Toxicological profile for

Honey and or honey extract

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

8028-66-8: Honey; UNII-Y9H1V576FH; Honey, purified; MEL; FENG MI; FENGMI (PubChem)

91052-92-5: Extract of honey; Honey extract; EINECS 293-255-4; Honey, ext. (PubChem)

1.3. Molecular formula

Unspecified

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

8028-66-8, 91052-92-5

1.7. Properties

1.7.1. Melting point

(°C): No data available to us at this time.

1.7.2. Boiling point

(°C): No data available to us at this time.

1.7.3. Solubility

“A typical product with Honey Extract, prepared in water … is soluble in any proportion of water” (CIR, 2020).

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

Occurrence in tobacco products

In the burnt part?	Yes
In tobacco naturally?	No evidence

Honey (CAS RN 8028-66-8) is used as a flavouring, humectant and skin conditioning agent in cosmetics in the EU;

Mel (CAS RN 8028-66-8) is used as a skin conditioning - humectant, moisturising and skin conditioning - emolient;

Honey extract (CAS RN 91052-92-5) is used as a flavouring, humectant and skin conditioning agent;

Mel extract (CAS RNs “8026-66-8” and 91052-92-5) is used as a moisturising agent;

and Mel powder (CAS RNs “8026-66-8” and 91052-92-5) is used as an abrasive, depilatory, bulking, binding and flavouring agent.

As taken from CosIng (Cosmetic substances and ingredients database).

Honey (CAS RN 8028-66-8) and honey extract (CAS RN 91052-92-5) are listed as ingredients in a number of personal care products (0.5-1.5%) by the CPID.

“A scenario analysis in regard to the risk of chronic exposure of consumers to residues through the consumption of contaminated honey and beeswax was conducted. Twenty-two plant protection products and veterinary substances of which residues have already been detected in beeswax in Europe were selected. The potential chronic exposure was assessed by applying a worst-case scenario based on the addition of a "maximum" daily intake through the consumption of honey and beeswax to the theoretical maximum daily intake through other foodstuffs. For each residue, the total exposure was finally compared to the acceptable daily intake. It is concluded that the food consumption of honey and beeswax contaminated with these residues considered separately does

not compromise the consumer's health, provided proposed action limits are met. In regard to residues of flumethrin in honey and in beeswax, "zero tolerance" should be applied." As taken from Wilmart O et al. 2016. J. Agric. Food Chem. 64(44), 8425-8434. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27741395

Honey distillate (CAS RN 91052-92-5) is listed as a fragrance ingredient by the International Fragrance Association IFRA.

"According to 2020 VCRP [Voluntary Cosmetic Registration Program] survey data, Honey is reported to be used in 1059 formulations (671 of which are leave-on formulations), and Honey Extract is reported to be used in 398 formulations (192 of which are leave-on formulations…)… All other in-use ingredients are reported to be used in 6 formulations or less. The results of a 2018 concentration of use survey conducted by the Council indicate Honey also has the highest concentration of use; it is used at up to 22% in paste masks and mud packs (which are considered rinse-off formulations) The highest concentration of use reported for leave-on products was in formulations containing Honey Extract at up to 7% in body and hand products."

"Honey is reported to be used in baby products, products that would be used near the eye, and products that could result in incidental ingestion and mucous membrane exposure. Honey is reported to be used in 13 baby products and at up to 0.01%. It is also reported to be used in 20 lipstick formulations (up to 3%), 1 dentifrice formulation (up to 0.00035%), 5 "other" oral hygiene product formulations (up to 0.1%), and 1 mouthwash and breath freshener formulation (concentration unknown). Honey could result in mucous membrane exposure as it is used at up to 3% in bath soaps and detergent formulation.

Additionally, Honey and Honey Extract are used in cosmetic sprays and could possibly be inhaled; for example, Honey is reported to be used in colognes and toilet waters and in hair sprays at up to 0.25% and 0.1%, respectively. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays. Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Honey is reportedly used in face powders at concentrations up to 3%, and could possibly be inhaled. Honey Extract is also reported to be used in powders (dusting and talcum) at up to 0.0001%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the air."

"Honey is commonly used as a sweetener and flavoring agent in many foods."

"Honey can be found as an ingredient in over-the-counter (OTC) cough and cold medications."

"A compound that is not naturally present in honey, 5-hydroxymethylfurfural (HMF), may be formed during the heating (via the Maillard reaction) or preservation (e.g., via acid-catalyzed dehydration of hexoses) of honey. HMF is a compound that may be mutagenic, carcinogenic, and cytotoxic."

As taken from CIR, 2020.

According to Health Canada's Natural Health Products Database, the following substances are used in non-medicinal products for the indicated purposes:

Honey (no CAS RN listed) is used as a binder, humectant and sweeting-agent for oral or topical use, a flavour enhancer for oral use and a skin-conditioning agent - humectant for topical use.

Honey extract (no CAS RN listed) is used as a skin-conditioning agent and skin-conditioning agent - humectant for topical use.

Honey powder (no CAS RN listed) is used as a binder, bulking agent and sweetening agent.

As taken from Health Canada, 2021.

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
formic acid	43183864	2.11	36950274	1.93
acetic acid	138631567	6.79	123372757	6.44
acetol	20637640	1.01	21094031	1.10
furfural	163518925	8.00	163252667	8.52
furfuryl alcohol	34933387	1.71	22570510	1.18
2,5-dimethyl-4-hydroxy-3(2H)-furanone + unknown	22568306	1.11	15181899	0.79
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	172856295	8.46	129024575	6.73
5-hydroxymethylfurfural	672647411	32.93	763282692	39.82
levoglucosan + unknown	108888620	5.33	85934655	4.48
1,6-anhydro-beta-D-glucofuranose	60476524	2.96	60451866	3.15
5,5'-oxy-dimethylene-bis(2-furaldehyde)	36207805	1.77	18654564	0.97
Total ion chromatogram	2045638701	100	1923150928	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74, pp. 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Honey 8028-66-8	34,000	Hydroxymethylfurfural + ?(28.7) Furfural (24.3) Acetic acid (6.3) Methylfurfural (5.6) Methylbenzenediol (4.1) Toluene (0.4) Styrene + ? (0.2)	4,900 4,100 1,100 950 700 68 34
Honey, absolute 91052-92-5	30	Hydroxymethylfurfural (19.4) Acetic acid (18.3) Sucrose (15.7) Furfural (13.1) Dihydroxyacetone (11.5)	3 3 2 2 2

2.3. Ingredient(s) from which it originates

Natural product.

As taken from Khan IA and Abourashed EA, 2010.

“A complex substance composed predominantly of low molecular weight sugars (except sucrose) but including invert sugar. Obtained by solvent extraction or distillation from honey.”

As taken from ChemIDplus (record for CAS RN 91052-92-5)

Honey and “mel” (both CAS RN 8028-66-8) are a saccharic secretion gathered and stored by honey bees, Apis mellifera.

Honey Extract (CAS RN 91052-92-5) is the extract obtained from Honey.

Mel Extract (CAS RNs “8026-66-8” and 91052-92-5) is an extract obtained from honey.

Mel Powder (CAS RNs “8026-66-8” and 91052-92-5) is the powder obtained from dehydrated, ground honey.

As taken from CosIng (Cosmetic substances and ingredients database).

“Honey (CAS RN 8028-66-8) is a saccharic secretion gathered and stored by honey bees of the species, Apis mellifera, Tetragonisca angustula, Scaptotrigona pectoralis, or Melipona becheii”.

As taken from CIR, 2020

3. Status in legislation and other official guidance

Honey, ext. (CAS RN 91052-92-5) is not registered under REACH (ECHA).

Honey (CAS RN 8028-66-8) is not registered under REACH (ECHA).

Honey, ext. (CAS RN 91052-92-5) and honey (CAS RN 8028-66-8) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023a)

Honey (CAS RN 8028-66-8) is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products.

Honey (CAS RN 8028-66-8) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” (AICIS, 2017).

The Cosmetic Ingredient Review (CIR) Expert Panel concluded that honey-derived ingredients (including honey, honey powder and honey extract) are safe in cosmetics in the present practices of use and concentration described in the safety assessment (CIR, 2020).

“The Codex Alimentarius has established that the HMF [5-hydroxymethylfurfural] concentration in honey should be lower than 80 mg/kg; however, the European Union recommends a lower limit of 40 mg/kg.”

“[The] FDA requires proper labeling of honey and honey products to ensure that these products are not adulterated and misbranded. All honey and honey products must be labeled in accordance with sections 402 and 403 of the Federal Food, Drug, and Cosmetic Act (21 USC 342 and 343). The international FAO/WHO Codex Alimentarius Standard requires that: Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavor, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the remove of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or quality is impaired.”

“Although rare, infant botulism has been reported after ingestion of honey due to Clostridium botulinum spores. Because of this, the FDA, the Centers for Disease Control and Prevention, and the American Academy of Pediatrics, recommend not feeding honey to infants younger than 12 months.”

As taken from CIR, 2020.

Honey (no CAS RN listed) is classified as a Natural Health Product (NHP) for medicinal use under Schedule 1 item 1 (non-human animal material) of the NHP Regulations. When used in non-medicinal natural health products, it “must meet Standardized Food quality specifications as outlined in Part B of the Canadian Food and Drug Regulations”.

As taken from Health Canada, 2021.

Honey (CAS RN 8028-66-8) is approved for use as a biocidal active substance (Annex I) under the Biocidal Products Regulation (Regulation (EU) No 2019/1822). (ECHA, 2023b)

Honey (Honey, fermented, CAS RN 8028-66-8) and Honey, extract (CAS RN 91052-92-5) are listed on Australian Invenory of Industrial Chemicals (AICIS, formerly NICNAS). AICIS, undated.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“….In this review, specific attention is focused on absorption, metabolism, and beneficial biological activities of honey compounds in human. Honey is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes, vitamins and polyphenols. Among polyphenols, flavonoids are the most abundant and are closely related to its biological functions….” As taken from Alvarez-Suarez JM et al. 2013. Curr. Med. Chem. 20(5), 621-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23298140

“Over the last 150 years a number of people in New Zealand have been incapacitated, hospitalised, or died from eating honey contaminated with tutin, a plant-derived neurotoxin. A feature of the most recent poisoning incident in 2008 was the large variability in the onset time of clinical signs and symptoms of toxicity (0.5-17 h). To investigate the basis of this variability a pharmacokinetic study was undertaken in which 6 healthy males received a single oral dose of tutin-containing honey giving a tutin dose of 1.8 µg/kg body weight. ..... A novel analytical method subsequently revealed the presence of glycoside conjugates of tutin in addition to unconjugated tutin in honey. .....” As taken from Fields BA et al. 2014. Food Chem. Toxicol. 72, 234-241. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25084484

4.2. Absorption, distribution and excretion

“….In this review, specific attention is focused on absorption, metabolism, and beneficial biological activities of honey compounds in human. Honey is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes, vitamins and polyphenols. Among polyphenols, flavonoids are the most abundant and are closely related to its biological functions….” As taken from Alvarez-Suarez JM et al. 2013. Curr. Med. Chem. 20(5), 621-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23298140

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tutin-containing honey giving a tutin dose of 1.8 μg/kg body weight. The serum concentration-time curve for all volunteers exhibited two discrete peaks with the second and higher level occurring at approximately 15 h post-dose. ..... Pharmacokinetic analysis using a two-site absorption model resulted in a good fit to the observed concentration data. ..... These pharmacokinetic data will be important to better define a safe maximum tutin concentration in honey." As taken from Fields BA et al. 2014. Food Chem. Toxicol. 72, 234-241. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25084484

4.3. Interactions

"…There has been a renewed interest in the use of honey in the treatment of diabetes mellitus, partly due to an increase in the availability of evidence-based data demonstrating its benefits in diabetic rodents and patients. This commentary aims to underscore some of the research implications, issues and questions raised from these studies which show the beneficial effects of honey in the treatment of diabetes mellitus. Some of the issues highlighted in this article include: considering honey is sweet and rich in sugars, how could it be beneficial in the management of diabetes mellitus? Are the observed effects of honey or combined with anti-diabetic drugs exclusive to certain honey such as tualang honey? Could these beneficial effects be reproduced with other honey samples? Anti-diabetic drugs in combination with honey improve glycemic control, enhance antioxidant defenses and reduce oxidative damage. These effects are believed to be mediated partly via antioxidant mechanism of honey…." As taken from Erejuwa OO. 2014. J. Diabetes Metab. Disord. 13(1), 23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476150

A study, the first of its kind, reported the beneficial effects of combining anti-diabetic drugs with honey in diabetes mellitus. Honey administration was found to increase serum levels of insulin while it reduced serum concentrations of glucose and fructosamine in diabetic rats [14]. Even though glibenclamide or metformin reduced hyperglycemia, the administration of these drugs in combination with honey resulted in much lower glycemic levels. On the other hand, unlike honey, these anti-diabetic drugs produced no effect on serum fructosamine concentrations. However, when each of these drugs in combination with honey was administered, there was a significant reduction in serum fructosamine, creatinine, bilirubin, triglycerides and very low-density lipoprotein (VLDL) cholesterol in the diabetic rats. These effects were not observed when glibenclamide or metformin was administered alone [14]. Furthermore, the combination of anti-diabetic drugs with honey also enhanced antioxidant defenses and reduced oxidative damage in the kidney and pancreas of diabetic rats [15-17]. In brief, though data from in vivo studies are still limited, these studies reveal that honey could be used as an adjunct to diabetes therapy to achieve better glycemic control, improve metabolic derangements and mitigate oxidative stress-linked diabetic complications.As taken from Erejuwa OO. 2014. J. Diabetes Metab. Disord. 13(1), 23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476150

In a recent study, metformin combined with Ilam honey markedly produced lower levels of hyperglycemia, bilirubin, triglycerides, total cholesterol, VLDL and LDL and increased high density lipoprotein (HDL) cholesterol. On the other hand, metformin alone neither reduced bilirubin and triglycerides nor increased HDL in diabetic rats [30]. These data obtained with Ilam honey are similar to those obtained with tualang honey and thus suggest administration of other honey samples in combination with anti-diabetic drugs could replicate similar effects. However, there might still be some differences in pharmacological effects due to variations among honey samples [29]. As taken from Erejuwa OO. 2014. J. Diabetes Metab. Disord. 13(1), 23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476150

"Tualang honey (TH) is rich in flavonoids and phenolic acids and has significant anticancer activity against breast cancer cells comparable to the effect of tamoxifen (TAM), in vitro. The current study evaluated the effects of TH when used in combination with TAM on MCF-7 and MDA-MB-231 cells. We observed that TH promoted the anticancer activity of TAM in both the estrogen receptor-(ER-

)responsive and ER-nonresponsive human breast cancer cell lines. Flow cytometric analyses indicated accelerated apoptosis especially in MDA-MB-231 cells and with the involvement of caspase-3/7, -8 and -9 activation as shown by fluorescence microscopy. Depolarization of the mitochondrial membrane was also increased in both cell lines when TH was used in combination with TAM compared to TAM treatment alone. TH may therefore be a potential adjuvant to be used with TAM for reducing the dose of TAM, hence, reducing TAM-induced adverse effects." As taken from Yaacob NS et al. 2013. Evid. Based Complement. Alternat. Med. 2013, 989841. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23476711

"Manuka honey has been recognized for its anti-bacterial and wound-healing activity but its potential antitumor effect is poorly studied despite the fact that it contains many antioxidant compounds. In this study, we investigated the antiproliferative activity of manuka honey on three different cancer cell lines, murine melanoma (B16.F1) and colorectal carcinoma (CT26) as well as human breast cancer (MCF-7) cells in vitro. The data demonstrate that manuka honey has potent anti-proliferative effect on all three cancer cell lines in a time- and dose-dependent manner, being effective at concentrations as low as 0.6% (w/v). This effect is mediated via the activation of a caspase 9-dependent apoptotic pathway, leading to the induction of caspase 3, reduced Bcl-2 expression, DNA fragmentation and cell death. Combination treatment of cancer cells with manuka and paclitaxel in vitro, however, revealed no evidence of a synergistic action on cancer cell proliferation. Furthermore, we utilized an in vivo syngeneic mouse melanoma model to assess the potential effect of intravenously-administered manuka honey, alone or in combination with paclitaxel, on the growth of established tumors. Our findings indicate that systemic administration of manuka honey was not associated with any alterations in haematological or clinical chemistry values in serum of treated mice, demonstrating its safety profile. Treatment with manuka honey alone resulted in about 33% inhibition of tumor growth, which correlated with histologically observable increase in tumor apoptosis. Although better control of tumor growth was observed in animals treated with paclitaxel alone or in combination with manuka honey (61% inhibition), a dramatic improvement in host survival was seen in the co-treatment group. This highlights a potentially novel role for manuka honey in alleviating chemotherapy-induced toxicity." As taken from Fernandez-Cabezudo MJ et al. 2013. PLoS One 8(2), e55993. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23409104

"Effect of Acacia honey from north-west Nigeria on sodium arsenite-induced oxidative damage and clastogenicity in male Wistar rats was investigated. Animals were divided into four groups and were treated daily via oral gavage for one week before they were sacrificed. Brain, liver and blood serum were collected for antioxidant and protein assays. Clastogenicity, in vitro antioxidant activity, vitamins and minerals were also evaluated. From the results, co-administration of Acacia honey with sodium arsenite on the animals increased ($P < 0.05$) glutathione peroxidase, superoxide dismutase and catalase activities with concomitant decrease in malondialdehyde levels and anti-clastogenic effects relative to the group treated with sodium arsenite only. The honey possesses reducing power, high hydrogen peroxide scavenging activity, good amount of vitamins (A, C and E), flavonoids (5.08 ± 0.92 mg QE/100 g) and phenolics (5.40 ± 0.69 mg GAE/100 g). The minerals present include zinc, iron, sodium, magnesium, potassium and calcium. In conclusion, Acacia honey from Nigeria may mitigate oxidative stress and clastogenicity." As taken from Muhammed A et al. 2015. Nat. Prod. Res. 29(4), 321-6. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25105348

"BACKGROUND: Radiotherapy is frequently used in treatment approaches of pelvic malignancies. Nevertheless, it has some known systemic effects on blood cells and the immune system that possibly results in their susceptibility to infection. Probiotics are live microbial food ingredients that provide a health advantage to the consumer. Honey has prebiotic properties. The aim of this clinical trial was to investigate probable effects of probiotic or probiotics plus honey on blood cell counts and serum IgA levels in patients receiving pelvic radiotherapy. MATERIALS AND METHODS: Sixty-seven adult patients with pelvic cancer were enrolled. Patients were randomized to receive either: (1) Probiotic capsules (including: Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus

rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophiles) (n = 22), (2) probiotic capsules plus honey (n = 21) or (3) placebo capsules (n = 24) all for 6 weeks. Blood and serum samples were collected for one week before radiotherapy and 24-72 h after the end of radiotherapy. RESULTS: White blood cells (WBC), red blood cells (RBC), platelet counts, and serum IgA level were not significantly changed in patients taking probiotic (alone or plus honey) during pelvic radiotherapy. The mean decrease in RBC count was 0.52, 0.18, and 0.23 × 10(6) cells/μL, WBC count was 2.3, 1.21, and 1.34 × 10(3) cells/μL and platelet count was, 57.6, 53.3, and 66.35 × 10(3) cells/μL for the probiotic, probiotic plus honey, and placebo groups, respectively. The mean decrease of serum IgA was 22.53, 29.94, and 40.73 mg/dL for the probiotic, probiotic plus honey, and placebo groups, respectively. CONCLUSION: The observed nonsignificant effect of probiotics may be in favor of local effects of this product in the gut rather than systemic effects, however, as a trend toward a benefit was indicated, further studies are necessary in order to extract effects of probiotics or probiotic plus honey on hematologic and immunologic parameters in patients receiving pelvic radiotherapy." As taken from Mansouri-Tehrani HA et al. 2015. J. Res. Med. Sci. 20(7), 679-83. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26622258

"Glycemic Index (GI) is a measure of the effect foods have on blood glucose (i.e., blood sugar). In this study, it was aimed to find out the GI of highbush cranberry juice (HCJ) in four forms of use (juice with no added sugar and juice sweetened with corn syrup, sucrose or honey) based on data from a total of 20 healthy volunteers. After not eating for 12 hours and consuming the test foods and the reference food, the participants were subject to blood drawing by automatic lancet, 15, 30, 45, 60, 90, and 120 minutes into the application. GI values of HCJ samples were found by calculating incremental areas for each individual. GI of HCJ with no added sugar had the lowest value (40.95), followed by that of the sucrose added HCJ (42.75). GIs of HCJ with corn syrup and honey were found to be similar, 54.16 and 56.98, respectively. Moreover, those with no added sugar and those with sucrose and corn syrup fell into the low GI category, while the GI of HCJ with honey fell into the medium GI category. In conclusion, it is suggested that consuming HCJ with low GI values might be a healthier choice for individuals with chronical illnesses." As taken from Soylu M. 2018. Biochemistry 9(2), 20253-20258. Available at http://www.ijcrr.in/index.php/ijcrr/article/view/453

"BACKGROUND/AIM: Various honey samples exhibited protective effect against drug and chemical induced toxicity. The study was designed to determine the antioxidant content and activity of carob honey and to investigate its hepato-renal protective effect in carbon tetrachloride (CCl4) induced kidney and liver injury in rats. MATERIAL AND METHODS: Phenolic, flavone and flavonol in carob honey were quantified. DPPH, ABTS•+, ferric reducting antioxidant power, and total antioxidant activity were used to evaluate the antioxidant activity. Rats were used for the experiment, and received either intraperitoneal injection of CCl4 (1 mL/kg.b.wt); honey (orally, 2 g/kg.b.wt) and CCl4; or honey. Liver and kidney function parameters were assessed. Oxidative parameters including lipid peroxidation (MDA), protein carbonyl formation (PCO), advanced protein oxidation products (AOPP), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and ascorbic acid were measured in the kidney and liver tissues. RESULTS: CCl4 caused a significant elevation of liver enzymes, lactic acid dehydrogenase, blood glucose, uric acid, blood urea and serum creatinine as compared to the control group. Also, it significantly increased MDA, PCO and AOPP level, and markedly decreased GHS, ascorbic acid, CAT and GPx in the liver and kidney tissues. These changes were significantly ameliorated by carob honey before and after CCl4 administration. Honey alone did not cause significant changes as compared to the control group. CONCLUSION: The data showed for the first time that carob honey has high antioxidant content, antioxidant property, and protective effect against CCl4 induced kidney and liver toxicity by maintaining the activity of antioxidant defense system." As taken from El-Haskoury R et al. 2018a. Arch. Med. Res. 49(5), 306-313. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30342848

"The protective effects of both manuka and talh honeys were assessed using a rat model of cisplatin (CISP)-induced hepatotoxicity and nephrotoxicity. The results revealed that both honeys

exerted a protective effect against CISP-induced hepatotoxicity and nephrotoxicity as demonstrated by decreasing liver and kidney function. Manuka honey also prevented CISP-induced histopathological changes observed in the liver and decreased the changes seen in the kidneys. Talh honey decreased CISP-induced liver histopathological changes but had no effect on CISP-induced kidney histopathological changes. Both honeys reduced the oxidative stress in the liver. Conversely, they have no effect on kidney oxidative stress, except that manuka honey increased CAT activity. GC-MS analysis showed the presence of the antioxidant octadecanoic acid in talh honey while heneicosane and hydrocinnamic acid were present at a higher content in manuka honey. The molecular mechanism was to limit the expression of inflammatory signals, including COX-2 and NF-κB, and the expression of the apoptotic signal, BAX and caspase-3 while inducing Bcl-2 expression." As taken from Neamatallah T et al. 2018. Food Funct. 9(7), 3743-3754. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29897076

"Apis cerana honey (honey of Apis cerana Fabricius), widely distributed in the mountain areas of East Asia, has not been studied fully. The hepatoprotective activity of A. cerana honey was evaluated against bromobenzene-induced liver damage in mice. In high dose, A. cerana honey can significantly alleviate liver injury, as is indicated by the depressed levels of serum alanine aminotransferase (ALT) (59.13%) and aspartate aminotransferase (AST) (79.71%), the inhibited malondialdehyde (MDA) content (63.30%), the elevated activities of superoxide dismutase (SOD) (73.12%) and glutathione-Px (57.24%), and the decreased expression of Transforming growth factor β1 (51.83%) induced by bromobenzene ($P < 0.05$). The quantitative analysis of twelve major constituents (1 to 12) of A. cerana honey was executed by high performance liquid chromatography-diode array detector. The results indicate that treatment with A. cerana honey can prevent bromobenzene-induced hepatic damage in mice. Polyphenols might be the bioactive substances attributed to its antioxidant properties and intervention of oxidative stress." As taken from Zhao H et al. 2018. J. Food Sci. 83(2), 509-516. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29337369

"Honey is traditionally used in burns, wound healing, ulcers, boils, and fistulas. Honey was tested to prevent tartrazine toxicity in male rats for 8 weeks. The 18 rats of the experiment were randomly divided into three 6-rat groups. The negative control group (G1) fed diet with sulfanilic acid, the tartrazine positive group (G2) fed diet containing tartrazine and sulfanilic acid and the honey-treated group (G3) fed diet as in G2 and cotreated with honey. Tartrazine decreased antioxidants, high-density lipoproteins and proteins, and increased liver enzymes, kidney indices, lipid peroxidation, triglycerides, total cholesterol, and low- and very-low-density lipoproteins. In addition, tartrazine-treated group showed drastic damage of the tissues of stomach, liver, kidney, and testis. Honey treatment increased antioxidants and high-density lipoproteins, and decreased lipid peroxidation, liver enzyme and kidney parameters. Honey treatment also improved stomach, liver, kidney, and testis tissues. In conclusion, honey protects male rats against tartrazine toxicity." As taken from El Rabey HA et al. 2019. J. Fd Biochem. 43(4), e12780. Available at https://onlinelibrary.wiley.com/doi/pdf/10.1111/jfbc.12780

"Aim of the work: The present study was carried out to evaluate the role of bee honey and bee venom (BV) separately or in combination in ameliorating promotion of colon carcinogenesis induced by 1,2 dimethylhydrazine (DMH) in albino rats. Materials and methods: Rats were subcutaneously injected by DMH (20 mg/kg b. wt.) once a week for 15 weeks. DMH-treated animals received either oral administration of bee honey (500 mg/kg b. wt.) or intraperitoneal injection of BV (3 mg/kg b. wt.) or both together every other day along the period of DMH-treatment. At the end of 15th week treatment, blood samples and colon tissues were taken for biochemical analysis of lipid peroxide, glutathione peroxidase (GPx), alakaline phosphatase (ALP), carcinoemberyonic antigen (CEA) and alpha fetoprotein (AFP); and also for histopathological and immunohistochemical investigations. Results: The results showed an increase in the levels of lipid peroxide, ALP, CEA and AFP and a decrease of GPx level in DMH-treated rats as compared to control, while honey and BV treatments modulated the DMH-induced changes of these parameters. Moreover, they showed remarkable reduction in dysplasia, inflammatory cells infiltration and loss of

acinar patterns of colon glands and abnormalities of P53 expression which were clearly observed in DMH-treated group. Conclusion: Findings of the present study indicate significant roles for reactive oxygen species (ROS) in pathogenesis of DMH-induced colon toxicity and initiation of colon cancer. Also, it suggested that honey, BV or the combination of both have a positive beneficial effect against DMH induced colonic cancer in rats. Honey and BV inhibit oxidative stress and enhance antioxidant status suggesting a growing application of these natural compounds as an alternative medicine treatment of colon tumor." As taken from Nagy S et al. 2018. Cancer Biology 8(4), 9-20. Available at http://www.cancerbio.net/cb/cb080418/02_34105cbj080418_9_20.pdf

"Background: Honey as a natural product exhibits a variety of biological and pharmacological activities. Its anti-inflammatory, antioxidant, antibacterial, and antihypertensive effects have already been proven. Objectives: In this study, the inhibitory effects of honey on the 7,12-dimethylbenz(a)anthracene-initiated and croton oil-promoted mice skin carcinogenesis were studied. Methods: Albino Swiss mice were pretreated with multiple topical applications of honey. After nine hours, the carcinogenesis was initiated by a single dose of DMBA. Topical croton oil, as for a promoting agent, was applied biweekly for a period of 30 weeks. Results: The tumor incidences were observed. Compared to the control group, the honey pre-treated mice showed a significant inhibition in tumor incidences. In addition, the enhanced uptake of [3H]-thymidine in mice skin DNA was inhibited in honey-pretreated animals as compared to the control group. Conclusions: Taken together, the results suggest that the antioxidants existed in honey have diminishing effects on croton oil-mediated murine skin tumor promotion. In conclusion, we suggest that honey as an effective natural preventive agent may provide protection against skin cancer." As taken from Milani SM et al. 2018. Jundishapur Journal of Natural Pharmaceutical Products 13(3), e57992. Available at http://jjnpp.com/en/articles/57992.html

"Abstract: Background: Streptococcus mutans is a Gram-positive bacterium found in the oral cavity. As a cariogenic bacterium, Streptococcus mutans can cause dental caries through its ability to produce an acidic environment that can demineralize tooth structures so that the tooth layer is destroyed. Objective: To determine the optimal combination of probiotic milk Lactobacillus paracasei and calliandra honey which has antibacterial activity in Streptococcus mutans bacteria. Method: This study uses diffusion method on Nutrient Agar media. The study began with an examination of the physical properties of probiotic milk and calliandra honey including color, odor, taste, pH, specific gravity and viscosity. Antibacterial activity was indicated by the diameter of the Zone of inhibition (mm) in the form of clear areas around the well on the media so that containing Streptococcus mutans inoculums 0.25µl/ml. Result: The combination of probiotic milk Lactobacillus paracasei and 50% calliandra honey solution produced the highest activity at a ratio of 8: 2 with Zone of inhibition diameters of 16.40 ± 0.71 mm Conclusion: The combination of probiotic milk and calliandra honey with 5% concentration and 8: 2 ratio has the highest antibacterial activity against Streptococcus mutans that causes tooth cavities." As taken from Chasanah U et al. 2020. Indian Journal of Public Health Research & Development 11(1), 1441-1445. Available at https://bit.ly/3e8Us1n

"Abstract: Background: Streptococcus mutans commonly found in oral cavity and can be a pathogenic bacteria that leads to dental caries. Rinsing the oral cavity with antibiotic oral therapy is not suggested as the treatment of dental caries, because it has side effects. It can cause resistance of Streptococcus mutans towards antibiotic. Objective: To analyze the antibacterial activity of honey of mango, prebiotic milk, and the combination of both against Streptococcus mutans bacteria Method: The antibacterial activity test was performed by agar diffusion method with Müeller Hinton agar medium to determine the minimal inhibitory concentration inhibition (MIC). A study had been conducted on the antibacterial activity of the combination of honey of mango and probiotic milk of Lactobacillus paracasei ATCC BAA52 on the growth of Streptococcus mutans. Fermented milk was made by inoculating Lactobacillus paracasei ATCC BAA52 fermented milk, mango honey and their combination at optimum ratio (propotition) into fresh milk at 45°C, then incubated for 24 hours at room temperature Result: The result of probiotic milk characterization showed that the pH of probiotic milk decreased compared to fresh milk from from pH 6.33 to 3.89. Furthermore, the MIC

of each samples against Streptococcus mutans were determined Conclusion: Combination between mango honey (Mangifera indica) and probiotic mlik (Lactobacillus paracasei ATCC BAA52 can give optimum anti bacteria activities against Streptococcus mutans." As taken from Azzulfiyyah IW et al. 2020. Indian Journal of Public Health Research & Development 11(1), 913-917. Available at https://bit.ly/3aJpBWt

"Background: Juniperus procera and Majra honey are well-known as a folk medicine in many countries. Objectives: This work aimed to study the immunomodulatory effects after mixing Majra honey, J. procera water leaves extract and silver Nanoparticles (AgNPs) on immune or cancer cells. Methods: Juniperus procera water leaves extract and 20% Majra honey were prepared. Both the extract and honey were used separately to synthesize AgNPs. AgNPs were characterized using UV/Vis spectrophotometry and electron microscopy. Bioactive molecules in honey and the extract were explored using Fourier Transform Infrared (FT-IR) spectroscopy. Protein profile of honey was explored using Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) and honey sugar content was determined using High- Performance Liquid Chromatography (HPLC). Biological activities of honey and the extract were tested. Results: The results demonstrated the ability of the extract/honey to produce AgNPs in a spherical shape. The extract/honey contained many functional groups. SDS-PAGE of Majra honey showed many protein bands. HPLC revealed honey is of good quality and no external additives are added to it. The extract and extract+ AgNPs inhibited the growth of normal rat splenic cells while honey stimulated it. The extract+honey turned stimulatory to the splenic cells' growth and significantly diminished the inhibitory potential of the extract containing AgNPs. Both the extract and honey have antimicrobial activities, this potential increased in the presence of AgNPs. Honey and Honey+AgNPs inhibited HepG2 cancer cell proliferation while Hela cell growth inhibited only with honey+AgNPs. Conclusion: Both honey and the extract have antibacterial and immunomodulatory potentials as well as the power to produce AgNPs. Majra honey alone showed anticancer activity against HepGe2 cells, but not against Hela cells, and when contained AgNPs had anticancer activity on both cell lines. Mixing of Majra honey with J. procera extract showed characterized immunomodulatory potentials that can be described as immunostimulant." As taken from Ghramh HA et al. 2020. Anticancer Agents Med. Chem. 20(8), 970-981. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32053084/

"Honey and ghee are an essential component of our diet. They play an important role like anti-inflammatory, antioxidative, antimicrobial, etc. It is written in Charak Samhita that an equal mixture of honey and ghee turn into a harmful component for health. This study was designed to explore the mechanism of toxicity through the biochemical and histological parameters in Charles foster rats (24 rats were used). We have divided these rats into four groups (n = 6) - normal, honey (0.7 ml/100 g bw), ghee (0.7 ml/100 g bw), and honey + ghee (1:1) (1.5 ml/100 g bw). Treatment was given orally for 60 days. All rats were sacrificed on 61 days. Biochemical parameters like liver function test, kidney function test, Oxidative stress, Glycemic, and some protein modification parameters were done in blood plasma. We found weight loss, hair loss, red patches on ear, and increased liver function test, oxidative stress, Amadori product formation, advanced glycation end-product formation, dipeptidyl protease (DPP-4) and decreased incretins (glucagon-like peptide-1(GLP-1) and gastric inhibitory polypeptide (GIP)) in honey + ghee group. H&E and immunohistochemistry results showed mild inflammation in liver tissue but no changes in the kidney, intestine and, pancreas. Thus it concluded that the increased formation of Amadori product, DPP-4 activity and low incretins (GLP-1, GIP) activity resulting high postprandial hyperglycemic response could be collectively responsible for oxidative stress-mediated toxicity of honey and ghee in the equal mixture." As taken from Aditi P et al. 2020. Toxicol. Rep. 7, 624-636. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32455119/

5. Toxicity

5.1. Single dose toxicity

Toxicant	LD50	Animal	Route
Acetylandromedol	1.28	Mouse	IP
	0.15	Mouse	SC
	1	Mouse	IP
	3.9	Frog	SC
Andromedol	3.47	Mouse	SC
	0.908	Mouse	IP
	5.08	Frog	SC
Desacetylpieristoxin B	0.65	Mouse	SC
Gelsemine	0.5	Rabbit	SC
Gelsemine, HCl	4	Mouse	IP
	0.8	Rabbit	IP LD75
Tutin	1.2	Guinea-pig	Stomach tube
	0.2	Rat	Stomach tube
	0.75	Guinea-pig	SC
	0.4	Rat	SC
	0.7	Guinea-pig	IP
	0.5	Rat	IP
Hyenanchin	12	Guinea-pig	Stomach tube
	0.40-90	Rat	Stomach tube
	9	Guinea-pig	SC
	0.3	Rat	SC
	9	Guinea-pig	IP
	0.3	Rat	IP

(White 1981)

Record for Apis mellifera (Malaysian), honey (no CAS RN):

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	800 mg/kg	Behavioral - analgesia Biochemical - Metabolism	FTRPAE Fitoterapia. (Inverni della Beffa SpA, via Ripamonti, 99, 20141 Milan, Italy) V.18-1947- Volume(issue)/page/year:

				(Intermediary) - effect on inflammation or mediation of inflammation	81,1196,2010
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	60 mg/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	FTRPAE Fitoterapia. (Inverni della Beffa SpA, via Ripamonti, 99, 20141 Milan, Italy) V.18-1947- Volume(issue)/page/year: 83,1054,2012

As taken from RTECS, 2013.

Records for Apis mellifera (Malaysian), honey, ethyl acetate extract and Apis mellifera (Malaysian), honey, methanol extract (no CAS RNs):

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	180 mg/kg	Behavioral - analgesia Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	FTRPAE Fitoterapia. (Inverni della Beffa SpA, via Ripamonti, 99, 20141 Milan, Italy) V.18-1947- Volume(issue)/page/year: 81,1196,2010

As taken from RTECS, 2011.

Record for honey, grayanotoxin-contaminated (no CAS RN):

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Human	0.286 mg/kg	Vascular - BP lowering not characterized in autonomic section Cardiac - pulse rate Gastrointestinal - nausea or vomiting	AJEMEN American Journal of Emergency Medicine. (WB Saunders, Philadelphia, PA) V.1- 1983- Volume(issue)/page/year: 24,595,2006
TDLo - Lowest published toxic dose	Oral	Human	0.29 mg/kg	Cardiac - arrhythmias (including changes in conduction) Vascular - BP lowering not characterized in autonomic section Gastrointestinal - nausea or vomiting	AJEMEN American Journal of Emergency Medicine. (WB Saunders, Philadelphia, PA) V.1- 1983- Volume(issue)/page/year: 24,595,2006
TDLo - Lowest published toxic dose	Oral	Rodent - rat	0.1 gm/kg	Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - catalases Biochemical - Enzyme	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979-

				inhibition, induction, or change in blood or tissue levels - other oxidoreductases Biochemical - Metabolism (Intermediary) - lipids including transport	Volume(issue)/page/year: 156,155,2014

As taken from RTECS, 2017

“Over the last 150 years a number of people in New Zealand have been incapacitated, hospitalised, or died from eating honey contaminated with tutin, a plant-derived neurotoxin. A feature of the most recent poisoning incident in 2008 was the large variability in the onset time of clinical signs and symptoms of toxicity (0.5-17 h). To investigate the basis of this variability a pharmacokinetic study was undertaken in which 6 healthy males received a single oral dose of tutin-containing honey giving a tutin dose of 1.8 µg/kg body weight. ..... Two subjects reported mild, transient headache at a time post-dose corresponding to maximum tutin concentrations. There were no other signs or symptoms typical of tutin intoxication such as nausea, vomiting, dizziness or seizures. .....” As taken from Fields BA et al. 2014. Food Chem. Toxicol. 72, 234-241. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25084484

“The substance honey from rhododendron is proposed to be used as rodenticide in baits. The applicant claims that field studies performed on their own, demonstrate that mice die as a result of the grayanotoxins in the honey. However, no proper scientific report has been provided to substantiate these claims.”

As taken from EFSA, 2017.

“Studies on compounds present in plant or animal matter helps in identifying compounds responsible for harmful and beneficial effect in plants and animal by-products. This study was designed to identify and quantify phytochemical compounds in Mitracarpus villosus methanolic leaf extract, and determine the toxicity of M villosus ointment and honey. The results show that M. villosus leaves contains phenolics, saponins, flavonoids, cardiac glycosides and tannins but no alkaloids. Quantitative phytochemical analysis showed varied percentage content of these compounds with saponins being highest (14.0%) and tannins lowest (1.41%). Acute oral toxicity was determined to be 15066 mg/kg and 7542 mg/kg as LD50 for honey and M. villosus ointment respectively, inferring that both honey and M. villosus ointment belong to the nontoxic class group of substances. Primary irritation indices were recorded as 0.16 and 0.33 for honey and ointment respectively. Therefore, honey and M. villosus ointment belong to the category of negligible irritants. These findings is indicative of the promising potentials of M. villosus ointment as a topical remedy for diseases and further confirms reasons for high demand of honey for various uses despite its alarming cost increase.” As taken from Jato JA et al. 2018. International Journal of Modern Science and Technology 3(11), 230-237. Available at https://www.researchgate.net/publication/328967732_Phytochemical_Analysis_of_Mitracarpus_villosus_and_Comparative_Toxicity_of_Mitracarpus_villosus_Ointment_and_Honey

5.2. Repeated dose toxicity

Natural honey lowers plasma prostaglandin concentrations in normal individuals (Abstract). Twelve normal, healthy adult individuals, 9 men and 3 women, 25-48 years of age (mean, 38 years), were recruited in the study. After 12 hours of fasting, blood specimens were collected at 8:00 AM for prostaglandin E (2) (PGE (2)), PGF(2alpha), and thromboxane B(2) assays. Each individual then drank 250 ml of water containing 1.2 g/kg body weight of natural unprocessed honey, after which collection of blood was repeated at 1, 2, and 3 hours for estimation of prostaglandins. Each individual was asked to drink the same amount of honey diluted in water once

a day for a maximum of 15 days. After 12 hours of fasting, morning blood specimens were collected on day 16, and plasma prostaglandin concentrations were measured. The quantitative analysis of prostaglandins was performed with use of an enzyme-linked immunosorbent (ELISA) test. Results showed that the mean plasma concentration of thromboxane B(2) was reduced by 7%, 34%, and 35%, and that of PGE(2) by 14%, 10%, and 19%, at 1, 2, and 3 hours, respectively, after honey ingestion. The level of PGF (2alpha) was decreased by 31% at 2 hours and 14% at 3 hours after honey ingestion. At day 15, plasma concentrations of thromboxane B (2), PGE(2), and PGF(2a) were decreased by 48%, 63%, and 50%, respectively. It may be concluded that honey can lower the concentrations of prostaglandins in plasma of normal individuals. As taken from Al-Waili NS and Boni NS.J Med Food. 2003 Summer; 6(2):129-33. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/12935324?dopt=AbstractPlus

Safety of intravenous (i.v.) or intrapulmonary administration of different concentrations of honey and their effects on blood sugar, renal and liver function tests, bone marrow function, lipid profile, and carbon tetrachloride (CCl(4))-induced liver damage were studied. Healthy sheep of either sex, 6-8 months old, were assigned randomly into the following groups: sheep received i.v. infusion of 5% honey in normal saline at 10-day intervals for 50 days and were compared with sheep that received 5% dextrose; sheep received higher doses of honey (50 g of honey) by i.v. infusion daily for 10 days; sheep received four higher doses of honey (80 g each dose) for 2 weeks; sheep received subcutaneous injection of CCl(4) after four doses of i.v. infusion of 80 g of honey, and estimations of serum gamma-glutamyl transpeptidase (SGGT), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) were performed daily for 10 days postinjection; sheep received i.v. infusion of 40 g of honey, and blood sugar estimation was performed for 3 h at 30-min intervals after infusion and compared with sheep that received 5% dextrose; sheep received rapid i.v. injection of 40% honey or 40% dextrose, and blood sugar was estimated before and after injection; sheep received various concentrations of honey in distilled water (0.5 mL/1.5 mL, 0.75 mL/1.75 mL and 1.2 mL/2.2 mL), and blood sugar estimation was performed before and after inhalation. Results showed that i.v. or intrapulmonary administration of honey did not cause any adverse effect. Intravenous delivery of honey by slow infusion caused improvement of renal and hepatic function, bone marrow function, and lipid profile. It reduced SGOT, SGPT, triglyceride, cholesterol, blood urea nitrogen, and blood sugar and elevated serum protein, serum albumin, hemoglobin, white blood cell, and neutrophil percentage. Similar results were obtained with the use of higher doses of honey. CCl(4) caused mild elevation of SGPT and SGGT and lowering of SGOT in sheep that received repeated i.v. administration of honey before administration of CCl(4), whereas in control sheep CCl(4) caused significant elevation of all the liver enzymes. Intravenous infusion of 40 g of honey caused elevation of blood sugar for 90 min postinfusion, whereas it decreased blood sugar at 2 and 3 h postinfusion as compared with fasting blood sugar. Dextrose caused significant elevation of blood sugar at all time intervals. Similar results were obtained with the use of 10% dextrose or 80 g of honey. Addition of honey to dextrose caused less hyperglycemia as compared with dextrose alone. Acute injection of 20 mL of 40% dextrose significantly elevated blood sugar for 3 h postinjection, whereas little elevation in blood sugar was obtained after injection of 40% honey; the difference between honey and dextrose was significant. Inhalation of honey caused significant lowering of blood sugar during and after inhalation as compared with fasting blood sugar and water inhalation. The effect was greater with a higher concentration of inhaled honey. It might be concluded that slow i.v. infusion or rapid i.v. injection of honey in different concentrations was safe and could lower blood sugar and improve renal, hepatic, and bone marrow functions and lipid profile. Intravenous honey had a hepatoprotective effect against CCl(4)-induced liver injury. Inhaled honey was safe and reduced blood sugar significantly. As taken from Ai_Waili NS. J Med Food. 2003a. Fall; 6(3):231-47. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed?term=14585190

“Colon cancer has been a major problem worldwide. Kelulut honey (KH) is produced by the stingless bees from Trigona species and has strong antioxidant activities that could be one of the potential chemopreventive agents from natural resources. Aim of This Study. This study

investigated the chemopreventive properties and toxicity of KH in Sprague Dawley rats induced with azoxymethane (AOM). Material and Method. Twenty-four male Sprague Dawley rats aged 5 weeks were divided into 4 groups: (G1) untreated group not induced with AOM, (G2) untreated group induced with AOM, (G3) treated group induced with AOM, and (G4) treated group not induced with AOM. Injection of AOM (15 mg/kg) was via intraperitoneal route once a week for two subsequent weeks. The treatment groups were given oral administration of KH (1183 mg/kg body weight) twice daily for 8 weeks. Results. Treatment with KH significantly reduced the total number of aberrant crypt foci (ACF) and aberrant crypts (AC) and crypt multiplicity. KH was not toxic to the animals since the level of blood profile parameters, liver enzymes, and kidney functions was in normal range. Conclusions. The current finding shows that KH has chemopreventive properties in rats induced with colorectal cancer and also was found not toxic towards the animals." As taken from Saiful Yazan L et al. 2016. Biomed. Res. Int. 2016, 4036926. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27525267

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	280 gm/kg/3W	Liver - other changes Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - multiple enzyme effects	TROEF9 Toxicology Reports. (Elsevier Inc.) V.1-2014- Volume(issue)/page/year: 6,875,2019

As taken from RTECS, 2020

5.3. Reproduction toxicity

Treatment of male albino rats with 5% honey for 20 days had no significant effect on total body weight or on the relative weight of other organs like the testis, seminal vesicles, spleen, kidneys, liver, heart, or brain. The only significant change was a 17% increase in the relative weight of the epididymis (P < or = .01). The relative weight of all the other organs was similar to those in control animals treated for the same period with drinking water. Treatment of rats for the same period with the same concentration of 5% sucrose produced no significant changes in absolute or relative weight of tested organs compared to control animals. The same treatment with Palestinian honey increased significantly the epididymal sperm count by 37% (P < or = .05). The activity of testicular marker enzymes for spermatogenesis such as sorbitol dehydrogenase (SDH) was increased by 31% (P < or = .05), and lactate dehydrogenase (LDH) was reduced by 48% (P < or = .05), which indicates that treatment with honey induces spermatogenesis. Similar treatment with sucrose had no significant effect on any of the key enzymes or epididymal sperm count. In conclusion, our results show that ingestion of honey induces spermatogenesis in rats by increasing epididymal sperm count, increasing selectively the relative weight of the epididymis, and increasing SDH activity and reducing LDH activity (Abdul-Ghani et al. 2008. Journal of Medicinal Food 11, 799-802). As taken from http://www.ncbi.nlm.nih.gov/pubmed/19053876?dopt=AbstractPlus

"BACKGROUND: To investigate the potential protective effects of Tualang honey against the toxicity effects induced by Bisphenol A (BPA) on pubertal development of ovaries. METHODS: This study was conducted on pre-pubertal female Sprague Dawley rats. Animals were divided into four groups (n = 8 in each group). Group I was administered with vehicle 0.2 ml of corn oil (Sigma-Aldrich, USA) using oral gavage daily for six weeks; these animals served as negative control (CO group), Group II was administered with BPA suspended in corn oil at 10 mg/kg body weight and served as positive control (PC group), Group III was administered with 200 mg/kg body weight of

Tualang honey 30 min before the administration of BPA at 10 mg/kg (TH group) while Group IV was administered with 200 mg/kg body weight of Tualang honey 30 min before the administration of corn oil (THC group). Body weight of all animals were monitored weekly. RESULTS: The BPA-exposed animals exhibited disruption of their estrus cycle, while those animals treated with BPA together with Tualang honey, exhibited an improvement in percentage of normal estrous cycle. Their ovaries had lower numbers of atretic follicles compared to the PC group but higher than the CO group. CONCLUSIONS: Tualang honey has a potential role in reducing BPA-induced ovarian toxicity by reducing the morphological abnormalities of the ovarian follicles and improving the normal estrous cycle." As taken from Zaid SS et al. 2014. BMC Complement. Altern. Med. 14, 509. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25519484

"Exposure to prenatal stress is associated with impaired reproductive function in male rat offspring. Honey is traditionally used by the Malays for enhancement of fertility. The aim of this study was to determine the effect of honey on reproductive system of male rat offspring exposed to prenatal restraint stress. Dams were divided into four groups (n = 10/group): control, honey, stress and honey + stress groups. Dams from honey and honey + stress groups received oral honey (1.2 g kg(-1) body weight) daily from day 1 of pregnancy, meanwhile dams from stress and honey + stress groups were subjected to restraint stress (three times per day) from day 11 of pregnancy until delivery. At 10 weeks old, each male rat offspring was mated with a regular oestrus cycle female. Male sexual behaviour and reproductive performance were evaluated. Then, male rats were euthanised for assessment on reproductive parameters. Honey supplementation during prenatal restraint stress significantly increased testis and epididymis weights as well as improved the percentages of abnormal spermatozoa and sperm motility in male rat offspring. In conclusion, this study might suggest that supplementation of honey during pregnancy seems to reduce the adverse effects of restraint stress on reproductive organs weight and sperm parameters in male rat offspring." As taken from Haron MN and Mohamed M. 2016. Andrologia 48(5), 525-31. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26289766

"Background and Objective: Honey is comprise high amount of variety of simple sugars which might serve nutrition to sperm cells. The objective of this study was to evaluate the effects of honey addition into the extender on the quality of frozen thawed in Bali bull spermatozoa. Materials and Methods: A total of four Bali cattle bulls were used in this study. Honey solution was added at the concentration of 0.1, 0.2, 0.3 and 0.4% to bovine semen cryoprotective medium. The cryoprotective extender (skim milk-egg yolk) for the control group was the same as that for the treatment groups except that it was not supplemented with honey solution. Sperm parameters were assessed including motility, abnormality and viability. The data were statistically analyzed pre and post-thawing. Results: The results indicated that percentage of the sperm motility before freezing was significantly lower ($p>0.05$) among control and treatment groups. Furthermore, the percentage of the abnormality and viability were no significantly different ($p>0.05$) among control and treatment groups. The sperm abnormality frozen thawed was significantly higher ($p<0.05$) between control and treatment groups. Whereas, the percentage of the motility and viability of frozen thawed was no significantly different ($p>0.05$) among control and treatment groups. Conclusion: It is concluded that honey supplementation into the extender was significantly effect on the sperm motility before freezing and sperm abnormality on the frozen thawed." As taken from Malik A. 2018. Asian Journal of Animal and Veterinary Advances 13(2), 109-113. Available at http://docsdrive.com/pdfs/academicjournals/ajava/2018/109-113.pdf

5.4. Mutagenicity

In vitro					
Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**

Salmonella typhimurium TA98	Ames test with honey at up to 20 mg/ml (seven different types). Also antimutagenicity study with a known mutagen (Trp-P-1)	Mutation	With S9	-ve (for all seven honeys) All honeys were antimutagenic [Limited study: guidelines recommend the use of at least 4 strains, with and without S9]	Wang et al. 2002

Honey has been used since ancient times as a flavorful sweetener and for its therapeutic and medicinal effects. Consumers' demand for natural, healthy products has driven renewed interest in honey's health benefits. The commonly encountered food mutagen, Trp-p-1, has been demonstrated to be mutagenic in bacteria and carcinogenic in animals. Chemically, honey is quite complex. Honey is comprised primarily of sugars; however, it contains many other potentially biologically active components, such as antioxidants. Sugars have been reported to display both mutagenic and antimutagenic effects in different systems; antioxidants often display antimutagenic activity. Little information exists about potential antimutagenic effects of honey. Antimutagenicity of honeys from seven different floral sources against Trp-p-1 was tested via the Ames assay and compared to that of a sugar analogue and to individually tested simple sugars. All honeys exhibited significant inhibition of Trp-p-1 mutagenicity; most demonstrated a linear correlation between percentage inhibition and log transformed honey concentration from 10 microg/mL to 20 mg/mL. Each displayed significant degrees of inhibition of mutagenicity above concentrations of 1 mg/mL, with individual variations in degree of effectiveness. Buckwheat honey displayed the greatest inhibition at 1 mg/mL, with slightly less effectiveness at higher concentrations. A sugar analogue demonstrated a pattern of inhibition similar to that of the honeys, with enhanced antimutagenicity at concentrations greater than 1 mg/mL. Glucose and fructose were also similar to honeys and were more antimutagenic than maltose and sucrose. As taken from Wang XH eta l., J Agric Food Chem. 2002 Nov 6; 50(23):6923-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/12405798

Honey, both unifloral (Syzygiumcumini) and bifloral, demonstrated strong antimutagenicity against physical (UV, γ) and chemical (ethylmethane sulfonate) mutagens as ascertained by rpoB/RifR and Ames tests. The effect of honey was evaluated in radiation (UV or γ) exposed Escherichia coli cells for SOS response, a well known error prone repair pathway known to significantly contribute to mutagenicity by quantifying LexA repressor level, measuring cell filamentation frequency, and prophage induction by SIVET (Selectable--In-Vivo Expression Technology) assay. LexA was almost completely degraded, phenotypically long filamentous cells (~30 μm) were formed, and SIVET induction frequency was increased in radiation exposed E. coli cultures, however, these changes were significantly inhibited in presence of honey confirming its strong antimutagenic nature. Further, rpoB/RifR mutation frequency upon UV exposure in E. coli recA- cells was found to be negligible, whereas, E. coliumuC- and umuD- knockouts showed comparatively higher mutation frequency. Honey did not show any effect on mutagenesis in these knockouts, indicating the SOS dependence of the observed mutagenesis. Honey was also found to suppress EMS induced mutagenesis but through SOS independent mechanism. Phenolics present in honey were found to be one of the important factors contributing to the antimutagenicity of honey (Saxena et al. 2012. Food and Chemical Toxicology 50, 625-633). As taken from http://www.ncbi.nlm.nih.gov/pubmed/22269905?dopt=AbstractPlus

Mutagenicity, the ability to induce genetic mutation, is interlinked with carcinogenicity [78]. Honey is shown to have a strong antimutagenic agent and hence has anticarcinogenic property [79]. The effect of honey on radiation (UV orγ) exposedEscherichia colicells shows SOS response (SOS is an error prone repair pathway contributing to mutagenicity) [79]. A study was performed to knock out some important genes such asumuC,recA,andumuDinvolved in SOS mediated mutagenesis. These changes are significantly inhibited in the presence of honey confirming its strong antimutagenic effect [79]. Honeys from different floral origins exhibit inhibition of Trp-p-1 mutagenicity [11]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

“The Malaysian Tualang honey (TH) is not only cytotoxic to human breast cancer cell lines but it has recently been reported to promote the anticancer activity induced by tamoxifen in MCF-7 and MDA-MB-231 cells suggesting its potential as an adjuvant for the chemotherapeutic agent. However, tamoxifen produces adverse effects that could be due to its ability to induce cellular DNA damage. Therefore, the study is undertaken to determine the possible modulation of the activity of 4-hydroxytamoxifen (OHT), an active metabolite of tamoxifen, by TH in non-cancerous epithelial cell line, MCF-10A, in comparison with MCF-7 cells. MCF-7 and MCF-10A cells were treated with TH, OHT or the combination of both and cytotoxicity and antiproliferative activity were determined using LDH and MTT assays, respectively. The effect on cellular DNA integrity was analysed by comet assay and the expression of DNA repair enzymes was determined by Western blotting. OHT exposure was cytotoxic to both cell lines whereas TH was cytotoxic to MCF-7 cells only. TH also significantly decreased the cytotoxic effect of OHT in MCF-10A but not in MCF-7 cells. TH induced proliferation of MCF10A cells but OHT caused growth inhibition that was abrogated by the concomitant treatment with TH. While TH enhanced the OHT-induced DNA damage in the cancer cells, it dampened the genotoxic effect of OHT in the non-cancerous cells. This was supported by the increased expression of DNA repair proteins, Ku70 and Ku80, in MCF-10A cells by TH. The findings indicate that TH could afford protection of non-cancerous cells from the toxic effects of tamoxifen by increasing the efficiency of DNA repair mechanism in these cells.” As taken from Yaacob NS and Ismail NF. 2014. BMC Complement. Altern. Med. 14, 106. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24646375

“Effect of Acacia honey from north-west Nigeria on sodium arsenite-induced oxidative damage and clastogenicity in male Wistar rats was investigated. Animals were divided into four groups and were treated daily via oral gavage for one week before they were sacrificed. Brain, liver and blood serum were collected for antioxidant and protein assays. Clastogenicity, in vitro antioxidant activity, vitamins and minerals were also evaluated. From the results, co-administration of Acacia honey with sodium arsenite on the animals increased ($P < 0.05$) glutathione peroxidase, superoxide dismutase and catalase activities with concomitant decrease in malondialdehyde levels and anti-clastogenic effects relative to the group treated with sodium arsenite only. The honey possesses reducing power, high hydrogen peroxide scavenging activity, good amount of vitamins (A, C and E), flavonoids (5.08 ± 0.92 mg QE/100 g) and phenolics (5.40 ± 0.69 mg GAE/100 g). The minerals present include zinc, iron, sodium, magnesium, potassium and calcium. In conclusion, Acacia honey from Nigeria may mitigate oxidative stress and clastogenicity.” As taken from Muhammed A et al. 2015. Nat. Prod. Res. 29(4), 321-6. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25105348

“Various samples of raw (unprocessed) floral honey collected from different geographical locations of India were assayed for its antimutagenicity against ethyl methanesulfonate in E. coli MG1655 cells through rifampicin resistance assay. A monofloral honey ("Pongammia pinnata", local name "Karanj") displayed maximum antimutagenicity (78.0 ± 1.7; P ≤ 0.05). Solid phase extraction (using Amberlite XAD-2 resin) followed by HPLC resulted into different peaks displaying varying antimutagenicity. Peak at retention time (Rt) 27.9 min (henceforth called P28) displayed maximum antimutagenicity and was further characterized to be abscisic acid (ABA) using ESI-MS and NMR. Its antimutagenicity was reconfirmed through human lymphoblast cell line (TK6) mutation assay using thymidine kinase (tk+/-) cell line. Although ABA from this honey displayed strong antimutagenicity, it lacked any in vitro antioxidant capacity indicating noninvolvement of any radical scavenging in the observed antimutagenicity.” As taken from Saxena S et al. 2017. J. Agric. Food Chem. 65(23), 4624-4633. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28535345

Rhododendron honey (RH) is obtained from the rhododendron plants are grown in many regions around the world, causes poisoning in humans due to the grayanotoxin (GTX) compound in its structure. It is used by the public as a therapeutic for some diseases. It was aimed to study the genotoxic and cytotoxic effects of RH in mouse bone-marrow and sperm cells by using three mammalian bioassays. 25, 50 and 75 mg kg^{-1} concentrations of RH given to male mice via gavage for 24 and 48 h treatment periods and its active ingredient Grayanatoxin (GTX-III) 0.01 mg kg^{-1} by i.p. injection. Chromosome aberrations (CA), polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE), micronucleated polychromatic erythrocytes (MNPCE) and sperm abnormalities were investigated. The results demonstrated that all the tested concentrations of RH significantly induced total abnormal cell frequency including chromosomal breaks for two time periods. In the MN assay, 75 mg kg^{-1} RH and 0.01 mg kg^{-1} GTX-III significantly increased % MNPCE and significantly reduced PCE/NCE ratios after 24 and 48 h treatments on mice demonstrating potential genotoxic and cytotoxic effect. Although there was a concentration-related increase in the percentage of total sperm abnormalities, this increase was not statistically significant compared to control. As a result, microscopic genotoxicity and cytotoxicity marker tests showed that RH and its active ingredient GTX-III have potential genotoxic and cytotoxic effect on mice bone marrow cells. It is understood that RH that is used to treat some diseases by public, should be handled carefully and used in a controlled manner.HighlightsChromosome aberration, micronucleus and sperm morphology assays are recommended as reliable biological indicators.RH and its active ingredient GTX-III have potential genotoxic and cytotoxic effect on mice bone marrow cells.Significant changes were observed upon the treatment of 75 mg kg^{-1} MH for MN assay. As taken from Pinar Goc Rasgele et al PubMed, 2021 Available at

5.5. Cytotoxicity

Record for Tualang honey (no CAS RN):

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - breast tumor	2.4 pph	In Vitro Toxicity Studies - cell membrane integrity: cytoplasmic enzymes leakage (lactate dehydrogenase, ATP enzymes etc.)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011

IC50 - Inhibitor Concentration 50	In vitro	Human - HeLa cell	2.4 pph	In Vitro Toxicity Studies - cell membrane integrity: cytoplasmic enzymes leakage (lactate dehydrogenase, ATP enzymes etc.)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011
ICLo - Inhibitor Concentration Low	In vitro	Human - breast tumor	2.4 pph/24H	In Vitro Toxicity Studies - apoptosis in vitro Biochemical - Metabolism (Intermediary) - effect on mitochondrial function	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011
ICLo - Inhibitor Concentration Low	In vitro	Human - HeLa cell	2.4 pph/24H	In Vitro Toxicity Studies - apoptosis in vitro Biochemical - Metabolism (Intermediary) - effect on mitochondrial function	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011
ICLo - Inhibitor Concentration Low	In vitro	Human - breast tumor	2.4 pph/6H	In Vitro Toxicity Studies - apoptosis in vitro	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011
ICLo - Inhibitor Concentration Low	In vitro	Human - HeLa cell	2.4 pph/6H	In Vitro Toxicity Studies - apoptosis in vitro	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,871,2011

As taken from RTECS, 2012.

“BACKGROUND: Current evidence supports that consumption of polyphenols has beneficial effects against numerous diseases mostly associated with their antioxidant activity. Honey is a good source of antioxidants since it contains a great variety of phenolic compounds. OBJECTIVE: The main objective of this work was to investigate the antiproliferative and apoptotic effects of three crude commercial honeys of different floral origin (heather, rosemary and polyfloral honey) from Madrid Autonomic Community (Spain) as well as of an artificial honey in human peripheral blood

promyelocytic leukemia cells (HL-60). MATERIAL AND METHODS: HL-60 cells were cultured in the presence of honeys at various concentrations for up to 72 hours and the percentage of cell viability was evaluated by MTT assay. Apoptotic cells were identified by chromatin condensation and flow cytometry analysis. ROS production was determined using 2´,7´-dichlorodihydrofluorescein diacetate (H2DCFDA). RESULTS: The three types of crude commercial honey induced apoptosis in a concentration and time dependent-manner. In addition, honeys with the higher phenolic content, heather and polyfloral, were the most effective to induce apoptosis in HL-60 cells. However, honeys did not generate reactive oxygen species (ROS) and N-acetyl-L-cysteine (NAC) could not block honeys-induced apoptosis in HL-60 cells. CONCLUSION: These data support that honeys induced apoptosis in HL-60 cells through a ROS-independent cell death pathway. Moreover, our findings indicate that the antiproliferative and apoptotic effects of honey varied according to the floral origin and the phenolic content.As taken from Morales P & Haza AI. 2013. Pharmacogn. Mag. 9(35), 231-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23930007

“Lung cancer is one of the leading causes of death worldwide. We investigated the molecular mechanism of antiproliferation potential of Acacia honey on NCI-H460 cells by cell cycle, viability, cytokines, calcium ion and gene expression analysis. Acacia honey inhibited cells proliferation, arrested G0/G1 phase, stimulated cytokines, calcium ion release as well as suppressed p53 and Bcl-2 expression in a dose-dependent manner. We proposed that the molecular mechanism of the antiproliferation potential of Acacia honey on NCI-H460 cell line is due to cell cycle arrest, stimulation of cytokines and calcium ion as well as downregulation of Bcl-2 and p53 genes.” As taken from Aliyu M et al. 2013. Nutr. Cancer 65(2), 296-304. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23441617

“Manuka honey has been recognized for its anti-bacterial and wound-healing activity but its potential antitumor effect is poorly studied despite the fact that it contains many antioxidant compounds. In this study, we investigated the antiproliferative activity of manuka honey on three different cancer cell lines, murine melanoma (B16.F1) and colorectal carcinoma (CT26) as well as human breast cancer (MCF-7) cells in vitro. The data demonstrate that manuka honey has potent anti-proliferative effect on all three cancer cell lines in a time- and dose-dependent manner, being effective at concentrations as low as 0.6% (w/v). This effect is mediated via the activation of a caspase 9-dependent apoptotic pathway, leading to the induction of caspase 3, reduced Bcl-2 expression, DNA fragmentation and cell death. Combination treatment of cancer cells with manuka and paclitaxel in vitro, however, revealed no evidence of a synergistic action on cancer cell proliferation. Furthermore, we utilized an in vivo syngeneic mouse melanoma model to assess the potential effect of intravenously-administered manuka honey, alone or in combination with paclitaxel, on the growth of established tumors. Our findings indicate that systemic administration of manuka honey was not associated with any alterations in haematological or clinical chemistry values in serum of treated mice, demonstrating its safety profile. Treatment with manuka honey alone resulted in about 33% inhibition of tumor growth, which correlated with histologically observable increase in tumor apoptosis. Although better control of tumor growth was observed in animals treated with paclitaxel alone or in combination with manuka honey (61% inhibition), a dramatic improvement in host survival was seen in the co-treatment group. This highlights a potentially novel role for manuka honey in alleviating chemotherapy-induced toxicity.” As taken from Fernandez-Cabezudo MJ et al. 2013. PLoS One 8(2), e55993. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23409104

Honey induces apoptosis in various types of cancer cells via depolarization of mitochondrial membrane [19]. Honey elevates caspase 3 activation level and poly (ADP-ribose) polymerase (PARP) cleavage in human colon cancer cell lines [20] which is attributed to its high tryptophan and phenolic content [20]. It also induces apoptosis by upregulating and modulating the expression of pro- and antiapoptotic proteins in colon cancer cell lines [23]. Honey increases the expression of caspase 3, p53, and proapoptotic protein Bax and downregulates the expression of antiapoptotic

protein Bcl2 [23] (Figure 2). Honey generates ROS (reactive oxygen species) resulting in the activation of p53 and p53 in turn modulates the expression of pro- and antiapoptotic proteins like Bax and Bcl-2 [23]. Honey as an adjuvant therapy with Aloe vera boosts the expression of proapoptotic protein Bax and decreases the antiapoptotic protein Bcl-2 expression in Wistar rats [14]. Manuka honey exerts its apoptotic effect on cancer cells through the induction of the caspase 9 which in turn activates the caspase-3, the executor protein. Apoptosis induced by Manuka also involves induction of DNA fragmentation, activation of PARP, and loss of Bcl-2 expression [31]. The apoptotic property of honey makes it a possible natural substance as antic-cancer agent as many chemotherapeutics currently used are apoptosis inducers. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

Honey has been shown to affect cell cycle arrest. Administration of honey mixed with Aloe vera solution showed a marked decrease in expression of Ki67-LI in tumor cells in rats [14]. It suggests that honey therapy could lead to lowering tumor cell proliferation by arresting cell cycle [14].Honey and its several components (like flavonoids and phenolics) are reported to block the cell cycle of colon [20], glioma [34], and melanoma [35] cancer cell lines in G0/G1 phase. This inhibitory effect on tumor cell proliferation follows the downregulation of many cellular pathways via tyrosine cyclooxygenase, ornithine decarboxylase, and kinase [20, 34– 36]. The results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and the trypan blue exclusion assays have confirmed that anti-proliferative effect of honey is a dose- and time-dependent manner [35]. Honey or its components mediate inhibition of cell growth due to its perturbation of cell cycle [35, 36]. Cell cycle is also regulated by p53 which is involved in tumor suppression. Honey is reported to be involved in modulation of p53 regulation [20]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

Royal jelly (RJ) proteins (apalbumin-1 and apalbumin-2) in honey have antitumor properties. These proteins stimulate macrophages to release cytokines TNF-α, interleukin-1(IL-1) and interlueken-6 (IL-6) [41, 42]. Pasture, jelly bush, and Manuka honeys (at concentrations of 1% w/v) stimulate monocytes to release tumor necrosis factor-alpha and interleukin- (IL-) 1β and IL-6 [43, 44]. The possible mechanism involves the binding of TNF-R to TNF-α and adaptor protein such as TNFR associated death domain protein (TRADD), TNF receptor associated factor (TRAF), and receptor-interacting protein (RIP) to regulate apoptosis and inflammation through these cytokines [45]. This TNF-α release can play a pivotal role as a key cytokine to regulate important cellular processes such as apoptosis, cell proliferation, and inflammation [41, 45]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

The antitumor effect of honey may be attributed to its antioxidant activity [75,76]. An enhanced antioxidant status with apoptosis has been observed inhepatocellular carcinoma cells[75]. Daily consumption of 1.2 g/kg body weight of honey has been shown to elevate the amount and the activity of antioxidant agents such as beta-carotene, vitamin C, glutathione reductase, and uric acid [60]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

“Type 2 diabetes consists of progressive hyperglycemia, insulin resistance, and pancreatic β-cell failure which could result from glucose toxicity, inflammatory cytokines, and oxidative stress. In the present study, we investigate the effect of pretreatment with Gelam honey (Melaleuca spp.) and the individual flavonoid components chrysin, luteolin, and quercetin, on the production of reactive oxygen species (ROS), cell viability, lipid peroxidation, and insulin content in hamster pancreatic cells (HIT-T15 cells), cultured under normal and hyperglycemic conditions. Phenolic extracts from a local Malaysian species of Gelam honey (Melaleuca spp.) were prepared using the standard extraction methods. HIT-T15 cells were cultured in 5 % CO2 and then preincubated with Gelam honey extract (20, 40, 60, and 80 μg/ml) as well as some of its flavonoid components chrysin, luteolin, and quercetin (20, 40, 60, and 80 μM), prior to stimulation by 20 and 50 mM of glucose. The antioxidative effects were measured in these cultured cells at different concentrations and time point by DCFH-DA assay. Pretreatment of cells with Gelam honey extract or the flavonoid components prior to culturing in 20 or 50 mM glucose showed a significant decrease in the production of ROS, glucose-induced lipid peroxidation, and a significant increase in insulin content and the viability of cells cultured under hyperglycemic condition. Our results show the in vitro antioxidative property of the Gelam honey and the flavonoids on the β-cells from hamsters and its cytoprotective effect against hyperglycemia.” As taken from Batumalaie K et al. 2014. Clin. Exp. Med. 14, 185-195. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23584372

“The Malaysian Tualang honey (TH) is not only cytotoxic to human breast cancer cell lines but it has recently been reported to promote the anticancer activity induced by tamoxifen in MCF-7 and MDA-MB-231 cells suggesting its potential as an adjuvant for the chemotherapeutic agent. However, tamoxifen produces adverse effects that could be due to its ability to induce cellular DNA damage. Therefore, the study is undertaken to determine the possible modulation of the activity of 4-hydroxytamoxifen (OHT), an active metabolite of tamoxifen, by TH in non-cancerous epithelial cell line, MCF-10A, in comparison with MCF-7 cells. MCF-7 and MCF-10A cells were treated with TH, OHT or the combination of both and cytotoxicity and antiproliferative activity were determined using LDH and MTT assays, respectively. The effect on cellular DNA integrity was analysed by comet assay and the expression of DNA repair enzymes was determined by Western blotting. OHT exposure was cytotoxic to both cell lines whereas TH was cytotoxic to MCF-7 cells only. TH also significantly decreased the cytotoxic effect of OHT in MCF-10A but not in MCF-7 cells. TH induced proliferation of MCF10A cells but OHT caused growth inhibition that was abrogated by the concomitant treatment with TH. While TH enhanced the OHT-induced DNA damage in the cancer cells, it dampened the genotoxic effect of OHT in the non-cancerous cells. This was supported by the increased expression of DNA repair proteins, Ku70 and Ku80, in MCF-10A cells by TH. The findings indicate that TH could afford protection of non-cancerous cells from the toxic effects of tamoxifen by increasing the efficiency of DNA repair mechanism in these cells.” As taken from Yaacob NS and Ismail NF. 2014. BMC Complement. Altern. Med. 14, 106. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24646375

“BACKGROUND: Cancer is one of the major fatal human diseases. Natural products have been used in the treatment of cancer for long time. Bee products including honey and propolis have been introduced for malignancy treatment in recent decades. In this study cytotoxicity of bee products and their effects on the expression of proapoptotic genes have been investigated. MATERIALS AND METHODS: Cytotoxic effects of Astragalus honey, ethanol extract of propolis and a sugar solution (as control) against HepG2, 5637 and L929 cell lines have been evaluated by the MTT assay. Total RNAs of treated cells were isolated and p53 and Bcl-2 gene expression were evaluated, using real-time PCR. RESULTS: Propolis IC50 values were 58, 30 and 15 μg/ml against L929, HepG2 and 5637, respectively. These values for honey were 3.1%, 2.4% and 1.9%, respectively. Propolis extract has increased the expression of the Bcl-2 gene in all cell lines whereas the honey decreased that significantly ($P < 0.05$). Also, we found that honey and propolis decreased p53 gene expression in HepG2 and 5637 significantly but not in L929 cells. The sugar solution increased the expression of p53 in two cancer cell lines but no significant changes were observed in the expression of this gene in L929 as normal mouse cell. CONCLUSION: By

downregulation of Bcl-2 expression it could be concluded that the cytotoxicity of honey was more than two fold against tested cancer cells compared with the sugar solution. No significant changes were observed in the expression of p53 in honey-treated cells. Propolis had no significant effect on Bcl-2 and p53 gene expressions (P > 0.05)." As taken from Sadeghi-Aliabadi H et al. 2015. Adv. Biomed. Res. 4, 42. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25789268

"Honey is a natural product known to modulate several biological activities including cancer. The aim of the present study was to examine the phytochemical content and the antioxidant activity of Strawberry tree (Arbutus unedo) honey (STH) and its cytotoxic properties against human colon adenocarcinoma (HCT-116) and metastatic (LoVo) cell lines in comparison with Manuka (Leptospermum scoparium) honey (MH). Several unifloral STH and MH were analyzed for their phenolic, flavonoid, amino acid and protein contents, as well as their radical scavenging activities. STH from the Berchidda area showed the highest amount of phenolic, flavonoid, amino acid and protein content, and antioxidant capacity compared to MH. Both STH and MH induced cytotoxicity and cell death in a dose- and time-dependent manner in HCT-116 and LoVo cells, with less toxicity on non-cancer cells. Compared to MH, STH showed more effect at lower concentrations on HCT-116 and LoVo cells. In addition, both honeys increased intracellular reactive oxygen species (ROS) generation. In HCT-116 cells, STH and MH induced similar ROS production but in LoVo cells STH induced a higher percentage of ROS compared to MH. Our results indicate that STH and MH can induce cell growth inhibition and ROS generation in colon adenocarcinoma and metastatic cells, which could be due to the presence of phytochemicals with antioxidant properties. These preliminary results are interesting and suggest a potential chemopreventive action which could be useful for further studies in order to develop chemopreventive agents for colon cancer." As taken from Afrin S et al. 2017. Int. J. Mol. Sci. 18(3), E613. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28287469

"Soft-tissue invasive fungal infections are increasingly recognized as significant entities directly contributing to morbidity and mortality. They complicate clinical care, requiring aggressive surgical debridement and systemic antifungal therapy. To evaluate new topical approaches to therapy, we examined the antifungal activity and cytotoxicity of Manuka Honey (MH) and polyhexamethylene biguanide (PHMB). The activities of multiple concentrations of MH (40%, 60%, 80%) and PHMB (0.01%, 0.04%, 0.1%) against 13 clinical mould isolates were evaluated using a time-kill assay between 5 min and 24 h. Concentrations were selected to represent current clinical use. Cell viability was examined in parallel for human epidermal keratinocytes, dermal fibroblasts and osteoblasts, allowing determination of the 50% viability (LD50) concentration. Antifungal activity of both agents correlated more closely with exposure time than concentration. Exophiala and Fusarium growth was completely suppressed at 5 min for all PHMB concentrations, and at 12 and 6 h, respectively, for all MH concentrations. Only Lichtheimia had persistent growth to both agents at 24 h. Viability assays displayed concentration-and time-dependent toxicity for PHMB. For MH, exposure time predicted cytotoxicity only when all cell types were analyzed in aggregate. This study demonstrates that MH and PHMB possess primarily time-dependent antifungal activity, but also exert in vitro toxicity on human cells which may limit clinical use. Further research is needed to determine ideal treatment strategies to optimize antifungal activity against moulds while limiting cytotoxicity against host tissues in vivo." As taken from Yabes JM et al. 2017. Med. Mycol. 55(3), 334-343. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27601610

"BACKGROUND: Antioxidant and anti-inflammatory properties of honey have been largely recognized by various studies. Almost all of the potential benefits are associated with polyphenol content. Honey varieties from the arid region are reported to be rich in polyphenols, but data related to its bioactivity in vitro is greatly lacking. This study aimed at establishing the antioxidant and anti-inflammatory properties of arid region honey. Four honey varieties from arid region (H1, H2, H3, and H4) and two popular non-arid region honey (H5 and H6) were tested in vitro in this study. METHODS: The erythrocyte membrane protection effect of honey varieties were measured by hemolysis assay after exposing erythrocytes to a peroxide generator. The subsequent production of MDA (malondialdehyde) content in erythrocytes was measured. Immunomodulatory effect of the

honey varieties was tested in prostate cancer cells PC-3 and PBMC (peripheral blood mononuclear cells) by measuring the IL-6 (interleukin 6) and NO (nitric oxide) levels in cell culture supernatant after incubation with the honey varieties. PC-3 cell viability was assessed after incubation with honey varieties for 24 h. RESULTS: Arid region honey exhibited superior erythrocyte membrane protection effect with H4 measuring 1.3 ± 0.042mMTE/g and H2 measuring 1.122 ± 0.018mMTE/g. MDA levels were significantly reduced by honey samples, especially H4 (20.819 ± 0.63 nmol/mg protein). We observed a significant decrease in cell population in PC-3 after 24 h in culture on treatment with honey. A moderate increase in NO levels was observed in both cultures after 24 h at the same time levels of IL-6 were remarkably reduced by honey varieties. CONCLUSION: The results demonstrate the antioxidant effect of arid region honey due to its erythrocyte membrane protection effect and subsequent lowering of oxidative damage as evident from lower levels of lipid peroxidation byproduct MDA. Arid region honey varieties were as good as non-arid region types at decreasing cell viability of prostate cancer cells. The moderate increase in NO levels in PC-3 and PBMCs were not significant enough to elicit any pro-inflammatory response. However, IL-6 secretion was remarkably reduced by all honey varieties in a comparable level indicating the potential anti-inflammatory property of arid region honey." As taken from Hilary S et al. 2017. BMC Complement. Altern. Med. 17(1), 177. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28356100

"Honey is a complex biological substance, consisting mainly of sugars, phenolic compounds and enzymes. Using five quick and accessible assays for measuring honey's cytotoxicity in vitro, we found honey is cytotoxic towards prostate cancer cells PC3 and DU145. However, the level of cell death varied with assay. The MTT assay was confounded by the reduction of the MTT reagent by honey's reducing sugars and phenolic compounds, and the lactate dehydrogenase assay was invalidated by honey oxidising the enzyme cofactor NADH. The sulforhodamine B assay gave valid results, but measures only protein content, providing no information about cell death in the remaining cells. The trypan blue assay and a microscope-based propidium iodide/Hoechst staining assay assess only late stage membrane permeability. However, the propidium iodide/Hoechst assay gives morphological information about cell death mechanism. A combination of the sulforhodamine B and propidium iodide/Hoechst assays would provide the most accurate quantification of honey cytotoxicity." As taken from Abel SDA and Baird SK. 2018. Food Chem. 241, 70-78. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28958561

"Natural products with bioactive components are widely studied on various cancer cell lines for their possible cytotoxic effects, recently. Among these products, honey stands out as a valuable bee product containing many active phenolic compounds and flavonoids. Numerous types of multifloral honey and honeydew honey are produced in Turkey owing to its abundant vegetation. Therefore, in this study, we investigated the cytotoxic effects of particular tree-originated honeys from chestnut, cedar, pine, and multifloral honey on cell lines representing different types of the most common cancer of women, breast cancer, MCF7, SKBR3, and MDAMB-231, and fibrocystic breast epithelial cell line, MCF10A as a control. All honey samples were analyzed biochemically. The dose- (1, 2.5, 5, 7.5, and 10 μg/mL) and time (24th, 48th, and 72nd hours)-dependent effects of ethanol/water solutions of the honey samples were scrutinized. Cell viability/cytotoxicity was evaluated by the water soluble tetrazolium Salt-1 (WST-1) method. Apoptotic status was detected by Annexin V-PI assay using FACSCalibur. The statistical analysis was performed using GraphPad Prism 6 and the clustering data analysis with the R programming language. The biochemical analyses of the honey samples showed that the tree-originated honey samples contained more total phenolic compounds than the multifloral honey. Phenolic content of the honey types increases in order of multifloral, pine, cedar, and chestnut, respectively, which is compatible with their cytotoxic affectivity and dark color. In addition, the antioxidant capacity of the studied honey types was observed to increase in order of multifloral < pine < cedar ≅ chestnut. According to the WST-1 data, chestnut honey induced cytotoxicity over 50% on all the cell lines, including the control MCF10A cells, even with low doses (honey concentrations starting from 1 μg/mL) ($P < 0.0001$). Similarly, Cedar honey was observed to be the second most effective honey in this study. Cedar honey, with the dose of 1 μg/mL, was

detected statistically highly significant on MCF10A, MCF7, and SKBR3. In contrast, pine honey showed dramatically significant cytotoxicity only on the MDAMB 231 cells with a 1 µg/mL dose at the same time point ($P = 0.018$). While pine honey caused an anticancer effect on the MCF-7 and SKBR3 cancer cell lines with a 2.5-5 µg/mL dose ($P < 0.0001$), like cedar and chestnut honeys, it increased the viability of the MCF10A control cells with the doses of 2.5-5 µg/mL. It only showed cytotoxicity with higher doses (10 µg/mL) on the MCF10A cell line ($P < 0.0001$). Moreover, we have observed that the multifloral and artificial honey samples were mostly ineffective or increased cell viability with the doses of 1-5 µg/mL. Apoptotic effects of the other honey samples on the MCF-7 cell line were found as chestnut> pine> cedar> multifloral in the Annexin V-propidium iodide (PI) analysis. Chestnut, cedar, and pine honey displayed a remarkably cytotoxic effect on breast cancer cell lines, MCF7, SKBR3, and even on the most aggressive MDAMB 231, representing the triple negative breast cancer, which lacks of targeted anticancer therapy. The chestnut and cedar honeys stand out to be the most cytotoxic on all cell lines, while pine honey was found to be the least toxic on control cells with appropriate toxicity on the cancer cells." As taken from Seyhan MF et al. 2017. IUBMB Life 69(9), 677-688. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28695656

"Honey originating from different floral sources exhibits the broad spectrum of antibacterial activity as a result of the presence of hydrogen peroxide as well as nonperoxide bioactive compounds. The mechanisms of antibacterial activity of Polish melilot honey were investigated for the first time. Polish melilot honey samples (Melilotus albus biennial = 3 and annual = 5, Melilotus officinalis = 1) were collected directly from beekeepers and analysed for pollen profile, basic physicochemical parameters, antioxidant capacity, radical scavenging activity, total phenolic contents as well as antibacterial properties against pathogenic bacteria Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella spp. The physicochemical properties of melilot honey were specific for light-coloured unifloral honey samples and were not dependent on its botanical and geographical origin ($P > 0{\cdot}05$). All tested honey samples exhibited inhibitory activity (above 90%) against Gram-positive bacteria at the concentration of 12·5-25%. Above 30-50% of antibacterial activity of melilot honey was connected with glucose oxidase enzyme action and was destroyed in the presence of catalase. Hydrogen peroxide-dependent antibacterial activity of honey was inversely correlated with its radical scavenging activity ($r = -0{\cdot}67$) and phenolic compounds ($r = -0{\cdot}61$). Antibacterial action of melilot honey depends not only on hydrogen peroxide produced by glucose oxidase, but also on other nonperoxide bioactive components of honey. SIGNIFICANCE AND IMPACT OF THE STUDY: Melilot honey is used in traditional medicine as an anticoagulant agent due to the possibility of the presence of the coumarin compounds which are specific for Melilotus plant. Melilotus albus is rarely used to produce honey, and antibacterial properties of this variety of honey had not been studied yet. Nine samples of melilot honey produced in different regions of Poland were analysed according to their antibacterial activity which was correlated with physiochemical parameters and antioxidant activity. It was shown that antibacterial activity of melilot honey is created by hydrogen peroxide and other bioactive compounds." As taken from Sowa P et al. 2017. Lett. Appl. Microbiol. 65(1), 82-89. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28426165

"Clostridium difficile is the cause of the nosocomial C. difficile infection (CDI). The conventional antibiotics used in CDI therapy are often unsuccessful, and recurrent infections may occur. Biofilm formation by C. difficile is associated with chronic or recurrent infections; biofilms may contribute to virulence and impaired antimicrobial efficacy. Manuka honey, derived from the Manuka tree (Leptospermum scoparium), is known to exhibit antimicrobial properties that are associated with its significant content of methylglyoxal, a natural antibiotic. The aim of the present study was to determine the antimicrobial effect of Manuka honey on clinical C. difficile strains belonging to four prominent polymerase chain reaction (PCR) ribotypes (RTs) (RT017, RT023, RT027 and RT046) and on their biofilm formation in vitro. Minimal inhibitory and bactericidal concentrations (MICs and MBCs, respectively) were determined using the broth dilution method. The biomass of the biofilm and the clearance of C. difficile biofilms by Manuka honey were determined using the crystal violet

staining method. The MIC and MBC of Manuka honey for C. difficile strains were equal at 6.25% (v/v). PCR RT027 strains produced more biofilm in vitro than the other examined strains. Manuka honey effectively inhibited biofilm formation by C. difficile strains of different PCR RTs." As taken from Piotrowski M et al. 2017. Eur. J. Clin. Microbiol. Infect. Dis. 36(9), 1661-1664. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28417271

"This work was directed to study the inhibitory effects of honey collected from different geographical regions of District Khairpur against certain pathogenic bacteria. It has been observed that the valuable use of honey in the management of bacterial infection is when it can be applied directly to the bacteria without dilution. There are few published reports on the physicochemical and antibacterial characteristics of honey from A. florea, the dwarf honeybee native to Pakistan. Current study explores the variation in physicochemical properties and the level of antimicrobial potential of honey samples collected from wild bee combs of A. florea shows potential genetic diversity from District Khairpur. The acacia honey found effective to stop the growth of isolates except Proteus and Shigella. The antibacterial action of honey was attained in high concentrations of honey both in well diffusion as well as disc diffusion methods." As taken from Naheed R and Farooqi SR. 2018. Journal of Entomology and Zoology Studies 6(1), 1564-1570. Available at http://www.entomoljournal.com/archives/2018/vol6issue1/PartV/5-5-61-351.pdf

"This study was aimed to determine the antibacterial activity of honey and/or lemon juice on strains of Streptococcus pneumoniae and Streptococcus pyogenes from respiratory tract infections. Clinical isolates were collected from Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and Ahmadu Bello University Health Services (ABUHS) Samaru campus, Zaria. The isolates were characterized by standard microbiological procedures. Antibacterial activity of the honey and lemon juice, as well as that of some standard antibiotic formulations were assayed using agar well diffusion and broth dilution method. Minimum Inhibitory and Bactericidal Concentrations were carried out. Rate of kill was also carried out to determine the death/survival rate of the bacterial isolates after exposure to the agents. Noticeable variations in the antibacterial activity of the agents were observed. Thus, inhibition zones (mm) ranging from 10 - 22 (100% Honey), 14 - 29 (100% Lemon) and 20 - 29 (Honey/Lemon juice mixture) were obtained. However, Minimum Inhibitory Concentrations (µg/ml) range between 1.95-125 (Ceftriaxone), 1.56-NI (Gentamicin), 31.5-NI (Amoxicillin-Clavulanic acid), 0.98-62.5 (Levofloxacin), 50.0-NI (Azithromycin), 20.0 - 75.0 (100% v/v Honey), 22.5 - 47.5 (100% v/v Lemon juice) and 17.5 - 25.0 (Honey/Lemon juice mixture). However, for the rate of kill, Honey/Lemon juice mixture, Lemon juice effected complete killing at 120 minutes; While, Ceftriaxone, Levofloxacin and Honey produced complete killing at 1440 minutes. Therefore, from the findings, honey/lemon juice mixture, Lemon juice, Levofloxacin, Ceftriaxone and Gentamicin had higher antibacterial activity than Azithromycin, Amoxicillin-Clavulanic acid and Honey. However, for the statistical analysis, at $p \geq 0.05$, there is significant difference between honey/lemon juice mixture and honey. In conclusion, the bacterial isolates were more susceptible to honey/lemon juice mixture, lemon juice, Levofloxacin, Ceftriaxone and Gentamicin; but less susceptible to Azithromycin, Amoxicillin-Clavulanic acid and Honey. Excellent bactericidal activity was observed with honey/lemon juice mixture, lemon juice compared to the honey alone. The findings in this research therefore provides scientific basis to the use of honey and lemon juice as an alternative medicine by the populace in the treatment of respiratory tract infections." As taken from Mshelia BM et al. 2018. Acta Scientific Microbiology 1(3), 22-27. Available at https://actascientific.com/ASMI/pdf/ASMI-01-0023.pdf

"Combination of natural products with chemodrugs is becoming a trend in discovering new therapeutics approach for enhancing the cancer treatment process. In the present study, we aimed to investigate the cytotoxic and apoptosis induction of Gelam honey (GH) combined with or without 5-Fluorouracil (5-FU) on HT-29 cells. The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay to assess cytotoxicity. Morphological changes and apoptosis were determined by the inverted microscope, Annexin V-FITC, and DNA fragmentation via flow cytometric analysis, respectively. Our results demonstrate that combined treatment revealed a remarkable and concentration-dependent cytotoxic effect on HT-29 cells in comparison

with GH and 5-FU alone. Flow cytometry analysis showed that early apoptosis event was more pronounced in combined treatment. In addition, compared to 5-FU alone, apoptosis of HT-29 cells treated with combinations of GH and 5-FU demonstrated increasing percentages of fragmented DNA. Our results suggest that GH has a synergistic cytotoxic effect with 5-FU in HT-29 cell lines in vitro. Although the actions of the molecular mechanisms are not yet clear, the results reveal that the combination of GH and 5-FU could have the potential as a therapeutic agent." As taken from T-Johari SAT et al. 2019. Int. J. Cell Biol. 3059687. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30923553 [A corrigendum has been issued relating to an incorrect spelling of one of the authors of this paper. See T-Johari SAT et al. 2019. Int. J. Cell Biol. 2019, 9050626. DOI: 10.1155/2019/9050626. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6668543/

"The paper examines the antiproliferative, antimicrobial and antioxidative effects of fir (Abies alba Mill.) honeydew honey from mountain region of Croatia (Gorski kotar) as a potential replacement for standard antibiotics and chemotherapeutic agents. Cell viability, annexin V assay and flow cytometry analysis served to analyse the antiproliferative effect on, apoptosis induction in and cell death of cancer cell lines: HeLa, MCF-7, SW620, CFPAC-1, MIA PaCa-2 and normal diploid human fibroblasts (BJ). Antimicrobial activity was tested against Staphylococcus and Acinetobacter strains by agar well diffusion and microdilution assays. The DPPH-Ö assay determined the radical scavenging activity, while mathematical models helped to evaluate the kinetic data of DPPH-Ö inhibition. Antiproliferative effect on all tested cell lines and the prominent effect on normal diploid human fibroblasts (BJ), colorectal adenocarcinoma (SW620, metastatic) and breast epithelial adenocarcinoma (MCF-7, metastatic) was observable. The mechanisms of antiproliferative effect included accumulation of cells in the sub-G1 phase in all tested cells and induction of apoptosis in SW620 and MCF-7 cells predominantly. The antibacterial assays showed that antibiotic-resistant strains of both bacteria, including multi-resistant strain A. baumannii ATCC® BAA-1605™, were sensitive to all tested honey samples. Radical scavenging assay suggests that antioxidants present in the honey possess different radical suppressing abilities and that they react at different rates with radicals, thereby causing two steps of reaction. The results of the study indicate that Croatian fir honeydew honey has a therapeutic potential due to the strong biological activity and can serve to protect human health." As taken from Brozni-ç D et al. 2018. Food Technol. Biotechnol. 56(4), 533-545. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30923450

"This study was designed to evaluate and correlate the pharmacological, phytochemical, and physicochemical properties of raw unifloral Mauritian eucalyptus honey (EH) and a commercially available honey (CH). The pharmacological activity was evaluated in terms of antibacterial, antioxidant (nitric oxide scavenging), antielastase, antityrosinase, antimelanogenic, and anticancer activity (MCF-7 and HeLa cell toxicity). The presence of phytochemicals including alkaloids, flavonoids, saponins, phenols, anthraquinones, and steroids were determined along with the total phenolic (TPC), total flavonoid (TFC), and tannin content (TC). Physicochemical properties including the pH, colour, total soluble solids, and density were also investigated. The results showed that EH displayed greater antibacterial, antioxidant, and anticancer activity against the MCF-7 cell line compared to CH, which also showed higher extracellular antimelanogenic activity. MH (IC50 = 532.75 µg/ml) displayed significantly greater scavenging activity than CH (IC50 = 647.6 µg/ml). To conclude, honey may be potentially exploited as complementary and alternative therapies for the management of infectious and chronic diseases." As taken from Aumeeruddy MZ et al. 2019. Biocatalysis and Agricultural Biotechnology 18, 101005. Available at https://www.sciencedirect.com/science/article/pii/S1878818118309915 "The aim of the present study was to evaluate the effects of Strawberry tree honey (STH) on oxidative stress, metabolic phenotype, migration, invasion and epithelial-mesenchymal transition in adenocarcinoma (HCT-116) and metastatic (LoVo) colon cancer cells as well as in human dermal fibroblasts (HDF). Significant oxidative stress was observed through the increase of intracellular ROS generation, lipid and protein damage and reduction of antioxidant enzyme activities in colon cancer cells; in HDF these effects were limited or none. The expression of NF--¦B, p-I-¦Bα, Nrf2 was suppressed after

STH treatment in colon cancer cells. All the parameters of mitochondrial respiration and glycolysis were reduced after STH treatment in cancer cells, while they were unchanged in HDF. Wound-closure percentages and the expression of MMP-2, MMP-9, N-cadherin, β-catenin decreased, while those of E-cadherin increased after STH treatment in colon cancer cells. Thus, STH can be used for its potential in cancer prevention." As taken from Afrin S et al. 2019a. Journal of Functional Foods 57, 477-487. Available at https://www.sciencedirect.com/science/article/pii/S175646461930221X "The aim of this work was to assess the phytochemical composition and anticancer effects of Strawberry-tree honey (STH) on cellular proliferation, cell cycle and apoptosis in human colon adenocarcinoma (HCT-116) and metastatic (LoVo) cancer cells. Kaempferol and gallic acid were the major phenolic compounds. STH showed higher cytotoxic and anti-colonogenic effects in a time- and dose-dependent manner; it arrested cell cycle in S and G2/M and regulated cell cycle genes, such as cyclin D1, cyclin E, CDK2, CDK4, p21Cip, p27Kip and p-RB. STH treatment promoted apoptosis by modulating key genes (p53, caspase-3, c-PARP) as well as intrinsic (Bax/Bcl2, Cyto C and caspase-9) and extrinsic (Fas L and caspase-8) apoptotic factors. STH also caused endoplasmic reticulum stress by increasing ATF-6 and XBP-1 expressions, suppressed EGFR, HER2 and downstream markers (p-Akt and p-mTOR) and elevated p-p38MAPK and p-ERK1/2. In conclusion, STH have shown a chemo-preventive action on different colon cancer cell models." As taken from Afrin S et al. 2019b. Journal of Functional Foods 57, 439-452. Available at https://www.sciencedirect.com/science/article/pii/S1756464619302221 "Stingless bees (Kelulut) can produce three major commercial products i.e. honey, propolis and beebread. These products are widely believed to have medicinal benefits, similar with products produced by stinging bees. However, there are very few scientific data available on the stingless bee's products to prove the claims. Thus, this study was conducted to investigate the characteristics of the products from the perspective of physicochemical analysis, activities of antioxidant and anti proliferation on cancer cells. Stingless bees honey, propolis and beebread were collected and their physicochemical and antioxidant properties were analysed prior to treatment on human breast adenocarcinoma (MCF-7) cell lines. Physicochemical analysis indicated that the samples are mostly not within the range reported by the Codex Standard for Honey. Honey, propolis and beebread exhibited antioxidant activity through the total phenolic content of 700 mg GAE/kg, 1600 mg GAE/kg and 300 mg GAE/kg respectively. Propolis has the highest antioxidant activity and inhibited MCF-7 cell growth at IC50 of 38.9 µg/ml. Meanwhile, stingless bee honey and beebread displayed the IC50 at 60 v/v and 64µg/mL respectively. The data is crucial to unveiled and prove medicinal properties and potential possessed by the stingless bee products. Subsequently, increase their commercial value in the future." As taken from Ismail WIW et al. 2018. IOP Conf. Ser.: Mater. Sci. Eng. 440(1), 012048. Available at https://iopscience.iop.org/article/10.1088/1757-899X/440/1/012048/meta

"Renal cell carcinoma cells (ACHN) were cultured in a medium containing 10% fetal bovine serum and 5, 10, or 15% honey for 3 consecutive days. Cell viability was determined by the 3-(4,5-dimethyliazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and apoptotic cells were determined using annexin V-fluorescein isothiocyanate (FITC) by flow cytometry. Honey decreased cell viability and induced apoptosis in malignant cells in a concentration- and time-dependent manner ($P < 0.001$). The half maximal inhibitory concentration (IC50) values at 48 and 72 hours were 1.7 ± 0.04 and 2.1 ± 0.03 µg/mL, respectively." [Referenced to Samarghandian S et al. 2011.]

"A similar study was performed on human breast cancer (MCF-7, MDA-MB-231), immortalized cervical cancer (HeLa), and normal breast epithelial cells. Cells were plated at a concentration of 1 x 105 cells/well. The cells were allowed to adhere overnight, and the culture medium was replaced with fresh assay medium supplemented with 2% fetal bovine serum. Cells were then treated with different concentrations of tualang honey (1 - 10%), and incubated for up to 72 hours. Tualang honey induced a statistically significant increase in cell death in MDA-MB-231, MCF-7, and HeLa cancer cell lines in a dose- and time-dependent manner. Treatment of the normal breast epithelial cell line did not show a clear cytotoxic effect, even after 72 hours of incubation. Flow cytometric analysis of cells stained with annexin V-FITC and propidium iodide showed that tualang honey

significantly increased apoptosis in all cancer cell lines compared to untreated cells." [Referenced to Fauzi A et al. 2011.]

"The potential cytotoxic effect of honey-impregnated wound dressings on human skin keratinocytes and dermal fibroblasts was studied. Five and 21 days after initiating the tissue culture, the honey-impregnated wound dressing was introduced directly onto the cells in the test wells to allow for cell growth. Small blocks of commercial dressings were then inserted into the wells, adjacent and distal to the tissue explants. The amount of test material used was not stated. Keratinocytes and fibroblasts treated with honey implants displayed a modest uniform increase in early cell proliferation and cell counts per mm. Nuclear and cytocavitary networks appeared normal, and cell proliferation was also evident immediately adjacent to the product. No cell toxicity was observed." [Referenced to Du Toit D and Page B, 2009.]

As taken from CIR, 2020.

"The aim of this in vitro study is to characterize the phenolic compounds of twelve honey samples collected from different locations in Palestine (H1-6) and Morocco (H7-12) and to evaluate their cytotoxic and cytostatic effects in cells from the human colorectal carcinoma cell line HCT-116 and breast cancer cell line MCF-7. Quantitative HPLC analysis revealed nine phenolic compounds in three Moroccan honey samples, namely, syringic acid, tannic acid, caffeic acid, ferulic acid, coumaric acid, gallic acid, rosmarinic acid, epicatechin, and pyrogallol. Syringic acid, abundant in numerous types of honey with strong antioxidant capacities, was present at values ranging between 0.10 mg/100 g and 1.24 mg/100 g of Daghmos (H11) and Kabbar (H10) samples, respectively. No significant reductions in cell viability were observed in both cell lines treated with the Palestinian samples as measured with MTT assay. Significant cytostatic effects were after treatment of HCT cells with Morar honey H1 with IC50 of 1789 µg/ml. Three Moroccan samples, H7 (Zaâtar), H9 (Bochnikha), and H10 (Kabbar), showed slight, but significant cytostatic effects in HCT cells. A strong correlation was observed between cytostatic activity of MCF cells and antioxidant content (phenols, flavonoids, and flavonol). Furthermore, a strong negative correlation was detected between the cytostatic activity in HCT cells and the contents of syringic acid (r= -0.756) and tannic acid (r= -0.610). These results indicate that the traditionally known anticancer effects of honey might be mediated in part through cytostatic effects." As taken from Imtara H et al. 2019. Evid. Based Complement. Alternat. Med. 2019, 8768210. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31263506/ "Honey is a nutrient rich natural product and has been utilized as traditional and complementary medicine since ancient times. In this study, antibacterial activity of Sider (Ziziphus spina-christi), Dharm (Lavandula dentata), and Majra (Hypoestes forskaolii) honey samples collected from Asir region of Saudi Arabia was in vitro evaluated at 80% and 50% w/v concentrations against five pathogenic bacteria i.e. Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Shigella flexneri, and Staphylococcus epidermidis. Well diffusion assays to measure the average zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) values were employed in the experiments. All the tested honey samples showed antibacterial activity in a dose-dependent manner. Sider and Dharm exhibited a good antibacterial activity at high concentrations while, Majra honey of Apis mellifera jemenitica and of Apis florea showed comparatively low antibacterial activity. The average MIC values of Sider, Dhram from Rijal Alma, Dharm from Al-Souda, Majra (A.m. jemenitica), and Majra (A. florea) honey against all tested bacteria were 22%, 16%, 18%, 32%, and 28% (v/v) respectively. Dharm and Sider honeys showed better antibacterial activity than Majra honey. Saudi honey can be considered as a promising future antimicrobial agent and should be further investigated as an alternative candidate in the management of resistant bacterial pathogens." As taken from Ghramh HA et al. 2019. Saudi J. Biol. Sci. 26(6), 1278–1284. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31516358/ "Numerous studies have explored the antibacterial properties of different types of honey from all around the world. However, the data available describing how honey acts against bacteria are few. The aim of this study was to apply a flow cytometry (FC) protocol to examine and characterize the

primary effects of three varieties of honey (avocado, chestnut and polyfloral) upon physiological status of Staphylococcus aureus and Escherichia coli cells to reveal their antibacterial action mechanisms. The effects of honey samples on membrane potential, membrane integrity, and metabolic activity were assessed using different fluorochromes, in a 180 min time course assay. Time-kill experiments were also carried out under similar conditions. Exposure of S. aureus and E. coli to the distinct honey samples resulted in physiological changes related to membrane polarization and membrane integrity. Moreover, honey induced a remarkable metabolic disruption as primary physiological effect upon S. aureus. The different honey samples induced quite similar effects on both bacteria. However, the depth of bacteria response throughout the treatment varied depending on the concentration tested and among honey varieties, probably due to compositional differences in the honey." As taken from Combarros-Fuertes P et al. 2020. Molecules 25(5), E1252. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32164305/ "Several studies have explored the antimicrobial properties of manuka honey (MkH). However, the data available regarding antibacterial action mechanisms are scarcer. The aim of this study was to scrutinize and characterize primary effects of manuka honey (MkH) upon the physiological status of Staphylococcus aureus and Escherichia coli (as Gram-positive and Gram-negative bacteria models, respectively), using flow cytometry (FC) to reveal its antibacterial action mechanisms. Effects of MkH on membrane potential, membrane integrity and metabolic activity were assessed using different fluorochromes in a 180 min time course assay. Time-kill experiments were carried out under the same conditions. Additionally, MkH effect on efflux pumps was also studied in an E. coli strain with an over-expression of several efflux pumps. Exposure of bacteria to MkH resulted in physiological changes related to membrane potential and membrane integrity; these effects displayed slight differences among bacteria. MkH induced a remarkable metabolic disruption as primary physiological effect upon S. aureus and was able to block efflux pump activity in a dose-dependent fashion in the E. coli strain." As taken from Combarros-Fuertes P et al. 2019. Microorganisms 7(8), 258. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31412630/ "Honey is an important animal product that is consumed by people of all ages and has become an important antimicrobial agent because it has both antibacterial properties and does not cause microbial resistance. Although, Turkey is among the most important honey producers of the world, there are not enough studies about the antibacterial activity of Turkish honey. According to their geographical area, honey exhibit considerable and variable antimicrobial activity. In this study, we investigated the in vitro antibacterial effect of honey obtained from Turkey, against Escherichia coli, Listeria monocytogenes, Salmonella Typhimurium and Staphylococcus aureus by using agar dilution, agar well diffusion and disc diffusion methods and compared the efficacy of these methods. Results showed the antibacterial effects of Turkish honey, collected from different regions against selected pathogens. Different concentrations of all honey samples displayed an antibacterial activity. Each microorganism exhibited different sensitivity to the honey tested. In addition, a significant difference was detected between the three methods for each microorganism and well diffusion method was found to be the most sensitive method." As taken from İplikçioğlu-Çil G et al. 2020. Ankara Univ. Vet. Fak. Derg. 67, 23 pp. Available at http://vetjournal.ankara.edu.tr/tr/issue/48904/674702 "Background: The present study was carried out to assess the antimicrobial effect of honey on bacterial isolates from sachet water sold within Eligbolo Community in Port Harcourt, Nigeria. Methodology: Five brands of sachet water commonly consumed by the people living in Eligbolo Community of Port Harcourt, Nigeria were purchased from different Vendors in the community. Nutrient and MacConkey agar plates were used for culturing of water samples using spread plate method. Ten-fold serial dilution and Most Probable Number (MPN) were among the methods used and the samples analyzed were according to standard procedures. Natural honey purchased from Ogbokolo in Benue State, Nigeria was used for susceptibility testing. Quality control, ant inhibition and water test methods were performed using the honey to confirm its originality before use. Antimicrobial sensitivity testing was done using the agar well diffusion method. Results: Results obtained showed the bacterial isolated from the 5 sachet brands of water. These include Bacillus species 5 (62.5%), Enterococcus faecalis 1 (12.5%), Staphylococcus epidermidis 1 (12.5%), and Escherichia coli 1 (12.5%). All of the 5 sachet water

samples analyzed failed to meet the WHO drinking water standard of zero coliform per 100 ml making them unsuitable for human consumption. Faecal coliform was isolated from sample C indicating faecal contamination of the drinking water. The sensitivity of the isolates to the honey sample showed higher zone of inhibition compared to the standard antibiotic used as control. Staphylococcus epidermidis showed the highest zone of inhibition (39 mm), followed by Escherichia coli (37 mm), Bacillus species (35 mm) and Enterococcus faecalis (32 mm) respectively. Conclusion: The results revealed that honey has a broad antimicrobial spectrum against Gram positive and Gram negative bacteria and could provide alternative agent to overcome the problem of increasingly bacteria resistance to synthetic antimicrobial agents. It is therefore, recommended that further work should be encouraged for the extraction of the crude components of honey and their use for antibiotic production." As taken from Agi VN et al. 2020. European Journal of Nutrition & Food Safety 12(2), 40-46. Available at http://journalejnfs.com/index.php/EJNFS/article/view/30191 "Honey exhibits antimicrobial activity against a wide range of bacteria. The aim of the present work was to evaluate the antimicrobial effects of the Libyan honeys harmal (Peganum harmala L.) , red camphor (Cinnamomum camphora), white camphor (Eucalyptus globule), sarou (Cupressus sempervirens), athl (Tamarix aphylla) and kharoub (Ceratonia silique) on Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeuroginosa, Escherichia coli, Bacteriods spp., Sarcina spp. and Candida albicans. Pathogens exhibited different sensitivities towards the honey samples. The results showed that C. camphora inhibited seven out of the nine tested microorganisms followed by T. aphylla honey, which inhibited six of them. The lowest effects were shown by P. harmala and C. semperviren honeys, where they only inhibited four different types of the tested microorganisms" As taken from Abouzeid AS et al. 2019. Egypt. J. Plant Prot. Res. Inst. 2(4), 617-723. Available at http://www.ejppri.eg.net/pdf/v2n4/24.pdf

"The antimicrobial properties of honey have stimulated interest in evaluating it as an alternative to antibiotics for cryopreserved buffalo semen. Acacia nilotica, Brassica campestris and Ziziphus jujuba honey were analyzed and Z. jujuba honey was found suitable in terms of quality and purity. Buffalo semen (24 ejaculates) was studied for in vitro dose tolerability to Z. jujuba honey (0.1%-1%), and up to 0.2% (v/v) was not toxic to buffalo spermatozoa. Afterward, semen from three bulls (24 ejaculates) was cryopreserved (four replicates) in tris-citric egg yolk extender supplemented with 0.1% or 0.2% honey, with or without streptomycin-penicillin (SP); extender with SP used as a control. After dilution and cooling, extender without antibiotics but with 0.2% honey was better ($p < 0.05$) than control in terms of sperm motility and plasma membrane integrity. After thawing, the extenders containing 0.1% honey with antibiotics and extender having 0.2% honey without antibiotics consistently yielded good results in terms of all parameters studied compared to control and other extenders. The extender containing 0.2% honey without antibiotics was better ($p < 0.05$) in terms of total aerobic bacterial count. In conclusion, 0.2% honey improves the post-thaw quality of buffalo spermatozoa and can replace the use of antibiotics in extender through its antimicrobial activity." As taken from Nasreen S et al. 2020. Biopreserv. Biobank. 18(1), 25-32. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31794675/

"Manuka honey (MH) is a natural food with many beneficial properties to human health, thanks to its high variety of bioactive compounds; however, little is known about its bioaccessibility. The aim of this study was to evaluate and compare the polyphenol compounds, the antioxidant capacity and the anticancer activity of MH subjected to an in vitro gastrointestinal digestion in human HCT-116 colon cancer cells. Raw MH and digested MH (DMH) were assessed for total polyphenols and flavonoids by spectrophotometric and HPLC-ESI-MS/MS analysis, and total antioxidant capacity (TAC) using different methods. Cell viability, intracellular ROS production, apoptosis, cell cycle and colony formation capacity were tested after treatment with MH or DMH. Results showed that total polyphenols, total flavonoids and TAC were significantly ($p < 0.05$) reduced after in vitro digestion. In addition, MH and DMH at 8, 16 and 24 mg/mL had similar effects in inducing intracellular ROS production and in inhibiting the colon formation ability; MH induced a more marked apoptosis compared to DMH, while cell cycle was blocked in S phase by MH and in Sub G1 phase by DMH.

Our results increase knowledge of the effect of gastrointestinal digestion on the biological effect of honey against colorectal cancer." As taken from Cianciosi D et al. 2020. Antioxidants (Basel) 9(1), 64. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31936782/

5.6. Carcinogenicity

"The study was conducted to determine the effect of Malaysian jungle Tualang Honey (TH) on development of breast cancer induced by the carcinogen 7,12-dimethylbenz(α)anthracene (DMBA) in rats. Forty nulliparous female Sprague-Dawley rats were given 80 mg/kg DMBA then randomly divided into four groups: Group 1 served as a Control while Groups 2, 3 and 4 received 0.2, 1.0 or 2.0 g/kg bodyweight/day of TH, respectively, for 150 days. Results showed that breast cancers in the TH-treated groups had slower size increment and smaller mean tumor size (≤ 2 cm3) compared to Controls (≤ 8 cm3). The number of cancers developing in TH-treated groups was also significantly fewer ($P<0.05$). Histological grading showed majority of TH-treated group cancers to be of grade 1 and 2 compared to grade 3 in controls. There was an increasing trend of apoptotic index (AI) seen in TH-treated groups with increasing dosage of Tualang Honey, however, the mean AI values of all TH-treated groups were not significantly different from the Control value ($p>0.05$). In conclusion, Tualang Honey exerted positive modulation effects on DMBA-induced breast cancers in rats in this preliminary study." As taken fron Kadir EA et al. 2013. Asian Pac. J. Cancer Prev. 14(3), 2249-54. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23725121

"Tualang honey (TH) is rich in flavonoids and phenolic acids and has significant anticancer activity against breast cancer cells comparable to the effect of tamoxifen (TAM), in vitro. The current study evaluated the effects of TH when used in combination with TAM on MCF-7 and MDA-MB-231 cells. We observed that TH promoted the anticancer activity of TAM in both the estrogen receptor-(ER-)responsive and ER-nonresponsive human breast cancer cell lines. Flow cytometric analyses indicated accelerated apoptosis especially in MDA-MB-231 cells and with the involvement of caspase-3/7, -8 and -9 activation as shown by fluorescence microscopy. Depolarization of the mitochondrial membrane was also increased in both cell lines when TH was used in combination with TAM compared to TAM treatment alone. TH may therefore be a potential adjuvant to be used with TAM for reducing the dose of TAM, hence, reducing TAM-induced adverse effects." As taken from Yaacob NS et al. 2013. Evid. Based Complement. Alternat. Med. 2013, 989841. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23476711

"The main treatment for cancer is by using chemotherapy and radiotherapy which themselves are toxic to other viable cells of the body. Recently, there are many studies focusing on the use of natural products for cancer prevention and treatment. Of these natural products, honey has been extensively researched. The mechanism of the anti-cancer activity of honey as chemopreventive and therapeutic agent has not been completely understood. The possible mechanisms are due to its apoptotic, antiproliferative, antitumor necrosis factor (anti-TNF), antioxidant, anti-inflammatory, estrogenic and immunomodulatory activities. We collate the findings of several studies published in the literature in order to understand the mechanism of its action." As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

"Manuka honey has been recognized for its anti-bacterial and wound-healing activity but its potential antitumor effect is poorly studied despite the fact that it contains many antioxidant compounds. In this study, we investigated the antiproliferative activity of manuka honey on three different cancer cell lines, murine melanoma (B16.F1) and colorectal carcinoma (CT26) as well as human breast cancer (MCF-7) cells in vitro. The data demonstrate that manuka honey has potent anti-proliferative effect on all three cancer cell lines in a time- and dose-dependent manner, being effective at concentrations as low as 0.6% (w/v). This effect is mediated via the activation of a caspase 9-dependent apoptotic pathway, leading to the induction of caspase 3, reduced Bcl-2 expression, DNA fragmentation and cell death. Combination treatment of cancer cells with manuka and paclitaxel in vitro, however, revealed no evidence of a synergistic action on cancer cell

proliferation. Furthermore, we utilized an in vivo syngeneic mouse melanoma model to assess the potential effect of intravenously-administered manuka honey, alone or in combination with paclitaxel, on the growth of established tumors. Our findings indicate that systemic administration of manuka honey was not associated with any alterations in haematological or clinical chemistry values in serum of treated mice, demonstrating its safety profile. Treatment with manuka honey alone resulted in about 33% inhibition of tumor growth, which correlated with histologically observable increase in tumor apoptosis. Although better control of tumor growth was observed in animals treated with paclitaxel alone or in combination with manuka honey (61% inhibition), a dramatic improvement in host survival was seen in the co-treatment group. This highlights a potentially novel role for manuka honey in alleviating chemotherapy-induced toxicity." As taken from Fernandez-Cabezudo MJ et al. 2013. PLoS One 8(2), e55993. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23409104

"….Honey also displays an important antitumoral capacity, where polyphenols again are considered responsible for its complementary and overlapping mechanisms of chemopreventive activity in multistage carcinogenesis, by inhibiting mutagenesis or inducing apoptosis….the evidence of the biological actions of honey can be ascribed to its polyphenolic contents which, in turn, are usually associated to its antioxidant and anti-inflammatory actions, as well as to its cardiovascular, antiproliferative and antimicrobial benefits." As taken from Alvarez-Suarez JM et al. 2013. Curr. Med. Chem. 20(5), 621-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23298140

"Antitumour potential of honey is attributed to its excellent antioxidant activity which in turn depends on the geographical origin. The present study focuses on exploration of antioxidant and antitumour potential as well as total phenolic contents (TPC) of 58 Pakistani honeys involving spectrochemical techniques and potato disk assay. Agrobacterium tumefaciens was used to induce tumours in potato disks. All analysed honey samples exhibited 1.33±0.00-155.16±0.98mg/100g of TPC, 50% 2,2-diphenyl picryl hydrazyl (DPPH) inhibition, >7.36±0.43-39.86±2.34mg/100g qurecitin equivalent antioxidant contents, >13.69±0.91-65.50±1.37mg/100g ascorbic acid equivalent antioxidant contents, 64.65±0.43-1780.74±11.79mM ferric reducing antioxidant power and 60% peroxide inhibition. Antitumour activity observed for 43 natural and 10 commercial samples was >20%. Two samples from Faisalabad region showed 87.50±5.50% and 79.00±5.56% antitumour activity which were reference standard. It was concluded that Pakistani honeys possessed excellent antioxidant and antitumour potential overall." As taken from Noor N et al. 2014. Food Chem. 143, 362-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24054252

"Honey is a natural product known for its varied biological or pharmacological activities-ranging from anti-inflammatory, antioxidant, antibacterial, antihypertensive to hypoglycemic effects. This review article focuses on the role of honey in modulating the development and progression of tumors or cancers. It reviews available evidence (some of which is very recent) with regards to the antimetastatic, antiproliferative and anticancer effects ofhoney in various forms of cancer. These effects of honey have been thoroughly investigated in certain cancers such as breast, liver and colorectal cancer cell lines. In contrast, limited but promising data are available for other forms of cancers including prostate, bladder, endometrial, kidney, skin, cervical, oral and bone cancer cells. The article also underscores the various possible mechanisms by which honey may inhibit growth and proliferation of tumors or cancers. These include regulation of cell cycle, activation of mitochondrial pathway, induction of mitochondrial outer membrane permeabilization, induction of apoptosis, modulation of oxidative stress, amelioration of inflammation, modulation of insulin signaling and inhibition of angiogenesis. Honey is highly cytotoxic against tumor or cancer cells while it is non-cytotoxic to normal cells. The data indicate thathoney can inhibit carcinogenesis by modulating the molecular processes of initiation, promotion, and progression stages. Thus, it may serve as a potential and promising anticancer agent which warrants further experimental and clinical studies." As taken from Erejuwa OO et al. 2014. Molecules 19(2), 2497-2522. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24566317

“Colon cancer has been a major problem worldwide. Kelulut honey (KH) is produced by the stingless bees from Trigona species and has strong antioxidant activities that could be one of the potential chemopreventive agents from natural resources. Aim of This Study. This study investigated the chemopreventive properties and toxicity of KH in Sprague Dawley rats induced with azoxymethane (AOM). Material and Method. Twenty-four male Sprague Dawley rats aged 5 weeks were divided into 4 groups: (G1) untreated group not induced with AOM, (G2) untreated group induced with AOM, (G3) treated group induced with AOM, and (G4) treated group not induced with AOM. Injection of AOM (15 mg/kg) was via intraperitoneal route once a week for two subsequent weeks. The treatment groups were given oral administration of KH (1183 mg/kg body weight) twice daily for 8 weeks. Results. Treatment with KH significantly reduced the total number of aberrant crypt foci (ACF) and aberrant crypts (AC) and crypt multiplicity. KH was not toxic to the animals since the level of blood profile parameters, liver enzymes, and kidney functions was in normal range. Conclusions. The current finding shows that KH has chemopreventive properties in rats induced with colorectal cancer and also was found not toxic towards the animals.” As taken from Saiful Yazan L et al. 2016. Biomed. Res. Int. 2016, 4036926. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27525267

“Breast cancer has been recognized as the leading cause of death in women worldwide. Research has shown the importance of complementary and alternative therapies in cancer. In this study, we investigated the antitumoural therapeutic effects of Malaysian Tualang honey (TH) and Australian/New Zealand Manuka honey (MH) against breast cancer in rats. Thirty syngeneic virgin female Sprague-Dawley (SD) rats were induced by the carcinogen 1-methyl-1-nitrosourea (MNU) 80 mg/kg. The treatment started when first palpable tumour reached 10-12 mm in size by dividing rats into following groups: Group 0 (negative control); Group 1 (positive control); and Groups 2 and 3 which received 1.0 g/kg body weight/day of TH and MH, respectively, for 120 days. The data demonstrate that cancer masses in TH and MH treated groups showed a lower median tumour size, weight, and multiplicity compared with the nontreated positive control ($p < 0.05$). Treatment also showed a dramatic slower growth rate (up to 70.82%) compared with the nontreated control (0%) ($p < 0.05$). The antitumoural effect was mediated through modulation of tumour growth, tumour grading, estrogenic activity, and haematological parameters. Our findings demonstrate that systemic administration of TH and MH increases the susceptibility of expression of proapoptotic proteins (Apaf-1, Caspase-9, IFN-γ, IFNGR1, and p53) and decreases the expression of antiapoptotic proteins (TNF-α, COX-2, and Bcl-xL 1) in its mechanism of action. This highlights a potential novel role for TH and MH in alleviating breast cancer.” As taken from Ahmed S et al. 2017. Evid. Based Complement. Alternat. Med. 2017, 5904361. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28479926

“Honey is a natural product known to modulate several biological activities including cancer. The aim of the present study was to examine the phytochemical content and the antioxidant activity of Strawberry tree (Arbutus unedo) honey (STH) and its cytotoxic properties against human colon adenocarcinoma (HCT-116) and metastatic (LoVo) cell lines in comparison with Manuka (Leptospermum scoparium) honey (MH). Several unifloral STH and MH were analyzed for their phenolic, flavonoid, amino acid and protein contents, as well as their radical scavenging activities. STH from the Berchidda area showed the highest amount of phenolic, flavonoid, amino acid and protein content, and antioxidant capacity compared to MH. Both STH and MH induced cytotoxicity and cell death in a dose- and time-dependent manner in HCT-116 and LoVo cells, with less toxicity on non-cancer cells. Compared to MH, STH showed more effect at lower concentrations on HCT-116 and LoVo cells. In addition, both honeys increased intracellular reactive oxygen species (ROS) generation. In HCT-116 cells, STH and MH induced similar ROS production but in LoVo cells STH induced a higher percentage of ROS compared to MH. Our results indicate that STH and MH can induce cell growth inhibition and ROS generation in colon adenocarcinoma and metastatic cells, which could be due to the presence of phytochemicals with antioxidant properties. These preliminary results are interesting and suggest a potential chemopreventive action which could be useful for further studies in order to develop chemopreventive agents for colon cancer.” As taken

from Afrin S et al. 2017. Int. J. Mol. Sci. 18(3), E613. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28287469

5.7. Irritation/immunotoxicity

Honey and royal jelly are complex etherogeneous mixtures of flowers' nectar, sugars, proteins and bee's glandular secretions. The existence of a type I hypersensitivity to honey is still matter of debate, while an aetiological role of Compositae pollens in the clinical manifestations following honey ingestion has been envisaged. We describe two cases of severe systemic reactions (anaphylaxis and generalized urticaria/angioedema) due to honey and royal jelly ingestion in patients sensitized to compositae (mugwort). Both patients had a skin and RAST positivity to mugwort and a positive prick-by-prick to the offending foods. Moreover, in one of the two patients the RAST-inhibition assay showed the strong cross-reactivity between the proteins of honey and mugwort and the SDS-PAGE analysis showed that the major proteic bands from honey and mugwort extracts are largely superimposable. Both the clinical data and the laboratory analysis support the hypothesis of a strict link between sensitization to compositae and adverse reactions to honey and jelly. As taken from Lombardi C et al. Allergol Immunopathol (Madr). 1998 Nov-Dec; 26(6):288-90. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/9934408

A 14-month-old boy presented with anaphylaxis after honey ingestion. He was given as much as one teaspoon of honey for several times until he was six months old. When he was 14 months old, his mother gave him approximately five teaspoons of honey. After five minutes, his lips were swollen and within 10 minutes urticaria, angio-oedema, cough and wheezing occurred. He was taken to a primary medical centre immediately. Systemic corticosteroid and antihistamines were administered. He was referred to an allergy centre for further evaluation..... Five weeks after anaphylaxis, prick-to-prick skin test was performed for the honey that was eaten and for another two species which are frequently consumed in our country. Honey which was eaten was found positive, flower honey was negative, and honey composed of mixed flower and pine honey was weak positive. Skin prick tests with common pollens and pinus pollen were also negative (Tuncel et al. 2011. Allergol Immunopathol (Madr) 39, 112-113). As taken from http://www.elsevier.es/en/revistas/allergologia-et-immunopathologia-105/anaphylaxis-caused-by-honey-ingestion-in-an-90003128-research-letters-2011

Honey is an established traditional medicine with a variety of putative nutritional and health effects, including antibacterial, antioxidant, anti-inflammatory and prebiotic. The aim of the present study was to investigate the safety of consuming manuka honey, UMF 20+, on healthy individuals by establishing whether UMF 20+caused an allergic response (as measured by IgE levels), changed major commensal and beneficial microbial groups in the gut and/or affected levels of one of the most common advanced glycation endpoints, N-(carboxymethyl)-lysine (CML). The study had a randomised, double-blind cross-over design. A total of twenty healthy individuals aged 42-64 years were recruited. We tested two different honeys- a multiflora honey and UMF 20+, both produced by Comvita New Zealand Ltd (Te Puke, New Zealand). Multiflora honey or UMF 20+(20 g) was consumed daily for 4 weeks, with a 2-week 'washout' period in between. Blood samples were collected every week for each intervention period and used to measure total IgE levels in serum and advanced glycation endproducts - a consequence of methyglyoxal accumulation. Faecal samples were collected at the beginning and end of each 4-week period. DNA was extracted from faecal samples and the levels of a number of microbial groups in the gut, both beneficial and commensal, were analysed. Neither product changed the levels of IgE or CML or altered gut microbial profiles during the trial, confirming that UMF 20+is safe for healthy individuals to consume (Wallace et al. 2010. British Journal of Nutrition 103, 1023-1028). As taken from http://www.ncbi.nlm.nih.gov/pubmed/20064284?dopt=AbstractPlus

PURPOSE: To investigate the effect of topically applied honey on intact corneas, surgically induced corneal abrasions and endotoxin induced keratitis. MATERIALS AND METHODS: The

effect of honey on the cornea was investigated by application of honey on intact corneas, wounded corneas and endotoxin-induced keratitis in Lewis rats. The corneas were wounded by creating an epithelial defect using a surgical blade, and the keratitis was induced by topically applying Pseudomonas aeruginosa endotoxin to scarified corneas. After treatment rats were sacrificed and cornea harvested in each case. Corneas were processed for paraffin embedding for histological and immuno-fluorescence staining. Corneas were also harvested and processed for total ribonucleic acid (RNA) isolation for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for various growth factors and inflammatory chemokines/cytokines). RESULTS: Histological analysis revealed that no inflammation or morphological changes occurred after honey treatment in naive intact corneas. Vascular endothelial growth factor (VEGF) levels were also not altered after honey treatment. Topical application of honey to injured corneas resulted in faster epithelial healing and decreased expression of VEGF, transforming growth factor beta (TGF-β), interferon gamma (IFN-γ), interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF-α) in injured corneas. Our results also established that honey treatment reduced the inflammation in endotoxin-induced keratitis by reducing the levels of angiogenic factors (VEGF and TGF-β), inflammatory cytokines (IL-12) and chemokines (CC chemokine receptor 5(CCR-5)). CONCLUSION: Short term use of honey on intact corneas can be safe. Honey has anti-angiogenic and anti-inflammatory properties that can be explored in several corneal inflammatory and infectious conditions (Uwaydat et al. 2011 Current Eye Research 36, 787-796). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21812661?dopt=AbstractPlus

METHODS: Using a rabbit animal model, a nonrandomized controlled trial of four treatment regimes was performed with two rabbits in each group. The left nasal cavity was irrigated with a 1.5-mL manuka honey solution once daily and the right nasal cavity was not treated. Groups 1-3 were treated for 3, 7, and 14 consecutive days, respectively, and killed the morning after the last treatment. Group 4 was treated for 14 consecutive days followed by a 14-day washout period and then killed the following morning. The nasal respiratory mucosa was immediately harvested after death. The mucosa was examined by light microscopy for histological change in comparison with the control side. RESULTS: Cilia were not measured quantitatively but were equally present on the treated and untreated mucosa. There was no histological evidence of inflammation, epithelial injury, or significant morphological changes. CONCLUSION: The application of a manuka honey solution to rabbit nasal respiratory mucosa over different treatment intervals did not show evidence of histological epithelial injury (Kilty et al. 2010. Journal of Rhinology and Allergy 24, e63-66). As taken from http://www.ncbi.nlm.nih.gov/pubmed/20338104?dopt=AbstractPlus

“The aim of this study was to identify the major allergens of wildflower honey in local patients with atopic disease. SDS-PAGE revealed ten protein bands of 25 to 110 kDa, with a heavy cluster in region of 40-75 kDa. Immunoblotting demonstrated seven IgE-binding bands of 39 to 110 kDa. The 60 kDa protein had the highest frequency of IgE-binding (100%) followed by 54 kDa protein (95%), thus identified as the major allergens of wildflowerhoney. Our findings indicate that the allergen extract used for diagnosis of honey allergy contains both the 54 kDa and 60 kDa proteins”. As

taken from Yadzir ZH et al. 2011. Southeast Asian J. Trop. Med. Public Health 42(2), 370-5. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21710860

"Honey allergy is a very rare, but serious health condition. In this study, we presented six patients who described systemic allergic reactions after ingestion of honey. Three of the six patients had suffered from anaphylaxis. Honey-specific IgE was measured and skin-prick tests for honey were performed to diagnose honey allergy. The results of honey-specific IgE of all patients were positive. Four patients had high serum-specific IgE forhoney bee venom and two of five patients had also experienced anaphylaxis due to bee stings. Skin-prick tests with honey and pollens were positive in five patients. Honey is one of the foods that can cause severe systemic reactions. Specific IgE and skin-prick tests are helpful for the diagnosis of honey allergy." As taken from Vezir E et al. 2014. Allergy Asthma Proc. 35(1), 71-4. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24433600

Manuka, Pasture, Nigerian Jungle, and royal jelly honeys are found to increase IL-1β, IL-6, and TNF-α production [16, 44, 58]. This immunomodulatory and immunoprotective activity of honey is often linked to anticancer action [16, 59]. Honey stimulates antibodies, B and T lymphocytes, neutrophils, monocytes, eosinophils, and natural killer cells (NK-cells) production during primary and secondary immune responses in tissue culture [59– 62]. It has been shown that honey stimulates macrophages, T-cells, and B-cells to provoke antitumor effect [59]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

Background: Honey is widely used in folk medicine to treat cough, fever, and inflammation. In this study, the effect of aerosolised honey on airway tissues in a rabbit model of ovalbumin (OVA)-induced asthma was investigated. The ability of honey to act either as a rescuing agent in alleviating asthma-related symptoms or as a preventive agent to preclude the occurrence of asthma was also assessed.

Methods: Forty New Zealand white rabbits were sensitized twice with mixture of OVA and aluminium hydroxide on days 1 and 14. Honey treatments were given from day 23 to day 25 at two different doses (25% (v/v) and 50% (v/v) of honey diluted in sterile phosphate buffer saline. In the aerosolised honey as a rescue agent group, animals were euthanized on day 28; for the preventive group, animals were further exposed to aerosolised OVA for 3 days starting from day 28 and euthanized on day 31. The effects of honey on inflammatory cell response, airway inflammation, and goblet cell hyperplasia were assessed for each animal.

Results: Histopathological analyses revealed that aerosolised honey resulted in structural changes of the epithelium, mucosa, and submucosal regions of the airway that caused by the induction with OVA. Treatment with aerosolised honey has reduced the number of airway inflammatory cells present in bronchoalveolar lavage fluid and inhibited the goblet cell hyperplasia.

Conclusion: In this study, aerosolised honey was used to effectively treat and manage asthma in rabbits, and it could prove to be a promising treatment for asthma in humans. Future studies with a larger sample size and studies at the gene expression level are needed to better understand the mechanisms by which aerosolised honey reduces asthma symptoms (Kamaruzaman NA et al., (2014).

BACKGROUND Bee honey has been an outstanding household remedy used for the treatment of cough and wheezing associated with bronchitis. The therapeutic use of honey in the form of inhalation dates from very early days. This method is particularly effective in the treatment of diseases of the upper respiratory tract. OBJECTIVE The present work attempted to study the effects of bee honey in the form of nebulization in infants and children with acute asthma.

SUBJECTS AND METHODS After obtaining consent from their parents, 300 infants and children with mild to moderate acute attacks of asthma were included in this study. The mean age of studied patients was 2.49 Â± 3.02 years with male to female ratio of 1.2 to 1. All studied patients received Bee Honey Nebulization (BHN) for 30 minutes. Neither corticosteroids nor bronchodilators were given. The response was judged 60 minutes after BHN by changes in respiratory rate (RR), heart rate (HR), O2 saturation at room air (SPO2), dyspnoea, use of accessory respiratory muscles and chest wheezes. RESULTS There was a significant increase of SPO2 and decrease of RR and HR 60 minutes after BHN. The dyspnoea improved in 94% of patients. The chest wheezes disappeared in 35% and decreased significantly in 31% of patients. Six (6) patients were admitted because of persistence of symptoms. During and after BHN increased frequency of productive cough occurred in 78.7% and it was severe and exhausting in 2%. The expectoration of sputum was followed by improvement in nearly all patients. Apart from severe exhausting cough, no side effects occurred during and after BHN. CONCLUSION BHN is an effective and safe treatment for mild and moderate acute attacks of asthma in infants and children (Rhman, Mamdouh Abdul Maksoud Mohamed Abdul, 2007).

“BACKGROUND: Radiotherapy is frequently used in treatment approaches of pelvic malignancies. Nevertheless, it has some known systemic effects on blood cells and the immune system that possibly results in their susceptibility to infection. Probiotics are live microbial food ingredients that provide a health advantage to the consumer. Honey has prebiotic properties. The aim of this clinical trial was to investigate probable effects of probiotic or probiotics plus honey on blood cell counts and serum IgA levels in patients receiving pelvic radiotherapy. MATERIALS AND METHODS: Sixty-seven adult patients with pelvic cancer were enrolled. Patients were randomized to receive either: (1) Probiotic capsules (including: Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophiles) (n = 22), (2) probiotic capsules plus honey (n = 21) or (3) placebo capsules (n = 24) all for 6 weeks. Blood and serum samples were collected for one week before radiotherapy and 24-72 h after the end of radiotherapy. RESULTS: White blood cells (WBC), red blood cells (RBC), platelet counts, and serum IgA level were not significantly changed in patients taking probiotic (alone or plus honey) during pelvic radiotherapy. The mean decrease in RBC count was 0.52, 0.18, and 0.23 × 10(6) cells/μL, WBC count was 2.3, 1.21, and 1.34 × 10(3) cells/μL and platelet count was, 57.6, 53.3, and 66.35 × 10(3) cells/μL for the probiotic, probiotic plus honey, and placebo groups, respectively. The mean decrease of serum IgA was 22.53, 29.94, and 40.73 mg/dL for the probiotic, probiotic plus honey, and placebo groups, respectively. CONCLUSION: The observed nonsignificant effect of probiotics may be in favor of local effects of this product in the gut rather than systemic effects, however, as a trend toward a benefit was indicated, further studies are necessary in order to extract effects of probiotics or probiotic plus honey on hematologic and immunologic parameters in patients receiving pelvic radiotherapy.” As taken from Mansouri-Tehrani HA et al. 2015. J. Res. Med. Sci. 20(7), 679-83. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26622258

“Immediate skin reactions are common in dermatological practice, but may often be overlooked. The main objective of this article is to provide an update of the literature concerning immediate-type reactions or contact urticaria/contact urticaria syndrome caused by cosmetic ingredients in terms of immediate clinical symptoms, positive reactions following open, scratch or, most often, prick testing, and sometimes the detection of specific IgE antibodies. To this end, a selective search in different medical literature databases was performed. This yielded a list of cosmetic ingredients causing immediate reactions, including hair dyes and bleaches, preservatives, fragrance and aroma chemicals, sunscreens, hair glues, plant-derived and animal-derived components, permanent makeup and tattoos, glycolic acid peel, lip plumper, and alcohols. Many of the reported cases, however, lack appropriate controls and detailed investigation. Contact urticaria may occur with or without systemic symptoms, which are sometimes life-threatening.” As taken from Verhulst L and Goossens A. 2016. Contact Dermatitis 75(6), 333-344. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=27593503

“BACKGROUND: Antioxidant and anti-inflammatory properties of honey have been largely recognized by various studies. Almost all of the potential benefits are associated with polyphenol content. Honey varieties from the arid region are reported to be rich in polyphenols, but data related to its bioactivity in vitro is greatly lacking. This study aimed at establishing the antioxidant and anti-inflammatory properties of arid region honey. Four honey varieties from arid region (H1, H2, H3, and H4) and two popular non-arid region honey (H5 and H6) were tested in vitro in this study. METHODS: The erythrocyte membrane protection effect of honey varieties were measured by hemolysis assay after exposing erythrocytes to a peroxide generator. The subsequent production of MDA (malondialdehyde) content in erythrocytes was measured. Immunomodulatory effect of the honey varieties was tested in prostate cancer cells PC-3 and PBMC (peripheral blood mononuclear cells) by measuring the IL-6 (interleukin 6) and NO (nitric oxide) levels in cell culture supernatant after incubation with the honey varieties. PC-3 cell viability was assessed after incubation with honey varieties for 24 h. RESULTS: Arid region honey exhibited superior erythrocyte membrane protection effect with H4 measuring 1.3 ± 0.042mMTE/g and H2 measuring 1.122 ± 0.018mMTE/g. MDA levels were significantly reduced by honey samples, especially H4 (20.819 ± 0.63 nmol/mg protein). We observed a significant decrease in cell population in PC-3 after 24 h in culture on treatment with honey. A moderate increase in NO levels was observed in both cultures after 24 h at the same time levels of IL-6 were remarkably reduced by honey varieties. CONCLUSION: The results demonstrate the antioxidant effect of arid region honey due to its erythrocyte membrane protection effect and subsequent lowering of oxidative damage as evident from lower levels of lipid peroxidation byproduct MDA. Arid region honey varieties were as good as non-arid region types at decreasing cell viability of prostate cancer cells. The moderate increase in NO levels in PC-3 and PBMCs were not significant enough to elicit any pro-inflammatory response. However, IL-6 secretion was remarkably reduced by all honey varieties in a comparable level indicating the potential anti-inflammatory property of arid region honey.” As taken from Hilary S et al. 2017. BMC Complement. Altern. Med. 17(1), 177. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28356100

“Objective: To assess the clinical safety and tolerability of a novel MGO Manuka Honey microemulsion (MHME) eye cream for the management of blepharitis in human subjects. Methods and analysis: Twenty-five healthy subjects were enrolled in a prospective, randomised, paired-eye, investigator-masked trial. The MHME eye cream (Manuka Health New Zealand) was applied to the closed eyelids of one eye (randomised) overnight for 2 weeks. LogMAR visual acuity, eyelid irritation symptoms, ocular surface characteristics and tear film parameters were assessed at baseline, day 7 and day 14. Expression of markers of ocular surface inflammation (matrix metalloproteinase-9 and interleukin-6) and goblet cell function (MUC5AC) were quantified using impression cytology at baseline and day 14. Results: There were no significant changes in visual acuity, eyelid irritation symptoms, ocular surface characteristics, tear film parameters and inflammatory marker expression during the 2-week treatment period in treated and control eyes (all $p>0.05$), and measurements did not differ significantly between eyes (all $p>0.05$). No major adverse events were reported. Two subjects experienced transient ocular stinging, presumably due to migration of the product into the eye, which resolved following aqueous irrigation. Conclusion: The MHME eye cream application was found to be well tolerated in healthy human subjects and was not associated with changes in visual acuity, ocular surface characteristics, tear film parameters, expression of markers of inflammation or goblet cell function. The findings support future clinical efficacy trials in patients with blepharitis.” As taken from Craig JP et al. 2017. BMJ Open Ophthalmol. 1(1), e000066. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29354710

“Foxp3 Treg, a transcription factor, plays an important role in the balance of immune system. Diets containing polyphenols and flavonoids could increase the expression of FOXP3 mRNA although some studies have contrary results. Trigona honey is a specific honey from Trigona bees containing polyphenols and could influence the immune homeostasis. There have never been studies proving its effects on the expression of Foxp3mRNA as the transcription factor of Regulatory T Cells. It was

a laboratory research. Mice Balb/c were divided into the reference, positive and treatment groups. The reference group was only given standard feed and positive control was intraperitonially injected with Salmonella Enterica serovar Typhi. The treatment group was divided into 2 groups and given Trigona honey using canule with both doses of 0.23 mL/20 g bw and 0.27 mL/20 g bw daily for 10 days respectively. Foxp3 mRNA expression was examined by real-time RT-PCR. Repeated Anova and One Way Anova were used as the statistical methods, a p-value of less than 0.050 at the final analysis was considered indicating statistical significance. Results indicated Foxp3 mRNA expression of the groups given by honey was higher than the control group. The highest Foxp3 mRNA expression in Trigona honey was the group given with a dose of 0.27 mL/20 g bw (p=0.000, however, the group given Trigona Honey with a dose of 0.23 mL/20 g Bw also had moderate Foxp3 mRNA expression. These data suggested that Trigona honey could induce Foxp3mRNAexpression. The higher dose given, the higher Foxp3 mRNA expression." As taken from Natzir R and Rahman F. 2018. Acta Biomedica Indonesiana. Available at http://www.jurnal.biomedicaindonesiana.com/index.php/JBKIBI/article/view/49

"Objective: To identify the differences in cytokine expression between sinonasal tissue from patients treated with Leptospermum (Manuka) honey (LH) irrigation versus normal saline irrigation twice-daily for twelve weeks following sinus surgery (FESS). Methods: Forty-six CRS patients were recruited. Sinus tissue biopsies were collected during FESS and then at 5 and 12 weeks postoperatively during the course of treatment. A multi-plex cytokine assay quantified the abundance of 17 cytokines in biopsied tissue. Cytokine expression fold-change was analyzed between each time point using a robust linear regression model and compared between the two treatment groups. Results: Compared to the saline irrigation group, five cytokines were differently expressed (CI = 95%) in sinonasal tissue obtained from subjects in the LH irrigation group during the 12-week treatment period. Cytokines IL-6 (P = 0.0400), IL-8 (P = 0.0398), MCP-1 (P = 0.0284), and MIP-1β (P = 0.016) were significantly increased in the LH irrigation group compared to the saline irrigation group. IL-13 was significantly increased in the saline irrigation group compared to the LH group (P = 0.0086). Conclusion: LH may potentially act to modulate the expression of IL-6, IL-8, IL-13, MCP-1 and MIP-1β in sinonasal tissue." As taken from Manji J et al. 2018. World J. Otorhinolaryngol. Head Neck Surg. 5(1), 19-25. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30775697

"Efficient diagnosis of allergy and proper treatment need identification of the causative allergens eliciting clinical symptoms. The present study was performed to identify the most common aero- and food allergens and determine the pattern of sensitization among people of Ahvaz (southwestern Iran), one of the most polluted cities worldwide. Based on the physical examination and medical records, patients were referred to the Allergy laboratory for "in vitro" IgE determination. Specific and total IgE was determined by the ImmunoCAP system (Thermo Fisher-Phadia, Uppsala, Sweden). A total of 666 consecutive patients (51.1% female) were tested for 202 different allergens. The majority of requests (57%) belonged to food allergens. Sensitization to at least one allergen was found in 47.6% of patients. In a selected group of allergens for which specific IgE had been tested in at least 100 patients, the most common sensitizing aeroallergens were Russian thistle, grass pollen, and willow; while wheat, honey, and shrimp were the most frequent food allergens, respectively. Sensitization profiles based on measurement of specific IgE indicated that Russian thistle, grasses, and wheat were the most prevalent allergens in people with allergic symptoms living in Ahvaz." As taken from Shahrooei M et al. 2018. Iran J. Allergy Asthma Immunol. 17(4), 393-397. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30537803

"A human repeated insult patch test (HRIPT) was performed on 112 subjects using a cosmetic product containing 7% Honey Extract. Approximately 0.2 mL of the test substance was applied to the upper back, under an occlusive patch. Patches were allowed to remain in direct skin contact for a period of 24 hours. Applications were made to the same site, three times a week, for a total number of 9 applications during the induction period. After a 2-week rest period, challenge patches were applied to previously untreated test sites. After 24 hours, patches were removed and test sites

were evaluated. The test substance did not demonstrate a potential for eliciting dermal irritation or sensitization." [Referenced to Chemical Research Laboratories Inc. 2015.]

"According to a summary report, an HRIPT was performed on 116 subjects using a product containing 0.01% Honey Extract according to the same procedure as above. The product was tested at a 1% dilution in water (effective test concentration, 0.0001% Honey Extract). Seven individuals displayed low-level reactions (mild erythema) during the induction phase, and one individual displayed a high-level reaction in the induction phase. Eight individuals displayed low-level reactions during the challenge phase. (Individual subject scores were not provided.) The test substance was considered by the researchers to be non-sensitizing." [Referenced to Anonymous. 2019.]

"A 40-year old woman was referred to a clinic after suspected allergy to honey. At the age of 36, she had two episodes of generalized urticaria 20 minutes after ingestion of foods with honey. At the age of 37, five minutes after an inadvertent contact with a teaspoon with traces of honey, the patient reported swollen lips, urticaria, and angioedema. After treatment with oral corticosteroids and antihistamines, symptoms were resolved. Skin prick tests with standard panel of extracts from aeroallergens and common allergenic foods yielded negative results. Prick-to-prick tests (PPT) were performed with the previously consumed honey, and eight other kinds of honey (eucalyptus, sunflower, orange-tree, Arbutus-tree, French lavender, heather, flower incense, and rosemary). Results were positive for all honey types. Thirty minutes after the administration of the PPT, the patients suffered from anaphylaxis, generalized urticaria, swollen lips, tongue, and uvula, and hypotension. The same PPT was performed with these honeys in 6 control volunteers (3 healthy individuals, and 3 atopic with pollen sensitization and rhinitis). None of the volunteers displayed a positive skin reaction." [Referenced to Aguiar R et al. 2017.]

"A 48-year-old woman had been washing her body and hair with products blended with edible honey, and she applied honey to the face as a face pack. After 8 years of use, the woman developed itching and redness on facial skin as well as conjunctival hyperemia following the use of the face pack containing honey. After washing her body with honey-containing soap, the subject reported urticarial symptoms on her extremities and un-exposed face. One year later, the subject developed abdominal pain and distention after eating yogurt with honey. The patient had positive results for honey-antigen specific IgE antibodies in serum (UA), equivalent to 1.44 UA/mL, but not for honey bee venoms or Api m 10 (Apis mellifera venom component). Results for specific IgE against three cross-reactive carbohydrate determinant marker allergens were negative. Prick tests with honey gave positive results. Fifteen minutes after oral challenge with 30 mL of honey, the patient developed eyelid swelling, abdominal pain, and oral tingling." [Referenced to Katayama M et al. 2016.]

As taken from CIR, 2020.

"Background. Small amounts of protein can be found in honey, including well known allergen sources, such as plant pollen and honeybee secretions. Despite this, there are few case reports describing allergic reactions following the consumption of honey. The aim of this study was to examine the allergenic properties of nectar honey collected throughout the entire beekeeping season from different provinces in Poland. Materials and methods. The immunoreactive properties of 20 Polish nectar honeys were analysed using the sera of IgE pollen allergenic patients (n = 5). The botanical origins and pollen of the anemophilous plants in the studied honeys were identified through palynological analysis. Results. The significant differences in the protein content between the five varieties of honey and the differences in protein pattern and pollen profiles were observed. All of the honey samples contained immunoreactive fractions reacting with IgE present in the sera of patients allergenic to different pollens. Conclusions. Although honey allergies are reported relatively rarely, all the tested samples of Polish nectar honeys contained many protein fractions which reacted with the IgE antibodies of allergenic patients. In all samples, the immunoreactive protein band with a molecular weight around 60 kDa, probably secreted by bees, was present. The

results do not allow the immunoreactive fractions characteristic for particular honey varieties to be identified." As taken from Burzyńska M et al. 2020. Acta Sci. Pol. Technol. Aliment. 19(1), 15-24. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32227694/

"Manuka honey, a wound treatment used to eradicate bacteria, resolve inflammation, and promote wound healing, is a current focus in the tissue engineering community as a tissue template additive. However, Manuka honey's effect on neutrophils during the inflammation-resolving phase has yet to be examined. This study investigates the effect of 0.5% and 3% Manuka honey on the release of cytokines, chemokines, and matrix-degrading enzymes from a dHL-60 neutrophil model in the presence of anti-inflammatory stimuli (TGF-β, IL-4, IL-4 +IL-13). We hypothesized that Manuka honey would reduce the output of pro-inflammatory signals and increase the release of anti-inflammatory signals. The results of this study indicate that 0.5% honey significantly increases the release of CXCL8/IL-8, CCL2/MCP-1, CCL4/MIP-1β, CCL20/MIP-3α, IL-4, IL-1ra, and FGF-13 while reducing Proteinase 3 release in the anti-inflammatory-stimulated models. However, 3% honey significantly increased the release of TNF-α and CXCL8/IL-8 while reducing the release of all other analytes. We replicated a subset of the most notable findings in primary human neutrophils, and the consistent results indicate that the HL-60 data are relevant to the performance of primary cells. These findings demonstrate the variable effects of Manuka honey on the release of cytokines, chemokines, and matrix-degrading enzymes of this model of neutrophil anti-inflammatory activity. This study reinforces the importance of tailoring the concentration of Manuka honey in a wound or tissue template to elicit the desired effects during the inflammation-resolving phase of wound healing. Future in vivo investigation should be undertaken to translate these results to a physiologically-relevant wound environment." As taken from Minden-Birkenmaier BA et al. 2020. Journal of Tissue Viability 29(2), 91-99. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32249090/

"Background: Globally, cancer ranks among the most common causes of death. Multiple experimental and clinical studies have investigated anticancer effects of honey with promising results. This study focused on potential background mechanisms of this effect. Methods: The current literature was reviewed for potential anticancer pathways which are suggested for honey and its ingredients. Results: Flavonoids (kaempferol, catechin, and quercetin) and phenolic acids (caffeic acid and gallic acid) are the most important ingredients of honey with known anti-cancer activity. The main suggested mechanisms for anti-cancer activity of honey and its ingredients are antioxidant, apoptotic, tumor necrosis factor inhibiting, antiproliferative, immunomodulatory, anti-inflammatory and estrogenic effects. Conclusion: This review collates the current scientific understanding on the mechanism of anti-cancer activity of honey." As taken from Waheed M et al. 2019. Clin. Nutr. 38(6), 2499-2503. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30639116/

"Honey contains flavonoids and phenolic acids, and because of their antioxidant and anti-inflammatory properties, they may play an important role in human health. The purpose of this review was to synthesize the effects of natural honey on pro- and anti-inflammatory cytokines. The effects of honey on wound healing and immunity appear to be inconsistent. The available databases)PubMed and Scopus) were searched and 42 studies were assessed. In patients with cancer, honey has been reported to inhibit the effects of pro-inflammatory factors such as TNF-α and IL-6. In patients with neuro-inflammatory disorders honey has been shown to inhibit the expression of pro-inflammatory markers. It has also been reported that honey can reduce TNF-α expression in conditions associated with liver injury, by suppressing TNF-α converting enzyme activity. Honey inhibits APAP-induced hepatocellular necrosis by modulating the expression of IL-10 and IL-1ß. Animal studies have shown that honey can reduce serum IL-1ß, IL-6 and TNF-α concentration and increase IL-10 concentrations in a model of gastric ulcer. Some studies in diabetics have shown that honey can reduce serum TNF-α, IL-6, IL-1ß and TGF-ß by inhibiting NF-Kß. The source and type of honey and its component have not been indicated in various clinical and practical studies, which are a limitation of these studies, in relation to reproducing them. Sigma, Manuka, Gelam and Tulang honey have been used in most of the in vitro and animal

studies. The animal studies have demonstrated similar effects on pro-inflammatory factors, which include reducing serum TNF-α, IL-6 and IL-1β as well as increasing IL-10. There are few human RCTs investigating the effects of honey on inflammatory cytokines. Only one RCT has reported the type of honey that they have used. Tulang honey has been reported to increase serum TNF-α and decrease hs-CRP, which is therefore controversial. Further high-quality studies are needed to firmly establish the clinical efficacy of honey. Because most studies had used different duration, type of honey and dosage, which make them difficult to contextualize, as the phytochemical content of a honey may depend on its source. Furthermore, it is unclear whether honey's anti-inflammatory effects are related to its phenolic or tocopherol compounds, and whether its effects are greater than these individual components." As taken from Navaei-Alipour N et al. 2021. Phytother. Res. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33751689/

Honey is an ancient food in the human diet, and the chemical composition of some types of honey has been associated with several beneficial biological effects. Among them, honey has been highlighted to improve health and control inflammatory processes. However, there is no study elucidating the mechanism of action of honey produced organically. Here, we separated organic honey (OH) samples from the Brazilian Atlantic Rainforest into eight different profiles (OH-1 to OH-8) and evaluated, in vitro and in vivo, their anti-inflammatory potential. To determine cell viability, RAW 264.7 macrophages were treated with several concentrations of OH-1 up to OH-8, and anti-inflammatory activity was assessed through NF-κB activation and TNF-α levels. All types of the studied honey up to a concentration of 4% (w/v) did not interfere with macrophage viability and decreased NF-kB activation and TNF-α levels in macrophage culture in vitro. OH-7 was selected as the most promising anti-inflammatory and used in subsequent assays. Mice pretreated orally with OH-7 showed a decrease in neutrophil migration and TNF-α level. Thus, these types of Brazilian organic honey show promising anti-inflammatory potential, particularly the OH-7 variety. Brazilian organic honey may lead to the development of new products and/or be incorporated into food for use in veterinary medicine and human health as well..

Romário-Silva, D., et al. (2022). Brazilian Organic Honey from Atlantic Rainforest Decreases Inflammatory Process in Mice. Veterinary sciences, 9(6), 268. https://doi.org/10.3390/vetsci9060268\

Honey stimulates cellular secretion of cytokines, which has been attributed to activation of lipopolysaccharide (LPS)-dependent and LPS-independent pathways. The objective of this study was to identify whether LPS is present in Australian honey samples at levels that can stimulate interleukin-6 (IL-6) secretion by fibroblasts and whether it can transduce cell signalling by activating toll-like receptor 4 (TLR4). IL-6 was measured in culture media of fibroblasts exposed to honey for 24 h. LPS was detected in a 0.125 mg/mL solution of grey ironbark honey (0.61 ± 0.05 ng/g honey). TLR4 signalling was observed in RAW264.7 macrophages that were exposed to honey and this was prevented by preincubating the honey with the LPS-neutralising agent, polymyxin B. Australian Eucalyptus, Leptospermum and Cyathode honeys stimulated IL-6 secretion in cultured human dermal fibroblasts. To examine whether the response was dependent on floral source, fibroblasts were exposed to four different samples of grey ironbark honey obtained from Queensland and New South Wales, Australia. The magnitude of the cytokine response to these honeys was highly varied. We conclude that Australian honeys contain endotoxin at levels that can stimulate IL-6 secretion by fibroblasts and that signalling in macrophages involves TLR4 activation. The IL-6 secretory response was independent of floral source.

Russell, F. D., et al. (2022). Secretion of IL-6 by fibroblasts exposed to Australian honeys involves lipopolysaccharide and is independent of floral source. Scientific reports, 12(1), 16628. https://doi.org/10.1038/s41598-022-21130-6

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Honey (8028-66-8) and Honey extract (91052-92-5) was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Honey (8028-66-8) and Honey extract (91052-92-5) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)
In vitro cytotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)

The incidence of human poisoning by honey consumption is extremely low although several sources of toxic honey exist. The major sources are members of the Ericaceae, including Rhododendron, Azalea, Andromeda and Kalmia species. Various toxicants have been isolated from honey or nectar and include acetylandromedol, andromedol, desacetylpieristoxin B, gelsemine, gelsemine HCL, tutin and hyenanchin. Symptoms of honey poisoning in human subjects include numbness in extremities, tingling weak pulse, loss of consciousness, indistinct vision, dizziness, nausea, vomiting and loss of enervation of voluntary muscles for andromedotoxins and related substances; delerium, giddiness, nausea, abdominal and head pain, vomiting, limb rigidity, convulsions, coma and loss of memory for Tutin and Hyenanchin; Giddiness, blindness, lassitude, nausea and convulsions for Gelsemine (White 1981).

Infant botulism, a disease that results in a blockade of voluntary motor and autonomic functions, was first recognized in the United States in the late 1970s. Since then, more than 1000 cases in this country have been reported to the Centers for Disease Control and Prevention (CDC). Numerous studies have shown that the ingestion of honey is linked with infant botulism. In addition, honey samples across the United States have tested positive for Clostridium botulinum spores and toxins. Such substantial evidence led the CDC to recommend that honey not be given to infants younger than 12 months old. It is important that clinicians be familiar with this risk and should not recommend honey-containing products or supplements or the use of honey as a flavoring agent for infants in this age group. As taken from Tanzi MG and Gabay MP. Pharmacotherapy. 2002 Nov; 22(11):1479-83. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed?term=Pharmacotherapy.%202002%20Nov%3B%2022(11)%3A1479-83

“There is a wealth of information about the nutritional and medicinal properties of honey. However, honey may contain compounds that may lead to toxicity. A compound not naturally present in honey, named 5-hydroxymethylfurfural (HMF), may be formed during the heating or preservation processes of honey. HMF has gained much interest, as it is commonly detected in honey samples, especially samples that have been stored for a long time. HMF is a compound that may be mutagenic, carcinogenic and cytotoxic. It has also been reported that honey can be contaminated with heavy metals such as lead, arsenic, mercury and cadmium. Honey produced from the nectar of Rhododendron ponticum contains alkaloids that can be poisonous to humans, while honey collected from Andromeda flowers contains grayanotoxins, which can cause paralysis of limbs in humans and eventually leads to death. In addition, Melicope ternata and Coriaria arborea from New Zealand produce toxic honey that can be fatal. There are reports that honey is not safe to be consumed when it is collected from Datura plants (from Mexico and Hungary), belladonna flowers and Hyoscamus niger plants (from Hungary), Serjania lethalis (from Brazil), Gelsemium sempervirens (from the American Southwest), Kalmia latifolia, Tripetalia paniculata and Ledum

palustre. Although the symptoms of poisoning due to honey consumption may differ depending on the source of toxins, most common symptoms generally include dizziness, nausea, vomiting, convulsions, headache, palpitations or even death. It has been suggested that honey should not be considered a completely safe food." As taken from Islam MN et al. 2014. J. Appl. Toxicol. 34(7), 733-42. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24214851

Estrogen is involved in number of cancers [80]. Honey modulates estrogen by its antagonistic action. It may be useful in estrogen-dependent cancers such as breasts and endometrial cancers [17]. Estrogen receptors tie to estrogens to dimerize and then translocate into the nuclei. These complexes then bind to the specific DNA base sequences called estrogen-response elements (EREs) resulting in transcription and translation of the estrogenic effect in the targeted tissue [80]. This signaling cascade induced by estrogens may be modulated at any stage [80]. Honeys from various floral sources are reported to mediate estrogenic effects via the modulation of estrogen receptor activity [17, 81]. This effect is attributed to its phenolic content [17]. Greek honey extracts exert estrogen agonistic effect at high concentrations (20–100lg/mL) and antagonistic effect at low concentrations (0.2–5μg/mL) [17]. As taken from Ahmed S & Othman NH. 2013. Evid. Based Complement. Alternat. Med. 2013, 829070. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24363771

"In this study honeys of Acacia modesta, Prunus persica, Zizyphus sativa and Isodon rogosus plants were tested against two Gram-positive bacterial strains (Staphylococcus aureus and Bacillus cereus), two Gram-negative bacterial strains (Klebsilla pneumonia and Escherichia coli) and two fungal strains (Alternaria alternata and Trichoderma harzianum) through Agar well diffusion method. The tested honeys showed high antimicrobial activities to the tested bacterial and fungal strains. All the tested honeys were more active against Gram-negative bacterial strains than the Gram-positive bacterial strains. They showed lower activity against the tested fungal strains as compared to all the tested bacterial strains. The given honeys showed free radical scavenging activity also." As taken from Zahoor M et al. 2014. Pak. J. Pharm. Sci. 27(1), 45-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24374434

"AIM: To characterize the effect of manuka honey on medically important wound bacteria in vitro, focusing on its antiadhesive properties. MATERIALS & METHODS: Crystal violet biofilm assays, fluorescent microscopy, protein adhesion assay and gentamicin protection assay were used to determine the impact of manuka honey on biofilm formation, human protein binding and adherence to/invasion into human keratinocytes. RESULTS: Manuka honey effectively disrupted and caused extensive cell death in biofilms of Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes. Sublethal doses of manuka honey inhibited bacterial adhesion to the fibronectin, fibrinogen and collagen. Manuka honeyimpaired adhesion of laboratory and clinical isolates of S. aureus, P. aeruginosa and S. pyogenes to human keratinocytes in vitro, and inhibited invasion by S. pyogenes and homogeneous vancomycin intermediate S. aureus. CONCLUSION: Manuka honey can directly affect bacterial cells embedded in a biofilm and exhibits antiadhesive properties against three common wound pathogens." As taken from Maddocks SE et al. 2013. Future Microbiol. 8, 1523-36. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24266353

"BACKGROUND: One of the most common causes of vaginitis is candidiasis. The aim of this study is to compare the effect of honey and miconazole against Candida albicans, in vitro. MATERIALS AND METHODS: The different W/V concentrations of honey were prepared at 20, 40, 60, 80, and 95% and different dilutions of miconazole were prepared in 0.05, 5, and 50 μg/ml. A microdilution of 100/000 cells per ml of a two-day old culture of Candida albicans was prepared in normal saline, after culturing the strain of PTCC 5027 in RPMI 1640 medium. Ten microliters of this dilution was added to 1 ml of the RPMI 1640 medium containing different concentrations of honey and to 1 ml of the RPMI 1640 medium containing different dilutions of miconazole. The cultures were incubated at

35°C for 12, 24, and 48 hours. RESULTS: The growth rate of Candida albicans was determined in the cultures. The results indicated that the honey prevented the growth of C. albicans greatly only at an 80% concentration, whereas, miconazole inhibited it completely. CONCLUSIONS: As Candida albicans is a normal vaginal flora, the inhibitory effect of honey without the fungicide effect is a very good trend in the treatment of vaginal candidiasis." As taken fromBanaeian-Borujeni Set al. 2013. Adv. Biomed. Res. 2, 57. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24223372

"Delayed healing associated with distal limb wounds is a particular problem in equine clinical practice. Recent studies in human beings and other species have demonstrated the beneficial wound healing properties of honey, and medical grade honey dressings are available commercially in equine practice. Equine clinicians are reported to source other non-medical grade honeys for the same purpose. This study aimed to assess the antimicrobial activity of a number of honey types against common equine wound bacterial pathogens. Twenty-nine honey products were sourced, including gamma-irradiated and non-irradiated commercial medical grade honeys, supermarket honeys, and honeys from local beekeepers. To exclude contaminated honeys from the project, all honeys were cultured aerobically for evidence of bacterial contamination. Aerobic bacteria or fungi were recovered from 18 products. The antimicrobial activity of the remaining 11 products was assessed against 10 wound bacteria, recovered from the wounds of horses, including methicillin resistant Staphylococcus aureus and Pseudomonas aeruginosa. Eight products were effective against all 10 bacterial isolates at concentrations varying from <2% to 16% (v/v). Overall, the Scottish Heather Honey was the best performing product, and inhibited the growth of all 10 bacterial isolates at concentrations ranging from <2% to 6% (v/v). Although Manuka has been the most studied honey to date, other sources may have valuable antimicrobial properties. Since some honeys were found to be contaminated with aerobic bacteria or fungi, non-sterile honeys may not be suitable for wound treatment. Further assessment of gamma-irradiated honeys from the best performing honeys would be useful." As taken from Carnwath R et al. 2014. Vet. J. 199(1), 110-4. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23962613

"BACKGROUND AND AIMS: Candida species, especially Candida albicans, are major fungal pathogens of humans that are capable of causing superficial mucosal infections and systemic infections in humans. The aim of this study was to evaluate the jujube (Zizyphus spina-christi) honey for its in vitro inhibitory activity against pre-formed biofilm and its interference with the biofilm formation of C. albicans. METHODS: The XTT reduction assay, scanning electron microscopy (SEM) and atomic force microscopy (AFM) were employed to determine the inhibitory effect of Jujube honey on C. albicans biofilm. Changes in the infrared spectrum after treatment with honey were also determined by Fourier transform infrared (FTIR) spectroscopy. RESULTS: Jujube honey affects biofilms by decreasing the size of mature biofilms and by disruption of their structure. At a concentration of 40% w/v, it interferes with formation of C. albicans biofilms and disrupts established biofilms. The SEM and AFM results indicated that this type of honeyaffected the cellular morphology of C. albicans and decreased biofilm thickness. CONCLUSIONS: The present findings show that jujube honey has antifungal properties against C. albicans and has the ability to inhibit the formation of C. albicans biofilms and disrupt established biofilms." As taken from Ansari MJ et al. 2013. Arch. Med. Res. 44(5), 352-60. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23867789

"BACKGROUND: Antibacterial activity of honey is mainly dependent on a combination of its peroxide activity and non-peroxide components. This study aims to investigate antibacterial activity of five varieties of Malaysian honey (three monofloral; acacia, gelam and pineapple, and two polyfloral; kelulut and tualang) against Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa. METHODS: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were performed for semi-quantitative evaluation. Agar well diffusion assay was used to investigate peroxide and non-peroxide activities of honey. RESULTS: The results showed that gelam honey possessed lowest MIC value against S. aureus with 5% (w/v) MIC and MBC of 6.25% (w/v). Highest MIC values were shown by pineapple honey against E. coli

and P. aeruginosa as well as acacia honey against E. coli with 25% (w/v) MIC and 50% (w/v) MBC values. Agar inhibition assay showed kelulut honey to possess highest total antibacterial activity against S. aureus with 26.49 equivalent phenol concentrations (EPC) and non-peroxide activity of 25.74 EPC. Lowest antibacterial activity was observed in acacia honey against E. coli with total activity of 7.85 EPC and non-peroxide activity of 7.59 EPC. There were no significant differences ($p > 0.05$) between the total antibacterial activities and non-peroxide activities of Malaysian honey. The intraspecific correlation between MIC and EPC of E. coli ($r = -0.8559$) was high while that between MIC and EPC of P. aeruginosa was observed to be moderate ($r = -0.6469$). S. aureus recorded a smaller correlation towards the opposite direction ($r = 0.5045$). In contrast, B.cereus showed a very low intraspecific correlation between MIC and EPC ($r = -0.1482$). CONCLUSIONS: Malaysian honey, namely gelam, kelulut and tualang, have high antibacterial potency derived from total and non-peroxide activities, which implies that both peroxide and other constituents are mutually important as contributing factors to the antibacterial property of honey." As taken from Zainol MI et al. 2013. BMC Complement. Altern. Med. 13, 129. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23758747

"OBJECTIVE: Honey has antibacterial activity. The aim of this study was to evaluate the antibacterial activity of honey on Streptococcus mutans and Lactobacillus. MATERIALS AND METHODS: In this in vitro study, solutions containing 0%, 5%, 10%, 20%, 50% and 100%(w/v) of natural Hamadan honey were prepared. Each blood (nutrient) agar plate was then filled with dilutions of the honey. The strains of bacteria were inoculated in blood agar for 24 hours at 37°C and were adjusted according to the McFarland scale (10×10 cfumcl(-1)). All assays were repeated 10 times for each of the honeyconcentrations. Data were analyzed by non parametric Chi-Square test. Statistical significance was set at $\alpha=0.05$. RESULTS: Significant antibacterial activity was detected for honey on Streptococcus mutans in concentrations more than 20% and on Lactobacillus in 100% concentration ($P<0.05$). CONCLUSION: It seems that antibacterial activity of honey could be used for prevention and reduction of dental caries." As taken from Ahmadi-Motamayel F et al. 2013. J. Dent. (Tehran) 10(1), 10-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23724198

"BACKGROUND AN AIMS: Antibiotic multiresistant microbes represent a challenging problem. Because honey has a potent antibacterial property, the antimicrobial effects of different honey samples against multiresistant pathogens and their compositions were investigated. METHODS: Five honey samples were used: Talah, Dhahian, Sumra-1, Sidr, and Sumra-2. Samples were analyzed to determine chemical composition such as fructose, glucose, sucrose, pH, total flavonoids, total phenolics, hydrogen peroxide concentration, minerals and trace elements. Antimicrobial activities of the samples against 17 (16 were multiresistant) human pathogenic bacteria and three types of fungi were studied. Specimens of the isolates were cultured into 10 mL of 10-100% (volume/volume) honey diluted in broth. Microbial growth was assessed on a solid plate media after 24 h and 72 h incubation. RESULTS: The composition of honey samples varied considerably. Sumra 1 and 2 contained the highest level of flavonoids and phenolics and the lowest level of hydrogen peroxide, whereas Dhahian honey contained the highest level of hydrogen peroxide. Sixteen pathogens were antibiotic multiresistant. A single dose of each honey sample inhibited all the pathogens tested after 24 h and 72 h incubation. The most sensitive pathogens were Aspergillus nidulans, Salmonella typhimurum and Staphylococcus epidermidis (S. epidermidis). Although there was no statistically significant difference in the effectiveness of honey samples, the most effective honey against bacteria was Talah and against fungi were Dhahian and Sumra-2. CONCLUSIONS: Various honey samples collected from different geographical areas and plant origins showed almost similar antimicrobial activities against multiresistant pathogens despite considerable variation in their composition. Honey may represent an alternative candidate to be tested as part of management of drug multiresistant pathogens." As taken from Al-Waili N et al. 2013a. Arch. Med. Res. 44(4), 307-16. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23684665

“BACKGROUND: Manuka honey originates from the manuka tree (Leptospermum scoparium) and its antimicrobial effect has been attributed to a property referred to as Unique Manuka Factor that is absent in other types of honey. Antibacterial activity of Manuka honey has been documented for several bacterial pathogens, however there is no information on Clostridium difficile, an important nosocomial pathogen. In this study we investigated susceptibility of C. difficile to Manuka honey and whether the activity is bactericidal or bacteriostatic. METHODS: Three C. difficile strains were subjected to the broth dilution method to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for Manuka honey. The agar well diffusion method was also used to investigate sensitivity of the C. difficile strains to Manuka honey. RESULTS: The MIC values of the three C. difficile strains were the same (6.25% v/v). Similarly, MBC values of the three C. difficile strains were the same (6.25% v/v). The activity of Manuka honey against all three C. difficile strains was bactericidal. A dose--response relationship was observed between the concentrations of Manuka honey and zones of inhibition formed by the C. difficile strains, in which increasing concentrations of Manukahoney resulted in increasing size of zone of inhibition formed. Maximum zone of inhibition was observed at 50% (v/v) Manuka honey and the growth inhibition persisted over 7 days. CONCLUSION: C. difficile is appreciably susceptible to Manuka honey and this may offer an effective way of treating infections caused by the organism.” As taken from Hammond EN & Donkor ES. 2013. BMC Res. Notes 6(1), 188. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23651562

“Honey has been widely accepted as food and medicine by all generations, traditions, and civilizations, both ancient and modern. For at least 2700 years, honey has been used by humans to treat a variety of ailments through topical application, but only recently have the antiseptic and antimicrobial properties of honey been discovered. Honey has been reported to be effective in a number of human pathologies. Clinical studies have demonstrated that application of honey to severely infected cutaneous wounds rapidly clears infection from the wound and improves tissue healing. A large number of in vitro and limited clinical studies have confirmed the broad-spectrum antimicrobial (antibacterial, antifungal, antiviral, and antimycobacterial) properties of honey, which may be attributed to the acidity (low pH), osmotic effect, high sugar concentration, presence of bacteriostatic and bactericidal factors (hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides), and increase in cytokine release, and to immune modulating and anti-inflammatory properties of honey; the antimicrobial action involves several mechanisms. Despite a large amount of data confirming the antimicrobial activity of honey, there are no studies that support the systemic use of honey as an antibacterial agent.” As taken from Israili ZH et al. 2014. Am. J. Ther. 21(4), 304-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23782759

“This study aimed to determine the factors (phenolic compounds, flavonoids, sugars or H2O2) that contribute the most to the antimicrobial activity of heather honey samples against four yeasts and four bacteria with medical importance. To discard the effect of H2O2 in the antimicrobial activity, catalase was added. To evaluate the osmotic pressure's effect, artificial honey was also used. Phenolic compounds and flavonoids were determined and Pearson's correlation analysis was performed to assess whether these correlated with antimicrobial activity. The amount of phenolic compounds ranged from 630.89 ± 5.21 GAE kg-1 to 718.92 ± 4.41 GAE kg-1, while the flavonoids varied between 450.72 ± 5.67 CAE kg-1 and 673.98 ± 4.33 CAE kg-1. For the bacteria, the minimum inhibitory concentration (MIC) of the honey without catalase ranged from 1.01 ± 0.50% to 10.00 ± 4.72% and was between 2.00 ± 0.94% and 13.27 ± 5.23% for honey with catalase. Concerning the yeasts, the MICs was between 13.16 ± 4.08% and 20.00 ± 5.09% for honey without catalase and between 14.95 ± 4.16% and 25.67 ± 5.50% for honey with catalase. The elucidation of the antimicrobial factors and action mechanisms is essential for the correct use of honey in therapeutic applications.” As taken from Feás X et al. 2013. Molecules 18(4), 4233-46. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23579991

“Biofilm growth and its persistence within wounds have recently been suggested as contributing factors to impaired healing. The goal of this study was to investigate the anti-biofilm effects of

several honey samples of different botanical origin, including manuka honey against Proteus mirabilis and Enterobacter cloacae wound isolates. Quantification of biofilm formation was carried out using a microtiter plate assay. All honeys at a sub-inhibitory concentration of 10% (w/v) significantly reduced the biofilm development of both isolates. Similarly, at a concentration of 50% (w/v), each of the honeys caused significant partial detachment of Pr. mirabilis biofilm after 24 h. On the other hand, no honey was able to significantly detach Ent. cloacae biofilm. In addition, treatment of Ent. cloacae and Pr. mirabilis biofilms with all honeys resulted in a significant decrease in colony-forming units per well values in a range of 0.35-1.16 and 1.2-7.5 log units, respectively. Of the tested honeys, manuka honey possessed the most potent anti-biofilm properties. Furthermore, methylglyoxal, an antibacterial compound of manuka honey, was shown to be responsible for killing biofilm-embedded wound bacteria. These findings suggest that manuka honey could be used as a potential therapy for the treatment of wounds containing Pr. mirabilis or Ent. cloacae." As taken from Majtan J et al. 2014. Phytother. Res. 28(1), 69-75. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23494861

"Recently renewed interest in the therapeutic properties of honey has led to the search for new antimicrobial honeys. This study was undertaken to assess the antimicrobial activity and composition of a locally produced Portobello honey (PBH) on three bacteria known to infect wounds. Manukahoney (MH) was used for comparative purposes. Broth culture and agar disc diffusion assays were used to investigate the antimicrobial properties ofhoney. The honeys were tested at four concentrations: 75%, 50%, 10% and 1% (v/v) and compared with an untreated control. The composition ofhoney was determined by measuring: polyphenol content by Folin Ciocalteau method, antioxidant capacity by ferric ion reducing power assay, hydrogen peroxide (H2 O2) by catalase test, pH and sugar content by pH strips and refractometer, respectively. Both honeys at 75% and 50% inhibited the majority of the three bacteria tested. 10% PBH exhibited antimicrobial activity to the lesser extent than 10% MH. The difference was very significant ($p \leq 0.001$). Both honeys were acidic with pH 4, and both produced H2 O2 . The sugar content of PBH was higher than MH, but the difference was not significant. The MH had significantly higher levels of the polyphenols and antioxidant activity than PBH." As taken from Schneider M et al. 2013. Phytother. Res. 27(8), 1162-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22991325

"The aims of this study were to determine grayanotoxin (GTX-III) toxin level in mad honey using liquid chromatography-tandem mass spectrometry and examine the dynamic changes of certain biochemical parameters in blood serum of rats that consumed mad honey. For the experimental animal study, 20 Sprague-Dawley female rats were divided into 5 groups of 4 rats each, with one group being the control group (Group 1) and the others being the experimental groups (Groups 2-5). Groups 2, 3, 4, and 5 were, respectively, given mad honey extract at doses of 0.3, 0.6, 1.2, and 2.4 mg/g body weight/day via oral gavage for 8 days. According to results, the quantity of GTX-III found in the honey sample as 39.949 ± 0.020 μg GTX-III/g honey, and the biochemical analysis of the tested parameters (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatine kinase, and creatine kinase muscle and brain) showed a significant elevation with increasing concentration of honey. In conclusion, the use of increasing concentrations of Rhododendron honey was seen as a source of enzymatic symptoms." As taken from Sahin H et al. 2016. J. Evid. Based Complementary Altern. Med. 21(4), 255-9. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26239637

"The aim of this study was to evaluate new natural inhibitor sources for the enzymes urease and xanthine oxidase (XO). Chestnut, oak and polyfloral honey extracts were used to determine inhibition effects of both enzymes. In addition to investigate inhibition, the antioxidant capacities of these honeys were determined using total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity assays. Due to their high phenolic content, chestnut and oak honeys are found to be a powerful source for inhibition of both enzymes. Especially, oak honeys were efficient for urease inhibition with 0.012-0.021 g/mL IC50 values, and also chestnut

honeys were powerful for XO inhibition with 0.028-0.039 g/mL IC50 values. Regular daily consumption of these honeys can prevent gastric ulcers deriving from Helicobacter pylori and pathological disorders mediated by reactive oxygen species." As taken from Sahin H. 2016. J. Enzyme Inhib. Med. Chem. 31(3), 490-4. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/25942364

"BACKGROUND: Antioxidant and anti-inflammatory properties of honey have been largely recognized by various studies. Almost all of the potential benefits are associated with polyphenol content. Honey varieties from the arid region are reported to be rich in polyphenols, but data related to its bioactivity in vitro is greatly lacking. This study aimed at establishing the antioxidant and anti-inflammatory properties of arid region honey. Four honey varieties from arid region (H1, H2, H3, and H4) and two popular non-arid region honey (H5 and H6) were tested in vitro in this study. METHODS: The erythrocyte membrane protection effect of honey varieties were measured by hemolysis assay after exposing erythrocytes to a peroxide generator. The subsequent production of MDA (malondialdehyde) content in erythrocytes was measured. Immunomodulatory effect of the honey varieties was tested in prostate cancer cells PC-3 and PBMC (peripheral blood mononuclear cells) by measuring the IL-6 (interleukin 6) and NO (nitric oxide) levels in cell culture supernatant after incubation with the honey varieties. PC-3 cell viability was assessed after incubation with honey varieties for 24 h. RESULTS: Arid region honey exhibited superior erythrocyte membrane protection effect with H4 measuring 1.3 ± 0.042mMTE/g and H2 measuring 1.122 ± 0.018mMTE/g. MDA levels were significantly reduced by honey samples, especially H4 (20.819 ± 0.63 nmol/mg protein). We observed a significant decrease in cell population in PC-3 after 24 h in culture on treatment with honey. A moderate increase in NO levels was observed in both cultures after 24 h at the same time levels of IL-6 were remarkably reduced by honey varieties. CONCLUSION: The results demonstrate the antioxidant effect of arid region honey due to its erythrocyte membrane protection effect and subsequent lowering of oxidative damage as evident from lower levels of lipid peroxidation byproduct MDA. Arid region honey varieties were as good as non-arid region types at decreasing cell viability of prostate cancer cells. The moderate increase in NO levels in PC-3 and PBMCs were not significant enough to elicit any pro-inflammatory response. However, IL-6 secretion was remarkably reduced by all honey varieties in a comparable level indicating the potential anti-inflammatory property of arid region honey." As taken from Hilary S et al. 2017. BMC Complement. Altern. Med. 17(1), 177. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28356100

"Soft-tissue invasive fungal infections are increasingly recognized as significant entities directly contributing to morbidity and mortality. They complicate clinical care, requiring aggressive surgical debridement and systemic antifungal therapy. To evaluate new topical approaches to therapy, we examined the antifungal activity and cytotoxicity of Manuka Honey (MH) and polyhexamethylene biguanide (PHMB). The activities of multiple concentrations of MH (40%, 60%, 80%) and PHMB (0.01%, 0.04%, 0.1%) against 13 clinical mould isolates were evaluated using a time-kill assay between 5 min and 24 h. Concentrations were selected to represent current clinical use. Cell viability was examined in parallel for human epidermal keratinocytes, dermal fibroblasts and osteoblasts, allowing determination of the 50% viability (LD50) concentration. Antifungal activity of both agents correlated more closely with exposure time than concentration. Exophiala and Fusarium growth was completely suppressed at 5 min for all PHMB concentrations, and at 12 and 6 h, respectively, for all MH concentrations. Only Lichtheimia had persistent growth to both agents at 24 h. Viability assays displayed concentration-and time-dependent toxicity for PHMB. For MH, exposure time predicted cytotoxicity only when all cell types were analyzed in aggregate. This study demonstrates that MH and PHMB possess primarily time-dependent antifungal activity, but also exert in vitro toxicity on human cells which may limit clinical use. Further research is needed to determine ideal treatment strategies to optimize antifungal activity against moulds while limiting cytotoxicity against host tissues in vivo." As taken from Yabes JM et al. 2017. Med. Mycol. 55(3), 334-343. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27601610

“A scenario analysis in regard to the risk of chronic exposure of consumers to residues through the consumption of contaminated honey and beeswax was conducted. Twenty-two plant protection products and veterinary substances of which residues have already been detected in beeswax in Europe were selected. The potential chronic exposure was assessed by applying a worst-case scenario based on the addition of a "maximum" daily intake through the consumption of honey and beeswax to the theoretical maximum daily intake through other foodstuffs. For each residue, the total exposure was finally compared to the acceptable daily intake. It is concluded that the food consumption of honey and beeswax contaminated with these residues considered separately does not compromise the consumer's health, provided proposed action limits are met. In regard to residues of flumethrin in honey and in beeswax, "zero tolerance" should be applied.” As taken from Wilmart O et al. 2016. J. Agric. Food Chem. 64(44), 8425-8434. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27741395

“Concentration values of 24 elements (Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Ge, Hg, Mn, Mo, Pb, Sb, Se, Sn, Sr, Ti, Tl, U, V, and Zn) were determined in 72 honey samples produced in Italy by inductively coupled plasma mass spectrometry (ICP-MS). Considering the recommended established heavy metal daily intakes for humans, in this perspective, an equilibrated and ordinary honey consumption should not be considered matter of concerns for human health, even if particular attention should be addressed if honey is consumed by children, due to different maximum daily heavy metal intakes. Chemometric analysis of the results obtained highlights heavy metal content differences in honey samples obtained from notoriously polluted zones, confirming then that honey can be considered a bio-indicator of environmental pollution. Finally, Pearson coefficients highlighted correlations among element contents in honey samples.” As taken from Quinto M et al. 2016. Environ. Sci. Pollut. Res. Int. 23(24), 25374-25384. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27696193

“Rhododendron honey intoxication’s symptoms are dose-related. In mild form dizziness, weakness, excessive perspiration, hypersalivation, nausea, vomiting and paresthesias are present.”

“Reported amount of honey causing poisoning is between 5 to 150 g.”

As taken from EFSA, 2017

“Background: Oral hygiene is an act of cleansing the entire area of the mouth, including teeth and gums to avoid infection. Purpose of hygiene to reduce dental plaque, reduce risk of oral cavity, eliminate tooth decay, gum, improve comfort in child.. Objective: This study aims to determine the effect of oral hygiene using 30% pure honey to the number of candida albicans in hospitalized children. Method: This study was a quasy experiment pre and post test with control group design. The data were analyzed with paired t-test dan independent paired samples t-test. The population of this study was all hospitalized children. The sample size is determined by purposive sampling technique, with a sample size of 20 (10 children were intervention group, 10 children were control group). Result: Mean number of candida pre test of 38.90 CFU / ml and post test A total of 27.40 CFU / ml. The result of statistical test of separate parametric test in pairs of t-test p value of 0.001 (α = 0,05), so it can be concluded that there is oral hygiene effect using 30% pure honey to number of candida albicans child's mouth. Discussion: Hospitalized children were high risk population of nosocomial infection. There were many source of secondary infection such as infection by candida albicans. The recommendation of this research is that all children treated in hospital are done orally hygiene by using 30% pure honey.” As taken from Alfiyanti D and Hidayanti T-N. 2018. Media Keperawatan Indonesia 1(1), 36-42. Available at http://jurnal.unimus.ac.id/index.php/MKI/article/view/3292

“Physicochemical properties, main mineral content, and antioxidant activity were determined for eight floral carob honeys collected from different geographical regions of Morocco. Moroccan honeys showed good chemical and nutritional qualities, fulfilling the criteria described in the standard codex for honey. The percentages obtained for ashes were (0.13-0.69%), electrical conductivity (0.36-1.35 mS/cm), water content (17.30-22.80%), pH (4.17-5.05), free acidity (11.0-

42.50 meq/kg), lactone acidity (4.0-16.50 meq/kg), and for total acidity (16.50-59.50 meq/kg). In addition, minerals such as K, Na, Mg, Cu, Zn, and Ca of honey samples were determined and potassium was the major mineral in all samples. The antioxidant activities based on the free radical scavenging, reducing power, and total antioxidant activity were investigated, and the antioxidant capacity of the honey samples was correlated with their biochemical constituents such as total phenol and flavonoids content, and the best antioxidant capacity was confirmed by the honey from Taounate." As taken from El-Haskoury R et al. 2018b. J. Food Drug Anal. 26(1), 67-73. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29389590

"This study was conducted with the aim of determining the chemical, biochemical properties, and antimicrobial capabilities of some of the monofloral honeys produced in Turkey. In this study, 23 different monofloral honey samples were obtained from diverse geographical regions of Turkey. Floral origin of the honey samples was determined by melissopalinological analyses. Additionally, antioxidant properties were determined. To determine the antioxidant properties of honey samples, four test methods of total phenolic content, DPPH, iron reduction power and β-carotene linoleic acid emulsion method were used. As a result of the antioxidant activity analysis among the honey samples, rhododendron and parsley honey showed most prominent results in terms of the amount of phenolic compounds and antioxidant activity. On the other hand, acacia and citrus honey samples showed least antioxidant activity. A positive correlation was determined between four methods. Differences between antioxidant activities of honey samples were significantly found ($P < 0.01$)." As taken from Gül A and Pehliva T. 2018. Saudi Journal of Biological Sciences 25(6), 1056-1065. Available at https://www.sciencedirect.com/science/article/pii/S1319562X18300469

"Honey is a food known for its medical properties. In this work, we have studied the impact of different types of honey on insulin signalling pathway. We found that honey extracts inhibit the enzyme PTP1B, one of the main negative regulators of insulin receptor signalling. HPLC-MS analysis allowed us to confirm the presence of several natural PTP1B inhibitors in the honey extracts analysed. Statistical analysis methods show a correlation between specific 1H-NMR resonance frequencies/HPLC peaks and the inhibitory power of the samples. This finding will allow the prediction of the biological properties of honey samples applying relative simple analytical methods. Finally, we demonstrated that the treatment of HepG2 cells with honey extracts enhances the expression of insulin receptor, and stimulates glucose uptake. For the first time, our results demonstrate that bioactive components of honey could improve glycaemic control by both inhibiting PTP1B and stimulating the expression of insulin receptor in liver cells." As taken from Lori G et al. 2019. Biomed. Pharmacother. 113, 108752. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30927676

"Introduction: The effect of honey consumption in diabetic patients has been contradictory. The aim of the present animal study was to compare the effect of different types of honey on the lipid profile in diabetic rats. Material and methods: Sixty-four male Wistar rats were divided into two main groups: a streptozotocin-induced diabetes mellitus (DM) group (including four subgroups) and a healthy group (including four subgroups), based on random allocation. Three subgroups of each main group were given 1 mg/kg of three different types of honey (acacia, astragalus, and artificial honey) by oral gavage for 10 weeks. The control groups were given distilled water. Blood samples were collected, and the lipid profile was measured and compared between the eight groups after the intervention. Results: The levels of LDL, triglycerides (TG), and total cholesterol (Tchol) in DM rats treated with astragalus honey were significantly lower and the HDL level was significantly higher compared to the other DM and healthy groups (all p-values < 0.05). LDL, TG, and Tchol levels in DM rats treated with artificial honey were significantly higher, and HDL levels were significantly lower than for other types of honey and for the control groups (all p-values < 0.05). LDL, HDL, TG, and Tchol levels in healthy rats were not significantly different between the groups (p-value > 0.05). Conclusions: Different types of honey (acacia, astragalus, and artificial honey) had various effects on serum lipid profiles in diabetic rats. The results of this study indicated that the effect of honey on diabetic patients can vary widely based on its source." As taken from

Mohammadimanesh A et al. 2019. Arch. Med. Sci. Atheroscler. Dis. 4, e113-e118. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31211278/

“Background: This study investigated long-term effect of the Obudu honey on selected biomarkers of energy storage regulation, compared to table sugar. Methods: Fifty Wistar rats assigned to 5 groups of 10 rats each, were fed rat chow only (NC), 8% table sugar (S8%), 16% table sugar (S16%), 10% honey (H10%) and 20% honey (H20%) diets respectively, for 29 weeks. On dry weight basis, the percentages of table sugar and honey for each level of incorporation were equivalent. Diet intake, body weights and fasting blood glucose (FBG) were measured fortnightly. At the end of the study, serum glucose, insulin, leptin and tissue necrosis factor - α (TNF-α), wet weight of white adipose tissues (WAT) were measured. Results: After an initial adjustment to the diets, there was no significant difference in diet consumed by female and male subgroups, except the female group fed H20% which was consistently lower than the NC and the corresponding S16% fed group ($P < 0.05$). Both honey and sugar incorporated diets caused significant body weight gain in the female animals compared to NC; an effect which was higher with the honey than sugar, and depended on the level of each sweetener used as well as feeding duration ($P < 0.05$). Furthermore, S8% and S16% diets increased leptin concentration in the female rats, by 35.8 and 45.3% respectively compared with NC and by 63.8 and 40.5% compared to H10% and H20% respectively ($P < 0.05$). Also, the S8% and S16% diets significantly increased serum insulin in the female subgroups compared to the corresponding honey-sweetened diets; and in both male and female rats when compared to NC ($P < 0.05$). Lastly, the S8% and S16% diets also caused a dose-dependent increase of TNF-α in both female and male rats compared to the H10% and H20% diets and the control ($P < 0.05$). Conclusion: Data obtained from the study associated table sugar with obesigenic and inflammatory mechanisms more than the Obudu honey, particularly in the females. However, the data did not exempt the honey from obesigenic effect. The effects were subtle and may require a longer time to precipitate obesity.” As taken from Atangwho IJ et al. 2020. BMC Nutr. 6, 3. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32153977/

6. Functional effects on

6.1. Broncho/pulmonary system

“A 63-year-old woman presented with a 4-hr history of sneezing, visual disturbance, and dyspnea after drinking foreign honey dissolved in hot water. Severe hypotension (56/30 mmHg) and bradycardia (55 beats/min) were identified on arrival. She was immediately administered intravenous atropine (0.5 mg) and a bolus injection of Ringer solution (2,000 mL). Circulatory abnormality dramatically improved immediately after atropine injection and she was discharged on hospital day 2. We speculate that the patient suffered from honey intoxication because of manifestations such as hypotension and bradycardia, which are commonly seen in patients intoxicated by honey.” As taken from Inagaki T et al. 2013. Chudoku Kenkyu 26(4), 310-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24483011

6.2. Cardiovascular system

Seven men and three women (mean age, 31.2 years; range, 20-45 years) received a strictly controlled regular diet during a 2-week control period, followed by the regular diet supplemented with daily consumption of 1.2 g/kg body weight honey dissolved in 250 ml of water during a 2-week test period. At the end of each period, overnight fasting blood samples were withdrawn for assays of blood glucose, blood minerals, vitamin C, beta-carotene, uric acid, glutathione reductase, immunoglobulin E, hemoglobin, blood indices and cells, serum ferritin, serum iron, and iron-binding capacity. Results showed that honey increased antioxidant agents. It increased blood vitamin C concentration by 47%, beta-carotene by 3%, uric acid by 12%, and glutathione reductase by 7%. Honey increased serum iron by 20% and decreased plasma ferritin by 11%. It increased the percentage of monocytes by 50%, and increased lymphocyte and eosinophil percentages slightly.

Honey reduced serum immunoglobulin E by 34% and increased serum copper by 33%. It decreased aspartate transaminase by 22% and alanine transaminase by 18%. Honey markedly reduced lactic acid dehydrogenase by 41%, decreased creatinine kinase by 33%, and reduced fasting blood sugar by 5%. It caused slight elevations in blood zinc and magnesium, hemoglobin, and packed cell volume. It may be concluded that honey increased antioxidant agents, serum iron and blood indices, and trace elements and decreased immunoglobulin E, liver and muscle enzymes, and fasting blood sugar in healthy subjects. As taken from Ai-Waili NS. J Med Food. 2003b. Summer; 6(2):135-40. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/12935325

Honey intoxication, a kind of food poisoning, can be seen in the Black Sea region of Turkey and in various other parts of the world as well. In this study, 66 patients were hospitalized with a variety of symptoms including nausea, vomiting, salivation, dizziness, weakness, hypotension, bradycardia and syncope several hours after the ingestion of small amounts of honey. All patients had hypotension, and majority had bradycardia. These features resolved completely in 24 h with i.v. fluids and atropine, and none died. In conclusion, honey poisoning should be taken into consideration in the differential diagnosis of acute myocardial infarction and in the patients with vomiting, hypotension and bradycardia. As taken from Yilmaz O. Resuscitation. 2006 Mar; 68(3):405-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/16457936

The effect of honey on blood alcohol metabolism and the accompanying changes in serum triacylglycerol and blood pressure were investigated using volunteers. Fifty consenting undergraduates in apparent good health, between the ages of 15 and 30 years (23.6 +/- 7.4), were recruited for the study. The subjects were moderate alcohol drinkers (<30 g ethanol/day), matched in body weight and frame size. The participants were given ethanol (0.5 g/kg) and ethanol + honey (0.5 g/kg + 1.25 ml/kg) on two different occasions separated by 1 week. The results show that honey significantly ($p < 0.01$) increased blood alcohol disappearance and elimination rates by 32.4 and 28.6%, respectively, but reduced the intoxication time (that is, the time taken to attain zero blood alcohol level) and its degree (the peak blood alcohol level) by 30.0 and 4.4%. Ethanol + honey further increased serum triacylglycerol and blood pressure by 20.8 and 1.3/1.4% when compared with the proportion induced by ethanol after about 10 h of ingestion. The occasional use of honey as an anti-intoxicating agent may be approved. Meanwhile, further studies on how to ameliorate or prevent the associated increase in serum triacylglycerol and blood pressure is required. As taken from Onyesom I. Ann Nutr Metab. 2005 Sep-Oct;49(5):319-24. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/16088097

An unusual type of food poisoning is commonly seen in the Black Sea coast of Turkey attributable to andromedotoxin containing toxic honey ingestion. This study is a retrospective case series of 19 patients admitted to an emergency department in 2002, poisoned by "mad" honey. All of the patients had the complaints of nausea, vomiting, sweating, dizziness, and weakness, several hours after ingesting "mad" honey. Physical examination showed hypotension in 15 patients, sinus bradycardia in 15, and complete atrioventricular block (AVB) in four patients on admission. Two patients with bradycardia and two with AVB fell and injured their heads. Three of them presented with local haematoma. One patient had a 6 cm cut on his head without any neurological deficit and his cranial computed tomography imaging was normal. Hypotension and conduction disorders resolved with atropine treatment, resulting in complete recovery within 24 hours. As taken from Ozhan H et al. Emerg Med J. 2004 Nov; 21(6):742-4. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/15496712

“AIMS: Although cases of acute mad honey intoxication have been reported earlier, chronic mad honey intoxication (CMHI) syndrome has not been described and we address this issue only in this study. METHODS AND RESULTS: We prospectively evaluated the history of non-commercial honey intake in all patients referred to our institution for investigation of slow heart rate or atrioventricular (AV) conduction abnormalities. Between April 2008 and December 2008, 173 patients were referred to our institution for assessment of sinus bradycardia and various degrees of

AV block and/or permanent pacemaker implantation. All patients were questioned about history of honey intake. Detailed evaluation revealed a history of daily honey intake for a long period of time in five of the patients (2.8%). This non-commercial honey was made by different amateur beekeepers in eastern Back Sea region of Turkey. Discontinuation of honey intake resulted in prompt normalization of conduction and significant symptomatic improvement. None of the patients were admitted to hospital and all were asymptomatic during 3 months follow-up. Holter monitoring for 24-h revealed no abnormality at first and third month. CONCLUSIONS: This is the first report of CMHI. This issue should be suggested during assessment of patients with unexpected conduction abnormalities, because abandonment of honey intake results in prompt symptomatic and electrocardiographic improvement". As taken from Aliyev F et al. 2009. Europace 11, 954-956. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/19502248?dopt=AbstractPlus.

"OBJECTIVE: This study was designed to analyze the characteristics of adult patients with mad honeyintoxication, with special emphasis on its effects on vital signs and blood glucose levels. METHODS: Patients admitted to the Emergency Department of urban hospital in the Black Sea region of Turkey over the 16-months study period due to madhoneyintoxication were included. Patients' demographic and clinical characteristics, including age, sex, systolic and diastolic blood pressure, rhythm at ECG, heart rate, blood glucose levels and clinical outcomes were recorded and analyzed. RESULTS: Forty-six patients with a presumptive diagnosis of madhoneypoisoning were recruited. Mean age was 52.2 (±17.2). Blood glucose level was normal in 28 cases (60.9%) and high in 18 (39.1%). Systolic blood pressure (SBP) was low in 40 patients (87%) and normal in six (13%). Diastolic blood pressure (DBP) was low in 42 cases (91.3%) and normal in four (8.7%). Mean glucose level in patients with low SBP was 116.1 (±52.9) mg/dL, vs. 120.7 (±23.0) mg/dL in those with normal or high SBP (p = 0.389). Mean glucose level in patients with low DBP was 118.7 (±51.4) mg/dL, compared to 96.0 (±22.8) mg/dL in those with normal or high DBP (p = 0.146). Heart rate was below or equal to 45 bpm in 28 patients (60.9%). Complete (third degree) heart block was diagnosed in one case. CONCLUSION:M Madhoneywas found not to cause significant decreases in blood glucose levels in humans. Hypotension, bradycardia and related clinical consequences are commonly encountered in patients diagnosed with madhoney or grayanotoxin poisoning." As taken from Uzun H et al. 2013. Eur. Rev. Med. Pharmacol. Sci. 17(20), 2728-31. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24174354

"The aims of this study were to evaluate the clinical characteristics and outcomes of patients with grayanotoxin poisoning due to mad honey brought from Nepal. Medical records of patients with mad honey poisoning admitted to the emergency department between 1 January 2004 and 31 May 2012 were retrospectively reviewed. A total of 15 patients were included in this study. In all patients, mad honey was brought from the Himalayan region of Nepal. The mean age was 52.2 years, and 66.7 % were men. The mean amount of mad honey ingested was 47 cc, and the mean time from ingestion to onset of symptoms was 36 min. In all patients, initial vital signs showed hypotension and bradycardia. The initial electrocardiogram showed sinus bradycardia in eight patients, junctional bradycardia in four patients, complete atrioventricular block in two patients, and atrial fibrillation with slow ventricular response in one patient. Four patients were treated with intravenous normal saline solution only. Eleven patients were treated with intravenous normal saline solution and intravenous atropine sulfate in a dose ranging from 0.5 to 2.0 mg. In all patients, the blood pressure and pulse rate returned to normal limits within 24 h. There were no deaths. The clinical characteristics and outcome of grayanotoxin poisonings caused by the ingestion of mad honey from Nepal are similar with those of mad honey from the Black Sea region of Turkey" As taken from Sohn N et al. 2014. Intern. Emerg. Med. 9(2), 207-11. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24026434

"A 63-year-old woman presented with a 4-hr history of sneezing, visual disturbance, and dyspnea after drinking foreign honey dissolved in hot water. Severe hypotension (56/30 mmHg) and bradycardia (55 beats/min) were identified on arrival. She was immediately administered intravenous atropine (0.5 mg) and a bolus injection of Ringer solution (2,000 mL). Circulatory

abnormality dramatically improved immediately after atropine injection and she was discharged on hospital day 2. We speculate that the patient suffered from honey intoxication because of manifestations such as hypotension and bradycardia, which are commonly seen in patients intoxicated by honey." As taken from Inagaki T et al. 2013. Chudoku Kenkyu 26(4), 310-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24483011

"Despite reports indicating anti-inflammatory effects of honey, the anti-angiogenic effect of honey and its impact on inflammatory mediators in the air pouch model of inflammation have not yet been studied. The aims of present study were to investigate the effects of honey on angiogenesis, inflammatory cytokine vascular endothelial growth factor (VEGF) level as an important marker of angiogenesis and prostaglandin E2 (PGE2) in the rat air pouch model of inflammation. Male Wistar rats were anesthetized, and then 20 ml and 10 ml of sterile air were injected subcutaneously in the back on days 0 and 3, respectively. On day 6, inflammation was induced by injection of 1 ml of carrageenan 1% into pouches. After 72 h, the rats were sacrificed; pouch fluid was collected in order to determine PGE2 concentration and VEGF level. The Pouches were dissected out and weighed. Angiogenesis of granulomatous tissue was assayed using a hemoglobin kit. Honey was able to reduce granulation tissue weight and angiogenesis as well as showing potent inhibitory activities against PGE2 and VEGF in air pouch model of inflammation. The decrease in angiogenesis correlates with the inhibition of PGE2 and VEGF. Honey is potentially useful in the treatment of granulomatous inflammatory conditions. It seems that the anti-angiogenic activities of honey are mediated through modulation of PGE2 and VEGF production." As taken from Eteraf-Oskouei T et al. 2014. Drug Res. (Stuttg.). 64(10), 530-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24357137

"Diabetes mellitus, hypercholesteremia, hypertension (HTN), and obesity are well-known risk factors for cardiovascular diseases (CVD). Various medications are currently in use for management of these comorbidities. Undesirable side effects are unavoidable and the ultimate and ideal goal is hardly achieved. Honey and other bee products are widely used in traditional medicine for management of many diseases. Others and the authors have found potent biological activities of these products. Honey is now reintroduced in modern medicine as part of wound and burn management.Honey has antioxidant, anti-inflammatory, and antimicrobial activities. More studies are exploring other aspects of honey activity such as its effect on blood sugar, body weight, lipid profile, C-reactive protein, nitric oxide, proinflammatory prostaglandins, and homocysteine. Growing evidence and scientific data support the use of honey in patients with diabetes, HTN, dyslipidemia, obesity, and CVD. This review discusses clinical and preclinical studies on potential influence of honey on diabetes mellitus and cardiovascular risk factors, and emphasizes the importance of conducting more clinical and controlled studies." As taken from Al-Waili N et al. 2013b. J. Food Sci. 16(12), 1063-78. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24328699

"….Honey positively affects risk factors for cardiovascular diseases by inhibiting inflammation, improving endothelial function, as well as the plasma lipid profile, and increasing low-density lipoprotein resistance to oxidation…. the evidence of the biological actions of honey can be ascribed to its polyphenolic contents which, in turn, are usually associated to its antioxidant and anti-inflammatory actions, as well as to its cardiovascular, antiproliferative and antimicrobial benefits." As taken from Alvarez-Suarez JM et al. 2013. Curr. Med. Chem. 20(5), 621-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23298140

"Mad honey poisoning occurs when honey containing grayanotoxin is digested. The most common clinical signs and symptoms of poisoning involve findings of digestive system irritation, severe bradycardia and hypotension and central nervous system reaction. In this review, we aimed to underline the cardiac effects of mad honey poisoning. We also aimed to raise the awareness of physicians about early diagnosis and treatment of this rare entity." As taken from Erenler AK. 2016. Cardiovasc. Toxicol. 16(1), 1-4. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/25613735

“Rhododendron honey poisoning caused by grayanotoxin is associated with autonomic nervous system symptoms, such as.....bradycardia.”

“Severe intoxication may lead to life threating cardiac complication such as complete atrioventricular block. Reported amount of honey causing poisoning is between 5 to 150 g.”

As taken from EFSA, 2017

6.3. Nervous system

Fifteen persons developed atropine poisoning following consumption of wasp honey. Clinical signs, antidotal response and the presence of Datura plants near the wasp nests supported that the intoxications were caused by ingestion of atropine-contaminated honey. Two deaths occurred from heatstroke because of the poisoning and high environment temperatures and intensive physical activity. As taken from Ramirez M et al. Vet Hum Toxicol. 1999 Feb; 41(1):19-20. PubMed available at http://www.ncbi.nlm.nih.gov/pubmed/9949478

“The use of honey for therapeutic purposes is on the increase and many studies have shown that honey has the ability to influence biological systems including pain transmission. Therefore, this study was designed to investigate the analgesic and anti-inflammatory effects of honey and the effects of concurrent administration of autonomic nervous system blocking drugs. Studies on analgesic activities was carried out using hotplate and formalin-induced paw licking models while the anti-inflammatory activity was by the carrageenan paw oedema method. Animals were distributed into six groups consisting of five animals each. They were administered saline, honey (600 mg/kg), indomethacin (5 mg/kg), autonomic blockers (3 μg/kg of tamsulosin, 20 mg/kg (intraperitoneally) of propranolol, 2 ml/kg of atropine or 10 mg/kg (intra muscularly) of hexamethonium) or honey (200 and 600 mg/kg) with one of the blockers. The results showed that honey reduced pain perception especially inflammatory pain and the administration of tamsulosin and propranolol spared the effect of honey. Hexamethonium also spared the effects of honey at the early and late phases of the test while atropine only inhibited the early phase of the test. However, atropine and hexamethonium spared the anti-inflammatory effects of honey but tamsulosin abolished the effects while propranolol only abolished the anti-inflammatory effects at the peak of the inflammation. The results suggest the involvement of autonomic receptors in the anti-nociceptive and anti-inflammatory effects of honey although the level of involvement depends on the different types of the receptors.” As taken from Owoyele BV et al. 2014. Metab. Brain Dis. 29(1), 167-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24318481

“Recently, our research team has reported that Tualang honey was able to improve immediate memory in postmenopausal women comparable with that of estrogen progestin therapy. Therefore the aim of the present study was to examine the effects of Tualang honey supplement on hippocampal morphology and memory performance in ovariectomized (OVX) rats exposed to social instability stress. Female Sprague-Dawley rats were divided into six groups: (i) sham-operated controls, (ii) stressed sham-operated controls, (iii) OVX rats, (iv) stressed OVX rats, (v) stressed OVX rats treated with 17β-estradiol (E2), and (vi) stressed OVX rats treated with Tualang honey. These rats were subjected to social instability stress procedure followed by novel object recognition (NOR) test. Right brain hemispheres were subjected to Nissl staining. The number and arrangement of pyramidal neurons in regions of CA1, CA2, CA3 and the dentate gyrus (DG) were recorded. Two-way ANOVA analyses showed significant interactions between stress and OVX in both STM and LTM test as well as number of Nissl-positive cells in all hippocampal regions. Both E2 and Tualang honeytreatments improved both short-term and long-term memory and enhanced the neuronal proliferation of hippocampal CA2, CA3 and DG regions compared to that of untreated stressed OVX rats.” As taken from Al-Rahbi B et al. 2014. Acta Histochem. 116(1), 79-88. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23810156

“Rhododendron honey poisoning caused by grayanotoxin is associated with autonomic nervous system symptoms, such as excessive perspiration, hypersalivation, vomiting and bradycardia. Animal study confirmed autonomic symptoms of grayanotoxin intoxication.”

“Rhododendron honey intoxication’s symptoms are dose-related. In mild form dizziness, weakness, excessive perspiration, hypersalivation, nausea, vomiting and paresthesias are present.”

As taken from EFSA, 2017

6.4. Other organ systems, dependent on the properties of the substance

The management of chronic wounds such as venous ulcers is a common and long-term issue with the aging population. Non-standard treatment that is both medically and financially effective needs to be identified. Honey has been used for its healing properties for centuries and has been used to successfully heal wounds including pressure-ulcers in our care facility. However, there is not much evidence for its use in treating venous ulcers. To this end, I trialed the use of a honey-impregnated alginate dressing on a man who had a long-standing history of venous ulcers on his leg with the aim of evaluating the effectiveness of honey as an alternative treatment to the current wound management therapies. The honey seemed to act as an effective antibacterial, anti-inflammatory and deodorizing dressing, with total healing of the ulcer achieved. This result, together with past successes with the use of honey alginate on ulcerated wounds, has led to this product becoming mainstream in the treatment of chronic wounds within our care facility. As taken from van der Veyden EA. Br J Community Nurs. 2005 Jun; Suppl:S21, S24, S26-7. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/15944502

Our study with honey for its possible immunomodulatory activity reveals the immunosuppressive activity on induction of murine humoral antibody responses against different allergens as determined by passive cutaneous anaphylaxis and Ouchterlony double immunodiffusion techniques. Ovalbumin (OVA)-specific IgE antibody responses elicited with various doses were completely suppressed by different sources of commercial honeys. Honey is also found to have suppressed the induction of OVA-specific humoral antibody responses in different strains of mice. The results obtained in this work confirm the immunosuppressive activity of honey and suggest its possible applicability in conditions requiring immunosuppression. As taken from Duddukuri GR et al. Int Arch Allergy Immunol. 1997 Dec; 114(4):385-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/9414144

Honey contains fructose in excess of glucose, which may lead to incomplete fructose absorption associated with abdominal symptoms and/or diarrhea. This hypothesis was investigated in 20 healthy volunteers (13 males, 7 females) with a mean (+/- SD) age of 35.9 +/- 12.1 y. Each subject drank the following aqueous solutions in random order: 20 g lactulose, 100 g honey, 50 g honey, and 35 g each of a glucose and fructose mixture. The breath-hydrogen concentration was measured every 15 min for 6 h. Semiquantitative estimates of carbohydrate malabsorption were assessed with lactose as a nonabsorbable standard. Breath-hydrogen concentrations increased by 52 +/- 6, 30 +/- 4, 20 +/- 3, and 4 +/- 1 ppm (mean +/- SEM) after each of the four test solutions, respectively. The estimated carbohydrate malabsorption was 10.3 +/- 1.8, 5.9 +/- 1.2, and 0.5 +/- 0.2 g after 100 g honey, 50 g honey, and the glucose-fructose mixture, respectively ($F[2,57] = 16.05$, $P < 0.001$). Within 10 h after the ingestion of 100 g honey, 50 g honey, and the glucose-fructose mixture, six, three and none of the volunteers, respectively, reported loose stools ($chi\ 2 = 7.1$, $df = 2$, $P < 0.03$). The results of this study suggest that carbohydrate malabsorption after ordinary doses of honey is frequent in healthy adults and may be associated with abdominal complaints. Honey may have a laxative effect in certain otherwise healthy individuals, probably because of incomplete fructose absorption. As taken from Ladas SD et al. Am J Clin Nutr. 1995 Dec; 62(6):1212-5. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/7491882

The purpose of this study was to determine and compare the cariogenicity of various fluids that are frequently fed to infants and toddlers. We chose to examine sucrose, cola drink, honey, human milk, cow milk, and water because some of these have been associated with development of early childhood caries, although direct experimental evidence is lacking. We used our desalivated rat model because the approach mimics the situation found in infants, whereby the flow of saliva is interrupted through mechanical effects of a nipple. The animals received basic nutrition by gavage, and the fluids being tested were available ad libitum. Thus, the only substances that came in contact with teeth were the test fluids. The investigation continued for 14 days. Cola, sucrose, and honey were by far the most cariogenic. In addition, cola and honey induced considerable erosion. Human milk was significantly more cariogenic than cow milk probably because of its lower mineral content and higher level of lactose. Our data show that the use of honey, cola, and sucrose water in nursing bottles should be discouraged. Although human milk is more cariogenic than cow milk, it is no more cariogenic than are common infant formulas. Protracted exposure to human milk or formula through allowing an infant to sleep on the nipple should be discouraged, and the need for oral hygiene after tooth eruption should be emphasized. As taken from Bowen WH and Lawrence RA. Pediatrics. 2005 Oct; 116(4):921-6. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/16199702

Honey solution decreased urinary prostaglandins concentration and increased total urinary nitrite content whilst artificial honey decreased urinary nitrite and increased urinary prostaglandins. As taken from Int Urol Nephrol. 2005; 37(1):107-11. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/16132771

This study investigated effects of oral honey solution on total nitrite, a stable nitric oxide metabolite, in saliva, plasma, and urine samples collected from normal subjects. Fourteen adult healthy volunteers, 25-50 years old, nine males and three females, were enrolled in the study. Total nitrite was estimated in saliva, plasma, and urine after 14 hours of food fasting. Each subject was then asked to drink honey solution (80 g of raw honey dissolved in 250 mL of water). Saliva and blood samples were collected at 1, 2, and 3 hours after ingestion of honey solution for total nitrite assay, while urine samples were collected after 3 hours for total nitrite assay. The mean total fasting nitrite in saliva was 108 +/- 61.3 micromol/L, which was increased to 130 +/- 62.9, 131.2 +/- 59, and 135.1 +/- 64.3 micromol/L at 1, 2, and 3 hours, respectively. Plasma total nitrite was 22.41 +/- 16.22 micromol/L before drinking honey, which was increased to 34.71 +/- 18.13, 29.38 +/- 14.29, and 33 +/- 13.09 micromol/L at 1, 2, and 3 hours, respectively, after drinking honey. Urine total nitrite before drinking honey was 75.8 +/- 54.79 micromol/L, which was increased to 107.8 +/- 70.83 micromol/L 3 hours after ingestion of honey solution. Although not statistically significant, honey solution showed a tendency to increase total nitrite concentration in different biological fluids from humans, including saliva, plasma, and urine. As taken from Ai-Waili NS and Boni NS. J Med Food. 2004 Fall;7(3):377-80. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/15383235

In healthy subjects, dextrose elevated PGL at 1 (53%) and 2 (3%) hours, and decreased PGL after 3 hours (20%). Honey elevated PGL after 1 hour (14%) and decreased it after 3 hours (10%). Elevation of insulin and C-peptide was significantly higher after dextrose than after honey. Dextrose slightly reduced cholesterol and low-density lipoprotein-cholesterol (LDL-C) after 1 hour and significantly after 2 hours, and increased TG after 1, 2, and 3 hours. Artificial honey slightly decreased cholesterol and LDL-C and elevated TG. Honey reduced cholesterol, LDL-C, and TG and slightly elevated high-density lipoprotein-cholesterol (HDL-C). Honey consumed for 15 days decreased cholesterol (7%), LDL-C (1%), TG (2%), CRP (7%), homocysteine (6%), and PGL (6%), and increased HDL-C (2%). In patients with hypertriglyceridemia, artificial honey increased TG, while honey decreased TG. In patients with hyperlipidemia, artificial honey increased LDL-C, while honey decreased LDL-C. Honey decreased cholesterol (8%), LDL-C (11%), and CRP (75%) after 15 days. In diabetic patients, honey compared with dextrose caused a significantly lower rise of PGL. Elevation of PGL was greater after honey than after sucrose at 30 minutes, and was lower after honey than it was after sucrose at 60, 120, and 180 minutes. Honey caused greater elevation

of insulin than sucrose did after 30, 120, and 180 minutes. Honey reduces blood lipids, homocysteine, and CRP in normal and hyperlipidemic subjects. Honey compared with dextrose and sucrose caused lower elevation of PGL in diabetics. As taken from Ai-Waili NS. J Med Food. 2004 Spring; 7(1):100-7. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/15117561

The effects of bee honey products, pollen, and clofibrate on oxygen uptake in isolated rat liver mitochondria were studied. Honey products and pollen caused a significant increase in oxygen uptake after 2 h incubation. They also showed a uniform increase in total oxygen consumption after the first h, while after the second h, pollen increased consumption by 102% and clofibrate increased consumption by 14%. Honey products showed a very highly significant increase after the first and second h incubation period over 8 wk. The smallest increase was seen with clofibrate. It was concluded that bee honey products, pollen, and clofibrate significantly increase oxygen consumption in isolated rat liver mitochondria after incubation for 2 h. As taken from Teleb ZA. J. Drug Res.; VOL 19 ISS 1-2 1990, P119-136.

Wound healing is a complex and highly regulated process that can be compromised by both endogenous factors (pathophysiological) and exogenous factors (micro-organisms). Microbial colonisation of both acute and chronic wounds is inevitable, and in most situations endogenous bacteria predominate, many of which are potentially pathogenic in the wound environment. The risk of wound infection increases as local conditions favour bacterial growth rather than host defence. Consequently a primary objective in wound management is to redress the host-bacterial balance, and this is most effectively achieved by ensuring that the wound is cleared of devitalised tissue and foreign bodies, the bacterial load and inflammation are controlled, and that adequate tissue perfusion is maintained. Although surgical debridement is the most rapid and effective technique for removing devitalised tissue, topical enzymes, moisture-retentive dressings, biosurgical therapy and vacuum therapy have been used as alternative approaches to wound cleansing and preparation. Topical antimicrobial agents continue to be used widely for preventing wound infection and current interest is focused on alternatives to antibiotics, such as antimicrobial moisture-retentive dressings, honey, essential oils and cationic peptides. In addition to the need to control wound microflora, unregulated inflammation caused by both micro-organisms and underlying abnormal pathophysiological conditions is a major factor associated with poor healing in chronic wounds. Consequently, therapeutic strategies that target chronic inflammatory processes are critical to wound progression. The success of future therapies will be dependent on a growing understanding of the pathophysiological processes and the host-bacterial interactions that significantly influence wound healing. As talen from Bowler PG. Wound pathophysiology, infection and therapeutic options. Ann Med. 2002; 34(6):419-27. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/pubmed/12523497

“Preterm, critically ill neonates represent a challenge in wound healing. Many factors predispose infants to skin injuries, including decreased epidermal-dermal cohesion, deficient stratum corneum, relatively alkaline pH of skin surface, impaired nutrition and presence of multiple devices on the skin. We present a case series describing the use of medical-grade honey-Leptospermum honey (Medihoney), for successful treatment of slowly healing neonatal wounds, specifically stage 3 pressure ulcer, dehiscent and infected sternal wound, and full-thickness wound from an extravasation injury.” As taken from Boyar V et al. 2014. J. Perinatol. 34(2), 161-3. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476663

“This study aimed to clarify the effects of honey on acute-phase deep burn wounds. Two deep burn wounds were created on mice which were divided into four groups: no treatment, silver sulfadiazine, manuka honey, and Japanese acacia honey. Wound sizes were calculated as expanded wound areas and sampled 30 minutes and 1-4 days after wounding for histological observation. The wound sections were subjected to hematoxylin and eosin and immunohistological staining to detect necrotic cells, apoptotic cells, neutrophils, and macrophages. The no treatment group formed a scar. The redness around the wound edges in the silver sulfadiazine group was the most intense. All groups exhibited increased wound areas after wounding. The proportions of

necrotic cells and the numbers of neutrophils in the manuka and acacia honey groups were lower than those in the no treatment and silver sulfadiazine groups until day 3; however, there were no significant differences between all groups on day 4. These results show that honeytreatment on deep burn wounds cannot prevent wound progression. Moreover, comparing our observations with those of Jackson, there are some differences between humans and animals in this regard, and the zone of hyperemia and its surrounding area fall into necrosis, which contributes to burn wound progression." As taken from Nakajima Y et al. 2013a. Evid. Based Complement. Alternat. Med. 2013, 784959. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24348720

"Although many previous studies reported that honey promotes wound healing, no study has examined the effects of Japanese honey. The aim of this study was to investigate the effects of three types of Japanese honey, Acacia, Buckwheat flour, and Chinese milk vetch honey, on wound healing in comparison with hydrocolloid dressing. Circular full-thickness skin wounds were produced on male mice. Japanese honey or hydrocolloid dressing was applied daily to the mice for 14 days. The ratio of wound area for the hydrocolloid dressing group increased initially in the inflammatory and early proliferative phases and then decreased rapidly to heal with scarring. However, the ratios of wound area for the Japanese honey groups decreased in the inflammatory phase, increased in the proliferative phase, and decreased in the proliferative phase, and some wounds were not completely covered with new epithelium. These findings indicate that using Japanese honey alone has limited benefit, but since it reduces wound size in the inflammatory phase, it is possible to apply a combined treatment in which Japanese honey is applied only in the inflammatory phase, followed by hydrocolloid dressing from the proliferative phase, which would effectively contract the wound." As taken from Nakajima Y et al. 2013b. Evid. Based Complement. Alternat. Med. 2013, 504537. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23401714

"BACKGROUND: Honey is a viscous, supersaturated sugar solution derived from nectar gathered and modified by the honeybee, Apis mellifera. Honeyhas been used since ancient times as a remedy in wound care. Evidence from animal studies and some trials has suggested that honey may accelerate wound healing. OBJECTIVES: The objective was to determine whether honey increases the rate of healing in acute wounds (e.g. burns, lacerations) and chronic wounds (e.g. skin ulcers, infected surgical wounds). SEARCH METHODS: For this first update of the review we searched the Cochrane Wounds Group Specialised Register (searched 13 June 2012); The Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2012, Issue 5); Ovid MEDLINE (2008 to May Week 5 2012); Ovid MEDLINE (In-Process & Other Non-Indexed Citations 12 June 2012); Ovid EMBASE (2008 to 2012 Week 23); and EBSCO CINAHL (2008 to 8 June 2012). SELECTION CRITERIA: Randomised and quasi-randomised trials that evaluated honey as a treatment for any sort of acute or chronic wound were sought. There was no restriction in terms of source, date of publication or language. Wound healing was the primary endpoint. DATA COLLECTION AND ANALYSIS: Data from eligible trials were extracted and summarised by one review author, using a data extraction sheet, and independently verified by a second review author. MAIN RESULTS: We identified 25 trials (with a total of 2987 participants) that met the inclusion criteria, including six new trials that were added to this update. In acute wounds, three trials evaluated the effect of honey in acute lacerations, abrasions or minor surgical wounds and 12 trials evaluated the effect of honey in burns. In chronic wounds, two trials evaluated the effect of honey in venous leg ulcers, and single trials investigated its effect in infected post-operative wounds, pressure injuries, cutaneous Lieshmaniasis, diabetic foot ulcers and Fournier's gangrene. Three trials recruited people into mixed groups of chronic or acute wounds. Most trials were at high or unclear risk of bias. In acute wounds, specifically partial-thickness burns, honey might reduce time to healing compared with some conventional dressings (WMD -4.68 days, 95%CI -4.28 to -5.09 days), but, when compared with early excision and grafting, honey delays healing in partial- and full-thickness burns (WMD 13.6 days, 95% CI 10.02 to 17.18 days). In chronic wounds, honey does not significantly increase healing in venous leg ulcers when used as an adjuvant to compression (RR 1.15, 95% CI 0.96 to 1.38), and may delay healing in cutaneous Leishmaniasis when used as

an adjuvant to meglumine antimoniate compared to meglumine antimoniate alone (RR 0.72, 95% CI 0.51 to 1.01). AUTHORS' CONCLUSIONS: Honey dressings do not increase rates of healing significantly in venous leg ulcers when used as an adjuvant to compression. Honey may delay healing in partial- and full-thickness burns in comparison to early excision and grafting, and in cutaneous Leishmaniasis when used as an adjuvant with meglumine antimoniate. Honey might be superior to some conventional dressing materials, but there is considerable uncertainty about the replicability and applicability of this evidence. There is insufficient evidence to guide clinical practice in other types of wounds, and purchasers should refrain from providing honey dressings for routine use until sufficient evidence of effect is available." As taken from Jull AB et al. 2013. Cochrane Database Syst. Rev. 2, CD005083. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23450557

Several studies investigating the interaction of honey and drug-metabolizing enzymes showed controversial results, with some suggesting that honey induces CYP3A-mediated metabolism in mammals and humans. This clinical trial was conducted to determine the effect of repeated honey administration on human CYP3A enzyme activity using midazolam as a marker substance. In a randomized, single-blind, parallel-group study, 20 healthy volunteers were randomly assigned to receive either honey (2 × 20 g/d) or artificial honey (2 × 20 g/d) over a period of 10 days. To determine intestinal and hepatic CYP3A activity, oral (4 mg) and intravenous (2 mg) midazolam was administered in a semi-simultaneous way before honey administration, after the last honey administration, and 1 and 6 days thereafter. At baseline after oral midazolam, the partial metabolic clearance was similar in both groups (honey: 917.8 ± 234.6 mL/min vs artificial honey: 973.5 ± 373.8 mL/min). Ten days of honey administration did not change partial metabolic clearance (honey: 1016 ± 268 mL/min vs artificial honey: 1043 ± 450 mL/min), which was also true 1 and 6 days later. Neither honey nor artificial honey in amounts usually consumed affected the intestinal and hepatic CYP3A activity in healthy volunteers (Fetzner et al. 2011. Journal of Clinical Pharmacology 51, 1223-1232). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21148046?dopt=AbstractPlus

Present study was conducted to determine the effects of honey on blood hemostasis, in-vitro effect of honey was observed on platelet aggregation and blood coagulation employing, activated partial prothrombin time (aPTT), prothrombin time (PT), thrombin time (TT) and fibrinogen levels in blood. Honey samples showed moderate inhibition of platelet aggregation with IC(50) 5-7.5%. The coagulation assays showed that at higher concentrations (>15%) honey samples increased whole blood clotting time. When assayed in platelet poor plasma (PPP), honey samples significantly (P>0.005) prolonged aPTT, PT, and TT. The honey samples (at 3.75% and 7.5% concentrations) cause mean increment of aPTT = 19±10% and 62±10%; PT 6±5% and 40±5%; TT 35±15% and 112±30% respectively. Moreover, PPP isolated from whole blood pre-incubated with honey samples (9.0% for 10 minutes) showed mean prolongation of aPTT, PT and TT of 45±21%, 26±9% and 105±24% respectively. Interestingly, incubation of honey at 6.25% and 11.75% concentrations in PPP considerably (P≥0.005) reduced fibrinogen levels i.e. 13±4% and 86±30% respectively. The present study outlines the inhibitory effect of natural honey on platelet aggregation and blood coagulation. These observations provide first line data for modulatory role(s) of honey on process of hemostasis (Ahmed et al. 2011. Pakistan Journal of Pharmaceutical Science 24, 389-397). As taken from http://www.ncbi.nlm.nih.gov/pubmed/21715274?dopt=AbstractPlus

"The high intake of refined sugars, mainly fructose has been implicated in the epidemiology of metabolic diseases in adults and children. With an aim to determine whether honey can substitute refined sugars without adverse effect, the long-term effects of natural honey and cane syrup have been compared on visceral morphology in growing rats fed from neonatal age. Honey increased the caecum and pancreas weights in male rats, which could enhance enzymatic activities of pancreas and digestive functions by intestinal microflora of caecum. Unlike honey, cane syrup caused fatty degenerations in the liver of both male and female rats. Honey enhanced intestinal villi growth, and did not cause pathology in the rodents' abdominal viscera, suggesting potential nutritional benefit

as substitution for refined sugars in animal feed." As taken from Ajibola A et al. 2013. Indian J. Exp. Biol. 51(4), 303-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24195350

"This study was a case control cross sectional study that was conducted on 50 patients with type 1 diabetes mellitus and 30 controls without diabetes. The mean age of patients was 10.02 years. Oral sugar tolerance tests using glucose, sucrose and honey and measurement of fasting and postprandial serum C-peptide levels were done for all subjects in three separate sittings. The glycemic index (GI) and the peak incremental index (PII) were then calculated for each subject. Honey, compared to sucrose, had lower GI and PII in both patients and controls ($P < 0.01$). In both patients and controls, the increase in the level of C-peptide after honey was significant when compared with either glucose or sucrose ($P < 0.01$). Conclusion: Because of its possible stimulatory effect on diseased beta cells, honey might be considered in future therapeutic trials targeting beta cells of pancreas." As taken from Abdulrhman M et al. 2013. Complement. Ther. Clin. Pract. 19(1), 15-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23337559

"The antimicrobial and anti-biofilm properties of manuka honey (MH) are currently being explored in the treatment of chronic recalcitrant rhinosinusitis. Due to similarities between chronic rhinosinusitis and chronic otitis, manuka honey may find applications in the management of challenging cases of chronic otitis media implicating biofilms. The goal of this study was to investigate the safety of topical application of 4 % MH in the middle ear. Eleven adult female chinchillas had one of their ears randomly assigned to receive transtympanic 4 % MH, while the contralateral ear served as control. Auditory brainstem-evoked response (ABR) was performed before and after MH application. The facial nerve function and vestibular system were assessed clinically. The animals were euthanized one month following the last application, and the cochleae samples were processed for light and scanning electron microscopy. There was no statistically significant differences between ABR thresholds in both control and experimental ears before and after the application of MH. No morphological differences were seen in both groups of cochleae. The outer hair cell counts for both groups were comparable. Our results suggest that 4 % MH appears not toxic to the cells of the cochlea after 4 weeks of application. The long-term effects of prolonged contact on the structure and function of the cochlea however need further investigations." As taken from Aron M et al. 2015. Eur. Arch. Otorhinolaryngol. 272(3), 537-42. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/24337897

Honey has been shown to scavenge reactive oxygen species, ameliorate oxidative stress and reduce hyperglycemia [6, 7]. While honey supplementation in diabetic rats ameliorates renal oxidative stress independent of the dose, its hypoglycemic effect is dose-dependent [8]. This is a bit startling as honey is sweet and rich in sugars and it would not have been expected to exert a dose-dependent hypoglycemic effect. To explain this surprising finding, it is hypothesized that the fructose and oligosaccharides present in honey might in some way contribute to the observed hypoglycemic effect [9, 10]. In addition to its effects on oxidative stress and hyperglycemia, honey alvaresupplementation ameliorates several metabolic derangements commonly observed in diabetes. These include reduced levels of hepatic transaminases, triglycerides and glycosylated hemoglobin (HbA1c) as well as increased HDL cholesterol [. As taken fromErejuwa OO (2014). Effect of honeyin diabetes mellitus: matters arising. J. Diabetes Metab. Disord. 13(1), 23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476150

Moreover, honey positively modulates the glycemic response by reducing blood glucose, serum fructosamine or glycosylated hemoglobin concentrations and exerts antibacterial properties caused by its consistent amount of hydrogen peroxide and non-peroxide factors as flavonoids, methylglyoxal and defensin-1 peptide. Alvarez-Suarez JM et al. (2013).

"Gastric ulcers are among the most common diseases affecting humans. This study aimed at investigating the gastroprotective effects of manuka honey against ethanol-induced gastric ulcers in rats. The mechanism by which honey exerts its antiulcer potential was elucidated. Four groups of rats were used: control, ethanol (ulcer), omeprazole, and manuka honey. Stomachs were examined macroscopically for hemorrhagic lesions in the glandular mucosa, histopathological changes, and

glycoprotein detection. The effects of oxidative stress were investigated using the following indicators: gastric mucosal nitric oxide (NO), reduced glutathione (GSH), lipid peroxide (MDA, measured as malondialdehyde) glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase. Plasma tumour necrosis factor-α, interleukin-1β, and IL-6 were also measured. Manuka honey significantly decreased the ulcer index, completely protected the mucosa from lesions, and preserved gastric mucosal glycoprotein. It significantly increased gastric mucosal levels of NO, GSH, GPx, and SOD. Manuka honey also decreased gastric mucosal MDA and plasma TNF-α, IL-1β, and IL-6 concentrations. In conclusion, manuka honey likely exerted its antiulcer, effect by keeping enzymatic (GPx and SOD) and nonenzymatic (GSH and NO) antioxidants as well as inflammatory cytokines (TNF-α, IL-1β, and IL-6) in a reduced form, inhibited lipid peroxidation (MDA), and preserved mucous glycoproteins levels." As taken from Almasaudi SB et al. 2016. Oxid. Med. Cell. Longev. 2016, 3643824. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26770649

"The aim of the study is to evaluate the acute biochemical and histological changes in rat kidneys after treatment with grayanotoxin (GTX) of rhododendron honey (RH). A total of 60 Sprague-Dawley female rats were divided into five groups of 12 rats each, one being a control group (group 1) and group 2 was treated with 0.015 mg/kg/bw of GTX standard preparation via intraperitoneal injection. Groups 3, 4, and 5 were given RH at doses of 0.1, 0.5, and 2.5 g/kg/bw, respectively, via oral gavage. Compared to the control group, significant increases were observed in glucose, blood urea nitrogen (BUN), and creatinine levels of the GTX-injected groups after 1 h. However, in low dose RH group, such an increase was not observed and had a normal appearance histologically. Therefore, low dose (1 g/kg/bw) of RH produces no acute adverse effects on renal functions of rats." As taken from Silici S et al. 2016. Environ. Sci. Pollut. Res. Int. 23(4), 3300-9. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26490905

"Gastric ulcers are a major problem worldwide with no effective treatment. The objective of this study was to evaluate the use of manuka honey in the treatment of acetic acid-induced chronic gastric ulcers in rats. Different groups of rats were treated with three different concentrations of honey. Stomachs were checked macroscopically for ulcerative lesions in the glandular mucosa and microscopically for histopathological alterations. Treatment with manuka honey significantly reduced the ulcer index and maintained the glycoprotein content. It also reduced the mucosal myeloperoxidase activity, lipid peroxidation (MDA), and the inflammatory cytokines (TNF-α, IL-1β, and IL-6) as compared to untreated control group. In addition, honey-treated groups showed significant increase in enzymatic (GPx and SOD) and nonenzymatic (GSH) antioxidants besides levels of the anti-inflammatory cytokine IL-10. Flow cytometry studies showed that treatment of animals with manuka honey has normalized cell cycle distribution and significantly lowered apoptosis in gastric mucosa. In conclusion, the results indicated that manuka honey is effective in the treatment of chronic ulcer and preservation of mucosal glycoproteins. Its effects are due to its antioxidant and anti-inflammatory properties that resulted in a significant reduction of the gastric mucosal MDA, TNF-α, IL-1β, and IL-6 and caused an elevation in IL-10 levels." As taken from Almasaudi SB et al. 2017. Evid. Based Complement. Alternat. Med. 2017, 5413917. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28250794

"Mad honey poisoning has been reported in many countries, and it seldom results in death. We describe a rare case series of fatal honey poisoning caused by Tripterygium wilfordii Hook F (TwHF) in Southwest China. Three male construction workers were delivered to the emergency department with symptoms of food poisoning after ingestion of wild raw honey. Laboratory results showed that the 3 patients were at different degrees of renal damage, and 1 patient with severe symptoms died of acute renal failure 1 day after admission. Pollen analysis indicated that the suspected honey was heavily contaminated with TwHF pollen. Early diagnosis and prompt treatment are crucial for such poisoning. Pollen analysis is a practical approach to help diagnosis in remote areas where such honey poisoning occurs." As taken from Zhang Q et al. 2016. Wilderness Environ. Med. 27(2), 271-3. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27132027

“Objective: To assess the clinical safety and tolerability of a novel MGO Manuka Honey microemulsion (MHME) eye cream for the management of blepharitis in human subjects. Methods and analysis: Twenty-five healthy subjects were enrolled in a prospective, randomised, paired-eye, investigator-masked trial. The MHME eye cream (Manuka Health New Zealand) was applied to the closed eyelids of one eye (randomised) overnight for 2 weeks. LogMAR visual acuity, eyelid irritation symptoms, ocular surface characteristics and tear film parameters were assessed at baseline, day 7 and day 14. Expression of markers of ocular surface inflammation (matrix metalloproteinase-9 and interleukin-6) and goblet cell function (MUC5AC) were quantified using impression cytology at baseline and day 14. Results: There were no significant changes in visual acuity, eyelid irritation symptoms, ocular surface characteristics, tear film parameters and inflammatory marker expression during the 2-week treatment period in treated and control eyes (all $p>0.05$), and measurements did not differ significantly between eyes (all $p>0.05$). No major adverse events were reported. Two subjects experienced transient ocular stinging, presumably due to migration of the product into the eye, which resolved following aqueous irrigation. Conclusion: The MHME eye cream application was found to be well tolerated in healthy human subjects and was not associated with changes in visual acuity, ocular surface characteristics, tear film parameters, expression of markers of inflammation or goblet cell function. The findings support future clinical efficacy trials in patients with blepharitis.” As taken from Craig JP et al. 2017. BMJ Open Ophthalmol. 1(1), e000066. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29354710

“There is renewed interest in the potential use of natural compounds in cancer therapy. Previously, we demonstrated the anti-tumor properties of manuka honey (MH) against several cancers. However, the underlying mechanism and molecular targets of this activity remain unknown. For this study, the early targets of MH and its modulatory effects on proliferation, invasiveness, and angiogenic potential were investigated using two human breast cancer cell lines, the triple-negative MDA-MB-231 cells and estrogen receptor-positive MCF-7 cells, and the non-neoplastic breast epithelial MCF-10A cell line. Exposure to MH at concentrations of 0.3-1.25% (w/v) induced a dose-dependent inhibition of the proliferation of MDA-MB-231 and MCF-7, but not MCF-10A, cells. This inhibition was independent of the sugar content of MH as a solution containing equivalent concentrations of its three major sugars failed to inhibit cell proliferation. At higher concentrations (>2.5%), MH was found to be generally deleterious to the growth of all three cell lines. MH induced apoptosis of MDA-MB-231 cells through activation of caspases 8, 9, 6, and 3/7 and this correlated with a loss of Bcl-2 and increased Bax protein expression in MH-treated cells. Incubation with MH induced a time-dependent translocation of cytochrome c from mitochondria to the cytosol and Bax translocation from the cytosol into the mitochondria. MH also induced apoptosis of MCF-7 cells via the activation of caspases 9 and 6. Low concentrations of MH (0.03-1.25% w/v) induced a rapid reduction in tyrosine-phosphorylated STAT3 (pY-STAT3) in MDA-MB-231 and MCF-7 cells. Maximum inhibition of pY-STAT3 was observed at 1 h with a loss of >80% and coincided with decreased interleukin-6 (IL-6) production. Moreover, MH inhibited the migration and invasion of MDA-MB-231 cells as well as the angiogenic capacity of human umbilical vein endothelial cells. Our findings identify multiple functional pathways affected by MH in human breast cancer and highlight the IL-6/STAT3 signaling pathway as one of the earliest potential targets in this process.” As taken from Aryappalli P et al. 2017. Front. Oncol. 7, 167. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28856117

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open

literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing honey and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of honey. Table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	35 (absolute, 91052-92-5)	Baker et al., 2004a
	10,000 (8028-66-8)	JTI KB Study Report(s)
	62,200	Gaworski et al., 2011 & Coggins et al., 2011a
In vitro genotoxicity	45,400 (8028-66-8) 35 (absolute, 91052-92-5)	Baker et al., 2004c
	10,000 (8028-66-8)	JTI KB Study Report(s)
	2,300 (8028-66-8)	fGLH Study Report (2010)
	62,200	Gaworski et al., 2011 & Coggins et al., 2011a
In vitro cytotoxicity	45,400 (8028-66-8) 35 (absolute, 91052-92-5)	Baker et al., 2004c
	10,000 (8028-66-8)	JTI KB Study Report(s)
	2,300 (8028-66-8)	fGLH Study Report (2010)
	62,200	Gaworski et al., 2011 & Coggins et al., 2011a
Inhalation study	300 (8028-66-8)	Gaworski et al., 1998
	45,400 (8028-66-8) 35 (absolute, 91052-92-5)	Baker et al., 2004c
	10,000 (8028-66-8)	JTI KB Study Report(s)
	62,200	Gaworski et al., 2011 & Coggins et al., 2011a
Skin painting	300 (8028-66-8)	Gaworski et al., 1999
	10,000 (8028-66-8)	JTI KB Study Report(s)

A sister chromatid exchange (SCE) test using Chinese hamster ovary cells (with and without S9) was carried out on:

(a) cigarette smoke condensate (CSC) generated from cigarettes with a casing that included honey at 5% wet weight (4.4% dry weight) and

(b) CSC from cigarettes with invert sugar in place of honey in the casing.

There was no demonstrable difference in the level of SCE induced

The same CSCs were tested for ability to induce bacterial mutagenicity in Salmonella typhimurium strains TA98 and TA100, in the presence of S9. Again, there was no demonstrable difference between the two CSCs (Stavanja et al. 2003).

Stavanja et al., (2003) conducted a study in which the main objective was to summarize and interpret chemical and toxicological studies conducted for the evaluation of honey on the biological activity of mainstream smoke or cigarette smoke condensate. Cigarettes contained 5% wet weight honey (rather than invert sugar as a casing material). The researchers studied selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-wk dermal tumor promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 13-wk inhalation study of cigarette smoke in Sprague-Dawley rats. The authors concluded 'in vitro and in vivo studies demonstrated that cigarettes containing tobacco cased with honey had comparable biological activity to cigarettes containing invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufacture of cigarettes does not alter the potential toxicity of cigarette smoke condensate (CSC) or cigarette smoke; therefore the use of honey as an ingredient added to cigarette tobacco is acceptable from a toxicological perspective.

In a tumour promotion test carried out on groups of 40 female SENCAR mice, the skin was initiated with a known skin carcinogen. In the promotion phase, 9, 18 or 36 mg cigarette smoke condensate (CSC) was applied to the skin 3 times per week for 29 weeks. CSC was generated from cigarettes with a casing that included honey at 5% wet weight (4.4% dry weight) and the incidence of skin tumours was compared with that induced by CSC from cigarettes with invert sugar in place of honey in the casing. There was no difference between the two CSCs in their ability to induce skin tumours (Stavanja et al. 2003).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Honey (8028-66-8) and Honey extract (91052-92-5) was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Honey (8028-66-8) and Honey extract (91052-92-5) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)
In vitro cytotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Honey and/or honey extract was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.0052 mg/(tobacco portion; 310 mg)	Logic (2019)

In vitro genotoxicity	0.0052 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro cytotoxicity	0.0052 mg/(tobacco portion; 310 mg)	Logic (2019)
In vivo genotoxicity	0.0052 mg/(tobacco portion; 310 mg)	Logic (2019)
Inhalation study	0.0052 mg/(tobacco portion; 310 mg)	Logic (2019)

Aerosol from heated tobacco stick(s) containing Honey and/or honey extract was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.52	Labstat International Inc. (2021a)
In vitro genotoxicity	0.52	Labstat International Inc. (2021b)
In vitro cytotoxicity	0.52	Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that honey (CAS RN 8028-66-8) and honey extract (CAS RN 91052-92-5) are of uncertain persistence in the environment.

Data accessed June 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that honey (CAS RN 8028-66-8) and honey extract (CAS RN 91052-92-5) are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

Data accessed June 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

“The substance honey from rhododendron is proposed to be used as rodenticide in baits. The applicant claims that field studies performed on their own, demonstrate that mice die as a result of the grayanotoxins in the honey. However, no proper scientific report has been provided to substantiate these claims.

Because of the bactericidal effect of honey from rhododendron it has to be ensured that the bait boxes are impervious (especially when the honey is applied pure and not in capsules) thus to prevent the honey entering the soil.”

As taken from EFSA, 2017

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that honey (CAS RN 8028-66-8) and honey extract (CAS RN 91052-92-5) are of uncertain bioaccumulative potential in the environment.

Data accessed June 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

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ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 7 honey-derived ingredients. All of these ingredients are reported to function in cosmetics as skin-conditioning agents. The Panel considered the available data relating to the safety of these ingredients in cosmetic formulations. Because impurities, particularly pesticides and endotoxins, may be present in these ingredients, formulators should continue to use good manufacturing practices to monitor and limit these possible impurities. The Panel concluded the honey-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This is a safety assessment of the following 7 honey-derived ingredients as used in cosmetic formulations:

Honey	Hydrogenated Honey
Honey Cocoates	Hydrolyzed Honey
Honey Powder	Hydrolyzed Honey Protein
Honey Extract	

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary*), all of these ingredients function as skin-conditioning agents.[1] Other functions include, but are not limited to, use as a flavoring agent, anti-acne agent, abrasive, binder, depilating agent, exfoliant, hair-conditioning agent, and nail-conditioning agent (Table 1). Use as an anti-acne agent is not considered a cosmetic function in the United States (US) and, therefore, does not fall under the purview of the Panel.

The *Dictionary* defines Honey Cocoates as a complex mixture of esters produced by the reaction of honey with coconut acid.[1] In 2017, the Panel published a safety assessment with the conclusion that coconut acid is safe in cosmetics in the present practices of use and concentration [as described in that safety assessment].[2] In addition, the main components of Honey (i.e., fructose, glucose, maltose, and sucrose)[3] were reviewed by the Panel; in 2019, a safety assessment was published with the conclusion that these component ingredients are safe in the present practices of use and concentration [as described in that safety assessment].[4]

Some of the ingredients reviewed in this safety assessment may be consumed as food, and daily exposure from food would result in much larger systemic exposures than those from use in cosmetic products. Although oral studies are included herein, the primary focus of this safety assessment is on the potential for effects from topical exposure to these ingredients as used in cosmetics.

It should be noted that there are multiple species of bees that produce honey; however, Honey, used as a cosmetic ingredient, has been reported to be produced by the honeybee species *Apis mellifera*, *Tetragonisca angustula*, *Scaptotrigona pectoralis*, and *Melipona Becheii.*[1] In several studies, the honey used for testing was not produced by these species, but produced by a different honeybee species (e.g., *Apis dorsata*). Data from these studies have been included in the report as these may be helpful in drawing a conclusion of safety for this ingredient group. In most cases, information regarding the type of honey being tested (i.e., method of manufacture, floral source, species of producing bee) was not specified. However, if this information was available, it has been included in the report.

It is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention will be used (i.e., the names of cosmetic ingredients are capitalized (e.g., Honey Extract)). If it is not known that the test substance is the same as the cosmetic ingredient, the generic terminology, in all lowercase (e.g., honey extract), will be used.

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that Panel evaluates, is available on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline).

CHEMISTRY

Definition

According to the *Dictionary*, Honey (CAS No. 8028-66-8) is a saccharic secretion gathered and stored by honey bees of the species, *Apis mellifera*, *Tetragonisca angustula*, *Scaptotrigona pectoralis*, or *Melipona becheii*.[1] All ingredients reviewed in this report are derived from honey. The definitions of the ingredients included in this report are provided in Table 1.

Physical and Chemical Properties

Honey (CAS No. 8028-66-8) may be fluid, viscous, or solid, and ranges in color from clear to dark amber or black.[5] Honey is acidic by nature; however, the pH and acidity levels vary depending upon botanical origin and geographic origin of

the honey.[6] The usual pH of honey ranges from 2 - 6. According to a manufacturer, a typical product with Honey Extract, prepared in water, is light to medium yellow in color, has a pH level of approximately 2.5 - 6.5 at 25°C, and is soluble in any proportion of water.[7]

Natural Occurrence

Honey is commercialized in most countries of the world.[8] In the US alone, there are more than 300 types of honey, each with a unique flavor and color depending on the nectar source. Although there are many varieties of honey, the most common types of commercialized honey include those from botanical sources such as acacia, alfalfa, avocado, blueberry, buckwheat, clover, eucalyptus, fireweed, manuka, orange blossom, sage, tupelo, and wildflower.

Honeybee Species

Apis mellifera, also known as the Western honey bee, is the most common honeybee species worldwide.[9] This species was historically present across sub-Saharan Africa, Europe, parts of Western Asia, and the Middle East, and has now migrated westward to many countries, including the US. Honeybees of the *Tetragonisca angustula* species are stingless honeybees that are widely distributed in the neotropics, from Mexico to Northern Argentina.[10] The *Scaptotrigona pectoralis* and *Melipona becheii* species are both stingless honeybee species found in South America.[11,12]

Honey Production and Extraction

To produce honey, forager bees collect sugar-rich nectar from plant sources.[13,14] Once brought back to the hive, the nectar is distributed, ingested, and regurgitated multiple times. This process involves the physiochemical transformations of nectar, during which sucrose is inverted into dextrose and fructose by enzymes originating from the hypopharyngeal glands of the bees. The regurgitation process also aids in the process of dehydration of the solution. The altered nectar solution is then spread over an empty comb. Further dehydration occurs by the draft created by the flapping of bee wings in the hive. Once approximately 80% of the water content is evaporated, the honeycomb cells are capped with wax for preservation.

Traditionally, honey is collected by first introducing smoke into the beehive to sedate or remove bees.[14] The combs are then removed and squeezed to drain honey. Honey can also be extracted by placing combs in a metallic bowl containing a drainage hole. Burning embers are placed on top of the comb, and melted honey is drained and collected. In order to mechanically extract honey, caps are removed from combs, and placed in an extractor where centrifugation is performed. The honey is then sieved and collected.

Method of Manufacture

Information on the manufacture of Honey Extract and of a tradename mixture containing Honey Extract was provided by suppliers. The methods below regarding Honey Powder and honey protein are general to the processing of these ingredients, and it is unknown if they apply to cosmetic ingredient manufacture.

Honey Extract

According to one supplier, to produce Honey Extract, the honey is first extracted with a specified eluent under appropriate temperature conditions to yield a concentrate.[7] Typical eluents include water, butylene glycol, glycerin, and propylene glycol. The concentrate containing the phytochemical constituents is then blended with the desired diluent and preservation system to produce the final ingredient.

The manufacturing process of a tradename mixture containing 10.6% Honey Extract, 82.9% water, 4.4% propylene glycol dicaprylate/dicaprate, 1.5% phenoxyethanol, 0.3% xanthan gum, and 0.3% potassium sorbate was reported.[15] A mixture of demineralized water, propylene glycol dicaprylate/dicaprate, and honey is combined with xanthan gum to create the final product. The manufacturing process of a different tradename mixture containing 16.5% Honey, 27.6% water, and 55.9% propylene glycol was also reported. Honey is extracted by a mixture of propylene glycol and water.[16] The resulting product is then filtered.

Honey Powder

A honey powder, for food use, is produced by the combination of honey, an emulsifier, an anti-caking agent, and filler materials of high molecular weight that increase the glass transition temperature.[17] Filler materials include starch, carboxymethyl cellulose, gum Arabic, maltodextrin, and gelatin. The mixture is then powdered by using a either a spray or vacuum drying method with a filler to honey ratio of 50:50.

Honey protein

Honey proteins can be extracted via physical and chemical methods.[18] When physically extracting proteins, honey undergoes ultrafiltration and ultracentrifugation to isolate amylase before purification by ion exchange chromatography. A dialysis method can also be used to remove low molecular weight and interfering compounds by passive diffusion through a semipermeable membrane. Another physical extraction method involves the absorption of honey proteins by beads with specific properties. Combinatorial hexapeptide ligand library and C18 beads are used to capture honey peptidome from honey samples of chestnut, sunflower, eucalyptus, orange, and acacia. The honey peptidome is then filtered and eluted from

the beads using a solvent system. Microwave-assisted hydrolysis is another method used to extract proteins from honey. Chemical methods to extract honey involve co-precipitation using compatible precipitants, such as a sodium tungstate solution, trichloroacetic acid, sulfosalicylic acid, or ammonium sulfate.

Composition

Honey

Honey is a mixture of carbohydrates, proteins, enzymes, amino acids, vitamins, minerals, antioxidants, and other compounds.[14] Enzymes in honey include invertase, glucose oxidase, catalase, and acid phosphorylase. The sugar composition of honey is dependent upon the content of saccharides in the nectar used to produce the honey.[5] Generally, fructose and glucose are found in honey in similar amounts, with D-fructose as the prevalent sugar. Non-saccharide honey components include proteins, free amino acids (including proline), carboxylic acids (gluconic, citric, lactic, malic, succinic, butyric, propionic), essential oils, dyes, and vitamins. An overview of a chemical composition of honey can be found in Table 2.

Twenty-six amino acids have been reported in honey samples.[18] Proline is the most predominant amino acid in floral honey, followed by phenylalanine and glutamic acid. Amino acids account for approximately 0.3 – 1% of total honey by weight.

Phenolic acids and flavonoids are also present in honey.[3] The most common phenolic acids found in honey are 4-dimethylaminobenzoic acid, caffeic acid, *p*-coumaric acid, gallic acid, vallinic acid, syringic acid, and chlorogenic acid. Common flavonoids in honey include apigenin, genistein, pinocembrin, tricetin, chrysin, luteolin, quercetin, kaemferol, galangin, pinobanksin, and myricetin. The amounts of polyphenols in different honeys were quantified via high-performance liquid chromatography with diode-array detection (HPLC-DAD; Table 3). Generally, the quantity of a given polyphenol in the honey was approximately 0.2 mg/100 g honey, except for chestnut honey, which contained approximately 3 mg *p*-coumaric acid/100 g honey.[19]

Depending on the floral source, plant toxins may be transferred to the honey that is produced from their nectar, including secondary metabolites such as pyrrolizidine alkaloids, grayanotoxins, hyoscyamine, hyoscine, saponin, strychnine, gelsemine, tutin, hyenanchin, oleandrin, and oleandrigenin.[20] Honey collected from plants of the *Ericacea*e family (*Andromeda* sp., *Rhodendron ponticum*, *Kalmia* sp., *Lleucothoe* sp. *Lynoia* sp., *Pieris* sp.) has been shown to contain some of these toxins. Honey collected in areas where opium poppy cultivation is widespread has been reported to have narcotic effects.

Allergens, such as pollen, may also be present in honey.[21] Ten grams of honey contains approximately 20 to 100,000 grains of pollen, which retain their allergenic properties during the honey-making process. Other allergens include secretions of pharyngeal and salivary glands of honeybee heads, and honey bee venom.

Several studies included in this report involve the use of tualang honey, which is a Malaysian multi-floral jungle honey produced by *Apis dorsata*. A comparison of the physiochemical characteristics of tualang and manuka honey (a mono-floral honey formed by *Apis mellifera*; found in New Zealand and Australia) is provided in Table 4.[22]

Honey Extract

The phenolic content of acacia, chestnut, orange tree, and woodland honey extracts were evaluated by HPLC.[23] All honey extract samples had similar, but quantitatively different, phenolic profiles. The woodland honey extract was richer in polyphenols compared to the other three extracts, showing high levels of caffeic acid, coumaric acid, ferulic acid, iso-ferulic acid, pinobanksin, and pinocembrin.

Impurities

Environmental contaminants of honey include heavy metals (e.g., lead, cadmium, and mercury), radioactive isotopes, organic pollutants, polychlorinated biphenyls, pesticides (insecticides, fungicides, herbicides, and bactericides), pathogenic bacteria, and genetically modified organisms.[24] Beekeeping contaminants include acaricides (i.e., lipophilic synthetic compounds and nontoxic substances such as organic acids and components of essential oils), antibiotics (e.g., tetracyclines, streptomycin, sulfonamides, and chloramphenicol), and paradichlorobenzene.

A compound that is not naturally present in honey, 5-hydroxymethylfurfural (HMF), may be formed during the heating (via the Maillard reaction) or preservation (e.g., via acid-catalyzed dehydration of hexoses) of honey.[20,25] HMF is a compound that may be mutagenic, carcinogenic, and cytotoxic. The *Codex Alimentarius* has established that the HMF concentration in honey should be lower than 80 mg/kg; however, the European Union recommends a lower limit of 40 mg/kg.[26]

Honey Extract

According to one supplier, heavy metal testing was conducted on Honey Extract in a glycerin and water base.[7] No antimony, arsenic, cadmium, chromium, iron, lead, mercury, or nickel was detected. In addition, no residual pesticides were detected.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2020 VCRP survey data, Honey is reported to be used in 1059 formulations (671 of which are leave-on formulations), and Honey Extract is reported to be used in 398 formulations (192 of which are leave-on formulations; Table 5).[27] All other in-use ingredients are reported to be used in 6 formulations or less. The results of a 2018 concentration of use survey conducted by the Council indicate Honey also has the highest concentration of use; it is used at up to 22% in paste masks and mud packs (which are considered rinse-off formulations).[28] The highest concentration of use reported for leave-on products was in formulations containing Honey Extract at up to 7% in body and hand products. Use concentration data were reported for Honey Cocoates in response to the Council survey (it is used at up to 2% in skin cleansing formulations), but no uses were reported in the VCRP; it should be presumed there is at least one use in a skin cleansing formulation, for which the concentration is reported. Conversely, VCRP data are available for Honey Powder, but concentration of use data were not reported. The ingredients not in use according to the VCRP and industry survey are Hydrolyzed Honey and Hydrolyzed Honey Protein.

Honey is reported to be used in baby products, products that would be used near the eye, and products that could result in incidental ingestion and mucous membrane exposure. Honey is reported to be used in 13 baby products and at up to 0.01%. It is also reported to be used in 20 lipstick formulations (up to 3%), 1 dentifrice formulation (up to 0.00035%), 5 "other" oral hygiene product formulations (up to 0.1%), and 1 mouthwash and breath freshener formulation (concentration unknown). Honey could also result in mucous membrane exposure as it is used at up to 3% in bath soaps and detergent formulation.

Additionally, Honey and Honey Extract are used in cosmetic sprays and could possibly be inhaled; for example, Honey is reported to be used in colognes and toilet waters and in hair sprays at up to 0.25% and 0.1%, respectively. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays.[29,30] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[30,31] Honey is reportedly used in face powders at concentrations up to 3%, and could possibly be inhaled. Honey Extract is also reported to be used in powders (dusting and talcum) at up to 0.0001%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the air.[32-34]

The honey-derived ingredients in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[35]

Non-Cosmetic

Food

Raw honey has been consumed worldwide for centuries.[36] Honey is commonly used as a sweetener and flavoring agent in many foods. Honey is listed in the US Environmental Protection Agency (EPA) Inert Finder Database as approved for food and non-food use pesticide products.[37] For food use, it is regulated under 40 CFR 180.950a. In addition, the FDA requires proper labeling of honey and honey products to ensure that these products are not adulterated and misbranded. All honey and honey products must be labeled in accordance with sections 402 and 403 of the Federal Food, Drug, and Cosmetic Act (21 USC 342 and 343). The international FAO/WHO Codex Alimentarius Standard requires that:[38]

> Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavor, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the remove of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or quality is impaired.

Although rare, infant botulism has been reported after ingestion of honey due to *Clostridium botulinum* spores.[39] Because of this, the FDA, the Centers for Disease Control and Prevention, and the American Academy of Pediatrics, recommend not feeding honey to infants younger than 12 months. Neither *Clostridium botulinum* spores nor the neurotoxins are able to penetrate the skin; however, damaged skin may be affected.[40]

Medicine

Honey can be found as an ingredient in over-the-counter (OTC) cough and cold medications.[41] Currently, there is an FDA-approved dermal dressing containing manuka or *Leptospermum* honey, used for the management of wounds and burns.[42] Examples of wounds that are treated with this dressing are diabetic foot ulcers, leg ulcers, pressure ulcers, partial thickness burns, and surgical wounds.[43]

Traditionally, honey has been used as an antibacterial, antiseptic, anti-inflammatory, and apitherapeutic agent.[36] Honey is commonly used for treatment of cuts, eczema, dermatitis, skin diseases, Fournier's gangrene, burns, ulcers, surgical wounds, fungating wounds, pressure sores, and cancer or broken skin.[44] Traditional, Ayurvedic treatments utilize honey for cardiac pain, palpitations, and eye ailments.[45]

TOXICOKINETIC STUDIES

Toxicokinetic studies were not available regarding these honey derived-ingredients. However, toxicokinetic information on some of the relevant, primary components of honey (fructose, glucose, and maltose) can be found in the Panel's report on monosaccharides and disaccharides.[4]

TOXICOLOGICAL STUDIES

No general toxicological studies were found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

The effect of Palestinian honey on spermatogenesis was studied in male albino rats (12 rats/group) after 20 days of treatment.[46] Group A was given a 5% solution of Palestinian honey in drinking water, group B was treated with 5% sucrose in drinking water, and group C served as the control group and was given untreated drinking water. No significant effects on total body weight or weights of the testis, seminal vesicles, spleen, kidneys, liver, heart, or brain were noted. Rats treated with Palestinian honey displayed a significant increase in epididymal sperm count by 37% ($P \leq 0.05$). The activity of testicular marker enzymes for spermatogenesis such as sorbitol dehydrogenase was increased by 31%, and lactate dehydrogenase was reduced by 48%, indicating an induction of spermatogenesis.

A study was performed in order to examine the effect of honey on the reproductive system of rat male offspring.[47] Dams were divided into 10 rats/group. The control group received no treatment while treated animals were given honey (0.2 g/kg bw), daily, from day 1 of pregnancy to day 10, via gavage. In male offspring, testosterone levels were significantly lower in the treated group compared to the control group. Sperm counts, follicle stimulating hormone levels, and testes/epididymis weights were similar in control and honey-treated groups. The percentage of abnormal sperm was significantly higher in offspring of dams treated with honey compared to the control group.

GENOTOXICITY STUDIES

No genotoxicity studies were found in the published literature, and unpublished data were not submitted.

Anti-Mutagenicity

The potential anti-mutagenic effect of various honeys (fireweed, tupelo, Hawaiian Christmas berry, clover, acacia, buckwheat, and soybean) on 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-p-1), was studied.[48] Trp-p-1 is a commonly encountered food mutagen, and has been demonstrated to be mutagenic in bacteria and carcinogenic in animals. The anti-mutagenic effects of the honeys were assayed according to an Ames assay, with slight modification. All assays were performed in a in a final volume of 1 mL containing potassium phosphate buffer, Trp-p-1 (5 µL of 20 µg/mL in dimethyl sulfoxide), 4% S9 mix (500 µL), test strain *Salmonella typhimurium* TA98 (2×10^{10} cells/mL), and different honey solutions. Acacia, fireweed, soy, and tupelo honeys demonstrated enhanced anti-mutagenicity above 1 mg/mL, with inhibition between 40.3 and 62.9%; concentrations above 20 mg/mL did not further enhance anti-mutagenic effects. Clover and Hawaiian Christmas berry honey were most effective at 20 mg/mL, with 64.8 and 59.6% inhibition, respectively. The greatest inhibitory effect of buckwheat honey was observed at 1 mg/mL (52.1%).

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

ANTI-CARCINOGENICITY STUDIES

The potential anti-carcinogenic effect of tualang honey on breast cancer was studied in rats.[49] Forty female Sprague-Dawley rats were given 80 mg/kg 7,12-dimethylbenz[a]anthracene (DMBA) via gavage. Rats were then divided into four groups. Animals in group 1 were given only distilled water. Animals in groups 2, 3, and 4 were given 0.2, 1, and 2 g/kg bw/day tualang honey diluted in 0.5 mL water, respectively, via gavage, for 150 days. After treatment, animals were euthanized. Breast cancers in the honey-treated groups had smaller tumor size compared to controls. In addition, the number of cancers developed in honey-treated rats was significantly lower than control groups ($P < 0.05$). The majority of the cancers in the control groups were high grade, while cancers in honey-treated groups were of medium- or low-grade. These effects, however, were not dose-dependent.

Anti-Tumorigenicity

The anti-tumoral therapeutic effects of tualang honey and manuka honey was studied in rats (10/group).[50] Thirty female Sprague-Dawley rats were given an 80 mg/kg injection of the carcinogen 1-methyl-1-nitrosourea (MNU); an additional 10 female rats were left untreated. Treatment with honey started when the first tumor reached 10 - 12 mm in size. Positive (tumor induction and no honey treatment) and negative controls (no tumor induction or honey treatment) were included. Treatment groups were fed either tualang or manuka honey (1 g/kg bw/day) for 120 days. On the 120th day of treatment, rats were euthanized. Rats in the positive control group had the highest median number of tumors compared to groups treated with either honey. Groups treated with honey showed a significant reduction in tumor size and weight compared to the positive control group. The percent reduction in the size of primary tumors was greater with tualang honey (70.82%), as compared to manuka honey (57%). Tumor masses in the positive control group were solid, large in size, and hard in consistency, exhibiting areas of necrosis and hemorrhage. Both honey-treated groups had tumors which were softer, paler, and smaller in size. Tumors in the positive control group were observed to have increased heterogenous nuclei formation, which were hyperchromatic, vesicular, and highly pleomorphic, with moderate cytoplasm increased mitotic activity compared with the honey-treated groups, which had fatty tissue, small nuclei, and cystic spaces.

OTHER RELEVANT STUDIES

Airway Inflammation Reduction

New Zealand white rabbits (5/group) were dosed twice with an intraperitoneal injection of ovalbumin (OVA) and aluminum hydroxide on days 1 and 14.[51] Tualang honey was then given via a nebulizer from days 23 to 25 at concentrations of either 25 or 50%, diluted in sterile phosphate buffer saline (5 mL for 20 minutes). After treatment with aerosolized honey, animals were either euthanized, or, further exposed to aerosolized OVA for 3 days starting from day 28 and euthanized on day 31. The effects of honey on the inflammatory cell response, airway inflammation, and goblet cell hyperplasia were assessed. Treatment with aerosolized honey reduced the number of airway inflammatory cells present in bronchioalveolar lavage fluid and inhibited goblet cell hyperplasia. In addition, treatment with aerosolized honey led to a significant decrease in the thickening of the epithelial and mucosal regions.

Nasal Respiratory Mucosa

A study was performed in New Zealand white rabbits (2/group) to evaluate the effect of manuka honey on nasal respiratory mucosa.[52] The left nasal cavity of each rabbit was irrigated once daily with 1.5 mL of a 33% mixture of manuka honey with saline; groups were treated for either 3, 7, or 14 consecutive days, and then euthanized. The last group was treated for 14 days followed by a 14-day washout period, and then euthanized the following morning. The right nasal cavity of each rabbits served as a control, and was not treated. The mucosa were examined by light microscopy. No histological evidence of inflammation, mucosal injury, or significant morphological changes were observed.

Allergic Potential Following Ingestion

Twenty subjects were used in a 12-week study to determine the allergic potential of manuka and multi-floral honey.[53] The participants ate a normal diet with the inclusion of the allocated honey. For the first 2 weeks, all honey was excluded from the diet; then, participants consumed 20 g honey per day in two doses of 10 g each. After 4 weeks, there was another 2-week "washout" period, and the groups swapped to the other type of honey for 4 weeks. Fasting blood samples were collected at the beginning of the study, starting with the first sample after the initial 2-week washout, and then weekly during the 4-week interventions with honey. Immunoglobin E (IgE) measurements were carried out on frozen serum collected weekly during each of the honey interventions. IgE levels remained at a level consistent with a non-atopic response during the course of the study. The authors concluded that this level of consumption of manuka and multi-floral honey had no significant effect on allergic status.

Cytotoxicity of Honey-Impregnated Wound Dressing

The potential cytotoxic effect of honey-impregnated wound dressings on human skin keratinocytes and dermal fibroblasts was studied.[54] Five and 21 days after initiating the tissue culture, the honey-impregnated wound dressing was introduced directly onto the cells in the test wells to allow for cell growth. Small blocks of commercial dressings were then

inserted into the wells, adjacent and distal to the tissue explants. The amount of test material used was not stated. Keratinocytes and fibroblasts treated with honey implants displayed a modest uniform increase in early cell proliferation and cell counts per mm. Nuclear and cytocavitary networks appeared normal, and cell proliferation was also evident immediately adjacent to the product. No cell toxicity was observed.

Cytotoxicity in Cancer Cells

Renal cell carcinoma cells (ACHN) were cultured in a medium containing 10% fetal bovine serum and 5, 10, or 15% honey for 3 consecutive days.[55] Cell viability was determined by the 3-(4,5-dimethyliazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and apoptotic cells were determined using annexin V-fluorescein isothiocyanate (FITC) by flow cytometry. Honey decreased cell viability and induced apoptosis in malignant cells in a concentration- and time-dependent manner ($P < 0.001$). The half maximal inhibitory concentration (IC_{50}) values at 48 and 72 hours were 1.7 ± 0.04 and 2.1 ± 0.03 µg/mL, respectively.

A similar study was performed on human breast cancer (MCF-7, MDA-MB-231), immortalized cervical cancer (HeLa), and normal breast epithelial cells.[56] Cells were plated at a concentration of 1×10^5 cells/well. The cells were allowed to adhere overnight, and the culture medium was replaced with fresh assay medium supplemented with 2% fetal bovine serum. Cells were then treated with different concentrations of tualang honey (1 - 10%), and incubated for up to 72 hours. Tualang honey induced a statistically significant increase in cell death in MDA-MB-231, MCF-7, and HeLa cancer cell lines in a dose- and time-dependent manner. Treatment of the normal breast epithelial cell line did not show a clear cytotoxic effect, even after 72 hours of incubation. Flow cytometric analysis of cells stained with annexin V-FITC and propidium iodide showed that tualang honey significantly increased apoptosis in all cancer cell lines compared to untreated cells.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Human

Honey Extract

A human repeated insult patch test (HRIPT) was performed on 112 subjects using a cosmetic product containing 7% Honey Extract.[57] Approximately 0.2 mL of the test substance was applied to the upper back, under an occlusive patch. Patches were allowed to remain in direct skin contact for a period of 24 hours. Applications were made to the same site, three times a week, for a total number of 9 applications during the induction period. After a 2-week rest period, challenge patches were applied to previously untreated test sites. After 24 hours, patches were removed and test sites were evaluated. The test substance did not demonstrate a potential for eliciting dermal irritation or sensitization.

According to a summary report, an HRIPT was performed on 116 subjects using a product containing 0.01% Honey Extract according to the same procedure as above.[58] The product was tested at a 1% dilution in water (effective test concentration, 0.0001% Honey Extract). Seven individuals displayed low-level reactions (mild erythema) during the induction phase, and one individual displayed a high-level reaction in the induction phase. Eight individuals displayed low-level reactions during the challenge phase. (Individual subject scores were not provided.) The test substance was considered by the researchers to be non-sensitizing.

OCULAR IRRITATION STUDIES

Human

Use Study

A prospective, randomized, paired-eye, investigator-masked trial was performed on 25 subjects to determine the clinical safety of manuka honey eye cream on patients with blepharitis.[59] The cream (approximately $0.034 \text{ g} \pm 0.001 \text{ g}$) was placed on the periocular surface of the closed upper and lower eyelids of the affected eye. Applications occurred once a day, at night, for 2 weeks. The untreated eye served as a control. A questionnaire was given to grade the severity of dry eye symptomatology at baseline, and a telephone interview was conducted following the first day of cream application to check for immediate tolerability issues or adverse events. Clinical assessments were performed at baseline, day 7, and day 14 of the treatment period. There were no statistically significant differences in baseline clinical or impression cytology measurements between treated and control eyes. Twenty-three of 25 participants did not report any tolerability issues or adverse effects following the first day of product application. In two individuals, application too close to the eyelash margin and the use of excessive cream was presumed to result in a transient stinging sensation. Irrigation with water and reapplication of a modest quantity of cream resolved the issue in both cases. No other adverse effects were reported throughout the study.

CLINCAL STUDIES

Effect on Damaged Pediatric Skin

Eight pediatric patients ranging from 8 months to 13 years of age were evaluated in this study.[60] Five of the children had second-degree burns, and three had necrotic ulcers, circular skin lesions, and deep cervical trauma. Each child was treated with povidone iodine (10% solution), fusidic acid, and systemic antibiotics, followed by a honey-based ointment. After this initial treatment, patients were instructed to apply honey-containing ointment as well as a dressing impregnated with a polymer containing 20% medical grade honey, daily. The duration and amount of product used in this study were not stated. No adverse effects or allergic reactions were observed.

Case Studies

Anaphylaxis

A 40-year old woman was referred to a clinic after suspected allergy to honey.[61] At the age of 36, she had two episodes of generalized urticaria 20 minutes after ingestion of foods with honey. At the age of 37, five minutes after an inadvertent contact with a teaspoon with traces of honey, the patient reported swollen lips, urticaria, and angioedema. After treatment with oral corticosteroids and antihistamines, symptoms were resolved. Skin prick tests with standard panel of extracts from aeroallergens and common allergenic foods yielded negative results. Prick-to-prick tests (PPT) were performed with the previously consumed honey, and eight other kinds of honey (eucalyptus, sunflower, orange-tree, Arbutus-tree, French lavender, heather, flower incense, and rosemary). Results were positive for all honey types. Thirty minutes after the administration of the PPT, the patients suffered from anaphylaxis, generalized urticaria, swollen lips, tongue, and uvula, and hypotension. The same PPT was performed with these honeys in 6 control volunteers (3 healthy individuals, and 3 atopic with pollen sensitization and rhinitis). None of the volunteers displayed a positive skin reaction.

Epicutaneous Sensitization

A 48-year-old woman had been washing her body and hair with products blended with edible honey, and she applied honey to the face as a face pack.[62] After 8 years of use, the woman developed itching and redness on facial skin as well as conjunctival hyperemia following the use of the face pack containing honey. After washing her body with honey-containing soap, the subject reported urticarial symptoms on her extremities and un-exposed face. One year later, the subject developed abdominal pain and distention after eating yogurt with honey. The patient had positive results for honey-antigen specific IgE antibodies in serum (UA), equivalent to 1.44 UA/mL, but not for honey bee venoms or Api m 10 (*Apis mellifera* venom component). Results for specific IgE against three cross-reactive carbohydrate determinant marker allergens were negative. Prick tests with honey gave positive results. Fifteen minutes after oral challenge with 30 mL of honey, the patient developed eyelid swelling, abdominal pain, and oral tingling.

SUMMARY

The 7 honey-derived ingredients in this report all are reported to function in cosmetics as skin-conditioning agents. Other reported cosmetic functions include flavoring agent, abrasive, binder, depilating agent, exfoliant, hair-conditioning agent, and nail-conditioning agent. Honey derived for cosmetic purposes is reported to be produced by the honeybee species *Apis mellifera*, *Tetragonisca angustula*, *Scaptotrigona pectoralis*, and *Melipona becheii.*

Of the ingredients included in this report, Honey has the most reported uses, with a total of 1059; 671 of these are leave-on products. Honey Extract has the second greatest number of overall uses, with a total of 398 (192 are in leave-on formulations). Honey has the highest concentration of use, and is used at up to 22% in paste and mud packs. The highest concentration of use reported for leave-on products was in body and hand products containing Honey Extract at up to 7%. The ingredients not in use according to VCRP data and the industry survey are Hydrolyzed Honey and Hydrolyzed Honey Protein.

Honey is common in food and food products worldwide. Honey can be found in OTC cough and cold medications. Traditional medicine suggests the use of honey for various ailments and skin issues. Currently, there is an FDA-approved dermal dressing containing honey used for the management of wounds and burns.

The effect of Palestinian honey on spermatogenesis was studied in male albino rats. Rats treated with Palestinian honey displayed a significant increase in epididymal sperm count. The activity of testicular marker enzymes for spermatogenesis, such as sorbitol dehydrogenase, was increased, and lactate dehydrogenase was reduced, indicating an induction of spermatogenesis. The effect of honey on the reproductive system of rat male offspring was studied. Testosterone levels were significantly lower in the male offspring of treated animals, compared to control animals. The percentage of abnormal sperms were significantly higher in the offspring of dams treated with honey versus the control group. All other parameters were similar between treated and control group.

The potential anti-mutagenic effect of various honeys on Trp-p-1 was studied. Acacia, fireweed, soy, and tupelo honeys demonstrated enhanced antimutagenicity above 1 mg/mL, with inhibition between 40.3 and 62.9%. Concentrations above 20 mg/mL demonstrated no enhancement of the antimutagenic effects. Clover and Hawaiian Christmas berry honey were most

effective at 20 mg/mL, with 64.8 and 59.6% inhibition, respectively. The greatest inhibitory effect of buckwheat honey was observed at 1 mg/mL (52.1%).

In an anti-tumorigenicity study, Sprague-Dawley rats were given an injection of the carcinogen MNU and either given no treatment or treatment with manuka or tualang honey (1 g/kg bw/day) via diet. Groups treated with honey showed a significant reduction in tumor size and weight compared to the nontreated positive control. In addition, tumors in the positive control were large and hard, while tumors in honey-treated groups were small and soft.

In New Zealand white rabbits pre-treated with ovalbumin, treatment with aerosolized honey reduced the number of airway inflammatory cells present in bronchioalveolar lavage fluid and inhibited goblet cell hyperplasia. In addition, treatment with aerosolized honey led to a significant decrease in the thickening of the epithelial and mucosal regions. The nasal cavities of New Zealand white rabbits were irrigated with a honey and saline solution. No histological evidence of inflammation, epithelial injury, or significant morphological changes were observed.

Twenty subjects were used in a study to determine the allergic potential of manuka and multi-floral honey following ingestion. IgE levels remained at a level consistent with a non-atopic response during the course of the study.

The potential cytotoxic effect of honey-impregnated wound dressings on human skin keratinocytes and dermal fibroblasts was studied. Keratinocytes and fibroblasts treated with honey implants displayed a modest uniform increase in early cell proliferation and cell counts per mm. No cytotoxic effects were observed.

The anti-carcinogenic potential of honey (up to 15%) was studied using renal cell carcinoma cell lines. Honey decreased cell viability and induced apoptosis in malignant cells in a concentration- and time-dependent manner. A similar study was performed using tualang honey (1 - 10%) on human breast cancer, cervical cancer, and normal breast epithelial cell lines. Treatment with honey induced cell death in all cancer cell lines, but no clear cytotoxic effect was observed in the normal breast epithelial cells. In a different study, the effect of tualang honey (0.2 – 2 g/kg) on breast cancer-induced rats was observed. Smaller tumors were observed in honey-treated rats compared to control animals. In addition, the number of cancers developed in honey-treated rats was significantly lower than control groups.

An HRIPT that was performed on 112 subjects using a cosmetic product containing 7% Honey Extract applied using occlusive conditions yielded negative results. In an HRIPT performed on 116 subjects using a test substance containing 0.01% Honey Extract, tested as a 1% dilution, the test substance was considered to be non-sensitizing.

Twenty-five subjects were used in a 2-week prospective study to determine the safety of manuka honey eye cream on blepharitis patients. Twenty-three of 25 participants did not report any tolerability issues or adverse effects following the first day of product application. In two individuals, application too close to the eyelash margin and the use of excessive cream was presumed to result in a transient stinging sensation.

Honey-based ointment was used as part of a treatment of damaged skin in 8 children. No adverse effects or allergic reactions were reported after treatment.

Generalized urticaria was reported in a patient 20 minutes after ingesting foods with honey. After inadvertent contact with traces of honey, the same patient reported other allergic symptoms. Skin prick tests on common allergenic foods yielded negative results, however, prick-to-prick testing using different honey types yielded positive results.

After using cosmetics blended with edible honey and using honey as a face pack for 8 years, a woman reported allergic reactions after using a face pack containing honey and body soap containing honey. A year after these symptoms occurred, the patient experienced abdominal pain after ingestion of honey. Prick tests with honey yielded positive results.

DISCUSSION

The Panel reviewed the available, relevant data to assess the safety of these honey-derived ingredients as used in cosmetics in the present practices of use and concentration. The Panel noted the lack of sensitization data for six of the seven ingredients, but determined that the available sensitization data on Honey Extract could be used to support the safety of the remaining ingredients. The safety of these ingredients is further supported by historical food use and use in wound dressings.

As Honey Powder in foods has been reported to be 50% filler material, the Panel also discussed the safety of those possible filler ingredients (starch, carboxymethyl cellulose, gum Arabic, maltodextrin, and gelatin), and determined that these ingredients are not of concern. All of the named fillers have been previously reviewed by the Panel, and were considered safe as used in cosmetics.

The Panel expressed concern regarding pesticide residues and endotoxins that may be present in these ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities. In addition, the Panel noted the importance of avoiding the use of honey derived from toxic plant sources for use in cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure from formulations that may be aerosolized (e.g., colognes and toilet waters at up to 0.25% Honey). The Panel noted that in aerosol products, 95% – 99% of droplets/particles

would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. Respiratory safety of these ingredients was further supported by the lack of negative effects observed in a nasal irrigation and an inhalation study performed in rabbits. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following honey-derived ingredients are safe in cosmetics in the present practices of use and concentrations described in the safety assessment.

Honey	Hydrogenated Honey
Honey Cocoates	Hydrolyzed Honey*
Honey Extract	Hydrolyzed Honey Protein*
Honey Powder	

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

TABLES

Table 1. INCI names, definitions, and functions of the honey ingredients in this safety assessment[1,2]

Ingredient	Definition	Function
Honey 8028-66-8	Honey is a saccharic secretion gathered and stored by honey bees of the species, *Apis mellifera*, *Tetragonisca angustula*, *Scaptotrigona pectoralis*, or *Melipona becheii*	flavoring agent; humectant; skin-conditioning agent-humectant; solvent
Honey Cocoates	Honey Cocoates is a complex mixture of esters produced by the reaction of Honey with coconut acid [*The fatty acid composition of coconut oil (from which coconut acid* (CAS: 61788-47-4) *is derived) is 0-1% caproic, 5-9% caprylic, 6-10% capric, 44-52% lauric, 13-19% myristic, 8-11% palmitic, 0-1% palmitoleic, 1-3% stearic, 5-8% oleic, 0-2.5% linoleic*]	antiacne agent; film former; skin-conditioning agent – miscellaneous
Honey Extract 91052-95-5	Honey Extract is the extract of Honey	skin-conditioning agents-humectant; skin-conditioning agents-miscellaneous; solvents
Honey Powder	Honey Powder is the powder obtained from dehydrated, ground Honey	abrasives; binders; bulking agents; depilating agents; epilating agent; exfoliant; flavoring agent; hair conditioning agent; nail conditioning agent; skin-conditioning agent-miscellaneous
Hydrogenated Honey	Hydrogenated Honey is the end product of controlled hydrogenation of Honey	humectants; skin-conditioning agents-humectant; skin-conditioning agents-miscellaneous
Hydrolyzed Honey	Hydrolyzed Honey is the hydrolysate of Honey derived by acid, enzyme or other method of hydrolysis	skin-conditioning agents-humectant
Hydrolyzed Honey Protein	Hydrolyzed Honey Protein is the hydrolysate of honey protein derived by acid, enzyme or other method of hydrolysis	hair conditioning agents; skin-conditioning agents-miscellaneous

Table 2. Chemical Composition of Honey[3]

Constituent	g per 100 g honey
water	17.1
carbohydrates	82.4
fructose	38.5
glucose	31
maltose	7.2
sucrose	1.5
proteins, amino acids, vitamins, and minerals	0.5
calcium	0.0044 - 0.0092
potassium	0.0132 - 0.0168
copper	0.000003 - 0.0001
iron	0.00006 - 0.0015
magnesium	0.0012 - 0.0035
manganese	0.00002 - 0.0004
phosphorous	0.0019 - 0.0063
sodium	0 - 0.0076
zinc	0.00003 - 0.0004
ascorbic acid	0.002 - 0.0024
thiamin	< 0.000006
riboflavin	< 0.00006
niacin	< 0.00036
pantothenic acid	< 0.00011
pyridoxine (B6)	< 0.00032

Table 3. Honey polyphenols quantified with the HPLC-DAD method[19]

Honey	Polyphenol	Mean amount (mg per 100 g honey)
acacia	*p*-coumaric acid	0.077 ± 0.003
chestnut	*p*-coumaric acid	2.952 ± 0.004
eucalyptus	quercetin	0.164 ± 0.007
sunflower	caffeic acid	0.242 ± 0.001
sunflower	*p*-coumaric acid	0.107 ± 0
sunflower	kaempferol	0.205 ± 0.003
sunflower	chrysin	0.217 ± 0.002
thyme	*p*-coumaric acid	0.070 ± 0
wild carrot	*p*-coumaric acid	0.223 ± 0.001

Table 4. Physicochemical properties and constituents of tualang vs. manuka honey[22]

Property	tualang honey	manuka honey
appearance	dark brown	light to dark brown
pH	3.55 – 4	3.2 – 4.21
moisture content	23.3%	18.7%
total reducing sugars	67.5%	76%
fructose	29.6%	40%
glucose	30%	36.2%
sucrose	0.6%	2.8%
maltose	7.9%	1.2%
potassium	0.51%	1%
calcium	0.18%	1%
magnesium	0.11%	1%
sodium	0.26%	0.0008%
carbon	41.58%	-
oxygen	57.67%	-

- = not reported
-tualang honey: Malaysian multi-floral jungle honey produced by *Apis dorsata*
-manuka honey: Australian or New Zealand mono-floral honey produced by *Apis mellifera*

Table 5. Frequency (2020) and concentration (2018) of use of honey ingredients[27,28]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Honey		Honey Cocoates		Honey Extract	
Totals*	**1059**	**0.00001 - 22**	**NR**	**2**	**398**	**0.000002 – 7**
Duration of Use						
Leave-On	*671*	*0.0001 – 3*	*NR*	*NR*	*192*	*0.0000034 – 7*
Rinse-Off	*377*	*0.00001 – 22*	*NR*	*2*	*191*	*0.000002 – 0.01*
Diluted for (Bath) Use	*11*	*NR*	*NR*	*NR*	*15*	*NR*
Exposure Type						
Eye Area	23	3	NR	NR	10	NR
Incidental Ingestion	27	0.00035 – 3	NR	NR	17	NR
Incidental Inhalation-Spray	2; 186[a]; 353[b]	0.001 – 0.25; 0.01 – 0.75[b]	NR	NR	2; 70[a]; 73[b]	0.0000034 – 0.001; 0.00001 – 0.0021[b]
Incidental Inhalation-Powder	186[a]; 7[c]	3; 0.0005 – 3[c]	NR	NR	70[a]	0.0001; 0.001 – 7[c]
Dermal Contact	882	0.00001 – 22	NR	NR	291	0.000002 – 7
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	145	0.000039 – 10	NR	NR	62	0.0000034 – 0.005
Hair-Coloring	2	0.01 – 0.8	NR	NR	25	0.0001 – 0.006
Nail	1	NR	NR	NR	NR	NR
Mucous Membrane	209	0.00035 – 3	NR	NR	89	0.00028 – 0.01
Baby Products	13	0.01	NR	NR	NR	0.00051

	Honey Powder		Hydrogenated Honey	
Totals*	**6**	**NR**	**6**	**0.25**
Duration of Use				
Leave-On	*3*	*NR*	*1*	*NR*
Rinse Off	*3*	*NR*	*5*	*0.25*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]	NR	1[a]	NR
Incidental Inhalation-Powder	2[a]	NR	1[a]	NR
Dermal Contact	6	NR	6	0.25
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders
NR – no reported use

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TECHNICAL REPORT

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Outcome of the consultation with Member States and EFSA on the basic substance application for honey from rhododendron for use in plant protection as rodenticide

European Food Safety Authority (EFSA)

Abstract

The European Food Safety Authority (EFSA) was asked by the European Commission to provide scientific assistance with respect to the evaluation of applications received by the European Commission concerning basic substances. In this context, EFSA's scientific views on the specific points raised during the commenting phase conducted with Member States and EFSA on the basic substance application for honey from rhododendron are presented. The context of the evaluation was that required by the European Commission in accordance with Article 23 of Regulation (EC) No 1107/2009 following the submission of an application for approval of honey from rhododendron as a basic substance for use in plant protection as rodenticide. The current report summarises the outcome of the consultation process organised by EFSA and presents EFSA's scientific views on the individual comments received.

Keywords: honey from rhododendron, basic substance, application, consultation, plant protection, pesticide

Requestor: European Commission

Question number: EFSA-Q-2016-00571

Correspondence: pesticides.peerreview@efsa.europa.eu

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Summary

Honey from rhododendron is an active substance for which, in accordance with Article 23(3) of Regulation (EC) No 1107/2009, the European Commission received an application from Klaus Gasser + Partner for approval as a 'basic substance'. Regulation (EC) No 1107/2009 introduced the new category of 'basic substances', which are described, among others, as active substances, not predominantly used as plant protection products but which may be of value for plant protection and for which the economic interest in applying for approval may be limited. Article 23 of Regulation (EC) No 1107/2009 lays down specific provisions for consideration of applications for approval of basic substances.

In March 2013, the European Commission requested the European Food Safety Authority (EFSA) to provide scientific assistance with respect to the evaluation of applications received by the European Commission concerning basic substances. By a further specific request, received from the European Commission in September 2016, EFSA was asked to organise a consultation on the basic substance application for honey from rhododendron, to consult the applicant on the comments received, and to deliver its scientific views on the specific points raised in the format of a reporting table within three months of acceptance of the specific request.

A consultation on the basic substance application for honey from rhododendron, organised by EFSA, was conducted with Member States via a written procedure in June-August 2016. Subsequently, EFSA also provided comments and the applicant was invited to address all the comments received in the format of a reporting table and to provide an application update as appropriate, within a period of 30 days.

The current report summarises the outcome of the consultation process organised by EFSA on the basic substance application for honey from rhododendron and presents EFSA's scientific views on the individual comments received in the format of a reporting table.

It is acknowledged that the issue whether honey from rhododendron fulfils the criteria laid down in Article 23 (1a) of Regulation (EC) No 1107/2009 has been raised by some Member States during the commenting phase. EFSA considers this issue a risk management matter and does not provide an opinion in relation to that.

Proper batch analysis would be needed to determine the levels of grayanotoxins and other potential active or toxic substances, including relevant impurities in the honey from rhododendron intended to be used as pesticide. This would also allow demonstrating consistent composition and efficacy of the proposed product. Furthermore, specifications for content of grayanotoxins and other potential active or toxic substances including relevant impurities need to be proposed and agreed based on appropriate analysis of batches. Validated analytical methods for grayanotoxins and other potential active or toxic substances including relevant impurities in honey from rhododendron are not available and would need to be provided.

The substance honey from rhododendron is proposed to be used as rodenticide in baits. The applicant claims that field studies performed on their own, demonstrate that mice die as a result of the grayanotoxins in the honey. However, no proper scientific report has been provided to substantiate these claims.

For a basic substance no specific preparation should be needed since the raw technical product is supposed to be used. Therefore, EFSA did not assess the adequacy of the preparation proposed for the intended use (dosage gelatin capsules). However, the product would need to be conveniently labelled to prevent accidental human consumption.

Plant parts of rhododendron ssp. containing grayanotoxin are listed in the EFSA Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. The applicant claimed that authorisation as a food supplement, labelled with maximum dosage levels, has been obtained in the EU, but that was not demonstrated by evidence or supported by submission of details regarding the composition of such food supplement and its safety assessment for human health. Non-dietary exposure was not properly addressed and cannot be excluded.

Regarding consumer exposure no data with respect to residue behaviour is needed as long as it can be guaranteed that honey from rhododendron containing grayanotoxins ('mad honey') is only used in bait boxes and that any contact with trees or crops is excluded.

No data with respect to the fate and behaviour into the environment and concerning the effect on other non-target organisms are needed, as long it is guaranteed that i) the proposed uses are exclusively in baits and ii) the bait design is as such that the basic substance cannot be released from the bait box. The bait to be used should close after the entering of the mouse and guarantee the death of the trapped animal inside the bait box, preventing it from becoming a prey of non-target predatory vertebrates. It is noted that further data are needed to ensure that non-target terrestrial organisms could not access the bait. Furthermore, the target species are yet to be defined. No information or evidence has been provided to demonstrate that mice are not subject of unnecessary suffering during the 2 to 4 days until they are supposed to die.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1107/2009[1] (hereinafter referred to as 'the Regulation') introduced the new category of 'basic substances', which are described, among others, as active substances, not predominantly used as plant protection products but which may be of value for plant protection and for which the economic interest of applying for approval may be limited. Article 23 of the Regulation lays down specific provisions to identify a substance as a basic substance with a view to ensure that such active substances that do not have an immediate or delayed harmful effect on human and animal health nor an unacceptable effect on the environment can be approved as 'basic' and used for plant protection purposes.

Honey from rhododendron is an active substance for which, in accordance with Article 23(3) of the Regulation, the European Commission received an application from Klaus Gasser + Partner for approval as a 'basic substance' for use in plant protection as rodenticide.

The European Food Safety Authority (EFSA) organised a consultation with Member States on the basic substance application for honey from rhododendron, which was conducted via a written procedure in June-August 2016. The comments received, including EFSA's comments, were consolidated by EFSA in the format of a reporting table. Subsequently, the applicant was invited to address the comments in column 4 of the reporting table and to provide an application update as appropriate. The comments received and the response of the applicant thereon, together with the application update submitted by the applicant, were considered by EFSA in column 5 of the reporting table.

The current report aims to summarise the outcome of the consultation process organised by EFSA on the basic substance application for honey from rhododendron and to present EFSA's scientific views on the individual comments received in the format of a reporting table.

The application and, where relevant, any update thereof submitted by the applicant for approval of honey from rhododendron as a 'basic substance' in the context of Article 23 of the Regulation, is a key supporting documentation, therefore it is considered as a background documentation to this report and will also be made publicly available, excluding its appendices (Klaus Gasser + Partner; 2016 a,b).

1.2. Interpretation of the Terms of Reference

On 6 March 2013 the European Commission requested EFSA to provide scientific assistance with respect to the evaluation of applications received by the European Commission concerning basic substances. By a further specific request, received by EFSA on 20 September 2016, EFSA was asked to organise a consultation on the basic substance application for honey from rhododendron, to consult the applicant on the comments received, and to deliver its scientific views on the specific points raised in the format of a reporting table.

To this end, a technical report containing the finalised reporting table is being prepared by EFSA. The agreed deadline for providing the finalised report is 20 December 2016.

On the basis of the reporting table, the European Commission may decide to further consult EFSA to conduct a full or focussed peer review and to provide its conclusions on certain specific points.

[1] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1-50.

2. Assessment

The comments received on the basic substance application for honey from rhododendron and the conclusions drawn by EFSA are presented in the format of a reporting table.

The comments received are summarised in columns 2 and 3 of the reporting table. The applicant's considerations of the comments, where available, are provided in column 4, while EFSA's scientific views and conclusions are outlined in column 5 of the table.

The finalised reporting table is provided in Appendix A of this report. In addition, an overview table on the identity and biological properties of the substance and the list of intended uses in plant protection (GAP table) are provided in Appendix B and C, respectively.

Documentation provided to EFSA

1. Klaus Gasser + Partner, 2016a. Basic substance application on honey from rhododendron submitted in the context of Article 23 of Regulation (EC) No 1107/2009. January 2016. Documentation made available to EFSA by the European Commission.

2. Klaus Gasser + Partner, 2016b. Basic substance application update on honey from rhododendron submitted in the context of Article 23 of Regulation (EC) No 1107/2009. September 2016. Documentation made available to EFSA by the applicant.

References

EFSA (European Food Safety Authority), 2009. Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern on request of EFSA. EFSA Journal 2009; 7(9):281,100 pp. doi:10.2903/j.efsa.2009.281

Abbreviations

a.s.	active substance
ADI	acceptable daily intake
CLP	Classification, Labelling and Packaging
DG SANTE	Directorates-General - Health and Food Safety
EU	European Union
GTX	grayanotoxin
LC_{50}	lethal concentration, median
LD_{50}	lethal dose, median; dosis letalis media
MS	Member State

Appendix A – Collation of comments from Member States and EFSA on the basic substance application for honey from rhododendron and the conclusions drawn by EFSA on the specific points raised

1. Purpose of the application

General

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
1(1)	1	DE: Please see 5.13: "acceptable daily intake for humans: less than 5 g of honey from Rhododendron with grayanotoxin". "The oral LD_{50} for mice is approx. 1 mg/kg...", please compare to parathion LD_{50} (mice) approx. 5-25 mg/kg. Therefore, the substance applied for is a substance of concern, this honey cannot be considered as food. DE: The introduction implicates as if the toxin Grayanotoxin would have been proven to be "an essential use" for fruit production.	Honey from rhododendron as described in the application is a substance of concern, it does not meet the criteria of a basic substance. The applicant should clarify that he speculates about the desired effects or cite public available data or own experimental reports about it	In Turkey this honey is sold as food with food certificate without labelling maximum dosage intake. ADI (Acceptable Daily Intake) for humans is estimated to be less than 5 g of honey with approx. 50 mg/ kg Grayanotoxins. The LD_{50} (mice) is approx. 3-5 mg/kg due to the different grayanotoxin versions I-VIII with individual potency levels. In literature the LD_{50} (mice/ grayanotoxin I) is described at approx. 5,1 mg/ kg body weight. This product is not in contact with agro production. A sophisticated rodenticide for organic and conventional fruit production is essential. Mice are expanding fast and less fruit is a loss of yield.	Honey from rhododendron as described in the application is a substance of concern as it may be considered as toxic or containing toxic components (see Section 5).
1(2)		DE: No (literature) studies have		Basic substance application	Some references from peer

General					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		been provided with this application. A detailed evaluation of rhododendron honey only from the provided report is not possible.		(BSA) updated with online references and literature.	reviewed scientific literature on toxicological effects of honey from rhododendron have been submitted. However, explanations on how these references have been searched and selected, is not provided. A more systematic review would be necessary to guarantee that the search is exhaustive and unbiased.
1(3)	5.2	DK: We question if honey from Rhododendron fulfils the criteria laid down in Article 23 (1a). The severity off the acute effects presents an inherent capacity to cause an adverse effect on humans and this might not be completely neglected by applying a risk management perspective (bait boxes).		In Turkey this honey is sold as food with food certificate. It has been declared admissible by DG SANTE on this basis. The substance placed in a bait box secures zero contact with other animals + humans, even small insects like ants can't enter the bait box, although this substance is still a legal food product.	The issue whether honey from rhododendron fulfils the criteria laid down in Article 23 (1a) is considered by EFSA a risk management issue and EFSA does not provide an opinion in relation to that.
1(4)	9	DK: Please clarify/elaborate what is meant/implied by the sentence "*Also for other diseses, honey from rhododendron with grayanotoxins may be a plant protection product*		Baits are included in plant protection product (PPP) regulation. BSA corrected: "*diseases*" removed, modified.	Only uses in bait boxes which close after the entering of the mice and guarantee that the mice die inside the bait boxes, without release of basic substance into the environment, are considered

General					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		with perspectives." Rodents are not a disease, and any other application than bait boxes will likely not fulfil the criteria laid down in Article 23 (1a).			by EFSA for this application.
1(5)		NL: it is said that the honey from rhododendron should be used with a special bait box. If the bait box in combination with the honey is placed on the market as a PPP, it cannot be regarded a basic substance anymore as according to regulation 1107/2009 it is not allowed to market a basic substance as a plant protection product.		This application is for honey from rhododendron with GTX (grayanotoxins), to be placed in bait box.	Only uses in bait boxes that close after the entering of the mice and guarantee that the mice die inside the boxes, without release of basic substance into the environment, are considered by EFSA for this application. See also 1(3)
1(6)		PL: The LD_{50} value for mice specified by the applicant concerns the intraperitoneal route of administration not oral. Oral LD_{50} for mice is about 5-fold higher. Moreover, it is not specified for which grayanotoxin this value applies	EFSA: please clarify the source of information for the different endpoints mentioned in the report.	The oral LD_{50} for mice is approx. 5,1 mg/kg (Grayanotoxin I) and 4,9 mg/kg (Grayanotoxin III) according to literature.	The origin of the information on the oral toxicity (LD_{50}) for mice has not been clarified by the applicant in column 4. See Section 5 for further information.

2. Identity of the substance/product as available on the market and predominant use

2.1. Identity and Physical and chemical properties of the substance and product to be used

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
2(1)		DE: The numeration in the application sheet and in this commenting table are in different orders.	The applicant should modify his order to avoid misunderstandings	The numeration of chapters in the BSA has not been updated to avoid misunderstandings in the BSA.	Noted The numeration of chapters in the BSA has not been updated by the applicant.
2(2)	2.2.5 Description...	DE: Rhododendron honey is known in Turkey as "Mad Honey" because of its toxic effect. It is stated that the concentration of grayanotoxins in honey as food product ranges from 10-60 mg/kg. However the applicant intends to produce a special honey with higher toxin content. Therefore it is not clear which substance resp. specification is applied for; honey with food grade or honey which must not recommended for human consumption due to its content of toxins. The intended concentration of Grayanotoxins in the self-produced honey cover a	The applicant should state here why a more than doubled concentration of toxin could be still marked. For efficacy reasons a concentration would probably not be necessary. Please give proof that the achieved content of toxins (up to 150 mg/kg) is acceptable for a substance of no concern.	The concentration of grayanotoxin in the honey can have a range from 10-60 and 10-300mg/kg depending on final production and purpose of usage. This substance is still a legal food product. It does not make a challenging difference if you place honey with 10mg or 300mg/kg inside of a bait box. The mg/kg grayanotoxin level must be clearly labelled on the product.	Honey from rhododendron as described in the application is a substance of concern as it may be considered as toxic or containing toxic components (see Section 5).

2.1. Identity and Physical and chemical properties of the substance and product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		very wide range (10 – 150 mg/kg). Is a safe use ensured with such a high uncertainty in the concentration in the capsules? Is ensured that the mice eat enough of the capsules when the concentration of the toxins in the honey is low?		We developed a quality assurance system to deliver this honey in different grayanotoxin levels or mg/kg thus it is possible to measure the amount of honey one mouse must eat. (Updated BSA with reference to analytical method. (annex I; references in § 2)	
2(3)	2.2.5	DE: No information is provided regarding the composition of rhododendron honey especially with respect to the content of the different grayanotoxins and possible other substances which have effects regarding the proposed use.		The mice die from grayanotoxin. Grayanotoxin versions range from 1-8 and the most potent are GTX I + III. This substance is a legal food product. Composition of GTX is variable as all natural substances.	Proper batch analysis would be needed to determine the levels of grayanotoxins and other potential active or toxic substances, including relevant impurities in the honey from rhododendron intended to be used as pesticide. This would also allow demonstrating consistent composition and efficacy of the proposed product. Furthermore, specifications for content of grayanotoxins and other potential active or toxic substances including relevant impurities need to be proposed and agreed based on appropriate analysis of batches.

2.1. Identity and Physical and chemical properties of the substance and product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
2(4)	2.2.5	DE: No specification in terms of minimum and maximum contents for the different grayanotoxins has been provided.		We measure GTX I + III only. If other versions occur in the honey it's a surplus. This substance is a legal food product. Composition of GTX is variable as all natural substances.	Specifications for content of grayanotoxins and other potential active or toxic substances including relevant impurities need to be proposed and agreed based on appropriate analysis of batches.
2(5)	2.2.7	DE. No methods for the determination of grayanotoxins in rhododendron honey have been provided.		(Updated BSA with reference to analytical method. (annex I; (references in § 2)	It is not clear to which reference in Annex I the applicant is referring to. However, no proper validated analytical method report seems to be available as part of the application. A validated method for grayanotoxins in rhododendron honey is not available. See 2(10)
2(6)	2.2. IDENTITY AND PHYSICAL CHEMICAL PROPERTIES OF THE SUBSTANCE AND PRODUCT TO BE USED	NL: The product to be used is honey from rhododendron. The identity should reflect this. Information regarding all grayanotoxins and other compounds that are expected to contribute to the efficay should be given. Also the source for the		Grayanotoxin I+ III are the versions contributing to the executive effect. (Updated BSA with reference to analytical method. (annex I and references in § 2)	See 2(4)

2.1. Identity and Physical and chemical properties of the substance and product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
	(2.2.1, 2.2.2, 2.2.3)	information grayanotoxins should be stated. (references)			
2(7)	2.2.5 Description and specification	NL (July 2016): It is claimed that honey from rhododendron sold from food contain 10-60 mg/kg grayanotoxin. What is the source of this information?		We used honey from rhododendron with approx. 50mg/kg (grayantoxin I + III/ honey) but we used many different concentrations and of course if the grayanotoxin level is higher then mice must eat less in order to die.	See 2(4) and 2(5)
2(8)	2.2.5 Description and specification	NL: A specification should include a range for all compounds that contribute to the efficacy. Also other mayor components and any relevant impurities should be included. References should be given were the information was obtained.		Mice die from grayanotoxin only (reference in annex 1, BSA).	See 2(4) and 2(5)
2(9)	2.2.6 Identity of inactive isomers, impurities and additives	NL: Honey contains many compounds that do not contribute to the efficacy. The mayor components and any relevant impurities should be included. References should be given were the information was obtained.			See 2(4) and 2(5)
2(10)	2.2.7.1 Methods of	NL: the method of analysis		(Updated BSA with reference	See 2(3) and 2(5)

2.1. Identity and Physical and chemical properties of the substance and product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
	analysis	should be clearly described for all compounds that contribute to the efficacy. References should be given were the information was obtained.		to analytical method. (annex I and references in § 2)	Validated analytical methods for grayanotoxins and other potential active or toxic substances including relevant impurities in honey from rhododendron are not available and would need to be provided.
2(11)	2.2.7.2 Analytical methods for determination of relevant impurities	NL the applicability of these methods depends on the presence of relevant impurities (to be clarified at point 2.2.5 and 2.2.6). References should be given were the information was obtained.		Updated BSA, we measure grayanotoxin I + III only. Composition of GTX is variable as all natural substances.	See 2(10)
2(12)	Identity and physico-chemical properties	PL: Physico-chemical properties are given only for grayanotoxin I. Not given characteristics of grayanotoxin III, which can be even more toxic than grayanotoxin I, moreover, occurs in rhododendron honey in significant quantities		Grayanotoxin III is a bit more toxic than grayantoxin I, BSA application updated with the mg/ kg value of GTX I+ III.	No physico-chemical properties are available for grayanotoxins contained in honey from rhododendron.
2(13)	Molecular and structural formula	PL: As above; data was given only for grayanotoxin I		BSA application updated and added value for GTX 3.	Noted

2.2. Current Former and in case proposed trade names

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
2(14)	2.3. CURRENT, FORMER AND IN CASE PROPOSED TRADE NAMES OF SUBSTANCES/ PRODUCTS AS PUT ON THE MARKET	NL: The basic substance should be already on the market for other purposes that plant protection. In this case, honey from rhododendrons should be sold and be recognisable as honey from rhododendrons. The toxicity of the grayanotoxin makes honey from rhododendrons unsuitable for human consumption and therefore cannot be classified as foodstuff. This makes it unlikely for this honey to be sold for any other purpose than as a rodenticide. We therefore assume the honey cannot be accepted as basic substance. The name of the product(s) on the current market should be clear. According to regulation 1107/2009, it is not allowed to market a basic substance as a plant protection product. Therefore, produce honey from rhododendron cannot be produced solely as a plant protection product.		The honey is already on the market in Turkey and sold with food certificate. In the EU it is possible to sell it as food supplement and clearly label maximum dosage levels for human intake. Typical food stuff, no brand name except "Rhododendron Honey" or "Honey from Rhododendron" (made by Klaus Gasser + Partner for example) Its usage consists of the honey from rhododendron inside a bait box. The bait	The toxicity of the grayanotoxin makes honey from rhododendron unsuitable for human consumption and therefore cannot be classified as foodstuff. See Section 5

2.2. Current Former and in case proposed trade names

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
				box closes doors and keeps death mice inside.	
2(15)	2 General	EFSA: As presented it seems the product is not yet in the market and that it is intended to be produced as hoc for its use as rodenticide. At any case this honey could not be considered food and would need to be properly labelled to avoid accidental human consumption of it.		In the EU it is possible to sell this honey as food supplement and clearly label maximum dosage levels for human intake.	See 1(1) and Section 5
2(16)	2 General	EFSA: As already indicated by MS the content of grayanotoxins and other potential toxins in this honey would need to be clearly specified.		The concentration of grayanotoxin will be measured for each batch and clearly labelled. (Updated BSA with reference to analytical method. (annex I and references in § 2)	See 2(4) and 2(5)
2(17)	2 General	EFSA: validated methods of analysis of grayanotoxins and other active components in the honey would need to be provided.		(Updated BSA with reference to analytical method. (annex I and references in § 2)	See 2(5) and 2(10)

2.3. Manufacturer of the substance/products

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
2(18)	2.4	DE: There is a clear wish to develop a product visible in the application.	The applicant should be cited here as manufacturer.	Klaus Gasser + Partner produce and/ or sell this honey.	Noted
2(19)	2.4. MANUFACTURER	NL: a manufacturer has to be included		Klaus Gasser + Partner	Applicant clarifies that the manufactures is: Klaus Gasser + Partner

2.4. Type of preparation

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
2(20)	2.5. TYPE OF PREPARATION	NL: In our opinion, pure honey can be best classified as an Any other Liquid (AL) type formulation. Only a product (or a simple diluent) which is already on the market but not predominantly used for plant protection purposes a can be regarded as a basic substance. In this case the honey is to be packaged in capsules which could imply that a product will be placed on the market especially for PPP purposes. The packaging in capsules solely		The honey is packaged in dosages and dosages can be labelled as maximum levels for human intake as well. It is sold as honey from rhododendron.	For a basic substance no specific preparation is needed since the raw technical product is supposed to be used. Therefore, EFSA does not assess the adequacy of the preparation proposed for the intended use (dosage gelatin capsules). Anyhow, the product would need to be conveniently labelled to prevent accidental human consumption since it is poisonous to humans.

2.4. Type of preparation					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		for the use as plant protection product cannot be accepted.			
2(21)	Type of preparation	PL : It is not clear whether the gelatin capsule of honey will be an attractive bait for mice			See 2(20)

2.5. Description of the recipe for the product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
2(22)	2.5 Type of preparation of the substance/product	DE: The honey shall be used pure and/or will be packaged in capsules made of gelatin. When the honey is used pure, are the bait boxes still impervious?		BSA application updated. The honey will be packaged in dosages only and the packaging consists of capsules (gelatine etc.), nylon, bio plastics, plastics, paper, bio degradable plastics etc.	See 2(20)
2(23)	2.6	DE: A product will be sold for use in baits.	The applicant should describe the use in baits in more details and point out how selective the baits probably are.	Open bait box, place honey packaged in dosages, close bait box. Bait boxes which close doors and keeps mice inside are fine. Air holes in Bait box must have a micro grid in order that small animals and even ants can't enter the bait box.	Addressed. It should be clarified that the use of bait boxes which close doors and keeps mice inside should be considered mandatory, not an option.

2.5. Description of the recipe for the product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
2(24)	2.6	DE: A capsulation of rhododendron honey with gelatin is planned. However, gelatin is not an approved basic substance. It seems that this would be an application as plant protection product.		Gelatine, nylon, plastics or bio plastics, bio degradable plastics, paper are just the ordinary packaging materials used for bait independently – honey is the basic substance.	See 2(20)
2(25)	2.6. DESCRIPTION OF THE RECIPE FOR THE PRODUCT TO BE USED	NL: It is stated that the product will not be sold as plant protection product since it proceeds with no contact with agricultural productions. However, this cannot be accepted. If the product is sold for the elimination of mice a regular product authorisation as a plant protection product or biocide is required (depending on the place of use)		The product is sold as honey from rhododendron and accepted as food stuff.	See 2(20)
2(26)	2.6. DESCRIPTION OF THE RECIPE FOR THE PRODUCT TO BE USED	EFSA: It does not seem that the product as presented (capsules) may be prepared by the farmers directly from a honey available in the EU market. Ad hoc honey conveniently formulated needs to be used. Also the		We want to produce the honey as well and label maximum dosage levels for human intake. In the EU we can market this honey as food supplement and clearly label max dosage levels for human intake.	See 2(20)

2.5. Description of the recipe for the product to be used					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		product would need to be conveniently labelled to prevent accidental human consumption since it would be poisonous to humans.			

3. Uses of the substance and its product

3.1. Field of use					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
3(1)		DE: No correct field of use has been provided here; no reasoning of the intended use has been provided.	Please indicate Harmful organism, cultivated plant and so on. Provide publications which reason the layout of the intended use.	We have done real on field tests/ studies with positive results of its intended use. BSA Updated.	Applicant claim that field studies performed in-house demonstrate that mice die as a result of honey with grayanotoxins. However, no proper scientific report has been provided to substantiate these claims.
3(2)	3.2	DE: Mice are said to die within 2-4 days after ingestion. Due to confusion they would be more prone to predators. However, it is stated in other chapters, that mice should be trapped in bait boxes after having entered	The applicant should give evidence for his statement that mice will not suffer unnecessarily.	Mice stay in bait box and die in bait box.	Since the effect on other non-target predatory animals that could eat the dying mice has not been assessed, the type of bait box should be such the mice stay in bait box and die in bait box. No information or evidence

3.1. Field of use

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		the bait box.			has been provide to demonstrate that the mice are not subject of unnecessary suffering during the 2 to 4 days until they are supposed to die.
3(3)	3.3	DE: The description of intended uses is inconsistent with GAP table e.g. number of capsules per bait box, use with or without bait boxes. Please see also 2.6: "natural honey from Rhododendron ... used pure and/ or ... in capsules".		BSA application updated. The honey is packaged in dosages.	See 2(20)
3(4)		NL: No comments.			Noted

3.2. Effects on harmful organisms or on plants

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
3(5)	Ibid. and 3.1	DE: In the application it says that mice die within 2-4 days after eating the honey and that the bait boxes keep dead mice in the box. Does this mean that the mice are		Please indicate the corresponding law in Germany. The application is for the EU, in case we would need to find another solution for Germany.	See 3(2)

3.2. Effects on harmful organisms or on plants					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		held in the boxes dying for 2-4 days? In DE such traps are not allowed. Either the mice have to die instantly in the traps or they have to be able to leave the traps and die after 2-4 days somewhere outside.			
3(6)	Ibid.	DE: Here it is stated that the mice are more accessible to predators after eating honey from rhododendron because they show signs of confusion. When the mice are accessible to predators they cannot be in the traps anymore. That's a contradiction to the statement that dead mice are kept in the boxes.		BSA application updated. Mice stay in bait box and die in bait box. Air holes in the bait box must have a micro grid to avoid that small insects or even ants can enter the bait box.	See 3(2)
3(7)		DE: The applicant did not provide any public available publications. Only some temporary Internet-links were cited showing indirectly aspects cited in the application.	The applicant should include publications to verify his assumptions. Especially data about the pain of mice should be provided.		See 3(2)
3(8)		NL: No comments.			Noted
3(9)		PL: It is not clear how poisoned mice can get out of the bait		Mice stay in bait box and die in bait box.	See 3(2)

3.2. Effects on harmful organisms or on plants

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		box and be available to predators			

3.3. Summary of intended uses

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
3(10)		DE: Member state is unclear.	Please clarify whether Italy or EU is meant.		No further clarification provided by the applicant.
3(11)		DE: Column "Application" seems not to be correct concerning b).	Please clarify.		No further clarification provided by the applicant.
3(12)		DE: Column "Application rate" seems not to be correct concerning a).	Please clarify Min/Max.		No further clarification provided by the applicant.
3(13)		NL: No comments.			Noted
3(14)		EFSA: If poisoned mice can leave the bait and be available to predators, the potential indirect poisoning of non-target wild predators (eg. eagles, foxes etc…) needs to be carefully considered in the eco-toxicological assessment.		Mice stay in bait box and die in bait box.	See 3(2)

4. Classification and labelling of the substance

Classification and labelling of the substance					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
4(1)		DE: The reported acute oral LD_{50} for grayanotoxin is in the range of a very toxic compound (Acute Tox. 1).		Still food stuff allowed	See 1(1) and Section 5
4(2)		NL: No comments.			Noted
4(3)		PL : Grayanotoxin I and III are not classified according to Regulation (EC) No 1272/2008[2] as amended		Still food stuff allowed	Noted. See also 2(14), 2(20) and Section 5

5. Impact on Human and Animal Health

5.1. Toxicokinetics and metabolism in humans					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
5(1)		EFSA: a more robust literature review should be conducted on the impact on human and animal health of the components of honey from rhododendron. The outcome of the published literature		Still food stuff allowed	A robust literature review was not conducted on human and animal health.

[2] 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.

5.1. Toxicokinetics and metabolism in humans

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		should be well reported with clear reference to the studies.			
5(2)		EFSA: Rhododendron spp is included in the Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2009).		Still food stuff allowed BSA updated.	Rhododendron spp is included in the Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2009).
5(3)	5.12 5.13	DE: No evidence is given that honey with grayanotoxins is used as food. DE: It is stated that the acceptable daily intake for humans is less than 5 g/day. This clearly indicates that the substance must not be considered as food.	It must be doubted that the substance applied for can be considered as food. It rather seems a substance of concern.	In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern.	Evidence has not been submitted to underpin the claim that honey from rhododendron containing alkaloids (grayanotoxin) is a food product in the EU. As food poisoning is associated with grayanotoxin-contaminated honey (also called 'mad honey') honey with such properties cannot be considered compliant with provisions in EU food law. Plant parts of rhododendron ssp. that containing grayanotoxin are listed in the EFSA Compendium of botanicals that have been

5.1. Toxicokinetics and metabolism in humans

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
					reported to contain toxic, addictive, psychotropic or other substances of concern, which is part of the Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. The applicant claimed that despite of this, listing authorisation as a food supplement (labelled with maximum dosage levels) has been obtained but that was not demonstrated by evidence; nor supported by submission of details regarding the composition of such food supplement and its safety assessment for consumers.

5.2. Acute toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(4)	5.3	DE: The reported acute oral LD_{50}		In Turkey this honey is sold	See 5(1), 5(2) and 5(3).

5.2. Acute toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		for grayanotoxin is in the range of a very toxic compound (Acute Tox. 1).		with food certificate. Therefore it is not a substance of concern.	
5(5)		PL: The LD_{50} value for mice specified by the applicant concerns the intraperitoneal route of administration not oral. Moreover, it is not specified for which grayanotoxin this value applies. Oral LD_{50} for mice is reported as 5.1 mg/kg for grayanotoxin I and 4.9 mg/kg for grayanotoxin III.	EFSA: the applicant should clearly indicate the values for each grayanatoxin and the route of exposure.	In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern.	See 5(1), 5(2) and 5(3).

5.3. Short-term toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA		Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(6)		PL: Not specified whether these symptoms relate to human or animals poisoning. Moreover, there are acute toxicity studies of grayanotoxins in rats especially regarding hepatotoxicity and		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).

5.3. Short-term toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA		Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		nephrotoxicity			

5.4. Genotoxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(7)		DE: Not sufficient data reported to allow a firm conclusion.	References could be added.		See 5(1), 5(2) and 5(3).
5(8)		PL: Only preliminary studies were conducted in vitro. Grayanotoxins II and III did not cause chromosomal damage in cultured human lymphocytes. However, grayanotoxins structure provide these compounds a possible mutagenic activity, thus further studies should be performed.		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).

5.5. Long-term toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application

No comments.

5.6. Reproductive toxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(9)		PL: The applicant has not entered data about the effects on reproduction. Existing data from a study in mice and chicken embryos indicate that grayanotoxin I did not show embryotoxicity or teratogenic effects even at maternally toxic doses		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).

5.7. Neurotoxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 4 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(10)	5.4/5.8	DE: The symptoms described in Sections 5.2 and 5.4 are neurotoxic symptoms. This would be in line with effects reported in the Internet (https://en.wikipedia.org/wiki/Grayanotoxin).		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).
5(11)	5.7 neurotoxicity	NL: grayanotoxins are known neurotoxins which prevent inactivation of sodium channels and hereby cause persistent activation. Regulation 1107/2009 states that basic substances should not have		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for	See 5(1), 5(2) and 5(3).

5.7. Neurotoxicity

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 4 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		an inherent capacity to cause neurotoxic effects. Although, we do agree that considering the intended use in bait boxes there is no actual concern related to the potential neurotoxic effects.		environmental uses (spray) but confined in bait box.	
5(12)		PL: Rhododendron honey poisoning caused by grayanotoxin is associated with autonomic nervous system symptoms, such as excessive perspiration, hypersalivation, vomiting and bradycardia. Animal study confirmed autonomic symptoms of grayanotoxin intoxication.		In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).

5.8. Toxicity studies on metabolites

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application

No comments.

5.9. Medical Data: adverse effects reported in humans

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(13)		DE: In the Internet there are anecdotal reports of uses and effects in humans (https://en.wikipedia.org/wiki/Grayanotoxin) also from grayanotoxin containing honeys from other plants.	A systematic review of open literature should be done.	References in annex I.	See 5(1), 5(2) and 5(3).
5(14)		PL: Rhododendron honey intoxication's symptoms are dose-related. In mild form dizziness, weakness, excessive perspiration, hypersalivation, nausea, vomiting and paresthesias are present. Severe intoxication may lead to life threating cardiac complication such as complete atrioventricular block. Reported amount of honey causing poisoning is between 5 to 150 g		Quantities described here are largely above uses in bait, no contact with people is possible. In Turkey this honey is sold with food certificate. Therefore it is not a substance of concern. Substance not intended for environmental uses (spray) but confined in bait box.	See 5(1), 5(2) and 5(3).

5.10. Additional Information related to therapeutic properties or health claims

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(15)		DE: The applicant reported that honey from rhododendron is used as treatment in traditional medicine and as a health product.	References should be submitted.	BSA updated.	See 5(1), 5(2) and 5(3).

5.11. Additional information related to use as food

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
5(16)		DE: It is unclear whether honey from rhododendron is available as food on the EU market.	References should be added clarifying whether it is available as food on the EU market.	In Turkey it is sold with food certificate. In EU it is possible to sell it as food supplement and label maximum dosage levels for human intake.	See 5(1), 5(2) and 5(3).
5(17)		EFSA: It is doubted that honey from rhododendron that contains alkaloids (grayanotoxins) can be considered a food product. Not all rhododendrons produce grayanotoxins and therefore rhododendron honey may indeed be marketed, but this type of honey is not the same product that is subject to this application. In fact, honey that contains grayanotoxins (mad honey) is considered contaminated and it is associated with food poisoning.	It is suggested be more distinctive and the application concerning the food use of honey from rhododendron, or submit evidence for authorised marketing as a food product of honey that contains grayanotoxins.	In Turkey it is sold with food certificate. In EU it is possible to sell it as food supplement and label maximum dosage levels for human intake.	See 5(1), 5(2) and 5(3).

5.12. Acceptable daily intake, acute reference dose, acceptable operator exposure level					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
5(18)		DE: From the description of the intended use, it is unclear how the baits come into the box. In case there is an exposure of the user, a reference dose might be necessary.	Please clarify the handling of the baits and the box. Where relevant, please derive a reference dose.	Open box, place packaged honey dosage, close box. The operator must not eat packaged dosages.	The applicant clarified the product handling; however non-dietary exposure was not properly addressed and therefore cannot be excluded.
5(19)		DE: Not sufficient data reported to allow a firm conclusion whether the basic substance has an inherent capacity to cause endocrine disrupting or immunotoxic effects.	References could be added.	In Turkey it is sold with food certificate and consumed since centuries.	See 5(1), 5(2) and 5(3).
5(20)		DE: Based on the few summarised data, it is difficult to draw a firm conclusion whether the basic substance is not a substance of concern.	References could be added.	In Turkey it is sold with food certificate and consumed since centuries.	See 5(1), 5(2) and 5(3).

5.13. Impact on human and animal health arising from exposure to the substance or impurities contained in it					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
5(21)		DE: With respect to the high oral toxicity of grayanotoxin it should be ruled out that any	Description of the bait boxes, the bait (honey only in capsules or pure as well) and the	Packaged honey dosages will be placed in the bait box.	See 5(1), 5(2), 5(3) and 5(18).

5.13. Impact on human and animal health arising from exposure to the substance or impurities contained in it					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		exposure to operators, workers, bystanders or residents (especially children) occurs. Otherwise, a risk assessment is necessary.	handling of the bait boxes.		

6. Residues

Residues					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
6(1)		EFSA: The applicant states that rhododendron honey is used inside of bait boxes that would close in the trapped mice. Therefore, exposure of trees /crops is not relevant. However, it is also stated that the substance may have a positive influence on soil, roots and trees. This statement casts doubt over the non-relevance of exposure of the soil and the fruit trees, respectively, to grayanotoxins.	Clarification should be given on the possibility that soil and trees (via uptake from soil) could be exposed to the grayanotoxins of rhododendron honey under the use conditions intended. If this scenario cannot be excluded, evidence should be submitted that soil up-take and translocation of grayanotoxins in plants is not relevant.	BSA application updated. Honey is packaged in dosages and thus it is not relevant for soil, roots and trees. Honey can't leave the bait box even if it is raining the honey can't leave the bait box.	Addressed The applicant has clarified that the product design is as such that the honey cannot get out of the bait box and therefore soil and tree exposure is not considered a relevant scenario.

7. Fate and Behaviour in the environment

7.1 Fate and Behaviour in the environment

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
7(1)		EFSA: see comment 8(3) in relation to possible release from the baits when exposed to rain and comment 3(14) in relation to indirect poisoning of predators of the poisoned mice (when they leave the bait).		Bait box keeps mice inside and mice die in bait box.	Addressed Applicant has clarified that for the intended uses in bait, the bait to be used has to close after the entering of the mice and not to allow the trapped mice to leave the bait, guaranteeing the mice will die inside the bait box.

7.2 Estimation of the short and long-term exposure of relevant environmental media (soil, groundwater, surface water)

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
7(2)		No comments			No comments

8. Effects on non-target species

8.1. Effects on terrestrial vertebrates

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(1)	Ibid.	DE: It has to be ensured that the bait boxes do not attract other terrestrial vertebrates than mice.		Small animals, insects and even ants can't enter the bait box due to a micro grid covering air holes in the bait box.	See 8(2)
8(2)	3.2 Effects on harmful organisms or on plants	NL: According to the information provided by the applicant, the use of the substance is as a rodenticide to control the mice which can cause damage to orchards. the substance will be provided in a bait box. Upon oral exposure, the mice will die within 2-4 days. According to Article 23(2), a basic substance shall be approved where "any relevant evaluations show that the substance has neither an immediate effect on human or animal health nor an unacceptable effect on the environment". NL acknowledges that by the use of the substance in a bait box there is no further	A better solution for controlling the vole presence in orchards will be by mechanically removing or reducing the vegetative cover between the trees. This will create an unfavourable habitat for voles.	The solution of packaged honey in dosages placed in bait box is for any type of mice. It is recommended to place bait boxes around mice holes, especially if two or more holes are closed together.	The target species were not defined. More information on this point is needed. From the available information it cannot be excluded that non-target organisms could access the bait. Indeed the micro grid covering the air holes would not prevent non-target terrestrial vertebrates to access the bait from the same entrance as the one for the target organisms.

8.1. Effects on terrestrial vertebrates

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		exposure of the environment, birds, aquatic environment, non-target arthropods, bees, soil organisms and plants. The "mice" are not really defined by the applicant in terms of species. The meadow and pine vole are known to eat the bark and roots of fruit trees and thus NL assumes that the substance is meant to control these organisms. Furthermore by using the substance as a rodenticide exposure of other small mammals which inhabit the orchards cannot be excluded. Furthermore how many of these bait boxes will be placed in orchards and for how long the exposure of small mammals will be? What will the tree growers do with the carcasses, will there be a chance of secondary poisoning of bigger predators?		If mice would die outside box (which is not the case, because mice can't leave the bait box) GTX is consumed and metabolized therefore secondary poisoning is not expected. Furthermore quantities for mice are largely lower than these for higher animals.	
		The voles in general to not have the definition as "pest			

8.1. Effects on terrestrial vertebrates

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		organisms" under 1107/2009. In the Netherlands they are protected under a national law "Flora and Fauna Wet". Only in certain cases exemptions can be given to control to vole population.			
8(3)	8. Effects on non-target species	EFSA: From the information provided in the application, it is assumed that the exposure to non-target organisms is low, due to the use in bait boxes. It is, however, noted that under point 3.3 Summary of the intended uses it is reported that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry', does this mean that in case of rain/wet soil exposure in the environmental compartment can be expected and that the above mentioned conditions of use should be considered as risk mitigation measures? See also comment from DE 8(13) (under Section 8.5).	Further information on the potential exposure for non-target organisms needs to be provided. Applicant to clarify the reason why it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry.	BSA application updated. Even if it is raining honey can't leave the bait box and water can't touch honey in the bait box. Honey is packaged in nylon, plastic, bio degradable plastic or bio plastic, paper, capsules of gelatin.	Addressed. Applicant has clarified that the basic substance cannot leave the bait box even during rainfall events. See also 6(1) and 7(1).

8.1. Effects on terrestrial vertebrates

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(4)	8.1 Effects on non-target vertebrates	EFSA: The target/pests organisms should be better identified e.g. in term of species. Also, it is not demonstrated that the bait boxes are specific to the target organisms. See also comments from DE and NL.	Further details on the target species are needed. In addition, it should be demonstrated that the exposure potential for non-target small mammals or other non-target organism is expected to be low.		See 8(1) and 8(2)
8(5)		EFSA: More data are needed in order to assess the potential risk for terrestrial organisms, see also 8(4). It should be ensured that the risk assessment covers the representative uses of honey from rhododendron.	A risk assessment and/or a scientific justification should be given in order to address the risk to terrestrial vertebrates from the representative uses of honey from rhododendron.	Small insects, animals and even ants don't have a chance to access and enter the bait box due to a micro grid covering air holes in the bait box.	See 8(2)

8.2. Effects on aquatic organisms

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(6)		NL: No comments			Noted
8(7)		EFSA: From the information provided in the application, it is assumed that the exposure to non-target organisms is low due to the	Applicant to clarify the reason why it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry.	BSA application updated. Even if its raining honey can't leave the bait box and water can't touch honey in the bait box. Honey is packaged in	Further data are not needed as long as it is guaranteed that the basic substance is used in baits, that the affected target organism die

8.2. Effects on aquatic organisms

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		use in bait boxes. It is, however, noted that under point 3.3 Summary of the intended uses it is reported that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry', does this mean that in case of rain/wet soil exposure in the environmental compartment can be expected and that the above mentioned conditions of use should be considered as risk mitigation measures? See also comment 8(13) from DE (under Section 8.5) and 3(14) form EFSA in relation to potential indirect poisoning of predators. .	A risk assessment and/or a scientific justification (e.g. exposure based) should be given in order to address the risk to aquatic organisms from the representative uses of honey from rhododendron.	nylon, plastic, bio degradable plastic or bio plastic, paper, capsules of gelatin.	in the bait and not in the open environment and that the bait design is as such that the basic substance cannot be released from the bait box. See also 8(3)

8.3. Effects on bees and other arthropods species

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(8)		DE: The applicant wrote: "The substance is not expected to	The applicant should correct the sentence: "The substance is	BSA application updated. It is not relevant since trap baits	Addressed

8.3. Effects on bees and other arthropods species

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		be toxic for bees". This is not correct.	toxic for bees. For the intended use this is not relevant because the honey is applied in capsules and baits."	are not a target for bees and due to the micro grid covering air holes in the bait box bees can't enter the bait box.	
8(9)		NL: No comments			Noted
8(10)		EFSA: Considering also the comment from DE, a risk assessment and/or a scientific justification should be given in order to address the risk to bees from the representative uses of honey from rhododendron.	A risk assessment and/or a scientific justification should be given in order to address the risk to bees from the representative uses of honey from rhododendron, see also comment 8(8).	Bees are looking for flowers, honey is not a target for bees in environment, although they may do some pillage. Furthermore, this honey (under nectar) is already collected, concentrated stored and eaten by bees in corresponding beehives! if this honey (or nectar) was toxic the beehive would have die from this operation in ordinary beehives and logically, this honey would not exist! Bees can't enter the bait box.	Addressed, see 8(8)

8.4. Effects on earthworms and other soil macroorganisms

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(11)		NL: No comments			Noted
8(12)		EFSA: From the information provided in the application, it is assumed that the exposure to non-target organisms is low due to the use in bait boxes. It is, however, noted that under point 3.3 Summary of the intended uses it is reported that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry', does this mean that in case of rain/wet soil exposure in the environmental compartment can be expected and that the above mentioned conditions of use should be considered as risk mitigation measures? See also comment from DE 8(13) (under Section 8.5) and 3(14) form EFSA in relation to potential indirect poisoning of predators.	Applicant to clarify the reason why it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry. A risk assessment and/or a scientific justification (e.g. exposure based) should be given in order to address the risk to earthworms and other soil macroorganisms from the representative uses of honey from rhododendron.	Baits are a very good security to avoid environment drift or spilling, accessibility to higher animals or smaller animals and insects. This confined application is driven in order to reduce risk to minimum (or zero) environmental possible contamination.	See 8(3)

8.5. Effects on soil microorganisms

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(13)	Ibid.	DE: Because of the bactericidal effect of honey from rhododendron it has to be ensured that the bait boxes are impervious (especially when the honey is applied pure and not in capsules) thus to prevent the honey entering the soil.		Honey is packaged in capsules (gelatin), nylon, plastics, biodegradable plastics, bio plastics and paper.	See 8(3)
8(14)		NL: No comments			Noted
8(15)		EFSA: From the information provided in the application, it is assumed that the exposure to non-target organisms is low due to the use in bait boxes. It is, however, noted that under point 3.3 Summary of the intended uses it is reported that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry', does this mean that in case of rain/wet soil exposure in the environmental compartment can be expected and that the above mentioned conditions of use should be considered as risk	Applicant to clarify the reason why it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry. A risk assessment and/or a scientific justification (e.g. exposure based) should be given in order to address the risk to soil microorganisms from the representative uses of honey from rhododendron.	Honey is packaged in capsules (gelatine), nylon, plastics, paper, bio degradable plastics and bio plastics. BSA application updated.	See 8(3)

8.5. Effects on soil microorganisms

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		mitigation measures? See also comment from DE 8(1) (under Section 8.5)			

8.6. Effects on other non-target organisms (flora and fauna)

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(16)		No comments			No comments

8.7. Effects on biological methods of sewage treatment

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
8(17)		No comments			No comments

9. Overall conclusions with respect of eligibility of the substance to be approved as basic substance

Overall conclusions with respect of eligibility of the substance to be approved as basic substance					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
9(1)		DE: It is proposed to checked from a legal point of view, whether the applied use is covered by the definition of plant protection product in Article 23(1)(a). In line with Article 23(1)(c) and (d) such uses would be out of scope for a basic substance. Additionally it should be checked whether the applied use is covered by the definition of a biocidal product according to regulation 528/2012[3].		It is marketed as honey from rhododendron (food product) and labelled with maximum dosage level for human intake.	See 1(1) and 1(3)
9(2)		NL: Based on the comments above, honey from rhododendron cannot be regarded a basic substance.		Honey from Rhododendron is indeed sold as basic "food product"	See 9(1)

[3] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167. 27.6.2012. p. 1–123.

10. Other comments

Other comments

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
10(1)		DE: Due to the lack of citations in the evaluation report, it is difficult to perform a proper evaluation. It is noted that some references were listed in appendix I but they were not made available.		Uses are confined to bait box, no spray.	See 10(2)
10(2)		PL : Although the concept of the use of rhododendron honey as a natural rodenticide is interesting, however, the documentation presented for evaluation should to be clarified and supplemented		BSA application updated.	Noted. However see also 1(2) and 5(1)
10(3)		PL: In our opinion, we used the following references: 1. Koca I., Koca A.F. (2007): Poisoning by mad honey: a brief review. Food Chem. Toxicol. 45, 1315-1318 2. Gunduz A. et al. (2006): Mad honey poisoning. Am. J. Emerg. Med. 24, 595-598 3. Akinci S. et al. (2008): An unusual presentation of mad honey poisoning: acute myocardial infarction. Int. J.		BSA application updated References taken in consideration	See 10(2)

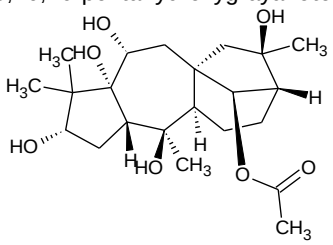
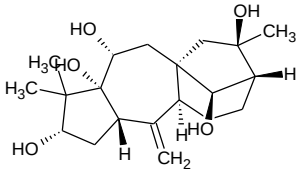
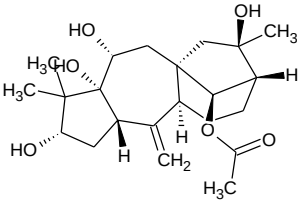
Other comments

No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		Cardiol. 129, e56-e58 4. Gunduz A. et al. (2008): Clinical review of grayanotoxin/mad honey poisoning past and present. Clin. Toxicol. 46, 437-442 5. Jansen S.A. et al. (2012): Grayanotoxin poisoning: "mad honey disease" and beyond. Cardiovasc. Toxicol. 12, 208-215 6. Ascioglu M. et al. (2000): Effects of acute grayanotoxin-I administration on hepatic and renal functions in rats. Turk. J. Med. Sci. 30, 23-27 7. Silici S. et al. (2016): Acuye effects of grayanotoxin in rhododendron honey on kidney functions in rats. Environ. Sci. Pollut. Res. 23, 3300-3309 8. Onat F. et al. (1991): Site of action of grayanotoxin in mad honey in rats. J. Appl. Toxicol. 11, 199-201 9. Kim S.E. et al. (2010): Presynaptic effects of grayanotoxin III on			

Other comments					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		excitatory and inhibitory nerve terminals in rat ventromedial hypothalamic neurons. Neurotoxicology 31, 230-238 10. Hikino H. et al. (1979): Subchronic toxicity of ericaceous toxins and rhododendron leaves. Chem. Pharm. Bull. 27, 874-879 11. Cucer N., Eroz R. (2010): Investigation of mutagenic effects of grayanotoxin II and III on cultured human lymphocytes. Al Ameen J. Med. Sci. 3, 293-299 12. Kobayashi T. et al. (1990): Developmental toxicity potential of grayanotoxin I in mice and chicks. J. Toxicol. Sci. 15, 227-234			
10(4)		EFSA: As highlighted in the comments from DE and PL, some references were listed but were not made available. A need to further supplement the provided documentation is identified. It is not clear whether a literature search in line with	Applicant to update the application by integrating the information at the basis of the application with information on any EU assessment (if available) and with a literature search in line with the EFSA Guidance on the submission of scientific peer-reviewed open literature	BSA application updated	See 10(2)

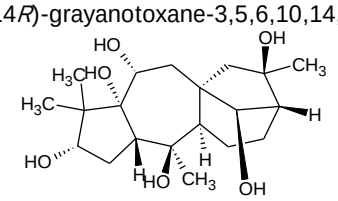
Other comments					
No.	**Column 1** **Reference to Application Template**	**Column 2** **Comments from Member States / EFSA**	**Column 3** **Proposal by Member States/EFSA on how the application should be updated to address the comment**	**Column 4** **Follow up response from applicant**	**Column 5** **EFSA's scientific views on the specific points raised in the commenting phase conducted on the application**
		the EFSA Guidance on the submission of scientific peer-reviewed open literature under Regulation (EC) No 1107/2009 was performed. Also, if available, EU assessments of honey from rhododendron should be reported. It is noted that additional references were provided by PL.	under Regulation (EC) No 1107/2009. The additional references indicated by PL should be considered further.		

Appendix B – Identity and biological properties

Common name (ISO)	Not applicable
Chemical name (IUPAC)	Not applicable
Chemical name (CA)	Not applicable
Common names	Honey from rhododendron, Mad Honey
CAS No	Not applicable
CIPAC No and EEC No	Not applicable
FAO specification	Not applicable
Minimum purity	Specifications for content of grayanotoxins and other potential active or toxic substances including relevant impurities are not available and need to be proposed
Relevant impurities	Active compounds grayanotoxins and other potential active or toxic substances and / or relevant impurities need to be determined.
Molecular mass and structural formula	For the active toxins found in the honey from rhododendron (secondary plant metabolites of rhododendron- *Rhododendron ponticum*-) Grayanotoxin I (3β,6β,14*R*)-3,5,6,10,16-pentahydroxygrayanotoxan-14-yl acetate  CC(=O)O[C@H]2[C@@]34C[C@@H](O)[C@@]1(O)[C@@H](C[C@H](O)C1(C)C)[C@](C)(O)[C@@H]4CC[C@H]2[C@](C)(O)C3 Grayanotoxin II (3β,6β,14*R*)-grayanotox-10-ene-3,5,6,14,16-pentol  C[C@@]3(O)C[C@]24C[C@@H](O)[C@@]1(O)[C@@H](C[C@H](O)C1(C)C)C(=C)[C@@H]4CC[C@@H]3[C@H]2O Grayanotoxin III (3β,6β,14*R*)-3,5,6,16-tetrahydroxygrayanotox-10-en-14-yl acetate  CC(=O)O[C@H]2[C@@]34C[C@@H](O)[C@@]1(O)[C@@H](C[C@H](

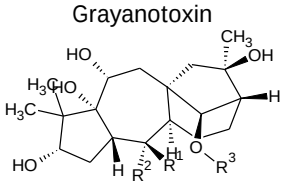
O)C1(C)C)C(=C)[C@@H]4CC[C@H]2[C@](C)(O)C3

Grayanotoxin IV
(3β,6β,14*R*)-grayanotoxane-3,5,6,10,14,16-hexol



C[C@@]3(O)C[C@]24C[C@@H](O)[C@@]1(O)[C@@H](C[C@H](O)C1(C)C)[C@](C)(O)[C@@H]4CC[C@@H]3[C@H]2O

Grayanotoxin



Grayanotoxin	R^1	R^2	R^3
Grayanotoxin I	OH	CH_3	Ac
Grayanotoxin II	CH_2		H
Grayanotoxin III	OH	CH_3	H
Grayanotoxin IV	CH_2		Ac

Ac =acetyl

Mode of Use	Mice baits boxes
Preparation to be used	According to the applicant, honey from rhododendron will be packaged in dosages consisting of capsules (gelatine etc.), nylon, bio plastics, plastics, paper, bio degradable plastics etc.
Function of plant protection	Rodenticide

Appendix C – List of uses

Use No	Member States	F G I	Pests or group of pests controlled (additionally: development stages of the pest)	Application Method/ Kind	Application Timing/ Growth stage of crop & season	Application Max Number (min interval between applications) a)Per use b)Per crop/ season	Application Rate kg, g product/ ha a)Max rate per appl. b)Max total rate per crop/ season	PHI (days)	Remarks (Safener, Synergist per ha)
1	Italy, EU	F	Mice	Basic substance is used inside of a bait box. Bait boxes close the door and keep death mice in bait box. Should water be inside bait box > operator must remove it from bait box	Autumn, Winter, Spring and when mice growth is visible.	a) 4-7 days	a) Min. 10 dosages per bait box b) Position 1 bait box each 5-15 m next to fruit trees.	Not relevant	

REASONED OPINION

EFSA Journal

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Modification of the existing maximum residue levels for fosetyl/phosphonic acid in chards/beet leaves and honey resulting from the use of potassium phosphonates

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Abstract

In accordance with Article 6 of Regulation (EC) No 396/2005, the applicant BASF SE submitted a request to the competent national authority in the Netherlands to modify the existing maximum residue levels (MRLs) for fosetyl/phosphonic acid (fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)) in chards/beet leaves and honey. The data submitted in support of the request were found to be sufficient to derive MRL proposals for the commodities under assessment. Adequate analytical methods for enforcement are available to control the residues of fosetyl and phosphonic acid in chards/beet leaves and honey. Based on the risk assessment results, EFSA concluded that the short-term and long-term intake of phosphonic acid residues resulting in chard/beet leaves and honey from the use of potassium phosphonates according to the reported agricultural practice is unlikely to present a risk to consumer health.

Keywords: fosetyl, phosphonic acid, potassium phosphonates, chards, beet leaves, honey, fungicide, MRL, consumer risk assessment

Requestor: European Commission

Question number: EFSA-Q-2021-00392; EFSA-Q-2021-00393

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Summary

In accordance with Article 6 of Regulation (EC) No 396/2005, BASF SE submitted two applications to the competent national authority in the Netherlands (evaluating Member State, EMS) to modify maximum residue levels (MRLs) for fosetyl/phosphonic acid in chards/beet leaves and honey resulting from the use of potassium phosphonates. The EMS drafted two evaluation reports in accordance with Article 8 of Regulation (EC) No 396/2005, which were submitted to the European Commission and forwarded to the European Food Safety Authority (EFSA) on 29 June 2021. To accommodate for the NEU/SEU intended uses of potassium phosphonates on chards/beet leaves, the EMS proposed to raise the existing MRL of 15 to 60 mg/kg or to 40 mg/kg according to the existing or proposed new residue definition for enforcement, respectively. Moreover, the EMS proposed to raise the existing MRL in honey from the limit of quantification (LOQ) of 0.5 to 150 mg/kg or to 100 mg/kg according to the existing or proposed new residue definition, respectively.

EFSA assessed both applications and evaluation reports as required by Article 10 of the MRL regulation. EFSA identified data gaps and points which needed further clarification, which were requested from the EMS. On 12 October 2021, the EMS submitted two revised evaluation reports, which replaced the previously submitted reports.

Based on the conclusions derived by EFSA in the framework of Directive 91/414/EEC, the data evaluated under previous MRL assessments, including the recent EFSA joint review of MRLs for fosetyl, disodium phosphonates and potassium phosphonates according to Article 12 and 43 of Regulation (EC) No 396/2005 (hereafter, joint MRL review) and the additional data provided by the EMS in the framework of this application, the following conclusions are derived.

The recent joint review of MRLs for fosetyl and phosphonates concluded that the data from public literature provide sufficient evidence to address the metabolism of potassium phosphonates in plants. In primary crops treated with salts of potassium phosphonate and in rotational crops, phosphonic acid is expected to be the main residue. The phosphonic acid is also the main metabolite of the active substances fosetyl and disodium phosphonate.

Studies investigating the effect of processing on the nature of potassium phosphonates (hydrolysis studies) demonstrated that the metabolite phosphonic acid is stable.

Based on the metabolic pattern identified in metabolism studies, hydrolysis studies and the toxicological significance of the metabolite phosphonic acid, the joint MRL review proposed a residue definition for potassium phosphonates in plant products as 'phosphonic acid and its salts, expressed as phosphonic acid' for both enforcement and risk assessment. The proposed enforcement residue definition has not been legally endorsed yet. The existing residue definition for enforcement set in Regulation (EC) No 396/2005 is 'fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)'. The residue definitions are applicable to primary crops, rotational crops and processed products.

EFSA concluded that for chards/beet leaves, assessed in this application, the metabolism of potassium phosphonates in plants and the possible degradation in processed products has been sufficiently addressed and that the residue definitions as proposed by the joint MRL review are applicable. In the absence of specific metabolism studies for honey, but considering the elementary nature of potassium phosphonates and the fact that metabolism of the active substance in primary and rotational crops proceeds according to the same metabolic pathway, EFSA concluded that the above-mentioned residue definitions are also applicable to honey.

Sufficiently validated analytical methods are available to quantify residues according to the existing residue definition for enforcement (i.e. fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)) in high water content commodities with an LOQ of 0.01 mg/kg. Moreover, the methods allow the monitoring of residues expressed in accordance with the proposed new residue definition for enforcement (i.e. phosphonic acid and its salts, expressed as phosphonic acid), and an LOQ of 0.1 mg/kg is achievable. For honey, a sufficiently validated analytical method is available with an individual LOQ of 0.05 mg/kg for phosphonic acid and fosetyl.

The occurrence of phosphonic acid residues in rotational crops was investigated in the framework of the joint review of MRLs for fosetyl and phosphonates. The MRLs derived during the MRL review and the present assessment for primary crops are expected to cover phosphonic acid residues in rotational crops from the soil uptake or from other sources.

Although phosphonic acid residues are expected to occur above 0.1 mg/kg in unprocessed chards/beet leaves and honey, considering the low contribution of phosphonic acid residues in these commodities to the total chronic consumers' exposure (below 1% of the theoretical maximum daily

intake (TDMI)), investigations on the effect of processing on the magnitude of residues in processed commodities were not deemed necessary.

The available residue trials are sufficient to derive MRL proposals for chards/beet leaves and honey according to the existing and the proposed new residue definition for enforcement.

Residues of phosphonic acid in commodities of animal origin were not assessed since chards/beet leaves and honey are normally not fed to livestock.

The toxicological profile of potassium phosphonates was assessed in the framework of the EU pesticides peer review under Directive 91/414/EEC and the data were sufficient to derive an acceptable daily intake (ADI) of 2.25 mg/kg body weight (bw) per day. An acute reference dose (ARfD) was deemed unnecessary. In the framework of the renewal of the approval for fosetyl, a revised ADI of 1 mg/kg bw per day has been derived, which was also recommended to be applied to phosphonic acid. Although this new ADI is not yet formally adopted, an indicative risk assessment was calculated based on this reference value as well.

The consumer risk assessment was performed with revision 3.1 of the EFSA Pesticide Residues Intake Model (PRIMo). In the framework of the joint review of MRLs for fosetyl, disodium phosphonate and potassium phosphonates, a comprehensive long-term exposure assessment was performed combining residue data originating from the use of the three active substances and the monitoring data, as well as certain codex maximum residue limits (CXLs) established for fosetyl and phosphonic acid. EFSA now updated exposure calculations with supervised trials median residue (STMR) values derived for commodities under assessment (chards/beet leaves and honey). In addition, the updated peeling factor for citrus fruits, derived from a previous assessment, was used to refine calculations.

Provided that the conclusions of the joint MRL review are implemented, the estimated long-term dietary intake considering the currently applicable ADI of 2.25 mg/kg bw per day (**scenario 1**), accounted for 36% of the ADI (Dutch toddler diet). Expressing the exposure as percentage of the revised ADI of 1 mg/kg bw per day as proposed by the EU pesticides peer review (**scenario 2**), the highest chronic exposure was calculated at 81% of the ADI (Dutch toddler diet). The contribution of residues in chard/beet leaves and honey to the total consumer intake was individually below 0.12% of the ADI, for both scenarios.

EFSA concluded that the proposed use of potassium phosphonates on chards/beet leaves and the consumption of honey, produced by bees foraging on melliferous crops treated with potassium phosphonates at the application rate considered in the present assessment, are not expected to result in a consumer exposure exceeding the toxicological reference values and therefore are unlikely to pose a risk to consumers' health.

EFSA proposes to amend the existing MRLs as reported in the summary table below.

Full details of all end points and the consumer risk assessment can be found in Appendices B–D.

Code[(a)]	Commodity	Existing EU MRL/new MRL proposal[(b)] (mg/kg)	Proposed EU MRL: existing enforcement RD/ Proposed new enforcement RD (mg/kg)	Comment/justification
Existing enforcement residue definition: Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl) **Proposed new enforcement residue definition (not yet implemented):** Phosphonic acid and its salts, expressed as phosphonic acid				
0252030	Chards/ beet leaves	15/70	60/40	The submitted data are sufficient to derive an MRL proposal for the NEU/SEU uses. The MRL proposal is lower than that of the joint MRL review for fosetyl and phosphonates, derived from NEU trials on spinaches treated with fosetyl (EFSA, 2021c). Risk for consumers unlikely.
1040000	Honey	0.5*/0.3	150/100	The MRL proposal reflects residues in honey from tunnel trials performed on buckwheat treated with potassium phosphonates.

Code[(a)]	Commodity	Existing EU MRL/new MRL proposal[(b)] (mg/kg)	Proposed EU MRL: existing enforcement RD/ Proposed new enforcement RD (mg/kg)	Comment/justification
				In the framework of the joint MRL review for fosetyl and phosphonates, an MRL for honey was derived from available monitoring data (EFSA, 2021c). Risk for consumers unlikely.

MRL: maximum residue level; NEU: northern Europe; SEU: southern Europe; GAP: Good Agricultural Practice.
*: Indicates that the MRL is set at the limit of analytical quantification (LOQ).
(a): Commodity code number according to Annex I of Regulation (EC) No 396/2005.
(b): MRL proposal, according to proposed new enforcement residue definition, derived in a recently published reasoned opinion of EFSA, not yet implemented (EFSA, 2021c).

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Assessment

The European Food Safety Authority (EFSA) received two applications to modify the existing maximum residue levels (MRL) for fosetyl/phosphonic acid in chards/beet leaves and honey resulting from the use of potassium phosphonates. The detailed description of the intended SEU/NEU use of potassium phosphonates in chards/beet leaves, which is the basis for the current MRL application, is reported in Appendix A. For honey, the MRL application is not linked to a specific GAP/crop but is related to intended uses on crops attractive to bees and that would be a potential source for residues of phosphonic acid in honey.

Potassium phosphonates are the name commonly used for the mixture of potassium hydrogen phosphonate and dipotassium phosphonate. The chemical structures of the components of the active substance and related compounds are reported in Appendix E.

Potassium phosphonates were evaluated in the framework of Directive 91/414/EEC[1] with France designated as rapporteur Member State (RMS); the representative use assessed was a foliar spray on grapes. The draft assessment report (DAR) prepared by the RMS has been peer reviewed by EFSA (EFSA, 2012). The active substance potassium phosphonates were approved[2] for the use as fungicide on 1 October 2013.

The EU MRLs related to the use of potassium phosphonates are established in Annex III of Regulation (EC) No 396/2005[3]. The current residue definition for enforcement is set as 'fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)'. Hence, the existing MRLs cover not only the uses of potassium phosphonates but also the uses of fosetyl and disodium phosphonate. A joint review of maximum residue levels (MRLs) for these three active substances (fosetyl, disodium phosphonate and potassium phosphonates) in accordance with Articles 12 and 43 of Regulation (EC) No 396/2005 has been performed recently (EFSA, 2021c); the proposed modifications have not yet been implemented in the EU MRL legislation.[4] It is noted that still a number of other modifications of the existing MRLs previously proposed by EFSA (EFSA, 2021a,b,d) have not yet been implemented in the MRL legislation, since the European Commission considered appropriate to await the MRL joint review for the related active substances. Certain Codex maximum residue limits (CXLs) have been taken over in the EU MRL legislation.[5]

In accordance with Article 6 of Regulation (EC) No 396/2005, BASF SE submitted two applications to the competent national authority in the Netherlands (Netherlands, 2021a,b) to modify maximum residue levels (MRL) for fosetyl/phosphonic acid in chards/beet leaves and honey resulting from the use of potassium phosphonates. The EMS drafted two evaluation reports in accordance with Article 8 of Regulation (EC) No 396/2005, which were submitted to the European Commission and forwarded to the European Food Safety Authority (EFSA) on 29 June 2021.

To accommodate for the intended use of potassium phosphonates on chards/beet leaves, the EMS proposed to raise the existing MRL of 15 to 60 mg/kg or to 40 mg/kg according to the existing or proposed new residue definition, respectively. Moreover, the EMS proposed to raise the existing MRL in honey from the limit of quantification (LOQ) from 0.5 to 150 mg/kg or to 100 mg/kg according to the existing or proposed new residue definition, respectively.

EFSA assessed both applications and evaluation reports as required by Article 10 of the MRL regulation. EFSA identified data gaps and points which needed further clarification, which were

[1] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32.

[2] Commission Implementing Regulation (EU) No 369/2013 of 22 April 2013 approving the active substance potassium phosphonates, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 111, 23.4.2013, p. 39–42.

[3] Regulation (EC) No 396/2005 of the Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

[4] For an overview of all MRL Regulations on this active substance, please consult: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as

[5] Commission Regulation (EU) 2019/552 of 4 April 2019 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for azoxystrobin, bicyclopyrone, chlormequat, cyprodinil, difenoconazole, fenpropimorph, fenpyroximate, fluopyram, fosetyl, isoprothiolane, isopyrazam, oxamyl, prothioconazole, spinetoram, trifloxystrobin and triflumezopyrim in or on certain products C/2019/2496. OJ L 96, 5.4.2019, p. 6–49.

requested from the EMS. On 12 October 2021, the EMS submitted revised evaluation reports (Netherlands, 2021a,b), which replaced the previously submitted reports.

EFSA based its assessment on the evaluation reports submitted by the EMS (Netherlands, 2021a,b), the draft assessment report (DAR) on potassium phosphonates and its addendum (France, 2005, 2012) prepared under Directive 91/414/EEC and the revised renewal assessment report (RAR) on fosetyl (France, 2018) prepared under Regulation (EU) No 1107/2009[6], the Commission review report on potassium phosphonates (European Commission, 2013), the conclusion on the peer review of the pesticide risk assessment of the active substances potassium phosphonates (EFSA, 2012) and fosetyl (EFSA, 2018b), as well as from the joint review of maximum residue levels (MRLs) for fosetyl, disodium phosphonate and potassium phosphonates according to Articles 12 and 43 of Regulation (EC) No 396/2005 (EFSA, 2021c).

For this application, the data requirements established in Regulation (EU) No 544/2011[7] and the guidance documents applicable at the date of submission of the application to the EMS are applicable (European Commission, 1997a–g, 2010, 2018, 2020, 2021; OECD, 2011). The assessment is performed in accordance with the legal provisions of the Uniform Principles for the Evaluation and the Authorisation of Plant Protection Products adopted by Commission Regulation (EU) No 546/2011[8].

A selected list of end points of the studies assessed by EFSA in the framework of both MRL applications including the end points of relevant studies assessed previously is presented in Appendix B.

The evaluation reports submitted by the EMS (Netherlands, 2021a,b) and the exposure calculations using the EFSA Pesticide Residues Intake Model (PRIMo) are considered as supporting documents to this reasoned opinion and, thus, are made publicly available as background documents to this reasoned opinion.

1. Residues in plants and honey

1.1. Nature of residues and methods of analysis in plants and honey

1.1.1. Nature of residues in primary crops

The metabolism of potassium phosphonates in primary crops was assessed during the EU pesticides peer review of this active substance (EFSA, 2012) and the joint review of MRLs for fosetyl and phosphonates (EFSA, 2021c). It was concluded that data from the public literature are sufficient to address the metabolism in plants. In crops treated with salts of potassium phosphonate, phosphonic acid is expected to be the main residue. No further studies on the metabolism of potassium phosphonates in primary crops were submitted in framework of the present MRL application. For the intended use on chards/beet leaves, the metabolic behaviour in primary crops is sufficiently addressed.

1.1.2. Nature of residues in rotational crops

Chards can be grown in rotation with other crops. According to the soil degradation studies evaluated in the framework of the EU pesticides peer review of fosetyl, moderate to high soil persistence (DT_{90} 91 to > 1,000 days) is reported for phosphonic acid, which is a common metabolite of fosetyl, disodium phosphonate and potassium phosphonates (EFSA, 2018b). Therefore, further investigation on the nature and magnitude of residues in rotational crops is required.

During the peer review of potassium phosphonates (EFSA, 2012), studies investigating the rate of degradation in soil of potassium phosphonates were not available. However, as highlighted for primary crops, considering the elementary nature of the active substance, the metabolic pathway of potassium phosphonates is expected to be similar also in rotational crops, with phosphonic acid being the main compound present in the treated soil and in the rotated crops (EFSA, 2021c).

[6] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

[7] Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances. OJ L 155, 11.6.2011, p. 1–66.

[8] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

Studies on the nature of phosphonic acid in rotational crops (root/tuber crops, leafy crops and cereals) were assessed in the framework of the EU pesticides peer review of fosetyl (EFSA, 2018b) (phosphonic acid applied to bare soil at 4.9 mg phosphonic acid/kg soil), confirming that the metabolite phosphonic acid is the major residue observed in rotational crops.

For the intended use on chards/beet leaves, the metabolic behaviour in rotational crops is sufficiently addressed.

1.1.3. Nature of residues in processed commodities

The effect of processing on the nature of phosphonic acid, which is the main metabolite of potassium phosphonates, was investigated in the framework of the EU pesticides peer review for fosetyl (EFSA, 2018b) and the joint review of MRLs for fosetyl and phosphonates (EFSA, 2021c). The available studies showed that phosphonic acid is hydrolytically stable under standard processing conditions representative of pasteurisation, baking/brewing/boiling and sterilisation.

1.1.4. Nature of residues in honey

Honey is a product originated from sugary secretions of plants (floral nectar mainly) through regurgitation, enzymatic conversion and water evaporation, followed by storage in the beehives for a certain time period.

In the absence of specific metabolism studies investigating the nature of phosphonic acid during formation of honey, data on the nature of residues in primary crops, rotational crops and processed commodities were considered to determine the nature of residues in honey (European Commission, 2018). Since the nature of residues is the same in primary and rotational crops and phosphonic acid is hydrolytically stable, it is expected that in pollen and nectar collected from primary and rotational crops, as well as in honey (resulting from the residues in floral nectar), the main residue will be phosphonic acid.

However, it would be desirable to further investigate whether enzymatic processes involved in the production of honey occurring in the bee gut or during the storage in the beehive have an impact on the nature of residues in honey.

1.1.5. Methods of analysis in plants and honey

In the framework of the joint review of MRLs for fosetyl and phosphonates, various analytical methods were reported. Sufficiently validated methods using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) are available to determine residues of phosphonic acid in plant matrices, including high water content matrices to which chards/beet leaves belong. The methods enable quantification of residues according to the current residue definition 'fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)' in high water content commodities with an LOQ of 0.01 mg/kg. Moreover, the methods allow the monitoring of residues expressed in accordance with the proposed new residue definition for enforcement 'phosphonic acid and its salts, expressed as phosphonic acid', and an LOQ of 0.1 mg/kg is achievable (EFSA, 2021c).

According to the information provided by the EURLs, during routine analysis, phosphonic acid can be enforced with an LOQ of 0.1 mg/kg in high water content commodities by means of a single residue method (Quick Polar Pesticides Method – QuPPe), using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (EURLs, 2020).

For honey, a sufficiently validated analytical method based on LC-MS/MS is available with an individual LOQ for phosphonic acid and fosetyl of 0.05 mg/kg (EFSA, 2021c). Although independent laboratory validation (ILV) and extraction efficiency data were not available, the EU pesticides peer review for fosetyl concluded that according to the data requirements applicable, the method was sufficiently validated (EFSA, 2018b).

1.1.6. Storage stability of residues in plants and honey

All available data on the storage stability of phosphonic acid residues under frozen conditions were assessed in the joint review of MRLs for fosetyl, disodium phosphonate and potassium phosphonates (EFSA, 2021c). In high water content commodities (relevant to chards/beet leaves), the available studies demonstrated acceptable storage stability for phosphonic acid for 25 months when stored at −18 to −25°C.

In the framework of the present application, a new study was submitted demonstrating the stability of phosphonic acid in honey and pollen for at least 6 months when stored at −18°C (Netherlands, 2021a).

1.1.7. Proposed residue definitions

The EU pesticides peer review of potassium phosphonates (EFSA, 2012) and the joint review of MRLs for fosetyl, disodium phosphonate and potassium phosphonates (EFSA, 2021c) proposed the following residue definitions for plant commodities:

- Residue definition for risk assessment: Phosphonic acid and its salts, expressed as phosphonic acid.
- Residue definition for enforcement: Phosphonic acid and its salts, expressed as phosphonic acid.

The residue definitions apply to primary crops, rotational crops and processed products. For honey, in the absence of specific metabolism studies, the proposed residue definitions for risk assessment and enforcement as derived by the joint MRL review are applicable.

The proposed residue definition for enforcement has not yet been implemented in Regulation (EC) No 396/2005; the current MRLs established in this regulation refer to the residue definition:

- Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl).

In the current reasoned opinion, the potassium phosphonate uses on chards/beet leaves and honey were assessed in view of deriving MRL proposals for the existing and the proposed new residue definition for enforcement.

1.2. Magnitude of residues in plants and honey

1.2.1. Magnitude of residues in primary crops

Chards/beet leaves

SEU/NEU, outdoor, foliar spray, 2 × 1.45 kg/ha potassium phosphonates/ha; interval between applications: 7–10 days; PHI: 7 days

In support of the present MRL application on chards/beet leaves, the applicant submitted 16 residue trials conducted on lettuces during growth seasons of 2018 and 2019. Trials were widespread in both EU zones (8 in NEU and 8 in SEU). All trials were designed as decline studies. Sampling was performed from the treated and the untreated plot at day 0 and 2–4, 6–8 and 13–14 days after the last application. Results indicate that phosphonic acid declined in lettuces by time.

Trial L180464 was disregarded by EFSA, as the plot was treated with a formulated product containing also fosetyl. Phosphonic acid is the common metabolite for fosetyl and potassium phosphonates; hence, the total residue was affected. In trials L190400 and L190401, phosphonic acid was present in samples obtained from untreated plots. Since residues in the samples from untreated plots were low compared to samples taken from treated plots, trials were deemed acceptable and residue data were considered for deriving risk assessment values and for the MRL calculation. EFSA notes that phosphonic acid residues have been also previously observed in samples from untreated plots (EFSA, 2020, 2021b, 2021d) and attributed to other possible sources (e.g. fertilisers, plant strengtheners, manure, soil amendments) (EFSA, 2021c).

The samples were analysed for phosphonic acid; to derive MRL proposals for the existing enforcement residue definition, the results were expressed as fosetyl by applying the molecular weight conversion factor. According to the assessment of the EMS, the methods used were sufficiently validated and fit for purpose. The samples of these residue trials were stored under conditions for which integrity of the samples has been demonstrated (Netherlands, 2021b).

According to the Technical guidelines on data requirements for setting maximum residue levels, comparability of residue trials and extrapolation on residue data on products from plant and animal origin (European Commission, 2020) residue data from trials conducted on lettuces (open leaf varieties) can be extrapolated to chards/beet leaves. Number of trials is sufficient to support the use on chards/beet leaves (minor crop; minimum 4 trials per zone required). Since residue data from trials in the NEU and SEU were similar (U-test, 5%), data were merged to derive a more robust MRL.

An MRL proposal of 40 mg/kg according to the proposed new residue definition for enforcement or 60 mg/kg according to the existing residue definition for enforcement, for chards/beet leaves were derived (see Appendix B.1.2.1). It is noted that during the joint review of MRLs for fosetyl and phosphonates, a higher MRL of 70 mg/kg was derived for the proposed new residue definition for enforcement on the basis of residue data extrapolation from five NEU trials on spinaches treated with fosetyl (EFSA, 2021c); these MRL proposals have not been yet legally endorsed.

1.2.2. Magnitude of residues in honey

Buckwheat (surrogate crop), tunnel trials, foliar spray: 3 × 2.36 kg potassium phosphonates/ha; 1st application at BBCH 55–59, 2nd at beginning of flowering at BBCH 61–63 and 3rd at full flowering at BBCH 63–65; PHI: 7–14 days.

In support of the MRL application on honey, the applicant submitted four independent residue trials performed on buckwheat treated with potassium phosphonates under semi-field conditions (tunnel trials). Trials were conducted in Germany during 2020. Hives were introduced in the tunnels just before the second application (beginning of flowering period). Tunnels were of the required size and access to water was provided. Honey was collected 7–14 days after the last application, at maturity (water content < 20%) before the end of flowering period. The sample size ranged from 21 to 57 g in the different trials, but this was considered as a minor deviation from the Technical Guidelines for honey requiring minimum of 100 g sample (European Commission, 2018), not affecting the validity of the trials. The samples of the residue trials were stored under conditions for which integrity of the samples was demonstrated. Samples were analysed for phosphonic acid; to derive MRL proposals for the existing enforcement residue definition, the results were expressed as fosetyl by applying the molecular weight conversion factor. According to the assessment of the EMS, the methods used were sufficiently validated and fit for purpose. Phosphonic acid residues were not present in honey samples from untreated plots (Netherlands, 2021a).

Phosphonic acid residues in honey ranged from 0.71 to 46 mg/kg, allowing to derive an MRL proposal of 100 mg/kg according to the proposed residue definition for monitoring or 150 mg/kg according to the existing residue definition for enforcement. It is noted that during the joint review of MRLs for fosetyl and phosphonates, an MRL of 0.3 mg/kg for honey was derived for the existing monitoring data using CI95 approach,[9] when considering 62 honey samples analysed during the 2015–2018 EU MS control programmes (EFSA, 2021c).

Data on residues in pollen and inflorescences of buckwheat were also presented in the evaluation report (Netherlands, 2021a). According to Commission Regulation (EU) 2018/62[10] MRLs are currently applicable only to honey; therefore, these additional results are considered as supplementary information only.

It is noted that the present MRL application for honey is related to intended uses on crops attractive to bees and that would be a potential source for residues of phosphonic acid in honey. EFSA notes that other uses of potassium phosphonates on melliferous crops authorised in the EU, might lead to higher phosphonic acid residues, however not expected when considering available monitoring data (EFSA, 2021c).

1.2.3. Magnitude of residues in rotational crops

Chards can be grown in rotation with other crops and phosphonic acid exhibits moderate to high soil persistence (see Section 1.1.2); hence, the presence of residues in succeeding crops should be investigated. In the framework of the present MRL application studies on rotational crops were not submitted. The possible transfer of phosphonic acid residues to crops that are grown in crop rotation was assessed in the joint MRL review (EFSA, 2021c), taking into consideration previous assessments of EFSA available for fosetyl and potassium phosphonates.

According to the confined rotational crops metabolism study evaluated in the framework of the peer review for the renewal of fosetyl (EFSA, 2018b), when phosphonic acid is applied to bare soil at a dose rate of 4.9 mg a.s./kg (equivalent to 14.7 kg phosphonic acid/ha), residues are taken up from the soil by the plant. Actually, based on the results of this study, residue concentrations of phosphonic

[9] Upper confidence interval (CI95) of the calculated P95. For honey (n > 59), CI95 was calculated. Residues below LOQ were included in the calculation by replacing them by the LOQ of the reporting laboratory (upper bound scenario).

[10] Commission Regulation (EU) 2018/62 of 17 January 2018 replacing Annex I to Regulation (EC) No 396/2005 of the European Parliament and of the Council. C/2018/0138. OJ L 18, 23.1.2018, p. 1–73.

acid accounted for 0.35 and 0.8 mg/kg in radish tops and roots, respectively, 0.76 mg/kg in lettuce leaves and 0.14 and 0.42 mg/kg in barley grain and straw, respectively, at 30-day PBI. Residues were not analysed at longer plant back intervals, but phosphonic acid residues in radish tops and roots planted 6 months after soil treatment were recovered at a level below 0.1 mg/kg.

Rotational crop field trials were considered in the framework of the peer review for the renewal of the approval of fosetyl (EFSA, 2018b). These field trials were conducted on lettuces, carrots and cereals (winter wheat and barley) following treatment of lettuces as a target crop three times with fosetyl at a total dose rate of 2.3 kg fosetyl/ha (corresponding to 1.73 kg phosphonic acid equivalents/ha) at plant back interval (PBI) of 30 days. Within 7 days after the last application (32–69 days after planting), the primary crop lettuce was destroyed, and the remaining plant parts were incorporated into the soil. Relevant rotational crops were sown/planted 30 days following the incorporation of lettuce in the soil. No other plant back intervals have been investigated. Residues of fosetyl and phosphonic acid were shown to be below the LOQ of the method in all rotational crop edible parts at the 30-day PBI, except in wheat grain (0.21 mg/kg for phosphonic acid). The rotational crop field trials have been performed with only slightly lower application rate than in the intended seasonal application on chards/beet leaves (1.9 kg phosphonic acid/ha).

In the framework of the joint MRL review, EFSA noted that rotational field trials conducted with fosetyl were under dosed compared to the critical GAPs authorised for potassium phosphonates, and the magnitude of residues of fosetyl and phosphonic acid was determined at the 30-day PBI only and not at later PBIs (EFSA, 2021c). A firm conclusion could not be derived on the actual residue levels of phosphonic acid in rotational crops and on the most appropriated risk mitigation measures, since these studies did not cover the maximum dose rates of application of the authorised GAPs and were also not expected to cover the possible accumulation of phosphonic acid residues following successive years of application as this compound is considered as highly persistent.

Therefore, additional rotational crops' field trials performed at a dose rate covering the maximum dose rates of application and the possible accumulation of phosphonic acid (max PEC_{soil} for phosphonic acid) are in principle required. Nevertheless, in the framework of the joint MRL review, monitoring data were also considered to derive MRL proposals covering all sources of phosphonic acid and their residues uptake from the soil. These data were expected to cover also the possible uptake of phosphonic acid in succeeding crops resulting from the use of fosetyl, potassium and disodium phosphonates in compliance with the authorised GAPs and from the use of other products of agricultural relevance (e.g. fertilisers, plant strengthens, manure, soil amendments). Therefore, additional rotational crops' field studies are only desirable (EFSA, 2021c).

For the intended use on chards/beet leaves, the seasonal application rate of potassium phosphonates is lower than application rates reported for the authorised uses in the joint MRL review; therefore, the previous conclusions are still valid and further investigations are not required.

1.2.4. Magnitude of residues in processed commodities

Although phosphonic acid residues are expected to occur above 0.1 mg/kg in unprocessed chards/beet leaves and honey, considering the low contribution of these commodities to the total consumers' chronic exposure (below 1% to the theoretical maximum daily intake (TDMI)) to phosphonic acid residues, investigations on the effect of processing on the magnitude of residues in processed commodities were not deemed necessary.

1.2.5. Proposed MRLs

The available data are considered sufficient to derive MRL proposals as well as risk assessment values for the commodities under evaluation (see Appendix B.1.2.1). In Section 3, EFSA assessed whether residues of phosphonic acid in chards/beet leaves resulting from the intended use of potassium phosphonates, and residues in honey resulting from the use of potassium phosphonates on melliferous crops (according to the use pattern assessed in the present application) are likely to pose a consumer health risk.

2. Residues in livestock

Not relevant as chards/beet leaves and honey are normally not used for feed purposes.

3. Consumer risk assessment

EFSA performed a dietary risk assessment using revision 3.1 of the EFSA PRIMo (EFSA, 2018a, 2019). This exposure assessment model contains food consumption data for different subgroups of the EU population and allows the acute and chronic exposure assessment to be performed in accordance with the internationally agreed methodology for pesticide residues (FAO, 2016).

The toxicological profile for potassium phosphonates was assessed in the framework of the EU pesticides peer review (EFSA, 2012). For phosphonic acid, which is the relevant component of residues in plant and animal products, an acceptable daily intake (ADI) of 2.25 mg/kg bw per day was derived (European Commission, 2013). An acute reference dose (ARfD) was not deemed necessary due to the low acute toxicity of phosphonic acid.

In 2018, in the framework of the renewal of the approval for fosetyl, a revised ADI of 1 mg/kg bw per day has been derived, which was also recommended to be applied to phosphonic acid (EFSA, 2018b). Although this new ADI is not yet formally adopted, an indicative risk assessment was calculated based on this reference value as well.

A short-term exposure assessment is not required since no ARfD is established or proposed for phosphonic acid.

In the framework of the joint review of MRLs for fosetyl, disodium phosphonate and potassium phosphonates, a comprehensive long-term exposure assessment was performed combining residue data originating from the use of the three active substances and the monitoring data as well as certain CXLs established for fosetyl and phosphonic acid (EFSA, 2021c). The input values were expressed as phosphonic acid equivalents. EFSA updated these exposure calculations with median residue values derived for commodities under assessment (chards/beet leaves and honey). In addition, the updated peeling factors for citrus fruits, which were not available for the joint MRL review and were derived from a recent reasoned opinion were used (EFSA, 2021d). All input values used in the exposure calculations are presented in Appendix D.1.

EFSA calculated two exposure scenarios: **scenario 1** using the existing ADI value for phosphonic acid of 2.25 mg/kg bw per day and **scenario 2,** with the proposed, lower ADI value of 1 mg/kg bw per day.

Considering the currently applicable ADI of 2.25 mg/kg bw per day (**scenario 1**), the estimated long-term dietary intake accounted for 36% of the ADI (Dutch toddler diet). Expressing the exposure as percentage of the revised ADI of 1 mg/kg bw per day as proposed by the peer review on fosetyl (EFSA, 2018b; **scenario 2**), the highest chronic exposure was calculated for Dutch toddler, representing 81% of the ADI (Dutch toddler diet). The contribution to the total consumer intake for both commodities under assessment was below 0.12% of the ADI for both scenarios.

For further details on the exposure calculations, screenshots of the Report sheet of the PRIMo are presented in Appendix C.

4. Conclusion and Recommendations

The data submitted in support of the MRL applications were found to be sufficient to derive MRL proposals for chards/beet leaves and honey. The MRL proposals were derived for the current enforcement residue definition as well as for the enforcement residue definition proposed by the EU pesticides peer review for potassium phosphonates and the joint MRL review. EFSA notes that the MRL proposal for chards/beet leaves as derived in the present assessment for the proposed residue definition is lower than the MRL proposal derived for chards/beet leaves by the joint MRL review; however, the value is not legally endorsed yet. For honey, a significantly lower MRL proposal was derived from available monitoring data (2015–2018 EU MS control programmes) during the joint MRL review.

Provided that the conclusions of the joint MRL review are implemented, EFSA concluded that the proposed SEU/NEU uses of potassium phosphonates on chards/beet leaves and the consumption of honey, produced by bees foraging melliferous crops treated with potassium phosphonates according to the use pattern assessed in the present application, will not result in a consumer exposure exceeding the toxicological reference value and therefore is unlikely to pose a risk to consumers' health.

The MRL recommendations are summarised in Appendix B.4.

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Abbreviations

a.s.	active substance
ADI	acceptable daily intake
AR	applied radioactivity
ARfD	acute reference dose
BBCH	growth stages of mono- and dicotyledonous plants
bw	body weight
CEN	European Committee for Standardisation (Comité Européen de Normalisation)
CF	conversion factor for enforcement to risk assessment residue definition
CXL	Codex maximum residue limit
DAR	draft assessment report
DAT	days after treatment
DT_{90}	period required for 90% dissipation (define method of estimation)
EC	emulsifiable concentrate
EMS	evaluating Member State
eq	residue expressed as a.s. equivalent
EURL	EU Reference Laboratory (former Community Reference Laboratory (CRL))
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly International Group of National Associations of Manufacturers of Agrochemical Products (GIFAP))
GC-MS	gas chromatography with mass spectrometry
HPLC	High-performance liquid chromatography
HPLC-MS	high performance liquid chromatography with mass spectrometry
HPLC-MS/MS	high performance liquid chromatography with tandem mass spectrometry
HPLC-UVD	high performance liquid chromatography with ultra-violet detector
HR	highest residue
IEDI	international estimated daily intake
ILV	independent laboratory validation

IPCS	International Programme of Chemical Safety
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
K_{oc}	organic carbon adsorption coefficient
LC	liquid chromatography
LOQ	limit of quantification
MRL	maximum residue level
MS	Member States
MS	mass spectrometry detector
MS/MS	tandem mass spectrometry detector
MW	molecular weight
NEU	northern Europe
OECD	Organisation for Economic Co-operation and Development
PBI	plant back interval
PF	processing factor
PHI	preharvest interval
P_{ow}	partition coefficient between n-octanol and water
PRIMo	(EFSA) Pesticide Residues Intake Model
RA	risk assessment
RAC	raw agricultural commodity
RD	residue definition
RMS	rapporteur Member State
SANCO	Directorate-General for Health and Consumers
SC	suspension concentrate
SCPAFF	Standing Committee on Plants, Animals, Food and Feed (formerly: Standing Committee on the Food Chain and Animal Health; SCFCAH)
SEU	southern Europe
STMR	supervised trials median residue
WHO	World Health Organization

Appendix A – Summary of intended GAP triggering the amendment of existing EU MRLs

For honey, the MRL application is not linked to a specific GAP/crop but is related to intended uses on crops attractive to bees and that would be a potential source for residues of phosphonic acid in honey. In the framework of the joint review of fosetyl, disodium phosphonate and potassium phosphonates (EFSA, 2021c), various uses were reported for crops that might be attractive to bees. These uses might lead to higher phosphonic acid residues in honey, however not expected when considering available monitoring data.

Crop and/or situation	NEU, SEU, MS or country	F G or I[a]	Pests or group of pests controlled	Preparation		Application				Application rate per treatment				PHI (days)[d]
				Type[b]	Conc. a.s.	Method kind	Range of growth stages and season[c]	Number max	Interval Between application (days) min–max	g a.s./hL min–max	Water (L/ha) min–max	Rate max	Unit	
Chards/beet leaves	NEU/SEU	F	*Bremia lactuca Peronospora sp*.	SC	453 g/L Potassium phosphonates	Foliar treatment – broadcast spraying	41–49	2	7–10	145–1,450	100–1,000	1450	g a.s./ha	7

MRL: maximum residue level; GAP: Good Agricultural Practice; NEU: northern European Union; SEU: southern European Union; MS: Member State; a.s.: active substance; SC: suspension concentrate.
(a): Outdoor or field use (F), greenhouse application (G) or indoor application (I).
(b): CropLife International Technical Monograph no 2, 7th Edition. Revised March 2017. Catalogue of pesticide formulation types and international coding system.
(c): Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including, where relevant, information on season at time of application.
(d): PHI: minimum preharvest interval.

Appendix B – List of end points

B.1. Residues in plants

B.1.1. Nature of residues and methods of analysis in plants/honey

B.1.1.1. Metabolism studies, methods of analysis and residue definitions in plants/honey

Primary crops (available studies)	**Crop groups**	**Crop(s)**	**Application(s)**	**Sampling (DAT)**	**Comment/Source**
	Fruit crops	No experimental studies submitted. The EU pesticides peer review and the joint review of MRLs for fosetyl and phosphonates concluded that, given the elementary nature of potassium phosphonates and according to the available data from public literature, the main residue resulting from the foliar and soil applications of potassium phosphonates in plants is phosphonic acid (EFSA, 2012, 2021c).			
	Root crops				
	Leafy crops				
	Cereals/grass				
	Pulses/oilseeds				
	Miscellaneous				

Rotational crops (available studies)	**Crop groups**	**Crop(s)**	**PBI (DAT)**	**Comment/Source**
	Root/tuber crops	Radish	32; 182	No experimental studies submitted for potassium phosphonates. Bridging data on studies with fosetyl (EFSA,) considered sufficient to assess the nature of potassium phosphonates in rotational crops. Residues of phosphonic acid are observed in plants grown only one month after application to the soil. Radish root: 0.8 mg/kg Lettuce: 0.76 mg/kg In all other crop parts phosphonic acid residues < LOQ (0.5 mg/kg) (EFSA, 2021c).
	Leafy crops	Lettuce	32	
	Cereals (small grain)	Barley	32	

Processed commodities (hydrolysis study)	**Conditions**	**Stable?**	**Comment/Source**
	Pasteurisation (20 min, 90°C, pH 4)	Yes	According to experimental studies provided in the EU pesticides peer review of fosetyl (EFSA, 2018b), fosetyl and phosphonic acid are hydrolytically stable (EFSA, 2021c).
	Baking, brewing and boiling (60 min, 100°C, pH 5)	Yes	
	Sterilisation (20 min, 120°C, pH 6)	Yes	
	Other processing conditions	–	–

Can a general residue definition be proposed for primary crops?	Yes	EFSA (2021c)
Rotational crop and primary crop metabolism similar?	Yes	EFSA (2021c)
Residue pattern in processed commodities similar to residue pattern in raw commodities?	Yes	EFSA (2021c)
Plant residue definition for monitoring (RD-Mo)	Existing residue definition: Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl) (Regulation (EC) No 396/2005) Proposed residue definition (not implemented yet): Phosphonic acid and its salts, expressed as phosphonic acid (EFSA, 2021c)	
Plant residue definition for risk assessment (RD-RA)	Phosphonic acid and its salts, expressed as phosphonic acid (EFSA, 2021c)	
Methods of analysis for monitoring of residues (analytical technique, crop groups, LOQs)	• HPLC–MS/MS (matrices: high water, dry/high starch, high acid, high oil). ILV provided and validated. Fosetyl LOQ: 0.01 mg/kg Phosphonic acid LOQ: 0.1 mg/kg (EFSA, 2021c) • GC-FPD (hops) Fosetyl LOQ: 2 mg/kg Phosphonic acid LOQ: 20 mg/kg (EFSA, 2021c) • Single residue method (QuPPe) for enforcement in routine analysis, LOQ 0.1 mg/kg (as phosphonic acid) for high water and high acid content commodities, and 0.2 mg/kg (as phosphonic acid) for high oil content and dry commodities (EURLs, 2020). • LC–MS/MS (Honey) Fosetyl LOQ: 0.05 mg/kg Phosphonic acid LOQ: 0.05 mg/kg (EFSA, 2018b, 2021c)	

DAT: days after treatment; PBI: plant-back interval; BBCH: growth stages of mono- and dicotyledonous plants; a.s.: active substance; MRL: maximum residue level; LOQ: limit of quantification; GC–MS: gas chromatography with mass spectrometry; QuPPe: Quick Polar Pesticides; LC–MS/MS: liquid chromatography with tandem mass spectrometry; ILV: independent laboratory validation

B.1.1.2. Stability of residues in plants and honey

Plant products (available studies)	**Category**	**Commodity**	**T (°C)**	**Stability period**		**Compounds covered**	**Comment/ Source**
				Value	**Unit**		
	High water content	Cucumbers	–18 to –25	25	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Lettuces		24	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Head cabbages		24	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Cherry tomatoes		24	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Wheat, whole plants		12	Months	Phosphonic acid	EFSA (2021c)
		Apples		12	Months	Phosphonic acid	EFSA (2021c)

Plant products (available studies)	Category	Commodity	T (°C)	Stability period		Compounds covered	Comment/ Source
				Value	Unit		
		Peaches		307	Days	Phosphonic acid	EFSA (2021c)
	High oil content	Avocados		25	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Almonds		218	Days	Phosphonic acid	EFSA (2021c)
		Pistachios		221	Days	Phosphonic acid	EFSA (2021c)
		Walnuts		146	Days	Phosphonic acid	EFSA (2021c)
	High protein content	Beans, dry		24	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
	High starch content	Potatoes		25	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
				12	Months	Phosphonic acid	EFSA (2021c)
		Wheat, grain		12	Months	Phosphonic acid	EFSA (2021c)
	High acid content	Grapes		25	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
		Oranges		24	Months	Phosphonic acid and its salts expressed as phosphonic acid.	EFSA (2021c)
	Processed products	Peach jam, puree, nectar and canned peaches		112–114	Days	Phosphonic acid	EFSA (2021c)
	Others	Wheat, straw		12	Months	Phosphonic acid	EFSA (2021c)
		Pollen	–18	6	Months	Phosphonic acid	Netherlands (2021a)
Products of animal origin (available studies)		Honey	–18	6	Months	Phosphonic acid	Netherlands (2021a)

B.1.2. Magnitude of residues in plants and honey

B.1.2.1. Summary of residues data from the supervised residue trials

Commodity	Region[a]	Residue levels observed in the supervised residue trials (mg/kg)	Comments/Source	Calculated MRL (mg/kg)	HR[b] (mg/kg)	STMR[c] (mg/kg)
RD-Mo (existing): Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl) **RD-Mo (proposed (**EFSA, 2021c**)):** Phosphonic acid and its salts, expressed as phosphonic acid **RD-RA (**EFSA, 2021c**):** Phosphonic acid and its salts, expressed as phosphonic acid						
Chards/beet leaves	NEU/SEU	**RD-Mo (existing)[d]:** 8.3; 8.4; 9.1; 11.3; 13.4; 14.7; 18.8; 2 × 20.1; 2 × 22.8; 24.1; 2 × 25.5; 26.8 **RD-RA=RD-Mo (proposed):** 6.2; 6.3; 6.8; 8.4; 10; 11; 14; 2 × 15; 2 × 17; 18; 2 × 19; 20	Residue trials on open-leaf lettuces compliant with GAP on chards. Extrapolation to chards/beet leaves possible. EFSA notes that in the joint MRL review, a higher MRL of 70 mg/kg was derived for the proposed RD-Mo from the use of fosetyl on spinach; the derived risk assessment values[e] were lower (STMR of 9 mg/kg and HR of 37 mg/kg) (EFSA, 2021c).	RD-Mo (existing): **60** RD-Mo (proposed): **40**	RD-RA: 20	RD-RA: 15
Honey	EU	**RD-Mo (existing)[d]:** 0.95; 0.98; 26.8; 61.6 **RD-RA=RD-Mo (proposed):** 0.71; 0.73; 27; 46	Semi-field (tunnel) trials with buckwheat treated with potassium phosphonates (3 × 2.36 kg/ha) at BBCH 55–65 via foliar application. The number of trials is sufficient to derive an MRL in honey.	RD-Mo (existing): **150** RD-Mo (proposed): **100**	RD-RA: 46	RD-RA: 10.37

MRL: maximum residue level; GAP: Good Agricultural Practice; RD: residue definition; Mo: monitoring; RA: risk assessment.
(a): NEU: Outdoor trials conducted in northern Europe, SEU: Outdoor trials conducted in southern Europe, EU: indoor EU trials or Country code: if non-EU trials.
(b): Highest residue. The highest residue for risk assessment refers to the whole commodity and not to the edible portion.
(c): Supervised trials median residue. The median residue for risk assessment refers to the whole commodity and not to the edible portion.
(d): Individual residues were recalculated to express them as fosetyl by applying the molecular weight (MW) conversion factor of 1.34 = MW fosetyl (110 g/mol)/MW phosphonic acid (82 g/mol)

B.1.2.2. Residues in rotational crops

Residues in rotational and succeeding crops expected based on confined rotational crop study?	Yes	Based on the results of the confined metabolism study with phosphonic acid applied to bare soil at 4.9 mg phosphonic acid/kg, residue concentrations of phosphonic acid accounted for 0.35 and 0.8 mg/kg in radish tops/leaves and roots, respectively, 0.76 mg/kg in lettuce leaves and 0.14 and 0.42 mg/kg in barley grain and straw, respectively at 30 day PBI. Residues were not analysed at longer plant back interval but phosphonic acid residues in radish tops and roots planted 6 months after soil treatment were recovered at a level of < 0.1 mg/kg (EFSA, 2018b; 2021c).
Residues in rotational and succeeding crops expected based on field rotational crop study?	Inconclusive	From the field trials conducted on lettuces, carrots and cereals (winter wheat and barley) following treatment of lettuces as a target crop with fosetyl at a total dose rate of 2.3 kg a.s./ha (corresponding to 1.73 kg phosphonic acid equivalents/ha), residues of fosetyl and phosphonic acid were shown to be below the LOQ in all rotational crops edible parts at the 30-day PBI, except in wheat grain (0.21 mg/kg for phosphonic acid) (EFSA, 2018b). However, no firm conclusion can be drawn on the actual residue levels of fosetyl and phosphonic acid in rotational crops since these trials do not cover the maximum dose rates of application of the GAPs currently authorized in Europe and are also not expected to cover the possible accumulation of phosphonic acid residues following successive years of application as this compound is considered as highly persistent. Nevertheless in the framework of the joint MRL review, monitoring data were also considered to derive MRL proposals which are expected to cover also the possible uptake of phosphonic acid in succeeding crops resulting from the use of fosetyl, potassium and disodium phosphonates in compliance with the authorized GAPs and from the use of other products of agricultural relevance. Additional rotational crops field studies are therefore only desirable (EFSA, 2021c).

a.s.: active substance; eq: equivalents; PBI: plant-back interval; LOQ: limit of quantification; GAP: Good Agricultural Practice.

B.1.2.3. Processing factors

No processing studies were submitted in the framework of the present MRL application.

B.2. Residues in livestock

Not relevant as chards/beet leaves and honey are not used for feed purposes.

B.3. Consumer risk assessment

Not relevant since no ARfD has been considered necessary.

ADI	Scenario 1 (TRV currently in place for phosphonic acid): 2.25 mg/kg bw per day (European Commission, 2013). Scenario 2 (TRV not yet endorsed): 1 mg/kg bw per day (EFSA, 2018b).
Highest IEDI, according to EFSA PRIMo	Scenario 1 (TRV currently in place for phosphonic acid): 36% ADI (NL toddler) Contribution of commodities assessed: Chards/beet leaves: 0.05% of ADI (ES adult diet) Honey: 0.05% of ADI (DE child diet) Scenario 2 (TRV not yet endorsed): 81% ADI (NL toddler) Contribution of commodities assessed: Chards/beet leaves: 0.12% of ADI (ES adult diet) Honey: 0.1% of ADI (DE child diet)
Assumptions made for the calculations	The long-term exposure assessment calculated during the joint review of MRLs for fosetyl, disodium phosphonate and potassium phosphonates (EFSA, 2021c) was updated with median residue levels derived from residue trials for chards/beet leaves and honey as derived from the residue trials submitted for the present assessment. Additionally, for citrus fruits the processing factors as derived in a recent EFSA opinion (not voted yet) (EFSA, 2021b) were applied to the input values for citruses. Calculations performed with PRIMo revision 3.1.

ARfD: acute reference dose; ADI: acceptable daily intake; TRV: toxicological refence values; bw: body weight; IEDI: international estimated daily intake; PRIMo: (EFSA) Pesticide Residues Intake Model; MRL: maximum residue level.

B.4. Recommended MRLs

Code[(a)]	Commodity	Existing EU MRL/new MRL proposal[(b)] (mg/kg)	Proposed EU MRL: existing enforcement RD/Proposed new enforcement RD (mg/kg)	Comment/justification
Existing enforcement residue definition: Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl) **Proposed new enforcement residue definition (not yet implemented):** Phosphonic acid and its salts, expressed as phosphonic acid				
0252030	Chards/beet leaves	15/70	60/40	The submitted data are sufficient to derive an MRL proposal for the NEU/SEU uses. The MRL proposal is lower than that of the joint MRL review for fosetyl and phosphonates, derived from NEU trials on spinaches treated with fosetyl (EFSA, 2021c). Risk for consumers unlikely.
1040000	Honey	0.5*/0.3	150/100	The MRL proposal reflects residues in honey from tunnel trials performed on buckwheat treated with potassium phosphonates. In the framework of the joint MRL review for fosetyl and phosphonates, an MRL for honey was derived from available monitoring data (EFSA, 2021c). Risk for consumers unlikely.

MRL: maximum residue level; NEU: northern Europe; SEU: southern Europe; GAP: Good Agricultural Practice.
*: Indicates that the MRL is set at the limit of analytical quantification (LOQ).
(a): Commodity code number according to Annex I of Regulation (EC) No 396/2005.
(b): MRL proposal, according to proposed new enforcement residue definition, derived in a recently published reasoned opinion of EFSA, not yet implemented (EFSA, 2021c).

Appendix C – Pesticide Residue Intake Model (PRIMo)

Scenario 1 (TRV currently in place for phosphonic acid)

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EFSA PRIMo revision 3.1; 2021/01/06

Phosphonic acid

LOQs (mg/kg) range from: 0.1 to: 0.10

Toxicological reference values

ADI (mg/kg bw per day):	2.25	ARfD (mg/kg bw):	Not necessary
Source of ADI:	EC	Source of ARfD:	EC
Year of evaluation:	2013	Year of evaluation:	2013

Input values

Details – chronic risk assessment | Supplementary results – chronic risk assessment

Details – acute risk assessment/children | Details – acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/(NEDI/IEDI) calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity/ group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity/ group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity/ group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
	36%	NL toddler	809.91	10%	Apples	5%	Potatoes	4%	Wheat		36%
	33%	DE child	751.07	11%	Apples	4%	Wheat	3%	Potatoes		33%
	24%	NL child	538.56	5%	Apples	4%	Wheat	4%	Potatoes		24%
	22%	GEMS/Food G06	505.76	7%	Wheat	2%	Potatoes	2%	Tomatoes		22%
	19%	GEMS/Food G08	431.35	5%	Potatoes	4%	Wheat	2%	Wine grapes		19%
	19%	GEMS/Food G11	428.50	5%	Potatoes	4%	Wheat	2%	Wine grapes		19%
	19%	GEMS/Food G07	419.70	4%	Potatoes	4%	Wheat	2%	Wine grapes		19%
	18%	PT general	411.63	6%	Potatoes	4%	Wheat	4%	Wine grapes		18%
	18%	RO general	398.77	5%	Wheat	4%	Potatoes	3%	Wine grapes		18%
	17%	GEMS/Food G15	385.28	5%	Wheat	4%	Potatoes	2%	Wine grapes		17%
	17%	IE adult	382.10	3%	Potatoes	2%	Wheat	2%	Wine grapes		17%
	17%	FR child 3 15 yr	381.12	5%	Wheat	3%	Oranges	2%	Potatoes		17%
	16%	GEMS/Food G10	369.92	4%	Wheat	4%	Potatoes	0.9%	Tomatoes		16%
	14%	SE general	322.96	5%	Potatoes	3%	Wheat	0.9%	Apples		14%
	14%	DK child	322.30	5%	Wheat	3%	Potatoes	2%	Apples		14%
	14%	UK toddler	320.50	4%	Potatoes	4%	Wheat	2%	Apples		14%
	14%	ES child	315.38	5%	Wheat	2%	Potatoes	2%	Oranges		14%
	14%	FR toddler 2 3 yr	308.10	3%	Wheat	3%	Apples	2%	Potatoes		14%
	13%	DE women 14-50 yr	289.38	2%	Apples	2%	Wheat	1%	Oranges		13%
	13%	IT toddler	286.48	7%	Wheat	1%	Potatoes	0.9%	Tomatoes		13%
	12%	FI 3 yr	274.74	6%	Potatoes	1%	Wheat	1%	Cucumbers		12%
	12%	NL general	270.95	3%	Potatoes	2%	Wheat	1%	Apples		12%
	12%	DE general	267.69	2%	Apples	2%	Wheat	1%	Potatoes		12%
	11%	UK infant	241.42	4%	Potatoes	3%	Wheat	1%	Apples		11%
	11%	FR adult	236.30	4%	Wine grapes	2%	Wheat	0.9%	Potatoes		11%
	10%	ES adult	227.43	2%	Wheat	1%	Potatoes	1.0%	Oranges		10%
	10%	FI 6 yr	219.06	5%	Potatoes	1%	Wheat	0.8%	Cucumbers		10%
	10%	IT adult	215.18	4%	Wheat	0.7%	Tomatoes	0.7%	Potatoes		10%
	9%	UK vegetarian	191.69	2%	Wheat	2%	Potatoes	1%	Wine grapes		9%
	8%	PL general	189.89	4%	Potatoes	2%	Apples	0.6%	Tomatoes		8%
	8%	LT adult	182.09	4%	Potatoes	2%	Apples	1%	Wheat		8%
	7%	UK adult	168.65	2%	Wheat	2%	Wine grapes	2%	Potatoes		7%
	7%	FR infant	166.29	2%	Potatoes	2%	Apples	0.8%	Wheat		7%
	7%	DK adult	162.76	2%	Potatoes	2%	Wine grapes	1%	Wheat		7%
	5%	FI adult	116.22	1%	Potatoes	0.5%	Apples	0.5%	Wine grapes		5%
	3%	IE child	62.32	1%	Wheat	0.7%	Potatoes	0.3%	Apples		3%

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of Phosphonic acid is unlikely to present a public health concern.

DISCLAIMER: Dietary data from the UK were included in PRIMO when the UK was a member of the European Union.

Acute risk assessment/children	Acute risk assessment/adults/general population
Details – acute risk assessment/children	Details – acute risk assessment/adults

As an ARfD is not necessary/not applicable, no acute risk assessment is performed.

Show results for all crops

Unprocessed commodities

Results for children No. of commodities for which ARfD/ADI is exceeded (IESTI): ---				**Results for adults** No. of commodities for which ARfD/ADI is exceeded (IESTI): ---			
IESTI				**IESTI**			
Highest % of ARfD/ADI	Commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)	Highest % of ARfD/ADI	Commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)
Expand/collapse list							
Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)							

Processed commodities

Results for children No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---				**Results for adults** No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---			
IESTI				**IESTI**			
Highest % of ARfD/ADI	Processed commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)	Highest % of ARfD/ADI	Processed commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)
Expand/collapse list							

Conclusion:

Scenario 2 (TRV not yet endorsed)

European Food Safety Authority

EFSA PRIMo revision 3.1; 2021/01/06

Phosphonic acid

LOQs (mg/kg) range from: 0.1 to: 0.10

Toxicological reference values

ADI (mg/kg bw per day):	1	ARfD (mg/kg bw):	Not necessary
Source of ADI:	EFSA	Source of ARfD:	EFSA
Year of evaluation:	2018	Year of evaluation:	2018

Input values

Details – chronic risk assessment | Supplementary results – chronic risk assessment

Details – acute risk assessment/children | Details – acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity/ group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity/ group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity/ group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
	81%	NL toddler	809.91	22%	Apples	11%	Potatoes	9%	Wheat		81%
	75%	DE child	751.07	25%	Apples	10%	Wheat	7%	Potatoes		75%
	54%	NL child	538.56	12%	Apples	10%	Wheat	9%	Potatoes		54%
	51%	GEMS/Food G06	505.76	17%	Wheat	5%	Potatoes	5%	Tomatoes		51%
	43%	GEMS/Food G08	431.35	11%	Potatoes	9%	Wheat	4%	Wine grapes		43%
	43%	GEMS/Food G11	428.50	11%	Potatoes	8%	Wheat	4%	Wine grapes		43%
	42%	GEMS/Food G07	419.70	10%	Potatoes	10%	Wheat	5%	Wine grapes		42%
	41%	PT general	411.63	14%	Potatoes	9%	Wheat	9%	Wine grapes		41%
	40%	RO general	398.77	12%	Wheat	10%	Potatoes	6%	Wine grapes		40%
	39%	GEMS/Food G15	385.28	11%	Wheat	10%	Potatoes	4%	Wine grapes		39%
	38%	IE adult	382.10	6%	Potatoes	5%	Wheat	4%	Wine grapes		38%
	38%	FR child 3 15 yr	381.12	11%	Wheat	6%	Oranges	4%	Potatoes		38%
	37%	GEMS/Food G10	369.92	9%	Wheat	8%	Potatoes	2%	Tomatoes		37%
	32%	SE general	322.96	11%	Potatoes	7%	Wheat	2%	Apples		32%
	32%	DK child	322.30	10%	Wheat	7%	Potatoes	5%	Apples		32%
	32%	UK toddler	320.50	9%	Potatoes	9%	Wheat	3%	Apples		32%
	32%	ES child	315.38	10%	Wheat	5%	Potatoes	4%	Oranges		32%
	31%	FR toddler 2 3 yr	308.10	7%	Wheat	6%	Apples	5%	Potatoes		31%
	29%	DE women 14-50 yr	289.38	5%	Apples	5%	Wheat	3%	Oranges		29%
	29%	IT toddler	286.48	15%	Wheat	2%	Potatoes	2%	Tomatoes		29%
	27%	FI 3 yr	274.74	13%	Potatoes	3%	Wheat	3%	Cucumbers		27%
	27%	NL general	270.95	7%	Potatoes	4%	Wheat	3%	Apples		27%
	27%	DE general	267.69	5%	Apples	4%	Wheat	3%	Potatoes		27%
	24%	UK infant	241.42	9%	Potatoes	6%	Wheat	3%	Apples		24%
	24%	FR adult	236.30	8%	Wine grapes	5%	Wheat	2%	Potatoes		24%
	23%	ES adult	227.43	5%	Wheat	3%	Potatoes	2%	Oranges		23%
	22%	FI 6 yr	219.06	10%	Potatoes	2%	Wheat	2%	Cucumbers		22%
	22%	IT adult	215.18	10%	Wheat	2%	Tomatoes	2%	Potatoes		22%
	19%	UK vegetarian	191.69	5%	Wheat	4%	Potatoes	3%	Wine grapes		19%
	19%	PL general	189.89	9%	Potatoes	4%	Apples	1%	Tomatoes		19%
	18%	LT adult	182.09	9%	Potatoes	4%	Apples	2%	Wheat		18%
	17%	UK adult	168.65	4%	Wheat	4%	Wine grapes	4%	Potatoes		17%
	17%	FR infant	166.29	5%	Potatoes	3%	Apples	2%	Wheat		17%
	16%	DK adult	162.76	3%	Potatoes	3%	Wine grapes	3%	Wheat		16%
	12%	FI adult	116.22	3%	Potatoes	1%	Apples	1%	Wine grapes		12%
	6%	IE child	62.32	3%	Wheat	2%	Potatoes	0.7%	Apples		6%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Phosphonic acid is unlikely to present a public health concern.
DISCLAIMER: Dietary data from the UK were included in PRIMO when the UK was a member of the European Union.

Acute risk assessment/children	Acute risk assessment/adults/general population
Details – acute risk assessment/children	Details – acute risk assessment/adults

As an ARfD is not necessary/not applicable, no acute risk assessment is performed.

Show results for all crops

Unprocessed commodities

Results for children No. of commodities for which ARfD/ADI is exceeded (IESTI): ---				**Results for adults** No. of commodities for which ARfD/ADI is exceeded (IESTI): ---			
IESTI				**IESTI**			
Highest % of ARfD/ADI	Commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)	Highest % of ARfD/ADI	Commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)
Expand/collapse list							
Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)							

Processed commodities

Results for children No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---				**Results for adults** No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---			
IESTI				**IESTI**			
Highest % of ARfD/ADI	Processed commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)	Highest % of ARfD/ADI	Processed commodities	MRL/input for RA (mg/kg)	Exposure (µg/kg bw)
Expand/collapse list							

Conclusion:

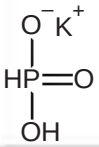
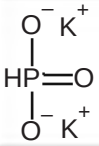
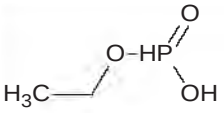
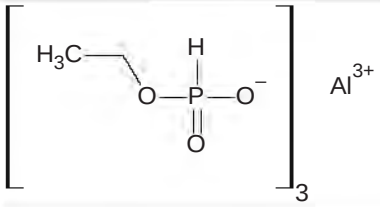
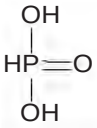
Appendix D – Input values for the exposure calculations

D.1. Consumer risk assessment

Commodity	Chronic risk assessment	
	Input value (mg/kg)	Comment
Risk assessment residue definition: phosphonic acid and its salts, expressed as phosphonic acid		
Chards/beet leaves	15	STMR-RAC
Honey	10.37	STMR-RAC
Grapefruits Oranges	17.11	STMR-RAC (23.44 mg/kg, potassium phosphonates, tentative; EFSA, 2021c) × PeF (0.73; EFSA, 2021d)
Lemons Limes Mandarins	17.11	STMR-RAC (23.44 mg/kg, potassium phosphonates; EFSA, 2021c) × PeF (0.73; EFSA, 2021d)
Other commodities of plant or animal origin	Input values derived from the joint review of maximum residue levels (MRLs) for fosetyl, disodium phosphonate and potassium phosphonates according to Articles 12 and 43 of Regulation (EC) No 396/2005 (see Appendix D.2; EFSA, 2021c).	

STMR-RAC: supervised trials median residue in raw agricultural commodity; PeF: peeling factor.

Appendix E – Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/ InChiKey[b]	Structural formula[c]
Potassium hydrogen phosphonate	potassium hydrogen phosphonate [K+].O[PH]([O-])=O GNSKLFRGEWLPPA-UHFFFAOYSA-M	
Dipotassium phosphonate	Dipotassium phosphonate [K+].[K+].[O-][PH]([O-])=O OZYJVQJGKRFVHQ-UHFFFAOYSA-L	
Fosetyl	ethyl hydrogen phosphonate O = P(O)OCC VUERQRKTYBIULR-UHFFFAOYSA-N	
Fosetyl-Al Fosetyl aluminium	aluminium tris(ethyl phosphonate) [Al+3].[O-]P(=O)OCC.[O-]P(=O)OCC.[O-]P(=O)OCC ZKZMJOFIHHZSRW-UHFFFAOYSA-K	
Phosphonic acid Phosphorous acid	phosphonic acid O = P(O)O ABLZXFCXXLZCGV-UHFFFAOYSA-N	

IUPAC: International Union of Pure and Applied Chemistry; SMILES: simplified molecular-input line-entry system; InChiKey: International Chemical Identifier Key.
(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2020.2.1 ACD/Labs 2020 Release (File version N15E41, Build 116563, 15 June 2020).
(c): ACD/ChemSketch 2020.2.1 ACD/Labs 2020 Release (File version C25H41, Build 121153, 22 March 2021).

Honey

Safety Data Sheet

according to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations Revision date: 01/3/2022 Supersedes: 0M/7/2021 Version: 1.

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1. Product Identifier

Product form: Substance

Substance name: Honey

CAS No.: 8028-66-8

1.2. Intended Use Of The Product

Use of the substance/preparation: Food grade

1.3. Name, Address, And Telephone Of The Responsible Party

Company:
Dutch Gold Honey
2220 Dutch Gold Drive
Lancaster, PA 17601
717-393-1716
www.dutchgoldhoney.com

Division:
McLure's of New England
46 North Littleton Road
Littleton, NH 03561

1.4. Emergency telephone number

Emergency number : 717-393-1716

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

Classification (GHS-US)

Not classified

2.2. Label elements

GHS-US labeling

No labeling applicable

2.3. Other hazards

Other hazards not contributing to the classification: Food product- may cause an allergic reaction in sensitive individuals.

2.4. Unknown acute toxicity (GHS US)

No data available

SECTION 3: Composition/information on ingredients

3.1. Substances

Name	Product identifier	%	Classification (GHS-US)
Honey	(CAS No.) 8028-66-8	100	Not classified

3.2. Mixtures

Not applicable

SECTION 4: First aid measures

4.1. Description of first aid measures

First-aid measures after inhalation: Not expected to present a significant inhalation hazard under anticipated conditions of normal use.

First-aid measures after skin contact: Gently wash with plenty of soap and water.

First-aid measures after eye contact: Rinse cautiously with water for several minutes.

First-aid measures after ingestion: This product is intended for food use. Ingestion is not expected to be harmful.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/injuries after inhalation: Not expected to present a significant inhalation hazard under conditions of normal use.

Symptoms/injuries after skin contact: None expected under normal conditions of use.

Symptoms/injuries after eye contact: May cause slight irritation to eyes.

Symptoms/injuries after ingestion: This product is intended for food use. Ingestion is not expected to be harmful.

4.3. Indication of any immediate medical attention and special treatment needed

No additional information available

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media: Use extinguishing media appropriate for surrounding fire.

Unsuitable extinguishing media: None known.

5.2. Special hazards arising from the substance or mixture

Fire hazard: Not considered flammable but may burn at high temperatures.

Explosion hazard: Product is not explosive.

Reactivity: Hazardous reactions will not occur under normal conditions.

5.3. Advice for firefighters

Protection during firefighting: Use normal individual fire protective equipment.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures: Avoid all contact with skin, eyes, or clothing.

Protective equipment: Not required for normal conditions of use.

6.2. Environmental precautions No additional information available

6.3. Methods and material for containment and cleaning up

Methods for cleaning up: Flush surfaces with hot water to clean. Collect by means of a non-combustible absorbent material.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Hygiene measures: Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for safe storage, including any incompatibilities

Storage conditions: Avoid temperature extremes.

7.3. Specific end use(s) Food grade.

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

No Occupational Exposure Limits (OELs) have been established for this product or its chemical components.

8.2. Exposure controls

Appropriate engineering controls	:	Not generally required. Site-specific risk assessments should be conducted to determine the appropriate exposure control measures. If applicable, use process enclosures, local exhaust ventilation, or other engineering controls to maintain airborne levels below recommended exposure limits.
Personal protective equipment	:	Not generally required. The use of personal protective equipment may be necessary as conditions warrant.
Hand protection	:	Not required for normal conditions of use.
Eye protection	:	Not required for normal conditions of use.
Skin and body protection	:	Not required for normal conditions of use.
Thermal hazard protection	:	If material is hot, wear thermally resistant protective gloves.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	:	Liquid
Appearance	:	Clear. Caramel. Amber.
Odor	:	Sweet. Floral.
Odor threshold	:	No data available
pH	:	3-7
Relative evaporation rate (butyl acetate=1)	:	No data available
Melting point	:	No data available
Freezing point	:	No data available

Boiling point	:	No data available
Flash Point	:	No data available
Auto-ignition temperature	:	No data available
Decomposition Temperature	:	No data available
Flammability (solid, gas)	:	No data available
Vapor pressure	:	No data available
Relative vapor density at 20 °C	:	No data available
Relative density	:	No data available
Solubility	:	No data available
Log Pow	:	No data available
Log Kow	:	No data available
Viscosity, kinematic	:	No data available
Viscosity, dynamic	:	No data available
Explosive properties	:	No data available
Oxidizing properties	:	No data available
Explosive limits	:	No data available

9.2. Other information No additional information available

SECTION 10: Stability and reactivity

Reactivity Hazardous reactions will not occur under normal conditions.

Chemical Stability Stable at standard temperature and pressure.

Possibility Of Hazardous Reactions Hazardous polymerization will not occur.

Conditions To Avoid Extremely high or low temperatures.

Hazardous Decomposition Products Carbon oxides (CO, CO2)

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity : Not classified

Skin corrosion/irritation: Not classified

Serious eye damage/irritation: Not classified

Respiratory or skin sensitization: Not classified

Germ cell mutagenicity: Not classified

Carcinogenicity: Not classified

Reproductive toxicity: Not classified

Specific target organ toxicity (single exposure): Not classified

Specific target organ toxicity (repeated exposure): Not classified

Aspiration hazard: Not classified

Symptoms/injuries after inhalation: Not expected to present a significant inhalation hazard under anticipated conditions of normal use.

Symptoms/injuries after skin contact: None expected under normal conditions of use.

Symptoms/injuries after eye contact: May cause slight irritation to eyes.

Symptoms/injuries after ingestion: This product is intended for food use. Ingestion is not expected to be harmful.

SECTION 12: Ecological information

12.1. Toxicity No additional information available

12.2. Persistence and degradability No additional information available

12.3. Bioaccumulative potential No additional information available

12.4. Mobility in soil No additional information available

12.5. Other adverse effects No additional information available

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Waste disposal recommendations: Dispose of waste material in accordance with all local, regional, national, and international regulations.

SECTION 14: Transport information

In accordance with ICAO/IATA/DOT/TDG

14.1. UN number Not regulated for transport

14.2. UN proper shipping name Not regulated for transport

14.3. Additional information

Other information : No supplementary information available.

Overland transport No additional information available

Transport by sea No additional information available

Air transport No additional information available

SECTION 15: Regulatory information

15.1. US Federal regulations Neither this product nor its chemical components appear on any US federal lists.

15.3. US State regulations Neither this product nor its chemical components appear on any US state lists.

SECTION 16: Other information

Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200.

This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.

SDS US (GHS HazCom) - US Only