were from various origins (e.g. Poland, Romania, Belarus, Nepal, Egypt and China). A member of the Swedish Board of Agriculture and a member of the Swedish Beekeepers Association were responsible for collecting the samples and sending them to the laboratory where the analyses were performed. The results show the percentages of hydrocarbons (of undefined origin) present in the different beeswax samples. (Ms Agneta Sundgren, Expert in Plant Protection from the Federation of Swedish Farmers, confirmed this by email on 10 July 2019 and the 19 March 2020).

As part of the Swiss 2017 annual wax monitoring programme, wax samples from 11 wax processors were analysed for adulteration with paraffins and stearin by the Länderinstitut für Bienenkunde in Hohen Neuendorf (Germany). Infrared spectroscopy was used to detect adulteration with more than 2.5% paraffin and more than 1% stearin. The samples of all 11 wax processors were not adulterated according to the criteria investigated (Christina Kast from Agroscope, Switzerland, email from 7 June 2019).

2.3. EFSA databases

Chemical contaminant data

EFSA collects data on the occurrence of chemical contaminants in food[14]. The data submission to EFSA follows the requirements of the EFSA Guidance on standard sample description for food and feed (EFSA, 2010); occurrence data are managed following the EFSA standard operational procedures on data collection and validation and on data analysis of food consumption and occurrence data.

The EFSA database on chemical contaminant occurrence data has no available data on beeswax. The working group consulted relevant EFSA Opinions to retrieve occurrence data on contaminants present in food commodities of interest that could be sources of adulterants, e.g. palm oil/fats and tallow.

Food consumption data

The EFSA Comprehensive European Food Consumption Database (EFSA, online) provides a compilation of national food consumption data at the individual level. All data are collected using FoodEx (Food classification and description system for exposure assessment) a food classification system developed to simplify the link between occurrence and food consumption data when assessing the exposure to hazardous substances. The FoodEx2 items relevant to beeswax for human consumption are the following:

- 'Comb honey'
- 'Bee-produced formulations as a food supplement' (including pollen, royal jelly, etc.).

The EFSA Comprehensive Database included only three records on 'comb honey' and 13 records of 'bee-produced formulation'. Information on the average proportion of beeswax in bee-produced formulations to estimate the human exposure to beeswax is not available. Data from 23 individuals who reported 32 occasions of eating food supplements containing propolis, according to the additional information from the data provider, were also retrieved. Due to the scarce data available on the food commodities, the working group did not consider it for the exposure assessment.

2.4. Eurostat

Eurostat is the statistical office of the European Union. Its mission is to provide high quality statistics for Europe. Several Eurostat product codes for commodities containing beeswax were identified (Table 3).

Table 3: Eurostat product codes and specifications

Product code	Specifications
152190	• beeswax • other insect wax spermaceti • wax for apiculture
15219091	• beeswax and other insect waxes, crude
9602 96020000	• worked vegetable or mineral carving material and articles of these materials • moulded or carved articles of wax, of stearin, of natural gums or natural resins or of modelling pastes • other moulded or carved articles, not elsewhere specified or included worked, unhardened gelatine (except gelatine of heading 3503) • articles of unhardened gelatine, N.E.S.[15]

The data on trade of product code 152190 (Appendix C) shows the EU Member States' imports from other EU countries ('EU28 intra') and from non-EU countries ('EU28 extra'), expressed in 100 kg and

[14] https://www.efsa.europa.eu/en/data/chemical-contaminants-data

[15] not elsewhere specified

includes annual data for the years 2013–2018. The extract shows the Member States that imported or exported the most beeswax, both from/to other EU countries and non-EU countries.

Germany, France and Greece are the three biggest importers in Europe. Although the country importing the largest amounts depends on the year, Germany was the largest importer of 'product code 152190' from outside Europe (3,405,000 kg in 2018), whereas France was the largest importer when the product originated in Europe. On exports, Germany and France were on top with 1,323,600 and 416,700 kg, respectively, exported to EU countries: the Netherlands being the second biggest exporter with 479,000 kg. Lower amounts are shown for exports outside Europe. However, code 152190 is not limited to human consumption; wax for apiculture is included, except for wax prepared in combs for beehives, which is part of Eurostat's 'product code 9602'.

'Product code 15219091' covers wax in the form of natural comb. Germany is the biggest importer (intra and extra EU) and the biggest exporter, exporting 10 times more within the EU than to outside Europe (Appendix C).

Data on the production of beeswax and honeycomb in Europe are not available.

2.5. MedISys

The Medical Information System (MedISys)[16] is an automated system for monitoring media. It was established by the JRC (Linge et al., 2009; Steinberger et al., 2013) and developed to be an effective early-warning system for food- and feed-borne hazards, in particular in the area of animal and plant health (Rortais et al., 2010).

MedISys was used to obtain information on beeswax adulteration using keywords related to the adulteration of beeswax, honey bee health or of human health issues connected to adulterated beeswax.

Before setting a proper filter on MedISys, *a posteriori* and broad searches were made on MedISys using the keyword 'beeswax' in three languages (English, Italian and German, i.e. 'beeswax', 'cera d'api' and 'Bienenwachs', respectively) over a period of approximately 2.5 years (from 1 January 2017 to 22 May 2019 for searches in English and to 23 May 2019 for searches in German and Italian). A total of 20,000, 3,210 and 656 articles were retrieved in Italian, English and German, respectively. As a pilot, only the English dataset was further assessed using a 'topic models' approach, a machine-learning technique which can automatically explore large collections of documents, connect those that exhibit similar patterns and deliver patterns of word use with the probabilistic model Latent Dirichlet Allocation (Blei and Lafferty, 2006; Blei, 2012). With this technique, noise from the media could be significantly reduced as well as the amount of information to be processed. The identification and monitoring of adulteration in beeswax and related emerging risks with a specific filter set on MedISys would merit further exploration (Rortais et al., in press).

2.6. Scientific literature

A comprehensive review of the scientific literature was conducted to investigate the presence of adulterants in beeswax.

The search was conducted in May 2019, using three databases: Scopus, CAB Abstracts and the Web of Science Core Collection. The search included papers published from 1 January 2000 to 22 May 2019.

The full list of databases searched and the search strings that were run in the databases are available in Appendix D.

Of the 866 articles retrieved, 138 were selected through title screening. The exercise excluded papers not related to the terms of reference for this mandate (e.g. archaeological studies on beeswax, novel nanostructured lipid carrier uses of beeswax or beeswax adulterated or contaminated with residues of plant protection products and veterinary substances). A validation of the screening was performed by the working group members.

Subsequently, two experts with experience in chemistry performed an abstract screening on 82 papers to retrieve relevant papers on, e.g. analytical methods, chemical characterisation, beeswax composition

[16] http://medusa.jrc.it

or identification of adulteration for beeswax. Two experts with expertise in bee biology and bee health screened 17 papers for topics related to honey bee health and beekeeping practices. In addition, all working members screened the 39 abstracts related to beeswax adulterated or contaminated with residues of plant protection products and veterinary substances through different pathways.

Finally, the 18 records from the inventory of studies (Section 2.2) and additional records already known to the members of the working group or collaborators, were also assessed (Fig. 3).

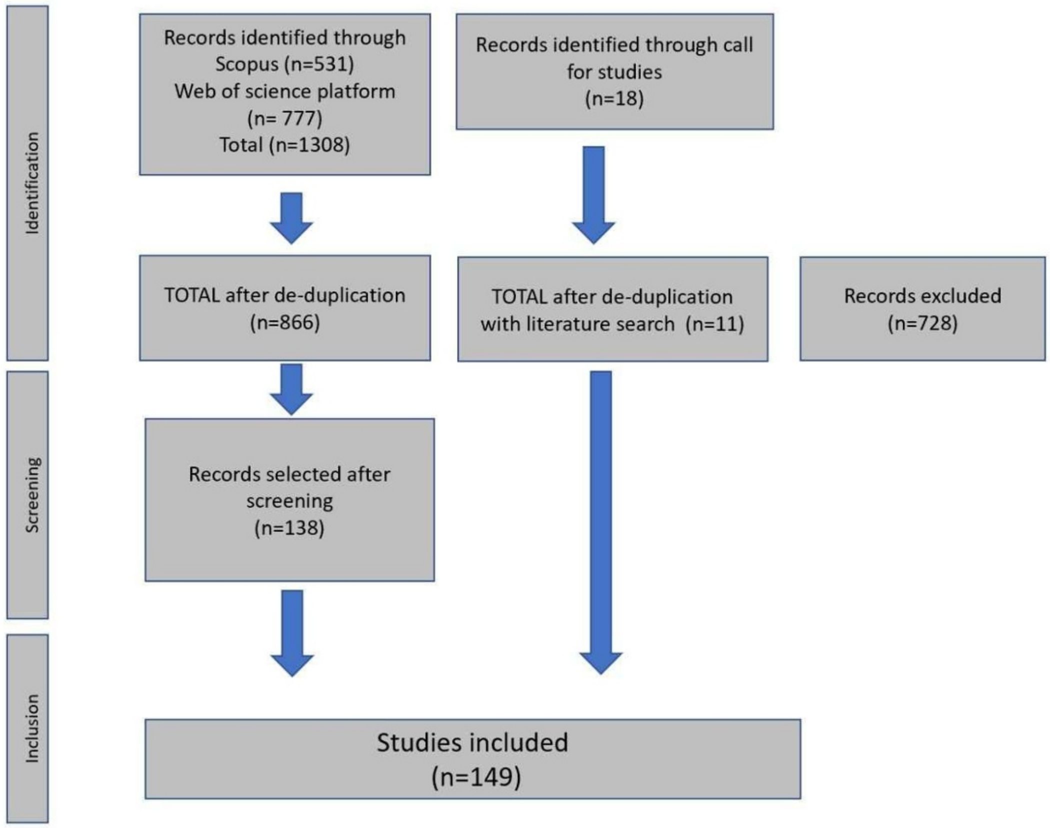


Figure 3: Flow chart for studies search results

3. Assessment

3.1. Purity criteria and analytical methods for the determination of beeswax authenticity

Various physico-chemical parameters (range values) defining pure beeswax are implemented by the legislation on beeswax for use in pharmaceuticals and the food industry. However, currently, there are no defined purity criteria or internationally standardised analytical methods for routine quality (purity) control of beeswax used in apiculture.

3.1.1. Overview of purity criteria and related classical physico-chemical methods

Classical physico-chemical methods have frequently been used for beeswax research over the last few decades, mostly determine beeswax quality (purity) criteria. Many of these criteria are implemented by the legislation on beeswax for its use as pharmaceutical-grade beeswax (Council of Europe, 2020) and in the food industry as food additive E901 (Commission Directive 2009/10/EC[17]). A comparative overview of these physico-chemical parameters (in range values) is presented in Table 4.

In 2009, the International Honey Commission (IHC) developed, for the first time, a proposal for quality criteria for beeswax used in apiculture based on 10 physico-chemical parameters (Bogdanov 2009, 2016). These criteria were mostly based on the *European Pharmacopoeia* (Council of Europe, 2011) standards. They were completed with additional parameters (water content, mechanical impurities, additives, and hydrocarbon content) determined in accordance with the methods recommended by the German Society for Fat Science (DGF, 1957). However, for most of the additional proposed parameters (i.e. water content, refractive index, and ester/acid ratio) there is no sufficient scientific evidence for their use as beeswax purity criteria. The IHC proposal was never implemented.

Table 4: Comparative overview of the physico-chemical parameters defining beeswax purity according to the FAO/WHO (JECFA, 2005), EFSA (2007), the European legislation on food additives (Commission Directive 2009/10/EC; Commission Regulation (EU) No 231/2012), the *European Pharmacopoeia* (Council of Europe, 2020), and the International Honey Commission proposal (Bogdanov, 2009, 2016). Table adapted from the COLOSS Beebook chapter on beeswax (Svečnjak et al., 2019a)

Purity criteria	**Chemical and technical assessment FAO/WHO (JECFA, 2005)**	**EFSA Scientific Opinion (EFSA, 2007)**	**European legislation on food additives (2009/10/EC)**	***European Pharmacopoeia* 10.2 (2020) Yellow beeswax**	***European Pharmacopoeia* 10.2 (2020) White beeswax**	**International Honey Commission (2016)**
Water content	-	-	-	-	-	<1%
Melting range (°C)	62–65	62-–65	62–65	61–66	61–66	61–65
Specific gravity (D2020):	-	0.96	0.96	0.960	0.960	
Refractive index (75°C)	-	-	-	-		1.4398–1.4451
Acid value (mg KOH/g)	17–24	17–24	17–24	17–22	17–24	17–22
Saponification value (mg KOH/g)	87–104	87–104	87–104	87–102	87–104	87–102

[17] Commission Directive 2009/10/EC of 13 February 2009 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 44, 14.2.2009, p. 62–78 [no longer in force].

Ester value (mg KOH/g)	-	-	-	70–80	70–80	70–90
Ester/acid ratio	-	-	-	-	-	3.3–4.3
Peroxide value (mM H_2O_2/kg)	<5	<5	<5	-	-	-
Glycerol and other polyols (%)	<0.5 (as glycerol)	<0.5 (as glycerol)	<0.5 (as glycerol)	Absent	Absent	Absent
Carnauba wax	Passes test			-	-	
Ceresin, paraffins and other waxes	Passes test	Absent	Absent	Absent	Absent	Absent
Fats, Japan wax, resin and soaps	Passes test	Absent	Absent	Absent	Absent	Absent
Arsenic (mg/kg)	-	<3	<3	-	-	-
Lead (mg/kg)	<2	<5	<5	-	-	-
Mercury (mg/kg)	-	<1	<1	-	-	-

The most commonly used methods and related range values cited in research on beeswax authentication are the determination of the melting point, the acid value, the saponification and the ester value (Table 5). However, analytical deviations (values outside the proposed ranges) on the acid, saponification, ester value, and ester/acid ratio have also been determined for authentic (non-adulterated) beeswax (Bennett, 1944; Tulloch and Hoffman, 1972; Tulloch, 1973; Serra Bonvehí, 1990; Puleo and Rit, 1992; Bernal et al., 2005; Serra Bonvehí and Orantes Bernejo, 2012; Maia and Nunes, 2013; Svečnjak, et al., 2015, 2019a).

Some other physico-chemical analytical methods have been used for beeswax authentication, namely, determination of beeswax density (specific gravity), peroxide value (Bogdanov, 2004; Bernal et al., 2005) ash content and iodine number (Serra Bonvehí, 1990; Puleo and Rit, 1992; Bernal et al., 2005; Svečnjak et al., 2019a). The use of these methods remains at the research development stage.

Table 5: Ranges of the physico-chemical parameters reported in the literature for authentic (unadulterated) beeswax (analytical deviations/anomalies compared with the proposed range values in Table 4)

Refence in literature	Melting point (°C)	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Ester value (mg KOH/g)	Ester/acid ratio	Peroxide value (mM H_2O_2/kg)	Iodine number
Bennett (1944)	61–65	16–23	85–101	72–79	3.6–4.3		4.0–12.0
Tulloch and Hoffman (1972)	63.4–65.1	17.4–21.8		70.3–75.4	3.38–4.12		
Tulloch (1973)		19.1		73.5	3.84		
Serra Bonvehí (1990)	61.9–64.1	17.4–19.8	90.1–90.8	70.3–79.0	3.54–4.34		9.6–17.3
Puleo and Rit (1992)	61–65	17–24	87–104	70–80			7–12
Bernal et al. (2005)	64–66	17.1–21.9	82.8–147.1	62.7–74.8	3.09–7.08	<0.01	7.6–13.1
Serra Bonvehí and Orantes Bernejo (2012)	61.9–64.1		90.1–98.3				
Maia and Nunes (2013)	63–67.3	14.4–23.0	65.5–124.2				

Svečnjak et al. (2015)	60–65	20.7–30.2	57.5–134.0	31.1–112.2	1.18–5.14		
Svečnjak et al. (2019a)				46.4–103.3	2.3–5.3	0–19.9	5.9–14.3

As reported by Bernal et al. (2005), the minimum amount of the most common beeswax adulterants (i.e. paraffin, stearic acid, tallow and carnauba wax) that can be detected by physico-chemical methods is relatively high and varies from 2% to 50%, depending on the type of adulterant (Table 6).

Table 6: Minimum adulteration percentages detected in beeswax by the measurement of reference physico-chemical parameters (Bernal et al., 2005)

Physico-chemical parameters	Paraffin (54–74°C)	Stearic acid	Tallow	Carnauba wax
Melting point	30–50%	30%	40%	5%
Acid value	10%	2%	10%	20%
Saponification value	10%	3%	15%	*
Ester value	5%	5%	10%	*
Ester/acid ratio	10%	15%	10%	40%
Iodine value	15%	15%	15%	*

***** Not useful for beeswax adulteration detection.

One of the factors that may affect the analytical range values (primarily saponification value, and consequently, ester value and ester/acid ratio) is an exposure of beeswax to a strong heat treatment (Tulloch, 1973). This represents an integral part of the comb foundation production process, given that beeswax used for this is commonly subject to different heat treatments (up to 140°C, most commonly 125–130°C) to kill the spores of the heat-resistant *Paenibacillus larvae*. Thus, deviations in range values can be partially explained by such a heat treatment (>100°C) applied during the recycling and processing of the beeswax (Tulloch, 1973; Svečnjak et al., 2015; Bogdanov, 2016). However, this does not explain the abnormal range values reported for pure beeswax samples collected directly from the beehives (i.e. wild-built combs not built upon comb foundation) (Bernal et al., 2005; Maia and Nunes, 2013; Svečnjak, et al., 2015). Some deviations may also arise from the different geographical origin of the beeswax (Beverly et al., 1995), as well as from minor variations between honey bee colonies (Svečnjak et al., 2015; 2019a).

Analytical deviations of physico-chemical parameters in the available body of scientific literature are not yet fully explained. Finally, as sampling details are often not available in the scientific literature, it is possible that abnormal analytical values are related to questionable sampling and/or origin of the beeswax samples.

Therefore, the deviations of the range values proposed for beeswax purity standards in existing legislation should be considered when performing physico-chemical tests (especially on comb foundations) for authentication purposes (Svečnjak et al., 2019a).

3.1.2. Overview of chromatographic and spectroscopic methods for the detection of adulteration in beeswax

Two modern analytical methods have been developed for the qualitative and quantitative (LoD: <5%) detection of adulterants in beeswax, quantifying paraffin and stearin/stearic acid in beeswax using gas chromatography (GC) with different detectors (mass spectrometry (MS), flame ionisation detection (FID)) and FTIR coupled with attenuated total reflectance (ATR) accessory (the FTIR-ATR technique).

GC-FID and GC-MS have been widely used in the last two decades for the chemical characterisation of beeswax. GC-MS is mainly used for the determination of the relative amounts of beeswax constituents (Jiménez et al., 2003, 2004; Serra Bonvehí and Ornantes Bermejo, 2012; Maia and Nunes, 2013), whereas GC-FID is preferable for the quantification of beeswax constituents (Jiménez et al., 2004, 2007, 2009; Serra Bonvehí and Ornantes Bermejo, 2012; Maia and Nunes, 2013).

3.1.2.1. Gas chromatography-based analytical techniques

Among different GC techniques, two methods have been established for quantifying paraffin and/or stearin/stearic acid in beeswax:

a) Gas chromatography–mass spectrometry

Waś et al. (2014a) described the GC-MS method for determining beeswax hydrocarbons. The method was further developed for the detection of beeswax adulteration with hydrocarbons of foreign origin, i.e. paraffin or ceresin (Waś et al., 2015, 2016). Quantitative analysis of *n*-alkanes is based on squalene used as the internal standard and detects paraffins with a detection limit of 3%. It allows the identification of beeswax hydrocarbons (alkanes, alkenes and dienes) and quantification of *n*-alkanes, but the method is not applicable for the detection of stearin and/or stearic acid.

In the case of beeswax adulterated with paraffin, a GC-MS chromatogram of hydrocarbons clearly shows the amount of alkanes with an odd number of carbon atoms (C_{21} to C_{35} dominate in pure beeswax) *versus* an increase of the peak intensities for the alkanes with even numbers of carbon atoms ($C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$, $C_{30}H_{62}$, $C_{32}H_{66}$, $C_{34}H_{70}$), which is detectable with the addition of only 3% paraffin.

b) Gas chromatography with flame ionisation detection

An analytical method for quantifying beeswax adulterants based on GC–FID has been presented in two studies (Jiménez et al., 2009; Serra Bonvehí and Orantes Bermejo, 2012).

Jiménez et al. (2009) determined the minimum percentage of adulterants in beeswax for three paraffins of different melting points, beef tallow, stearic acid and carnauba wax, using high-temperature gas chromatography (HTGC) with FID. The concentrations of 93 endogenous beeswax constituents (aliphatic hydrocarbons, olefins, acids, monoesters, alcohols and hydroxyacids) were measured in relation to an internal standard (octadecyl octadecanoate) in beeswax mixtures prepared with 2, 5, 10, 20 and 30% adulterants. Adulteration was determined by the decreased or increased concentrations of these endogenous substances (depending on the type of adulterant), with the advantage that the method was applicable to all types of adulterants. The authors concluded that adulteration as low as 1–4% could be detected in this way.

Serra Bonvehí and Orantes Bermejo (2012) determined adulteration with paraffins of different melting points, beef tallow, stearic acid and carnauba wax by the detection of adulterant-specific compounds, using HTGC–FID and HTGC-MS. The method focused on hydrocarbon waxes. The detection limits were determined using pure and adulterated beeswax with varied amounts of adulterants, such as hydrocarbon waxes of different melting points, beef tallow, stearic acid, and carnauba wax at 5%, 10%, 20% and 30%, respectively. The authors reported that percentages higher than 1% for stearic acid and 5% for paraffin could be detected in the beeswax-adulterated mixtures using HTGC–FID/MS.

Maia and Nunes (2013) used HTGC–FID with unsupervised data pattern recognition, i.e. cluster analysis and principal component analysis, to differentiate between authentic and paraffin-adulterated beeswax. However, the described procedure was limited to paraffin detection and involved data handling that is not suitable for routine beeswax adulteration detection.

3.1.2.2. Fourier-transform infrared spectroscopy – attenuated total reflectance

Analysis by FTIR–ATR for beeswax authentication is the most recently developed method. Maia et al. (2013) determined detection limits of around or below 5% for the adulterants hydrocarbon waxes, tallow and stearic acid. Svečnjak at al. (2015) described a method for routine detection of beeswax adulteration with paraffin, beef tallow, stearic acid and carnauba wax with a detection limit of below 3%. Svečnjak et al. (2019a) reported that stearic and palmitic acids, as well as commercially available stearin, exhibit almost equal infrared spectral features and that, therefore, the spectral regions indicative for stearic acid can also be used to detect palmitic acid and stearin in beeswax. The method was further elaborated by Tanner and Lichtenberg-Kraag (2019), showing that adulteration with as many as five adulterants (paraffin, stearic acid, tallow, carnauba wax and candelilla wax) could be determined with the same accuracy as adulteration with a single substance. Based on these results, it is possible to detect beeswax adulteration of less than 3% of these adulterants and their combinations by FTIR-ATR spectroscopy.

Table 7: Detection limits for paraffin and stearic acid (%) using chromatographic and spectroscopic analytical methods

Adulterant	GC–MS	GC–FID	GC–FID/MS	FTIR–ATR
Paraffin	<3%	<4%	<5%	<2.3%
Stearic acid	-	<1%	<1%	<1.2%

The chromatographic and spectroscopic methods currently used for the quantification of paraffin and stearin/stearic acid in beeswax are mostly based on the preparation of in-house reference standards (preparation of beeswax-adulterant mixtures). None of the methods has been validated by collaborative studies or inter-laboratory tests.

3.1.3. Purity criteria and technical specifications for beeswax when used in apiculture and as a food in honey pots (honey comb)

Most of the physico-chemical methods and corresponding purity criteria described for beeswax in existing legislation and the scientific literature have high detection limits for most common adulterants in beeswax, including paraffin and stearin/stearic acid. The minimum percentages of paraffin that can be detected in beeswax using physico-chemical methods range from 5% (ester value) to 50% (melting point), and from 2% (acid value) to 30% (melting point) for stearic acid (Bernal et al., 2005).

According to a recent review of the analytical methods and general recommendations of the Honey Bee Research Association, COLOSS, for the reliable detection of adulterants in beeswax (Svečnjak et al., 2019a), a set of at least three classical physico-chemical measurements should be complemented with chromatographic and/or spectroscopic analysis.

A recent study provides additional validation for the use of a comprehensive set of analytical methods (classic physico-chemical, and as well as advanced chromatographic and spectroscopic methods) as analytical methods for the detection of beeswax adulteration. The relative importance of these methods was assessed by three experts for feasibility and analytical performance in detecting targeted adulterants (paraffin and stearin/stearic acid). Based on the available data sets on the physico-chemical parameters determined for pure vs paraffin-adulterated beeswax, classic analytical methods were further combined and assessed by a receiver operating characteristic analysis. Calibration curves for detecting paraffin in beeswax were also validated. From the overall results of multiple simultaneous statistical tests, the authors recommended that at least two physico-chemical methods should be complemented with advanced chromatographic and/or spectroscopic analytical methods (GC-FID(MS) and/or FTIR-ATR) to ensure reliable detection (and quantification) of paraffin and stearin/stearic acid (and other adulterants) (Svečnjak et al., in press).

Routine purity testing (authenticity control) of beeswax used in apiculture should include the measurement of at least two physico-chemical parameters for screening purposes and be complemented with one or more advanced chromatographic/spectroscopic analyses (GC-MS, (HT)GC-FID/GC-FID/MS or FTIR-ATR method) for reliable detection (LOD<5%) and quantification of adulterants (paraffin, stearin/stearic acid). The selection of the methods would depend on adulterant type. A list of purity criteria proposed for beeswax used in apiculture and as a food (honeycomb) in honey pots is provided in Table 8.

Table 8: Purity criteria for beeswax used in apiculture (crude beeswax and comb foundations) and as a food (honeycomb) in honey pots

Purity criteria	Value	Method
Melting point	61–65°C	*European Pharmacopoeia* (10th Ed., 2020)
Specific gravity	0.950–0.960	*European Pharmacopoeia* (10th Ed., 2020)
Acid value	17–24	*European Pharmacopoeia* (10th Ed., 2020)
Ester value	70–90	*European Pharmacopoeia* (10th Ed., 2020)
Saponification value	87–104	*European Pharmacopoeia* (10th Ed., 2020)

Mechanical impurities	absent	visual inspection
Paraffin	absent	GC-MS, (HT)GC-FID, FTIR-ATR
Stearin/stearic acid	absent	(HT)GC-FID/GC-FID (MS), FTIR-ATR
Other adulterants than paraffin and stearin/stearic acid	absent	GC-MS, (HT)GC-FID/GC-FID (MS), FTIR-ATR

3.2. Hazard identification and characterisation

3.2.1. Hazard identification and characterisation for honey bee health

3.2.1.1. Paraffin adulteration of beeswax

Beeswax adulteration with hydrocarbon wax is a long-standing and growing problem worldwide (Tulloch, 1973; Bogdanov, 2004; Serra Bonvehí and Orantes Bermejo, 2012; Maia et al., 2013; Svečnjak et al., 2015; Waś, et al., 2016). The wide availability of hydrocarbon wax, its low price, and its physico-chemical properties (chemically inert, white or colourless, and odourless substance) makes it ideal for beeswax adulteration (Svečnjak et al., 2015).

Few reports have been published on the prevalence and level of adulteration of beeswax in the EU. A Spanish study investigated the prevalence and the level of adulteration by HTGC–FID/MS (Serra Bonvehí and Orantes Bermejo, 2012). The detection limits were 5% for hydrocarbon waxes and 1% for stearic acid. Hydrocarbon waxes were confirmed in 33 of the 90 samples analysed (37%) at concentrations between 5% and 30%.

Using FTIR–ATR spectroscopy, Svečnjak et al. (2018) checked the authenticity of 137 beeswax samples collected from the international market (15 European countries: 13 EU Member States and two non-EU countries, i.e. Bosnia and Herzegovina, and Serbia). Over 67.2% of the samples from most countries were adulterated with 5–93.5% paraffin, while stearic acid was detected sporadically (18.8–31.3%) and only in samples from Belgium and the Netherlands.

Negative effects of comb foundations adulterated with 50% of paraffin on brood were reported by Wallner (2005). The influence of beeswax comb foundation adulterated with different percentages of paraffin on comb construction, brood rearing and bee colony development was evaluated, but no impact was observed (Semkiw and Skubida, 2013). However, the authors noted that this result does not rule out harmful effects from other paraffins, as the paraffins available on the domestic market differ in chemical compositions and impurities.

3.2.1.2. Stearin/stearic acid adulteration of beeswax

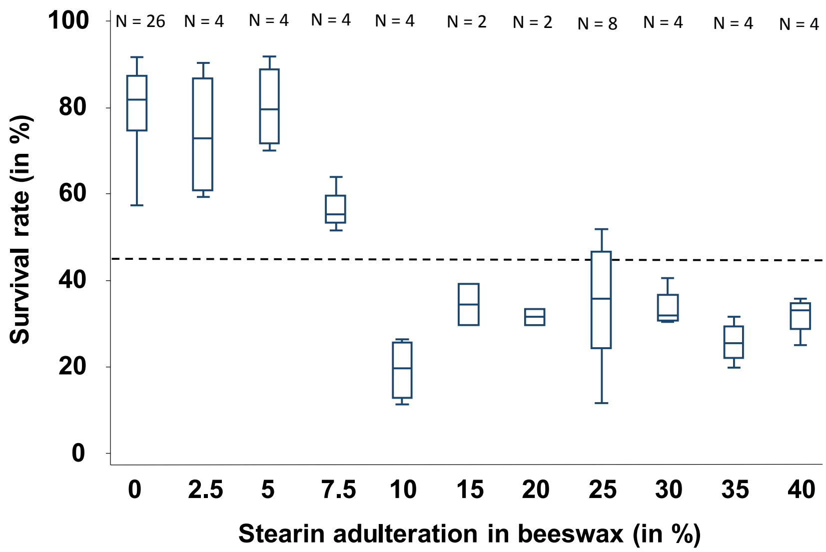
Adulteration by stearin/stearic acid occurs sporadically (Svečnjak et al., 2016, 2018; Reybroeck and van Nevel, 2018).

Recent studies demonstrated that beeswax comb foundation adulterated with stearic and palmitic acids affected brood development (Reybroeck, 2017), where mortality rates above 45% were observed with a minimum of 5% and 7.5% of stearic and palmitic acids, respectively (Fig. 4). Around 80% mortality rates were found with beeswax comb foundation containing 10% of mixtures of added fatty acids. Therefore, it was concluded that beeswax comb foundation made with stearic and palmitic acids was inappropriate for use in apiculture (FASFC, 2018).

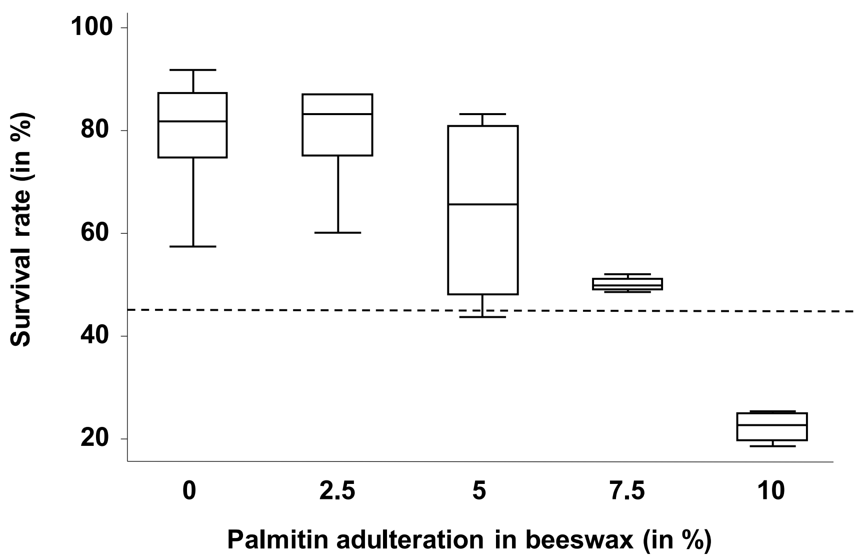
Bernal et al., 2005, reported that among 52 beeswax sheets offered to bees, 27 were rejected by them. Only rejected sheets were analysed and 93% of them were found positive for paraffin.

Figure 4: Brood survival rate in function of the percentage of adulterated beeswax by stearic acid (A) and palmitic acid (B) (Reybroeck Wim, 2019. Raw data used to calculate the data in the tables and figures presented in the reference Reybroeck W and van Nevel J, 2018. Effect of beeswax adulterated with stearin on the development of worker bee brood: results of a field trial. Message to Agnès Rortais, 20 November 2019. E-mail.

A Stearic acid



B Palmitic acid



3.2.2. Hazard identification and characterisation for human health

3.2.2.1. Paraffin as an adulterant in honeycomb

Dietary exposure to hydrocarbon waxes has been assessed by the EFSA CONTAM Panel in its Opinion on MOH in food (EFSA CONTAM Panel, 2012). Alkanes in MOH (MOSH), including those found in paraffin, are absorbed through the gastrointestinal tract. Some are efficiently biotransformed into the corresponding fatty alcohols and subsequently oxidised to fatty acids, but others are only slowly metabolised and still others seem not to be metabolised and are strongly accumulated, maybe for lifetime. Accumulation is mainly in adipose tissue, the lymph nodes, spleen and liver.

The EFSA evaluation from 2012 was based on hepatic microgranulomas associated with inflammation in Fischer 344 rats (EFSA CONTAM Panel, 2012). The NOAEL for the most potent mineral oil product, a low or medium melting point wax, was 19 mg/kg bw per day. It was used as a reference point for calculating MOEs and resulted in the evaluation of MOSH at the present human exposure as a 'potential concern'. However, there are doubts about the pertinence of the findings in Fischer 344 rats for humans (Pirow et al., 2019), but these doubts were substantiated only later by an EFSA project (Cravedi et al., 2017).

Barp et al. (2017a) found that Fischer 344 rats strongly accumulate *n*-alkanes in the range of C_{25}–C_{35}. It was hypothesised by these authors that crystallisation of these *n*-alkanes prevents biotransformation and plays a key role in triggering microgranuloma formation, sometimes with an inflammatory response seen in the hepatic microgranulomas (Barp et al. 2017a, Nygaard et al., 2019).

Only a few *n*-alkanes are detected in human tissues (Barp et al., 2014; Biedermann et al., 2015), indicating efficient metabolism. This means that the effects observed in Fischer 344 rats are not relevant to humans. This was confirmed by Pirow et al. (2019).

Barp et al. (2017b) showed that extrapolation from animal experiments underestimated the accumulation of MOSH in human tissues: the highest concentrations, notably in the spleen, were higher than in the animals exposed to the highest dose. Moreover, strong increases in the spleen weight were observed in rats at concentrations not even reaching those in the most exposed humans (Grob, 2018).

Waxes are made from mineral oil fractions containing substantial amounts of MOAH (typically 15–35%). Even though they cannot be totally considered as wax contaminants, MOAH are discussed in Section 3.3.2.2 as they are not paraffinic compounds.

3.2.2.2. Paraffin contaminants

There is a paucity of information regarding the possible presence of contaminants or toxic substances present in hydrocarbon waxes. Hydrocarbon waxes being produced from petroleum crude oil from different sources and having undergone several refining and purification steps may contain different contaminants.

In its Opinion on MOH in food, the EFSA CONTAM Panel (2012) also assessed hydrocarbon waxes. MOH cover both MOSH (including hydrocarbon waxes) and MOAH. MOAH are considered in the current report as the main contaminants in hydrocarbon waxes. Whereas food-grade MOH products are treated in such a way that the MOAH content is minimised, technical grade MOH typically contain 15–35% MOAH which could be assumed as the worst-case scenario of MOAH concentration in hydrocarbon waxes.

Based on hazard identification data on single substances and MOAH mixtures, the CONTAM Panel could only make qualitative conclusions on the possible hazards of MOAH. In particular, 3–7 ring MOAH with no alkylation or low degree of alkylation, were identified as the components of main concern in view of their genotoxic and carcinogenic nature. MOAH with a high degree of alkylation are not carcinogens, as recently reported by Van de Ven et al. (2018). However, they can act as tumour promoters following initiation with a genotoxic substance in skin-painting studies in mice. Finally, some MOAH with fewer than three rings like naphthalene could still act as carcinogens via non-genotoxic modes of action, involving cytotoxicity and regenerative cell proliferation.

In contrast to MOSH, aromatic hydrocarbons, including PAH, are not known to accumulate in tissues. No critical level could be established for the MOAH fraction of MOH because of their classification as genotoxic carcinogens and the lack of carcinogenicity studies performed on MOAH mixtures (EFSA CONTAM Panel, 2012).

PAH are known to be present in different types of hydrocarbon wax. Whereas pharmaceutical or food-grade highly refined paraffins have a PAH content of below 0.1%, the level of PAH in semi-refined or unrefined paraffins may be up to 1% (Suaria et al., 2018). In 2008, The EFSA CONTAM Panel based the risk assessment of PAH on a MOE approach with BMDL10 values derived from the two coal tar mixtures that were used in the carcinogenicity studies of Culp et al. (1998). A BMDL10 of 0.07 mg/kg bw per day was selected for benzo[a]pyrene as a marker for the carcinogenic PAH in food (Table 9). A BMDL10 of 0.34 mg/kg bw per day was chosen for PAH4 (Table 9) and a BMDL10 of 0.49 mg/kg bw per day was chosen for PAH8 (EFSA CONTAM Panel, 2008a).

There is no specific information on the type of wax used for the adulteration of beeswaxes. It is nevertheless likely that the cheapest and therefore the least refined waxes would mainly be used. Among the waxes commercially available, slack waxes (CAS# 64742-61-6) are poorly refined substances and may contain high PAH levels. According to the European Chemicals Agency, the carcinogenic potential of slack waxes from unknown feedstocks may vary depending on the degree of the refining process of the feedstocks and the resulting polycyclic aromatic compound content in waxes. Under the EU CLP Regulation[18], slack waxes from unknown or from carcinogenic feedstocks are classified as carcinogenic category 1B, H350, unless the base oil from which it is derived is not carcinogenic. In contrast, slack waxes from non-carcinogenic feedstocks are not considered to be carcinogenic and are, therefore, not classified.

3.2.2.3. Stearin as an adulterant in honeycomb

Food-grade stearin is a source of fat consumed in food products and, therefore, not expected to raise safety concerns in humans if consumed as such in beeswax. There is no specific information on the type of stearin used for the adulteration of beeswax, but it is likely that non-food-grade stearin would be used (see Section 1.4.4). However, it seems unlikely, for instance, that heavily contaminated oils will be fractionated to obtain stearin for beeswax. Furthermore, many materials of potential concern as a source of stearin are not easily available on the market.

3.2.2.4. Stearin contaminants

Stearin used for beeswax adulteration may be of plant origin (e.g. palm oil and fat) as well as of animal origin (e.g. tallow and lard). Stearin can be contaminated by lipophilic compounds, such as mineral oil products (Grob et al., 2001), PCBs (Bernard and Fierens, 2002), dioxins or 3-MCPD fatty acid esters.

Some of these contaminants are monitored at the EU level in various food commodities, including palm oil and fat, lard and, to a lesser extent, in tallow. Reference points determined by EFSA on several potential contaminants of stearin sources are summarised in Table 9.

Table 9: Reference points determined by EFSA on several potential contaminants of stearin source

Contaminant	Reference point	Value	Reference
B(a)P	BMDL10	0.07 mg/kg bw/d	EFSA, 2008a
PAH4	BMDL10	0.34 mg/kg bw/d	EFSA, 2008a
Dioxins and DL-PCBs	TWI	2 pg TEQ/kg bw/w	EFSA CONTAM Panel, 2018a
MOSH	NOAEL	19 mg/kg bw/d	EFSA CONTAM Panel, 2012
MOAH	-	-	
3-MCPD + related esters	TDI	2 µg/kg bw/d	EFSA CONTAM Panel, 2018b

3.3. Exposure assessment

3.3.1. Exposure assessment for honey bees

3.3.1.1. Exposures to beeswax

Honey bees are in contact with beeswax during their larval development and afterwards when manipulating and producing beeswax to build cells. Honey bees can be exposed to adulterants contained in beeswax via contact but also via the consumption of food that is stored in beeswax and might be contaminated by the migration of the adulterants from the beeswax to the food (i.e. royal jelly, pollen/beebread and nectar/honey). According to FASFC (2018), beeswax adulterants (paraffin, stearin and palmitin) being mostly lipophilic compounds (this is less the case for stearic acid), their migration

[18] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

is only expected through lipophilic media, which excludes nectar/honey matrices and, therefore, exposure of bees via the consumption of nectar and/or honey. Actually, to the knowledge of the working group, no published study was found on honey contaminated by paraffin, stearin and stearic acid or their contaminants.

Honey bee brood comprises three stages: eggs, larvae and pupae. In terms of exposure to beeswax, only the larval stage is considered because the surface area of the beeswax cell in contact with the egg is reduced whereas the surface area of the beeswax cell in contact with the pupa is unknown. Exposure of larvae to beeswax is considered via the liquid in which the larvae swim during their development. This liquid is called jelly (royal jelly for queen larvae and worker jelly for worker larvae).

The duration of the larval stage as well as the food composition and amounts provided by nurses to queens, workers and drones vary. During their entire development, queen larvae are surrounded by royal jelly (a glandular secretion produced by nurse bees) and feed exclusively on it, whereas worker and drone larvae receive only a limited quantity of royal jelly and feed exclusively on it during the first three days of their life. Then, during the next 2 days (for workers) or 3.5 days (for drones), they receive a mixture of royal jelly with honey and beebread (Haydak, 1943, 1968, 1970; Kunert and Crailsheim, 1988; Malone et al., 2002). It is assumed that worker and drone larvae consume about 30 mg of royal jelly (Nelson, 1924) and a total of 1.5–2 mg of pollen over a period of 5 days for worker and 2.04–2.72 mg of pollen over a period of 6.5 days for drones (EFSA PPR Panel, 2012). EFSA is currently revising these estimates in the process of revising the bee Guidance Document (EFSA, 2013). This work is not yet published, but it could provide new data that could be used to refine these exposure scenarios.

Royal jelly is a proteinaceous substance made from the nurses' hypopharyngeal glands and mandibular glands (Winston, 1987). The lipid content in royal jelly varies among studies, but it is in the range of 3–8% fresh matter (8–19% dry matter) (Sabatini et al., 2009; FASFC, 2018; Yeung and Argüelles, 2019). The composition of the royal jelly provided to queen and worker larvae is similar during the first 3 days of their development and then the lipid content in the worker jelly decreases on the fourth and fifth day whereas the content of the jelly provided to the queen larvae remains constant (Lercker et al., 1984). However, since worker and drone larvae only receive a limited (and unknown) amount of royal jelly after the third day, the total amount of food consumed by the larvae during the feeding period was defined by summing the amount of royal jelly consumed during the first 3 days and the amount of beebread provided after the third day (2 days for workers and 3.5 days for drones) (Table 10). The pollen that is stored in comb cells goes through a microbiological process of fermentation that takes a few days and results in a fermented pollen called beebread. According to the scientific literature, the lipid content of raw beebread varies widely, depending on the plant origin of pollen (Urcan, et al., 2017), but is in the range of 5.9–13.5%, whereas the total lipid content of bee pollen is in the range of 1–10% (Kaplan et al., 2016; Bobis et al., 2017).

Table 10: Amount of royal jelly and beebread (and lipid content) consumed by worker and drone larvae

		Larval development (in days)								
		1	2	3	4	5	6	7		
Larvae	Food type								Food amount (mg)	% lipid in food
Workers	RJ	3 days			2 days				30	3-8
	BB				2 days				1.5–2	5.9–13.5
Drones	RJ	3 days			3.5 days				30	3–8
	BB				3.5 days				2.04–2.72	5.8–13.5

RJ: royal jelly; BB: beebread.
Qualitative assessment for RJ:
High proportion (dark grey)
Low proportion (light grey)

3.3.1.2. Exposure scenarios

In the previous evaluation made by FASFC (2018), three scenarios were considered:

- **Scenario 1**: exposure of worker larvae following their close contact with adulterated beeswax constituting the cells in which they develop;
- **Scenario 2**: exposure of worker larvae via consumption of contaminated food (royal jelly) contained in adulterated beeswax;
- **Scenario 3**: exposure of adult bees via the manipulation of adulterated beeswax when building combs.

The first two scenarios focused on worker larvae (FASFC, 2018) and do not consider drones and queens. Queen larvae are not exposed to adulterated beeswax since they develop in cells that are not in contact with comb foundation (which might be adulterated) and commercially reared queens develop in cups which originate from capping beeswax (Laidlaw and Page, 1997). As for drones, considering the differences in the food composition surrounding, and consumed by, the worker and drone larvae, drones will present a different level of exposure. However, because of the lack of data, scenarios were presented for workers only. When the scenarios are tested, fine-tuned and validated with data, the reasoning can be used to extrapolate to drone larvae.

In the third scenario, the exposure of bees manipulating propolis was included as these bees might be in contact with beeswax too when mixing the propolis to beeswax.

The working group updated the above scenarios by providing new information (when available) and, in addition, a fourth scenario was established to assess exposure of nurses to adulterated beeswax via the consumption of fresh pollen.

Scenario 1: exposure of larvae via contact with beeswax

During the development of the worker larvae, it is assumed that the lipophilic contaminants diffuse gradually from the beeswax to the larvae over the larval feeding period, i.e. over 5 days, making one fifth of the amount of each of the adulterants and their contaminants migrating daily from the wax to the brood (FASFC, 2018)[19]. Knowing that a sheet of embossed wax fixed on a body frame of a simplex type hive measures 34.6 cm by 19.9 cm (= 6.88 dm^2), representing 65 g of wax at the origin and allows the construction of 5,504 cells, or 800 cells per dm^2, this represents **11.8 mg of wax per cell in contact with a worker larva** through the basal area of the cells (= 65 g / 5,504 cells = 0.0118 g) (Wilmart et al., submitted).

To determine the amount of adulterants (paraffin and stearin/stearic acid) and their contaminants that could migrate from the adulterated beeswax to the lipophilic matrices, FASFC (2018) suggested using the Log Po/W value (i.e. the logarithm of the concentration ratio of a chemical in octanol and water; chemicals with high Log Po/w values (e.g. >4) are hydrophobic, i.e. highly lipophilic).

Finally, the use of immobilised artificial membranes (which mimic the surface of a biological membrane) chromatography as a tool for the prediction of ecotoxicity of pesticides and to screen or rank chemicals with respect to their ecotoxicological risk, especially in the case of new chemical entities opens a new avenue that needs further exploration (Stergiopoulos et al., 2019).

Scenario 2: exposure of larvae via consumption of royal jelly and beebread stored in beeswax

FASFC (2018) considered the consumption of the worker larvae over one day with royal jelly (worst-case scenario, i.e. maximum amount consumed in a single day), but larvae are fed over several days (royal jelly, then beebread) and therefore exposure via consumption (both royal jelly and beebread) can be estimated over this period.

Exposure of larvae to paraffin and stearin/stearic acid via food is the amount of these adulterants that could potentially migrate to the larvae's food, being royal jelly and beebread. Given the food amounts (royal jelly and beebread) consumed by worker larvae and the proportion of lipids contained in these matrices (Table 10), it is possible to estimate the exposure of larvae to adulterants through the consumption of the contaminated lipids contained in beebread and royal jelly over the entire feeding period of the larvae:

[19] Migration for pesticides.

- Worker larvae: (30 mg royal jelly × 3–8% lipids = 0.9–2.4 mg lipids) + (1.5–2 mg pollen in beebread × 5.9–13.5% lipids =0.09–0.27 mg) = **0.99–2.67 mg lipids contained in larval food over a period of 5 days**

Scenario 3: exposure of adult bees via the mastication of beeswax

In-hive adult bees masticating beeswax when building cells and when mixing it with propolis might be exposed to adulterants contained in beeswax. Propolis is a resinous product collected by honey bees (*Apis mellifera* L.) from tree exudates, mainly resins of leaf bud mixed with approximately 25–30% beeswax (Tremolada and Vighi, 2014; Anjum et al., 2019) to form a sealing material in their honeycomb, smooth out the internal walls, and protect the entrance against intruders (Greenaway et al., 1990).

As a worst-case scenario, it is assumed that these bees ingest a quantity of adulterants that is equivalent to the amount of adulterants contained in the adulterated beeswax that they masticate (El Agrebi et al., 2019).

- For beeswax-producing bees: for a colony including approximately 50,000 bees and considering that 50% (or 25,000 bees) are foragers and 20% of these 25,000 bees (5,000 bees) develop the ability to produce wax during **7 days** (Winston, 1987). Worker honey bees build three sheets (34.6 cm × 19.9 cm = 6.88 dm^2 each) of wax (initially 65 g per sheet) within a simplex body in 2 days by stretching and incorporating newly produced wax (Winston, 1987). Once built, these three sheets consisting of 800 cells per dm^2, each weighing 0.0232 g will bring the weight of the three wax sheets to 383 g (6.88 dm^2 × 800 × 0.0232 g × 3 = 383 g). This amount of beeswax corresponds to **38.3 mg of masticated wax per bee and per day** (= 383 g/ (5,000 bees × 2 days).
- For bees masticating beeswax with propolis: considering a proportion of 25–30% of **beeswax in the propolis** (Tremolada and Vighi, 2014; Anjum et al., 2019) and the fact that a worker is able to masticate 0.0383 g of wax per day, which corresponds to **9.6–11.5 mg of masticated wax for propolis production per bee and per day** (= 38.3 mg of masticated wax per bee and per day × 0.25 or 0.3).

Scenario 4: exposure of nursing bees via the consumption of fresh pollen stored in beeswax

A nurse bee consumes on average a total of 65 mg of pollen over a period of 10 days of life (Pain and Maugenet, 1966; Crailsheim et al., 1992). As a worst-case scenario, we took into account the maximum consumption level of 12 mg of pollen per day, which can occur within a single day (Pain and Maugenet, 1966; Crailsheim et al., 1992) and the maximum lipid content in pollen. Considering the maximum amount of lipid that can be found in fresh pollen, nurses will consume a maximum of 12 mg × 10% lipids = **1.2 mg lipids contained in fresh pollen per bee and per day**.

3.3.2. Exposure assessment for humans

3.3.2.1. Exposure of the general population to pure beeswax as honeycomb in food

Beeswax is authorised as a food additive in food supplements, glazing and coatings, and in some water-based flavoured drinks in line with Regulation (EC) No 1333/2008[20]. The exposure to beeswax as a food additive (E901) was estimated by the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) (EFSA , 2007). On the basis of conservative assumptions, the Panel calculated a range from about 350 mg to 1,290 mg/person per day, i.e. up to 22 mg/kg bw/day (60 kg individual).

Although beeswax is not expected to be consumed as such by the general population, it is possible that certain users may ingest it in small amounts when consuming honey or comb honey. However, consumption data are not available. As reported by Hargrove et al. (2004), beeswax intake may average 4 g per day in certain populations. In the absence of any other information on beeswax consumption, the working group considered this value to be a worst-case scenario.

[20] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008 p. 16–33.

3.3.2.2. Exposure of the general population to beeswax adulterated with paraffin and/or stearin/stearic acid as honeycomb in food

Paraffin in food products

Cuticle waxes of fruits, such as apples and olives, contain considerable quantities of alkanes, mainly C_{27}, C_{29}, C_{31} and C_{33}. For instance, a kilogramme of olives or unpeeled apples provides up to 20 mg of *n*-alkanes (Salvayre et al., 1988; Belding et al.,1998; Pineda et al., 2017).

The CONTAM Panel estimated the dietary exposure to MOSH in the European general population to be between approximately 0.03 and 0.3 mg/kg bw per day and was higher in younger consumers than in adults and the elderly. The highest exposure estimate was for high consumers among children aged 3 to 10 years (P95 ranging from 0.14 to 0.32 mg/kg bw/day) (EFSA CONTAM Panel, 2012). Based on data collected in the period 1999–2009, the ANS Panel estimated the dietary exposure to high viscosity and medium viscosity mineral oils intended for use as food additives (EFSA ANS Panel, 2009, 2013b). For adults, the mean exposure to these saturated hydrocarbons was between 0.9 and 1.8 mg/kg bw/day and the highest exposure estimate was for toddlers (P95 ranging from 6 to 10.1 mg/kg bw/day).

Important differences are noted between mineral oils and hydrocarbon waxes, since *n*-alkanes are the main component of wax, whereas they usually represent a minor fraction in oils. No data specifically related to hydrocarbon wax were reported by EFSA. Tennant (2004) estimated that the total intake of hydrocarbon waxes in the EU ranged from 0.08 to 0.19 mg/kg bw/day for adults and from 0.23 to 0.64 mg/kg bw/day for pre-school children; whereas for oils the ranges were from 0.39 to 0.91 mg/kg bw/day for adults and from 0.75 to 1.77 mg/kg bw/day for children.

Although no reliable information exists regarding the consumption of honeycomb in the EU, the contribution of the consumption of adulterated beeswax to the dietary intake of hydrocarbon waxes can be based on the worst-case scenario described in Section 3.3.2.1, i.e. 4 g per day, corresponding to 57 mg/kg bw/day (for a default bw of 70 kg).

This scenario will consider the level of 94% paraffin reported for the adulteration of comb foundation by Svečnjak et al., 2018. However, because honey bees always add new amounts of virgin beeswax on foundation during their in-hive comb construction, the final level of adulterant in honeycomb can be reduced significantly. In an experiment based on comb foundation adulterated with 90% paraffin wax, Svečnjak et al. (2015) found that the final residual amount of paraffin in honeycomb when honey bees finished comb construction ranged from 47 to 67%, corresponding to a 1.3–1.9-fold decrease in the adulterant concentration in the final product. As a worst-case scenario, a 1.3-fold reduction of the paraffin level in honeycomb compared with comb foundation will apply, resulting in a paraffin exposure due to adulterated beeswax consumption of 2.9 g per day, corresponding to 41.4 mg/kg bw/day for a 70 kg individual.

Stearin in food products

Consumption of stearin and its fatty acid components due to adulteration will be very low (less than 1 g per day even considering a maximum consumption of 4 g) which is negligible compared with the daily consumption of stearin in fats in the diet.

3.3.2.3. Exposure of the general population to beeswax adulterated with paraffin and/or stearin/stearic acid contaminated with different levels of impurities – worst-case scenario (ingestion of beeswax)

Human exposure to contaminants present in hydrocarbon wax

The levels of MOAH and PAH in hydrocarbon waxes that could be used as adulterants strongly depend on the source and the refining process. Lachenmeier et al., (2017) found between 0.01 and 1.10% MOAH in refined mineral oil products used as raw material in cosmetics; the BfR found 1–5% MOAH in wax and vaseline used in cosmetics (BfR, 2018). These MOAH nearly exclusively consisted of hydrocarbons with 1–2 aromatic rings, i.e. of those not considered carcinogenic. No data were found on less refined waxes. However, crystallisation tends to discriminate the MOAH, and to be a wax (solid at temperatures up to somewhat above ambient temperature) the percentage of isoalkanes and MOAH must be low.

The fraction including the carcinogenic MOAH is low. Using a method based on adsorption chromatography, paper chromatography and spectrofluorometric analysis, Shubik et al., (1962) analysed 36 samples of petroleum waxes. No non-alkylated benzo(a)pyrene was detected in any wax, but pyrene, chrysene, benz(a)anthracene and fluoranthene were found in at least three waxes and benzo(e)pyrene in two waxes (LOD: approximately 10 µg/kg). It is noted, however, that, for example, the benzopyrenes in MOAH are more than 97% alkylated (Grob et al., 1991). Concentrations of total non-alkylated polyaromatic hydrocarbons of up to 670 µg/kg were found in two wax samples.

Based on elution chromatography, thin layer chromatography and subsequent spectrofluorometry detection, Monarca et al. (1981) determined the non-alkylated polyaromatic hydrocarbons in several hydrocarbon waxes, among which were two white soft paraffins of Italian pharmaceutical grade. Total content ranged from 6.1 to 82.6 µg/kg, benzo(a)pyrene from 0.9 to 11.6 µg/kg; fluoranthene was the most abundant compound. Lau et al. (1997) determined the total concentration of non-alkylated polyaromatic hydrocarbons (sum of the 16 indicators according to the EPA list N°610, 1984 (EPA, online)) in the hydrocarbon wax used by candle manufacturers in Germany. They reported a concentration of 462 µg/kg, with naphthalene being the most abundant compound. More recently, Conchione et al. (2015) measured the concentration of benzo(a)pyrene in 10 microcrystalline waxes used as food additives by solid-phase microextraction with GC-MS. Most of the samples had benzo(a)pyrene amounts below the limit of quantitation (7 µg/kg), except one sample (29.9 µg/kg). In the EFSA Scientific Opinion on the re-evaluation of microcrystalline wax (E905) as a food additive, concentrations of total non-alkylated polyaromatic hydrocarbons ranging from 0.8 to 4.9 µg/kg were reported (EFSA ANS Panel, 2013a). Of note, the chemical specifications for microcrystalline wax according to Commission Regulation (EU) No 231/2012 and JECFA (2000) indicate that the benzo(a)pyrene concentration should be not more than 50 µg/kg. It is noted that these determinations did not include the alkylated species, which strongly predominate in the MOAH (Grob et al., 1991; EFSA CONTAM Panel, 2012).

As a worst-case scenario, it can be considered that 94% of beeswax can be replaced by paraffin wax in comb foundation, corresponding to 72% in honeycomb, giving a concentration of MOAH in adulterated beeswax higher than 4% (according to data published by BfR, 2018). On the basis of the highest average consumption values reported by Hargrove et al. (2004) for beeswax (4 g per day), it can be estimated that the consumption of adulterated beeswax would result in an exposure to more than 160 mg MOAH per day. The exposure to the sum of PAH could be estimated based on the concentration of 462 µg/kg reported by Lau et al. (1997), corresponding to an exposure of 1.3 µg per day and may contribute substantially to the exposure to PAH of fossil origin for a part of the European population.

Human exposure to contaminants present in food stearin sources

Stearin may be of plant origin (e.g. from palm oil/fat) as well as of animal origin (e.g. from tallow and lard). From the food sector, related food categories can be contaminated by lipophilic compounds, such as hydrocarbons, PCB, dioxins, or glycidyl esters. These contaminants are in general monitored at the EU level in various food commodities, including palm oil and fat, lard, and to a lesser extent in tallow, and therefore they should not be of concern.

The dietary exposure to PAH across European countries was calculated both for mean and high consumers and varied between 1,168 ng/day (19.5 ng/kg bw per day) and 2,068 ng/day (34.5 ng/kg bw per day), respectively, for PAH4 (EFSA, 2008a). Lau et al. (1997) found a total PAH content (sum of the 16 indicators according to the Environmental Protection Agency (EPA) list N°610, 1984) of 205 µg/kg in stearin used by candle manufacturers in Germany. As a worst-case scenario, it is considered that beeswax intake may average 4 g per day in certain populations (Hargrove et al., 2004), corresponding to 0.95 g of stearin for the consumption of beeswax originating from adulterated honeycomb containing 31% stearin. A calculation based on the values reported by Lau et al. (1997) for PAH content in stearin used by candle manufacturers would result in an exposure estimate of 0.19 µg per day.

MOSH and MOAH were measured in a limited number of palm oil samples (n = 5 for MOSH and n = 4 for MOAH) with a maximum value of 9.6 and 25.2 mg/kg fat (EFSA occurrence database (EFSA, online)), respectively. An intake of 0.95 g of stearin per day, as a worst-case scenario, would result in maximum dietary exposure to 9 µg MOSH per day and 24 µg MOAH per day.

Tallow is the food category with the highest levels of dioxins and PCB. Adulteration of beeswax with stearin from tallow (assuming an intake of 0.95 g) at the highest reported level of 1.8 pg WHO_{2005}-TEQ/g[21] (EFSA, 2018a) would result in a dietary exposure of 1.7 pg per day.

2- and 3-MCPD fatty acid esters and glycidyl fatty acid esters form during the processing of fats and oils. The highest concentrations of 3-MCPD fatty acid esters in palm oil and palm kernel oil were 2,912 µg/kg and 624 µg/kg (as 3-MCPD equivalents), respectively. Glycidyl fatty acid ester concentrations were 3,955 and 428 µg/kg (as 3-MCPD equivalents) in palm oil/fat and palm kernel oil, respectively (EFSA CONTAM Panel, 2016). Palm oil/fat is the food category with the highest levels of 3-MCPD fatty acid esters and glycidyl fatty acid esters. On the basis of the P95 values, dietary exposures to 3-MCPDs and glycidol can be estimated at 2.8 µg per day and 3.7 µg per day, respectively, from an adulterated beeswax intake of 0.95 g.

Stearin from plant, and to a lesser extent of animal, origin may also contain traces of lipophilic mycotoxins such as aflatoxin, deoxynivalenol, T2 + HT2, zearalenone and ochratoxin. Previous EFSA Opinions reported mycotoxin concentrations in animal and vegetable fats and oils, but not specifically in palm oil and palm fat, tallow and lard (EFSA CONTAM Panel 2011; 2017; 2020). The highest levels were found for zearalenone. Due to the very limited data on mycotoxin occurrence in stearin sources such as palm oil, palm fat, lard and tallow, it is not possible to estimate the exposure to these contaminants from adulterated beeswax consumption.

3.4. Risk assessment

3.4.1. Risk assessment for honey bee health

The effect of paraffin adulteration of beeswax comb foundations on brood is controversial (Wallner, 2005; Semkiw and Skubida, 2013).However, the effect of beeswax adulterated by stearin, stearic and palmitic acids show effects on brood development (Aupinel, 2018; Reybroeck, 2017 and 2018).

The adulteration of beeswax with 7.5% of stearin or by 5% of palmitin induces a mortality rate of bee brood of above 45% (Reybroeck, 2017 and 2018). Higher percentages of adulteration have been reported in beeswax samples from the EU market (Svečnjak et al., 2018).

Currently, no data on the occurrence and concentration of contaminants in the adulterants are available.

3.4.2. Risk assessment for human health

It must be noted that the fraction of the population consuming beeswax is probably very small. In this assessment and in order to characterise the risk, a daily consumption of 4 g of beeswax has been assumed as a worst-case scenario (Hargrove et al., 2004) while the maximum adulteration of beeswax with paraffin and/or stearin/stearic acid has been found to be up to 94% and 31%, respectively (Svečnjak et al., 2018). However, because the residual level of adulterant in honeycomb can be substantially lower than in comb foundation (see Section 3.3.2.2), the values of 2.88 and 0.95 g per day were retained as the worst-case scenario for dietary exposure to paraffin and stearin as adulterants.

The consumption of stearin as such is not expected to raise safety concerns in humans.

The consumption of beeswax adulterated by paraffin and to a much lesser extent by MOSH- and MOAH-contaminated stearin would result in an increased exposure to MOSH and MOAH. In its previous Opinion on MOH in food, the EFSA CONTAM Panel (2012) considered that there is a ‘potential concern’ associated with the current exposure to MOSH in the EU. It was also concluded that it is not possible to characterise the hazards related to MOAH exposure in the absence of relevant dose–response data, but considering the potential carcinogenic risk, the EFSA CONTAM Panel estimated the dietary exposure to MOAH with three or more, non- or simple-alkylated, aromatic rings to be of ‘potential concern’ (EFSA CONTAM Panel, 2012). Consequently, a substantial contribution to MOSH and MOAH exposure resulting from the consumption of adulterated beeswax could be considered as a potential health concern.

The CONTAM Panel noted, however, that the evaluation of the MOSH in the Opinion from 2012 needs revision in light of the new data suggesting that the findings on the low and medium boiling point wax in Fischer 344 rats, on which the concern was based, may not be relevant for humans, while other

[21] WHO: World Health Organization; TEQ: toxic equivalents

potential end points have not been adequately investigated (Cravedi et al., 2017). The MOAH present in hydrocarbon waxes have low concentrations and almost exclusively consist of components with 1–2 aromatic rings.

The highest values of contaminants such as PAH, dioxins and dioxin-like PCB and 3-MCPDs measured in stearin food sources (e.g. palm oil or tallow) will lead to a negligible exposure when compared with the reference points and health-based guidance values identified in the relevant EFSA evaluations, even at the worst-case scenario of exposure considered. However, the consumption of beeswax adulterated with stearin might slightly contribute to the overall exposure to PAH, dioxins and dioxin-like PCB for which exposure has been considered to be a concern in previous EFSA Opinions.

As the grade of paraffin and stearin used for the adulteration is unknown due to a lack of data, the health risks from the adulteration of beeswax cannot be fully assessed.

No publications were identified concerning the potential transfer of paraffins from honeycomb to honey. However, because of their hydrophobic nature, paraffin waxes are not expected to migrate into non-fatty foods such as honey. The EFSA occurrence data of MOSH in honey confirm the absence of saturated hydrocarbons including paraffins.

4. Conclusions and recommendations

4.1. Conclusions

The following conclusions were made regarding the three terms of reference (ToR) as interpreted by EFSA:

- **ToR1: Establishment of purity criteria and technical specifications for beeswax when used in apiculture and as a food in honey pots (honeycomb);**

  Purity criteria for beeswax used in apiculture (crude beeswax and comb foundations) and as a food (honeycomb) in honey pots are proposed. Physico-chemical testing conducted with classical analytical methods does not guarantee the purity of beeswax and cannot be used alone for the detection of adulteration. Purity testing of beeswax used in apiculture should include the measurement of at least two physico-chemical parameters for screening purposes, complemented with one or more chromatographic or spectroscopic analyses, such as HTGC-FID, HTGC- MS or FTIR-ATR, for a sensitive (LoD <5%) and reliable detection and quantification of adulterants (paraffin, stearin/stearic acid).

- **ToR2: Evaluation of the possible health concerns for honey bees due to their exposure to adulterated beeswax and to other bee products contaminated with constituents of adulterated beeswax;**

  To assess all possible bee health impact of beeswax adulteration, exposure scenarios were presented however, toxicological data for different adulteration levels and endpoints comprising the testing of acute, chronic and sublethal toxicity are not available. This information is necessary to comprehensively assess the risks to honey bees from exposure to adulterated beeswax and their contaminants. A few studies tested the effect of hydrocarbon waxes, stearic and palmitic acids on bees. These studies show impacts on brood (mortality rates from 45% up to 80%) beeswax adulterated with 50% of hydrocarbon waxes, 5% of stearic acid, 7.5% of palmitic acid and 10% for mixture of fatty acids.

- **ToR3: Evaluation of the possible health concerns for humans due to the consumption of honey contaminated with constituents of adulterated beeswax or due to consumption of honeycombs contained in honey pots**

  In humans, the Working Group considers the exposure to waxes (largely consisting of n-alkanes and containing hardly any aromatic compounds with more than two aromatic rings) are of low concern. The consumption of beeswax adulterated by paraffin would result in an increased exposure to certain contaminants for which a potential concern has been already identified. Exposure to food-grade stearin and its contaminants would not be of concern, although the latter might slightly contribute to the overall exposure to some contaminants such as PAHs, dioxins and dioxin like PCBs. Beeswax adulterants and their contaminants are lipophilic, they are not expected to migrate to honey.

4.2. Recommendations

When conducting the assessment presented in this report, the working group identified several areas where information and data are missing (e.g. analytical methods, hazard identification and characterisation and exposure). For each of those areas, the working group made a list of recommendations with specific developments that could fill the gaps and support more evidence-based risk assessment of beeswax adulterants and their contaminants on honey bee and human health.

Analytical methods to detect and quantify adulterants and their contaminants in beeswax

- improve, combine and implement analytical tools and methodologies and standardise protocols (e.g. with specific LoD/LoQ, chemical analysis methods, sampling size);
- establish an open-access database on beeswax adulteration cases including availability of reference material/standard samples.

Hazard identification and characterisation related to adulterants and their contaminants in beeswax

- identify the origin, types, composition and levels of adulterants (paraffin, stearin and other adulterants such as carnauba wax) and their contaminants (e.g. PAH);
- determine the contamination pathways of adulterants during the beeswax recycling processes (from beekeeping production to commercial processing and marketing);
- determine mortality and other harmful effects on honey bees, including behavioural disorders, from exposure to low doses of adulterants, in order to generate toxicological reference endpoints (LD_{50} for oral, contact, chronic and acute exposures for larvae and adults).

Exposure assessment for honey bees and humans to adulterants and their contaminants in beeswax

Honey bees:

- develop research (laboratory and field trials) to assess external (contact through cuticle) and internal (oral through crop and gut) exposures in adults and larvae;
- develop methods and collect data on the migration of adulterants and their contaminants from beeswax towards bees and matrices in contact with bees (honey, nectar, pollen, beebread, propolis, royal jelly).

Humans:

- collect consumption data on beeswax as food (honeycomb) in the EU;
- collect data on migration of adulterants and their contaminants from beeswax to bee products (honey, royal jelly, beebread and propolis).

Additionally, EFSA recommends increased monitoring of production, import and use while ensuring traceability (e.g. including serial or lot/ batch number) of beeswax used in apiculture and for food (as honeycomb. Development of Good Manufacturing Practices for beeswax producers is also recommended.

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Abbreviations

ABP	animal by-product
ADI	acceptable daily intake
ATR	Attenuated total reflectance
EPA	Environmental Protection Agency from the United States
FID	flame ionisation detector
FTIR	Fourier-transform infrared spectrometers
GC	gas chromatography
GC–MS	Gas chromatography with mass spectrometry
GC–FID	Gas chromatography with flame-ionisation detection
HTGC–FID	High-temperature chromatography with flame ionisation detection
IHC	International Honey Commission
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MOAH	mineral oil aromatic hydrocarbons
MOH	mineral oil hydrocarbons
MOSH	mineral oil saturated hydrocarbons
MS	mass spectrometry
PAH	poly aromatic hydrocarbons
PCB	polychlorinated biphenyls
NOAEL	no-observed-adverse-effect level
RASFF	Rapid Alert System for Food and Feed
SFC	Scientific Committee on Food
SML	specific migration limit
TEQ	toxic equivalents
WHO	World Health Organization

Appendix A – RASFF news number 17-844

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Directorate G: Crisis management in food, animals and plants
Unit DDG2.G5.: Alert, traceability and Committees

RASFF

Brussels, 10 October, 2017

RASFF
NEWS

FOOD
INFORMATION EXCHANGE

NEWS: 17-844
ORIGINAL NOTIFICATION

SUBJECT: PARAFFIN IN BEESWAX FROM CHINA AND UKRAINE, VIA BELGIUM

PAGES: COVERPAGE(1)+5+2 file(s) attached

EMAIL: sante-rasff@ec.europa.eu

official control on the market - distribution to other member countries - informing authorities
Product distributed to Germany and Spain
notification flags: BE /CN O/CS N/DE D/ES D/UA O

The contact point from Commission Services has communicated to the Commission the following information:

RASFF

food - news - official control on the market - beeswax - European Commission
Notification Link https://webgate.ec.europa.eu/sanco_rasff/notification/view/340716

General Information

Notification number:	340716
Reference:	17-844
Notification type:	food
Notification basis:	official control on the market
Notification classification:	news
Product name:	beeswax
Product category:	honey and royal jelly
Notifying country:	European Commission
Notifying Country region:	
CP Reference:	
Date of notification:	10/10/2017
Infosan Informed:	No
eCommerce Related:	No
Notification status:	EC VALIDATED 10/10/2017 11:05:03
Follow-up status:	
Number of follow-ups:	0

Distribution Status

distribution to other member countries

Countries notified for the notification and associated follow-up

For follow-up: Belgium, Germany, Spain

Countries notified for the notification and associated follow-up

For attention:

Risk

Serious risk: Undecided

Impact on: human health

Motivate serious risk:

Number of people affected:

Illness / patient's symptoms:

Other hazards: Contaminates found are paraffin (between 1.7% to 5.8% with one sample of 52%) and stearin (up to 25%; suspicious samples showed 10 times higher concentration of stearic acid then Belgian wax samples).

Products

Product name: beeswax

Product category: honey and royal jelly

Product description

Product name on label: beeswax comb foundation

Brand/trade name:

Product aspect:

Barcode no.:

Other labelling:

Weight:

Temperature:

Traceability

Distribution status: distribution to other member countries

RASFF
Rapid Alert System for Food and Feed

Consignment

Consignment / lot number:	operators and lot numbers are confidential, under investigation
Origin:	China
Public health certificate number:	
Public health certificate date:	
CVED number:	
Other document:	
Number:	
Durability date:	
Description of the lot no. of units:	
Description of the lot total net weight:	

Consignment / lot number:	operators and lot numbers are confidential, under investigation
Origin:	Ukraine
Public health certificate number:	
Public health certificate date:	
CVED number:	
Other document:	
Number:	
Durability date:	
Description of the lot no. of units:	
Description of the lot total net weight:	

Related products

None

RASFF
Rapid Alert System for Food and Feed

Additional information

Date of last change of status:	
#340716 - ec validated - European Commission	
CP Reference:	
Organisation / ministry:	
Contact person:	Sante-food-fraud@ec.europa.eu
Additional information:	In June 2017 Belgian authorities informed German and Spanish authorities about contamination of the beeswax sent to Spain and Germany from a Belgian company [redacted]. Adulterated beeswax comes from China and from Ukraine. Contaminates found are paraffin (between 1.7% to 5.8% with one sample of 52%) and stearin (up to 25%; suspicious samples showed 10 times higher concentration of stearic acid then Belgian wax samples). On 03.07.2017 the case was transmitted in Food Fraud System ([redacted]) with request to Commission's services for coordination. There is a potential risk of adulterated beeswax entering the food chain in the form of honey combs. Companies are offering more often honeycomb instead of honey in jar to demonstrate the authenticity of the product. Contaminated wax sheets in those cases are integrated in the honey comb and can be potentially eaten by the consumers as indicated on the products label.

Notification reference: 17-844 (340716)
Document reference: 340716_1507626344156
10/10/2017 11:05:44
Page 4 of 5

Appendix B – Analytical methods to detect and quantify admixtures of paraffin and/or stearin to beeswax (Joint Research Centre)

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumer & Reference Materials (Geel)
Fraud Detection & Prevention

Geel, 28 August 2017

Analytical methods to detect and quantify admixtures of paraffin and/or stearin to beeswax

Background: *Beeswax is a rather expensive natural product; it can be fraudulently extended for economic gain with products having similar physico-chemical properties such as paraffin, stearin or hard fats.*

Results: *Identity and purity characteristics of beeswax and their assessment are described in various pharmacopoeias and in EU legislation. Those methods are fit-for-purpose for detecting gross adulteration and/or screening. Modern chromatographic methods exist to detect adulterated beeswax, with reported limits of detection of 1-4 %, depending on the nature of the added adulterant.*

Conclusion and recommendation: *For official control the harmonisation of an analytical method based on gas-liquid chromatography with flame-ionisation or mass spectrometric detection is needed as well as the establishment of purity criteria for beeswax applying the agreed analytical approach.*

Background

Beeswax is an authorised food additive (E 901) in the European Union, permitted in edible ices and as a glazing agent on confectionery (excluding chocolate), small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans and for the surface treatment only of certain fruits (fresh citrus fruits, melons, apples, pears, peaches and pineapples). It is also permitted in food supplements and as a carrier for colours[1].

[1] Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) on a request from the Commission on the safety in use of beeswax. The EFSA Journal (2007) 615, 1-28.

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Beeswax is obtained from the honeycombs of bees (*Apis mellifera* L) after removal of the honey. The combs are melted with hot water, steam, or solar heat. After removing the insoluble impurities, the liquid wax is cast into cakes for further purification to obtain food-grade yellow beeswax. Bleaching the latter with e.g. hydrogen peroxide, acids, sunlight, or bleaching earth yields white beeswax.

Beeswax consists primarily of five main groups of components, namely[2]:

1. Free fatty acids (typically 12-14%), most of which are saturated (ca. 85%) and have a chain length of C24-C32.

2. Free primary fatty alcohols (ca. 1%) with a chain length of C28-C35.

3. Linear wax monoesters and hydroxymonoesters (35-45%) with chain lengths generally of C40-C48. The esters are derived almost exclusively from palmitic acid, 15-hydroxypalmitic acid, and oleic acid. The variation in total chain length of the esters is mainly the result of the different chain lengths of the alcohol moiety (C24-C34).

4. Complex wax esters (15-27%) containing 15-hydroxypalmitic acid or diols, which, through their hydroxyl group, are linked to another fatty-acid molecule. In addition to such diesters, tri- and higher esters are also found.

5. Odd-numbered, straight chain hydrocarbons (12-16%) with a predominant chain length of C27-C33. With increasing chain length, the proportion of unsaturated species increases (above C33 only unsaturated species are present) and alkadienes and -trienes have been reported at only very low levels.

The composition of beeswax depends to some extent on the subspecies of the bees, the age of the wax, and the climatic circumstances of its production. However, this variation in composition occurs mainly in the relative amounts of the different components present, rather than in their chemical identity[3].

Identity and purity criteria for beeswax are laid down in several national pharmacopoeias as well as in the European Pharmacopoeia. Provisions for use as a food additive are given in Commission Regulation (EU) No 231/2012:

Melting range	Between 62 °C and 65 °C
Specific gravity	About 0,96
Solubility	Insoluble in water, sparingly soluble in alcohol, very soluble in chloroform and ether
Acid value	17-24
Saponification value	87-104

[2] Beeswax - Chemical and Technical Assessment 65th JECFA (http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/65/beeswax.pdf)

[3] R. Aichholz, E. Lorbeer. Journal of Chromatography A, 855 (1999) 601–615

2

Peroxide value	Not more than 5
Glycerol and other polyols	Not more than 0,5 % (as glycerol)
Ceresin, paraffins and certain other waxes	Transfer 3,0 g of the sample to a 100 ml round-bottomed flask, add 30 ml of a 4% w/v solution of potassium hydroxide in aldehyde-free ethanol and boil gently under a reflux condenser for 2 h. Remove the condenser and immediately insert a thermometer. Place the flask in water at 80 °C and allow to cool, swirling the solution continuously. No precipitate is formed before the temperature reaches 65 °C, although the solution may be opalescent.
Fats, Japan wax, rosin and soaps	Boil 1 g of the sample for 30 min with 35 ml of a 1 in 7 solution of sodium hydroxide, maintaining the volume by the occasional addition of water, and cool the mixture. The wax separates and the liquid remains clear. Filter the cold mixture and acidify the filtrate with hydrochloric acid. No precipitate is formed.

The International Honey Commission (IHC) has proposed the following criteria[4]:

Quality Criteria	*Value*	*Method*
Water content	< 1%	DGF-M-V-2*
Refractive index (at 75 °C)	1.4398-1.4451	Ph. Eur.**
Melting point	Melting point 61-65 °C	Ph. Eur.
Acid Number	17-22	Ph. Eur.
Ester Number	70-90	Ph. Eur.
Ester/Acid ratio	3.3-4.3	
Saponification Number	87-102 EP	Ph. Eur.
Mechanical impurities, additives	absent	DGF-M-V-3
Glycerols, polyols, fatty acids fats	absent	Ph. Eur.
Hydrocarbons	14.5 % for European	DGF-M-V-6

[4] http://www.bee-hexagon.net/files/file/fileE/Wax/WaxBook1.pdf

3

	beeswax 13.8 % for African and Asian beeswax	

* Methods of Deutsche Gesellschaft für Fettwissenschaft (DGF)
** European Pharmacopoeia

Beeswax is a comparatively expensive product and attempts exist to replace it partially with similar but cheaper products. Since the physical properties of paraffin and hard fats such as (hydrogenated) tallow and derivatives obtained from tallow (stearin) are close to beeswax, an incentive exist to use them as extenders to gain a financial advantage.

Question asked by DG SANTE to DG JRC

DG SANTE requested DG JRC to assess which analytical methods can be suggest to Member States to detect and quantify the amount of paraffin and stearin added to beeswax.

Analytical methods to detect and quantify paraffin and stearin in beeswax

Based on identity and purity criteria

The identity and purity criteria given in Commission Regulation (EU) No 231/2012 can be used for assessing purity of beeswax; quantification is not possible. Using those wet chemistry methods and the limits given in national pharmacopoeias, addition of as little as 2 % stearic acid and 5 % of paraffin with different melting ranges, tallow and carnauba wax was possible[5]. However, it appears that the lower limit of detection for the different adulterants was determined using only a restricted number of beeswax and adulterant samples, and may, therefore, not be generally applicable. Therefore, those compendial tests, though simple to carry out, have to be complemented by using more advanced analytical methods for confirmation of beeswax purity.

Based on infra-red spectroscopy

Infra-red spectrometry in combination with advanced data analysis has recently been introduced for purity control of beeswax[6,7]. The technique has the advantage of being rapid and, after appropriate calibration, quantitative and very well suited for routine control of beeswax purity. Limits of detection for commonly employed adulterants such as paraffin, microcrystalline wax, tallow and stearic acid were in the range of 0.5 to 5.0 %. However, it requires first a collection of reference spectra from authentic beeswax, and second, spectra of mixtures of different samples of beeswax and the targeted adulterants over the desired working range of the method. Unfortunately, spectra cannot easily be shared between different instruments; therefore, analysts wishing to use the method need to obtain authentic samples, prepare the required mixture, record the reference spectra, and develop the required statistical models for making

[5] J.L. Bernal, J.J. Jiménez, M.J. del Nozal, L. Toribio, M.T. Martín. Eur. J. Lipid Sci. Technol. 107 (2005) 158–166

[6] M. Maia, A.I.R.N.A. Barros, F.M. Nunes. Talanta 107 (2013)74–80

[7] L. Svečnjak, G. Baranović, M. Vinceković, S. Prđun, D. Bubalo, I. Tlak Gajger. J. Apic. Sci 59 (2015) 37–49

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predictions, before being able to start the actual work. This may limit the wider applicability of the technique for screening purposes in different laboratories unless a user consortium is formed for sharing samples, spectra and predictive models.

Based on chromatography

Gas-liquid chromatography (GLC) either with a flame-ionisation detector (FID) or with mass spectrometric (MS) detection is a useful tool to characterise the composition of beeswax and to determine its purity.

Researchers have used several sample preparation methods depending on the group of compounds (esters, fatty acids, hydrocarbons) they have used as purity markers, and different methods for quantifying the separated compounds (peak area normalisation, use of internal standard). Furthermore, in some publications only beeswax from a certain geographical region and/or a limited number of samples of different provenance has been used. For this reason published composition data for beeswax are difficult to compare.

Common to all methods is the principle that addition of paraffin, which is exclusively composed of hydrocarbons, will increase the amount of hydrocarbons and concomitantly decrease the relative proportion of wax esters and free fatty acids of the adulterated beeswax[8]. Genuine beeswax contains mostly odd-numbered hydrocarbons (more than 90 % of total hydrocarbons), whereas paraffins contain even- and odd-numbered hydrocarbons in roughly equal proportions. Addition of paraffin will, therefore, change the ratio of even- and odd-numbered hydrocarbons in beeswax extended with paraffin[9], in addition to an increase in the total amount of hydrocarbons.

Addition of stearin or hard fats (hydrogenated tallow or palm fat) can also be detected by GLC[10].

<u>Conclusions and recommendations</u>

1. The identity and purity criteria for beeswax listed in pharmacopoeias as well as in EU legislation are sufficient to detect gross adulteration with paraffin and stearin.
2. The compendial methods are simple but laborious.
3. They can be used for screening, but application of a confirmatory method is advisable.
4. Methods based on gas-liquid chromatography in combination with flame-ionisation or mass spectroscopic detection can confirm the presence of paraffin and stearin.
5. The methods currently used for this purpose are at the research level and have not been validated by collaborative study.

[8] J.J. Jiménez, J.L. Bernal, M.J. del Nozal, L. Toribio, J. Bernal. Eur. J. Lipid Sci. Technol. 109 (2007) 682–690

[9] E. Waś, T. Szczęsna, H. Rybak-Chmielewska. J. Apic. Sci. 60 (2016) 145-161

[10] J. Serra Bonvehi, F.J. Orantes Bermejo. Food Chemistry 132 (2012) 642–648

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6. Literature values on the detailed composition of beeswax differ in dependence of the applied analytical method and the associated data evaluation procedure.
7. If a chromatographic method shall be used for official control, its harmonisation will be needed.
8. The harmonisation effort has to include agreement which variant of the described chromatographic methods shall be used.
9. The method shall be validated by collaborative trial or at least in-house validated using an internationally accepted protocol.
10. A sufficient number of different samples of beeswax and adulterants (paraffin/stearin) have to be analysed with the agreed method to determine the biological variation of their composition.
11. Based on those data decision criteria for assessing beeswax purity have to be developed.
12. The potential of the method for (semi)quantification has to be explored.

Appendix C – Eurostat

Imports

FLOW	1 - IMPORT
INDICATORS	QUANTITY_IN_100KG - QUANTITY_IN_100KG
PRODUCT	152190 - BEESWAX, OTHER INSECT WAXES AND SPERMACETI, WHETHER OR NOT REFINED OR COLOURED

Back to TOC

PERIOD	Jan.-Dec. 2013	Jan.-Dec. 2013	Jan.-Dec. 2014	Jan.-Dec. 2014	Jan.-Dec. 2015	Jan.-Dec. 2015	Jan.-Dec. 2016	Jan.-Dec. 2016	Jan.-Dec. 2017	Jan.-Dec. 2017	Jan.-Dec. 2018	Jan.-Dec. 2018
REPORTER/PARTNER	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA
EU28 (AT, BE, BG, CY, CZ, DE, DK, EE, ES, F	52314	55068	71163	63182	94852	70727	85849	70910	81973	73522	93997	70621
AUSTRIA	217	55	212	100	169	225	165	165	1818	100	835	100
BELGIUM (and LUXBG -> 1998)	806	854	1361	1241	682	2767	478	1737	1448	6414	707	2891
BULGARIA	219	20	1066	50	938	304	40	1791	90	1361	45	556
CYPRUS	4	146	0	126	6	175	73	266	75	538	18	253
CZECH REPUBLIC (CS->1992)	165	240	58	360	96	310	143	130	186	995	246	868
GERMANY (incl DD from 1991)	5155	28962	6658	28849	6222	31562	5549	28385	5744	32198	5187	34050
DENMARK	153	10	121	78	74	105	140	136	198	1	194	3
ESTONIA	4		4		1	1	1		2	0	7	
SPAIN	2856	3861	2600	4310	4646	6961	3744	6622	2674	4313	2815	7028
FINLAND	507		487		495	0	482	0	639	0	599	
FRANCE	18693	9034	33439	11760	52827	10346	58715	8102	54239	11246	59103	8248
UNITED KINGDOM	1712	4203	2758	4288	3693	4402	2818	5945	1277	4602	1078	4765
GREECE	711	4408	1057	6032	813	6750	2018	4182	1238	5639	9937	4946
CROATIA	128	376	169	255	91	296	60	262	136	309	122	147
HUNGARY	121		62	10	28	55	144	427	144	0	89	20
IRELAND	46	0	42	0	36		29	5	41	2	79	1
ITALY	2429	2710	2757	4466	3352	4007	2781	3746	3314	4187	5184	3245
LITHUANIA	50		87		15	60	52	112	969	100	151	75
LUXEMBOURG	272		186		489		261		320	0	396	
LATVIA	26		16	162	10	20	8		7	50	12	50
MALTA	3		13		5		11		7	0	9	
NETHERLANDS	0	0	18	326	837	509	2041	5980	908	193	1003	1967
POLAND	2324	0	2869	179	2498	1276	2820	2215	4328	1242	3979	1311
PORTUGAL	590	0	398	3	574	200	172		274		378	20
ROMANIA	14142	66	13476	393	15103	270	1725	606	751	30	632	69
SWEDEN	148	121	169	127	169	126	170	1	112	3	130	5
SLOVENIA	99		174	67	151		179	95	227		239	
SLOVAKIA	734	1	909		832		1032	0	806	0	821	3

Exports

Extracted on	2019/07/18 10:33:13
FLOW	2 - EXPORT
INDICATORS	QUANTITY_IN_100KG - QUANTITY_IN_100KG
PRODUCT	152190 - BEESWAX, OTHER INSECT WAXES AND SPERMACETI, WHETHER OR NOT REFINED OR COLOURED

Back to TOC

PERIOD	Jan.-Dec. 2013	Jan.-Dec. 2013	Jan.-Dec. 2014	Jan.-Dec. 2014	Jan.-Dec. 2015	Jan.-Dec. 2015	Jan.-Dec. 2016	Jan.-Dec. 2016	Jan.-Dec. 2017	Jan.-Dec. 2017	Jan.-Dec. 2018	Jan.-Dec. 2018
REPORTER/PARTNER	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA	EU28_INTRA	EU28_EXTRA
EU28 (AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI,	16633	9947	22277	9509	22957	9772	27705	8989	35999	9027	34200	10016
AUSTRIA	1	20	1	0	2	1	14	3	32	4	46	9
BELGIUM (and LUXBG -> 1998)	920	101	1309	92	3368	64	2514	81	6682	95	3487	263
BULGARIA	185		511	0	498		894	161	1395	5	796	184
CZECH REPUBLIC (CS->1992)	6	2	13	0	4	2	2	2	599	51	516	57
GERMANY (incl DD from 1991)	7209	5055	9379	4723	7740	4506	8994	4419	11379	5212	13236	4716
DENMARK	250	36	243	84	82	225	66	239	350	141	1177	358
ESTONIA	1	2	0	2	1		0		0			
SPAIN	1386	181	1094	192	2423	120	2012	134	2914	127	1658	702
FINLAND	4	0	0	0	0	0	2	0	7	0	10	0
FRANCE	3911	2026	5435	2888	5035	2069	4124	1185	3436	1557	4167	1503
UNITED KINGDOM	798	1028	625	1033	732	1096	858	829	1204	955	1121	690
GREECE	1092		2257	23	1142	86	2012	2	1528	59	505	450
CROATIA		30		43	1	27	94	38	23	19	5	18
HUNGARY	0	37	1	5	12	16	1	2	0	62	0	32
IRELAND	70	13				1			2		0	
ITALY	208	456	684	163	944	231	595	485	661	275	1548	630
LITHUANIA	7	7	12		34	2	21	11	112	33	12	25
LUXEMBOURG	0		0		0		0		0		0	1
LATVIA				0			0	0	8	0	26	
NETHERLANDS	476	705	194	186	59	1121	5177	1304	5020	234	4790	245
POLAND	101	27	46	6	414	55	285	66	584	25	421	8
PORTUGAL	0	0		48	0	25	24	13	2	10	640	12
ROMANIA	2	185	469	0	451	100	2	0	54	151	2	103
SWEDEN	2	29	2	13	2	22	1	11	1	10	1	10
SLOVENIA	3	7	2	6	14	2	11	3	6	4	6	2
SLOVAKIA		0	0	0		0				0	29	0

Appendix D – Literature searches

Sources of information

Database	Platform	Time span
CAB Abstracts	Web of Science	2000–2019
Scopus	Scopus.com	2000–2019
Web of Science Core Collection • Science Citation Index Expanded (SCI-EXPANDED) • Social Sciences Citation Index (SSCI) • Arts & Humanities Citation Index (A&HCI) • Conference Proceedings Citation Index- Science (CPCI-S) • Conference Proceedings Citation Index- Social Science & Humanities (CPCI-SSH) • Book Citation Index– Science (BKCI-S) • Book Citation Index– Social Sciences & Humanities (BKCI-SSH) • Emerging Sources Citation Index (ESCI) • Current Chemical Reactions (CCR-EXPANDED) • Index Chemicus (IC)	Web of Science	2000–2019

Literature searches – summary of results

Platform	Results
Scopus	531
Web of Science platform	777
Results after de-duplication	866

Only review papers selected through screening will be selected.

Literature searches – search strings Scopus

Date of the search 22-05-2019

Set	Search terms	Results
15	((TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND ((TITLE-ABS-KEY (adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR alkane* OR (stearic* AND palmitic*) OR stearin*))))) AND (PUBYEAR > 1999)) OR ((TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND ((TITLE-ABS-KEY (adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR alkane* OR (stearic* AND palmitic*) OR stearin*))))) AND (TITLE-ABS-KEY (mortalit* OR health* OR brood* OR develop* OR surviv*)) AND (PUBYEAR > 1999)) OR ((TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND (TITLE-ABS-KEY (paraffin* OR (stearic* AND palmitic*) OR stearin* OR alkane*)) AND (PUBYEAR > 1999)) AND (LIMIT-TO (SUBJAREA, "AGRI") OR LIMIT-TO (SUBJAREA, "CHEM") OR LIMIT-TO (SUBJAREA, "PHAR") OR LIMIT-TO (SUBJAREA, "MEDI") OR LIMIT-TO (SUBJAREA, "ENVI") OR LIMIT-TO (SUBJAREA, "MULT") OR LIMIT-TO (SUBJAREA, "VETE"))	531 document results
14	(TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND (TITLE-ABS-KEY (paraffin* OR (stearic* AND palmitic*) OR stearin* OR alkane*)) AND (PUBYEAR > 1999)	219 document results
13	(TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND ((TITLE-ABS-KEY (adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR alkane* OR (stearic* AND palmitic*) OR stearin*))))) AND (TITLE-ABS-KEY (mortalit* OR health* OR brood* OR develop* OR surviv*)) AND (PUBYEAR > 1999)	191 document results
12	(TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND ((TITLE-ABS-KEY (adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR alkane* OR (stearic* AND palmitic*) OR stearin*))))) AND (PUBYEAR > 1999) AND (LIMIT-TO (SUBJAREA, "AGRI") OR LIMIT-TO (SUBJAREA, "CHEM") OR LIMIT-TO (SUBJAREA, "PHAR") OR LIMIT-TO (SUBJAREA, "MEDI") OR LIMIT-TO (SUBJAREA, "ENVI") OR LIMIT-TO (SUBJAREA, "MULT") OR LIMIT-TO (SUBJAREA, "VETE"))	469 document results
11	((TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))) AND (TITLE-ABS-KEY (paraffin* OR (stearic* AND palmitic*) OR stearin* OR alkane*))) AND (PUBYEAR > 1999)	219 document results
10	PUBYEAR > 1999	44,869,027 document results
8	TITLE-ABS-KEY (paraffin* OR (stearic* AND palmitic*) OR stearin* OR alkane*)	180,337 document results
6	PUBYEAR > 1999 AND (LIMIT-TO (SUBJAREA, "AGRI") OR LIMIT-TO (SUBJAREA, "CHEM") OR LIMIT-TO (SUBJAREA, "PHAR") OR LIMIT-TO (SUBJAREA, "MEDI") OR LIMIT-TO (SUBJAREA, "ENVI") OR LIMIT-TO (SUBJAREA, "MULT") OR LIMIT-TO (SUBJAREA, "VETE"))	21,098,217 document results
4	TITLE-ABS-KEY (mortalit* OR health* OR brood* OR develop* OR surviv*)	17,935,818 document results
2	(TITLE-ABS-KEY (adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR alkane* OR (stearic* AND palmitic*) OR stearin*))))	9,415,355 document results
1	TITLE-ABS-KEY (beeswax* OR (wax W/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) W/10 wax))	2,373 document results

Web of Science platform

Date of the search 21-05-2019

Set	Query	Results
# 10	#8 OR #6 OR #4 Refined by: Databases: (WOS OR CABI) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	797
# 9	#8 OR #6 OR #4 *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	923
# 8	#7 AND #1 *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	234
# 7	TS=(paraffin* OR (stearic* AND palmitic*) OR alkane* OR stearin*) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	144,757
# 6	#5 AND #1 *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	**392**
# 5	TS=(adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (alkane* OR paraffin* OR (stearic* AND palmitic*) OR stearin*))) AND TS=(mortalit* OR health* OR brood* OR develop* OR surviv*) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	3,212,600
# 4	#2 AND #1 Refined by: RESEARCH AREAS: (CHEMISTRY OR FOOD SCIENCE TECHNOLOGY OR BIODIVERSITY CONSERVATION OR BIOCHEMISTRY MOLECULAR BIOLOGY OR LIFE SCIENCES BIOMEDICINE OTHER TOPICS OR AGRICULTURE OR ENTOMOLOGY OR ENVIRONMENTAL SCIENCES ECOLOGY OR SCIENCE TECHNOLOGY OTHER TOPICS OR TOXICOLOGY OR ZOOLOGY OR ENDOCRINOLOGY METABOLISM OR PHARMACOLOGY PHARMACY OR MATERIALS SCIENCE OR INSTRUMENTS INSTRUMENTATION OR CELL BIOLOGY OR VETERINARY SCIENCES OR PHYSIOLOGY OR ELECTROCHEMISTRY) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	791
# 3	#2 AND #1 *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	879
# 2	TS=(adulter* OR purity OR pure OR characteriz* OR characteris* OR residue* OR contaminat* OR ((added OR addition) AND (paraffin* OR (stearic* AND palmitic*) OR alkane* OR stearin*))) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	8,833,994
# 1	TS=(beeswax* OR (wax NEAR/3 foundation) OR ((bee OR bees OR honeybee* OR apidae OR apis) NEAR/10 wax)) *Databases= WOS, BCI, CABI, CSCD, CCC, DRCI, FSTA, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=2000-2019* *Search language=Auto*	2,429