



# 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 (ChemIDplus)

**91052-92-5:** Extract of honey; Honey extract; EINECS 293-255-4 (ChemIDplus)

### *1.3. Molecular formula*

Unspecified (ChemIDplus)

### *1.4. Structural Formula*

No data available to us at this time.

### *1.5. Molecular weight (g/mol)*

No data available to us at this time.

### *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*

No data available to us at this time.

### *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 an emollient, humectant and moisturising agent; honey extract (CAS RN 91052-92-5) is used as a flavouring, humectant and skin conditioning agent; Mel extract (CAS RNs "8026-66-8" [this CAS RN is not valid] and 91052-92-5) is used as a moisturising agent; and Mel powder (CAS RNs "8026-66-8" [this CAS RN is not valid] and 91052-92-5) is used as an abrasive, depilatory, bulking, binding and flavouring agent. As taken from CosIng (Cosmetic substances and ingredients database). Accessed February 2018, available at <http://ec.europa.eu/growth/tools-databases/cosing/>

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, and honey extract (CAS RN 91052-92-5) as an ingredient in an auto product, by the US Department of Health and Human Services (2017).

"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 IFRA (2016).

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Specific Agents by Occupation\*

Honey (CAS RN 8028-66-8)

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
<a href="#">095</a>	REGISTERED NURSES	3,999	3,875
<a href="#">444</a>	MISCELLANEOUS FOOD PREPARATION OCCUPATIONS	482	301
<a href="#">447</a>	NURSING AIDES, ORDERLIES, AND ATTENDANTS	597	597
<a href="#">449</a>	MAIDS AND HOUSEMEN	890	463
<a href="#">453</a>	JANITORS AND CLEANERS	445	
<a href="#">547</a>	SPECIFIED MECHANICS AND REPAIRERS, N.E.C.	356	
<a href="#">637</a>	MACHINISTS	880	
<a href="#">687</a>	BAKERS	469	
<a href="#">688</a>	FOOD BATCHMAKERS	709	124
<a href="#">754</a>	PACKAGING AND FILLING MACHINE OPERATORS	320	320
<a href="#">756</a>	MIXING AND BLENDING MACHINE OPERATORS	998	
<a href="#">777</a>	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	1,295	1,117

<a href="#">889</a>	LABORERS, EXCEPT CONSTRUCTION	489	
TOTAL		11,928	6,797

Honey, powdered (CAS RN 8028-66-8)

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
<a href="#">453</a>	JANITORS AND CLEANERS	350	
<a href="#">633</a>	SUPERVISORS, PRODUCTION OCCUPATIONS	500	
<a href="#">637</a>	MACHINISTS	250	
<a href="#">754</a>	PACKAGING AND FILLING MACHINE OPERATORS	500	
<a href="#">768</a>	CRUSHING AND GRINDING MACHINE OPERATORS	1,816	
<a href="#">888</a>	HAND PACKERS AND PACKAGERS	2,251	2,251
TOTAL		5,667	2,251

\*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH, available at <https://web.archive.org/web/20111024125131/http://www.cdc.gov/noes/noes2/x5654occ.html> and <https://web.archive.org/web/20111028113756/http://www.cdc.gov/noes/noes2/x9109occ.html>

## 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)

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

### 3. Status in legislation and other official guidance

Honey, ext. (CAS RN 91052-92-5) is pre-registered under REACH (“envisaged registration deadline 30 November 2010”).

Honey (CAS RN 8028-66-8) is pre-registered under REACH (“envisaged registration deadline 31 May 2013”).

As taken from ECHA, 2018a.

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, 2018b)

Honey appears on the list of “Permitted Additives to Tobacco Products in the United Kingdom” (Department of Health, 2003) at a maximum level permitted for inclusion in cigarettes/RYO, cigars and pipe tobacco of 4% w/w tobacco.

Honey (CAS RN 8028-66-8) is listed in the US EPA Inert Finder Database (2018) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950a (Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2018).

Honey (CAS RN 8028-66-8) and honey extract (CAS RN 91052-92-5) are listed in the New Zealand Inventory of Chemicals and may be used as components in a product covered by a group standard but are not approved for use as chemicals in their own right (NZ EPA, 2006).

## 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

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### 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 ( $5.08 \pm 0.92$  mg QE/100 g) and phenolics ( $5.40 \pm 0.69$  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  $0.23 \times 10^6$  cells/ $\mu\text{L}$ , WBC count was  $2.3$ ,  $1.21$ , and  $1.34 \times 10^3$  cells/ $\mu\text{L}$  and platelet count was  $57.6$ ,  $53.3$ , and  $66.35 \times 10^3$  cells/ $\mu\text{L}$  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 chronic illnesses." As taken from Soylu M. 2018. Biochemistry 9(2), 20253-20258. Available at <http://www.ijcrr.in/index.php/ijcrr/article/view/453>

## 5. Toxicity

### 5.1. Single dose toxicity

Toxicant	LD50	Animal	Route
Acetyl andromedol	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
Desacetyl pieristoxin 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 (Intermediary) effect inflammation mediation inflammation	- - - on or of FTRPAE Fitoterapia. (Inverni della Beffa SpA, via Ripamonti, 99, 20141 Milan, Italy) V.18- 1947- Volume(issue)/page/year: 81,1196,2010
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	60 mg/kg	Biochemical Metabolism (Intermediary) effect inflammation mediation inflammation	- - on or of 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 inflammation mediation inflammation	- - - on or of 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 inhibition, induction, or change in blood or tissue levels - other oxidoreductases Biochemical - Metabolism (Intermediary) - lipids including transport	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- 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.

## 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<sub>4</sub>-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>

### 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 ( $P < \text{or} = .01$ ). 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% ( $P < \text{or} = .05$ ). The activity of testicular marker enzymes for spermatogenesis such as sorbitol dehydrogenase (SDH) was increased by 31% ( $P < \text{or} = .05$ ), and lactate dehydrogenase (LDH) was reduced by 48% ( $P < \text{or} = .05$ ), 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).	Mutation	With S9	-ve (for all seven honeys) All honeys were	Wang et al. 2002

	Also antimutagenicity study with a known mutagen (Trp-P-1)		antimutagenic [Limited study: guidelines recommend the use of at least 4 strains, with and without S9]	
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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 et al., 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,  $\gamma$ ) and chemical (ethylmethane sulfonate) mutagens as ascertained by *rpoB/RifR* and Ames tests. The effect of honey was evaluated in radiation (UV or  $\gamma$ ) 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  $\mu$ m) 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 or  $\gamma$ ) exposed *Escherichia coli* cells 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 as *umuC*, *recA*, and *umuD* involved 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 ( $5.08 \pm 0.92$  mg QE/100 g) and phenolics ( $5.40 \pm 0.69$  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 ( $78.0 \pm 1.7$ ;  $P \leq 0.05$ ). 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>

## 5.5. Cytotoxicity

Record for Tualang honey (no CAS RN):

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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

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 anticancer 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-L1 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- $\alpha$ , interleukin-1(IL-1) and interleukin-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-) 1 $\beta$  and IL-6 [ 43, 44]. The possible mechanism involves the binding of TNF-R to TNF- $\alpha$  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- $\alpha$  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 in *hepatocellular 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  $\beta$ -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 % CO<sub>2</sub> 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 IC<sub>50</sub> 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  $1.3 \pm 0.042$ mMTE/g and H2 measuring  $1.122 \pm 0.018$ mMTE/g.

MDA levels were significantly reduced by honey samples, especially H4 ( $20.819 \pm 0.63$  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  $\mu\text{g/mL}$ ) 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  $\cong$  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  $\mu\text{g/mL}$ ) ( $P < 0.0001$ ). Similarly, Cedar honey was observed to be the second most effective honey in this study. Cedar honey, with the dose of 1  $\mu\text{g/mL}$ , 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  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$ . It only showed cytotoxicity with higher doses (10  $\mu\text{g/mL}$ ) 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 ( $P > 0.05$ ). 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 ( $r = -0.67$ ) and phenolic compounds ( $r = -0.61$ ). 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 physicochemical 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 ( $\mu\text{g/ml}$ ) 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>

### 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( $\alpha$ )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 ( $\leq 2 \text{ cm}^3$ ) compared to Controls ( $\leq 8 \text{ cm}^3$ ). 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 from 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  $1.33\pm 0.00$ - $155.16\pm 0.98$ mg/100g of TPC, 50% 2,2-diphenyl picryl hydrazyl (DPPH) inhibition,  $>7.36\pm 0.43$ - $39.86\pm 2.34$ mg/100g quercetin equivalent antioxidant contents,  $>13.69\pm 0.91$ - $65.50\pm 1.37$ mg/100g ascorbic acid equivalent antioxidant contents,  $64.65\pm 0.43$ - $1780.74\pm 11.79$ mM 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  $87.50\pm 5.50\%$  and  $79.00\pm 5.56\%$  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 of honey 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 that honey 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- $\gamma$ , IFNGR1, and p53) and decreases the expression of antiapoptotic proteins (TNF- $\alpha$ , 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 heterogeneous 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- $\beta$ ), interferon gamma (IFN- $\gamma$ ), interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF- $\alpha$ ) 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- $\beta$ ), 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 (Kitty 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 wildflower honey. 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 for honey 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-1 $\beta$ , IL-6, and TNF- $\alpha$  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  $2.49 \pm 3.02$  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), O<sub>2</sub> saturation at room air (SPO<sub>2</sub>), dyspnoea, use of accessory respiratory muscles and chest wheezes. **RESULTS** There was a significant increase of SPO<sub>2</sub> 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  $0.23 \times 10^6$  cells/ $\mu$ L, WBC count was 2.3, 1.21, and  $1.34 \times 10^3$  cells/ $\mu$ L and platelet count was, 57.6, 53.3, and  $66.35 \times 10^3$  cells/ $\mu$ L 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  $1.3 \pm 0.042$  mMTE/g and H2 measuring  $1.122 \pm 0.018$  mMTE/g. MDA levels were significantly reduced by honey samples, especially H4 ( $20.819 \pm 0.63$  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 intraperitoneally 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>

### 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
<i>In vitro</i> genotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)
<i>In vitro</i> 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; delirium, 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](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 honey impaired 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 from Banaeian-Borujeni S et 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 honey affected 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 ( $10 \times 10^8$  cfumcl(-1)). All assays were repeated 10 times for each of the honey concentrations. 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 Manuka honey 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 H<sub>2</sub>O<sub>2</sub>) 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 H<sub>2</sub>O<sub>2</sub> 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  $630.89 \pm 5.21$  GAE kg<sup>-1</sup> to  $718.92 \pm 4.41$  GAE kg<sup>-1</sup>, while the flavonoids varied between  $450.72 \pm 5.67$  CAE kg<sup>-1</sup> and  $673.98 \pm 4.33$  CAE kg<sup>-1</sup>. For the bacteria, the minimum inhibitory concentration (MIC) of the honey without catalase ranged from  $1.01 \pm 0.50\%$  to  $10.00 \pm 4.72\%$  and was between  $2.00 \pm 0.94\%$  and  $13.27 \pm 5.23\%$  for honey with catalase. Concerning the yeasts, the MICs was between  $13.16 \pm 4.08\%$  and  $20.00 \pm 5.09\%$  for honey without catalase and between  $14.95 \pm 4.16\%$  and  $25.67 \pm 5.50\%$  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 of honey. The honeys were tested at four concentrations: 75%, 50%, 10% and 1% (v/v) and compared with an untreated control. The composition of honey was determined by measuring: polyphenol content by Folin Ciocalteu method, antioxidant capacity by ferric ion reducing power assay, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) 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 H<sub>2</sub>O<sub>2</sub>. 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  $39.949 \pm 0.020$  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  $1.3 \pm 0.042$  mMTE/g and H2 measuring  $1.122 \pm 0.018$  mMTE/g. MDA levels were significantly reduced by honey samples, especially H4 ( $20.819 \pm 0.63$  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 ( $\alpha = 0,05$ ), 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. 2018. 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 melissopalinalogical 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  $\beta$ -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. Epub ahead of print. Available at <https://www.sciencedirect.com/science/article/pii/S1319562X18300469>

## **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 Black 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 honey intoxication, 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 mad honey intoxication 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 mad honey poisoning were recruited. Mean age was 52.2 ( $\pm 17.2$ ). 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 ( $\pm 52.9$ ) mg/dL, vs. 120.7 ( $\pm 23.0$ ) mg/dL in those with normal or high SBP ( $p = 0.389$ ). Mean glucose level in patients with low DBP was 118.7 ( $\pm 51.4$ ) mg/dL, compared to 96.0 ( $\pm 22.8$ ) 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: Mad honey was 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 mad honey 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 threatening 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 honey treatments 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 taken 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 honey treatment 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*. Honey has 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 Leishmaniasis, 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:  $917.8 \pm 234.6$  mL/min vs artificial honey:  $973.5 \pm 373.8$  mL/min). Ten days of honey administration did not change partial metabolic clearance (honey:  $1016 \pm 268$  mL/min vs artificial honey:  $1043 \pm 450$  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 =  $19 \pm 10\%$  and  $62 \pm 10\%$ ; PT  $6 \pm 5\%$  and  $40 \pm 5\%$ ; TT  $35 \pm 15\%$  and  $112 \pm 30\%$  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  $45 \pm 21\%$ ,  $26 \pm 9\%$  and  $105 \pm 24\%$  respectively. Interestingly, incubation of honey at 6.25% and 11.75% concentrations in PPP considerably ( $P \geq 0.005$ ) reduced fibrinogen levels i.e.  $13 \pm 4\%$  and  $86 \pm 30\%$  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 from Erejuwa OO (2014). Effect of honey in 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- $\alpha$ , interleukin-1 $\beta$ , 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- $\alpha$ , IL-1 $\beta$ , 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- $\alpha$ , IL-1 $\beta$ , 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- $\alpha$ , IL-1 $\beta$ , 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- $\alpha$ , IL-1 $\beta$ , 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
<i>In vitro</i> genotoxicity	45,400 (8028-66-8)	Baker et al., 2004c
	35 (absolute, 91052-92-5)	
	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
<i>In vitro</i> cytotoxicity	45,400 (8028-66-8)	Baker et al., 2004c
	35 (absolute, 91052-92-5)	
	10,000 (8028-66-8)	JTI KB Study Report(s)
	2,300 (8028-66-8)	fGLH Study Report (2010)
		Gaworski et al., 2011 &

	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)	Baker et al., 2004c
	35 (absolute, 91052-92-5)	
	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
	1000 (8028 66 8)	

<i>In vitro</i> genotoxicity	1000 (0020-00-0) 13 (91052-92-5)	JTI KB Study Report(s)
<i>In vitro</i> cytotoxicity	1888 (8028-66-8) 13 (91052-92-5)	JTI KB Study Report(s)

## 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>

## 11. References for conventional products

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### **13. Last audited**

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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.

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

**Requestor:** European Commission

**Question number:** EFSA-Q-2016-00571

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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.

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## 1. Introduction

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

Regulation (EC) No 1107/2009<sup>1</sup> (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.

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<sup>1</sup> 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 <sub>50</sub>	lethal concentration, median
LD <sub>50</sub>	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	<p>DE: Please see 5.13: "acceptable daily intake for humans: less than 5 g of honey from Rhododendron with grayanotoxin". "The oral LD<sub>50</sub> for mice is approx. 1 mg/kg...", please compare to parathion LD<sub>50</sub> (mice) approx. 5-25 mg/kg. Therefore, the substance applied for is a substance of concern, this honey cannot be considered as food.</p> <p>DE: The introduction implicates as if the toxin Grayanotoxin would have been proven to be "an essential use" for fruit production.</p>	<p>Honey from rhododendron as described in the application is a substance of concern, it does not meet the criteria of a basic substance.</p> <p>The applicant should clarify that he speculates about the desired effects or cite public available data or own experimental reports about it</p>	<p>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<sub>50</sub> (mice) is approx. 3-5 mg/kg due to the different grayanotoxin versions I-VIII with individual potency levels. In literature the LD<sub>50</sub> (mice/grayanotoxin I) is described at approx. 5,1 mg/ kg body weight.</p> <p>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.</p>	<p>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).</p>
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 <b>small insects like ants can't</b> 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 <b>sentence</b> " <i>Also for other diseases, honey from rhododendron with grayanotoxins may be a plant protection product</i> "		Baits are included in plant protection product (PPP) regulation.  <b>BSA corrected: "diseases"</b> 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

<b>General</b>					
<b>No.</b>	<b>Column 1 Reference to Application Template</b>	<b>Column 2 Comments from Member States / EFSA</b>	<b>Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment</b>	<b>Column 4 Follow up response from applicant</b>	<b>Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application</b>
		<i>with perspectives.</i> " 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 <sub>50</sub> value for mice specified by the applicant concerns the intraperitoneal route of administration not oral. Oral LD <sub>50</sub> 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 <sub>50</sub> 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 <sub>50</sub> ) 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...	<p>DE: Rhododendron honey is <b>known in Turkey as "Mad Honey" because of its toxic effect.</b></p> <p>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 be recommended for human consumption due to its content of toxins.</p> <p>The intended concentration of Grayanotoxins in the self-produced honey cover a</p>	<p>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.</p> <p>Please give proof that the achieved content of toxins (up to 150 mg/kg) is acceptable for a substance of no concern.</p>	<p>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.</p> <p>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.</p>	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
		<p>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?</p>		<p>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)</p>	
2(3)	2.2.5	<p>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.</p>		<p>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.</p>	<p>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.</p>

<b>2.1. Identity and Physical and chemical properties of the substance and product to be used</b>					
<b>No.</b>	<b>Column 1 Reference to Application Template</b>	<b>Column 2 Comments from Member States / EFSA</b>	<b>Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment</b>	<b>Column 4 Follow up response from applicant</b>	<b>Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application</b>
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 <b>honey it's a surplus.</b> 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 efficacy 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	<p>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.</p> <p>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.</p>		<p>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.</p> <p>Typical food stuff, no brand name except "Rhododendron Honey" or "Honey from Rhododendron" (made by Klaus Gasser + Partner for example)</p> <p>Its usage consists of the honey from rhododendron inside a bait box. The bait</p>	<p>The toxicity of the grayanotoxin makes honey from rhododendron unsuitable for human consumption and therefore cannot be classified as foodstuff.</p> <p>See Section 5</p>

**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 <b>animals and even ants can't</b> 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 <b>the traps anymore. That's a contradiction</b> 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 <sub>50</sub> 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 <sup>2</sup> 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.

<sup>2</sup> 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 <sub>50</sub>		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 <sub>50</sub> 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 <sub>50</sub> 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 grayanotoxin 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 3	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 ( <a href="https://en.wikipedia.org/wiki/Grayanotoxin">https://en.wikipedia.org/wiki/Grayanotoxin</a> ).		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 ( <a href="https://en.wikipedia.org/wiki/Grayanotoxin">https://en.wikipedia.org/wiki/Grayanotoxin</a> ) 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 threatening 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**

<b>No.</b>	<b>Column 1 Reference to Application Template</b>	<b>Column 2 Comments from Member States / EFSA</b>	<b>Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment</b>	<b>Column 4 Follow up response from applicant</b>	<b>Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application</b>
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 <b>can't leave the bait box even if it is raining the honey can't leave the bait box.</b>	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 <b>even ants can't enter the bait box</b> 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	<p>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.</p> <p>According to Article 23(2), a basic substance shall be <b>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"</b>. NL acknowledges that by the use of the substance in a bait box there is no further</p>	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.	<p>The solution of packaged honey in dosages placed in bait box is for any type of mice.</p> <p>It is recommended to place bait boxes around mice holes, especially if two or more holes are closed together.</p>	<p>The target species were not defined. More information on this point is needed.</p> <p>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.</p>

**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
		<p>exposure of the environment, birds, aquatic environment, non-target arthropods, bees, soil organisms and plants. The <b>"mice" are not really defined</b> 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?</p> <p>The voles in general to not have <b>the definition as "pest</b></p>		<p>If mice would die outside box (which is not the case, <b>because mice can't leave the bait box</b>) GTX is consumed and metabolized therefore secondary poisoning is not expected. Furthermore quantities for mice are largely lower than these for higher animals.</p>	

**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
		<p>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.</p>			
8(3)	8. Effects on non-target species	<p>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 <b>that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry',</b> 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).</p>	<p>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 <b>in baits when it's not raining</b> and when the ground and soil are dry.</p>	<p>BSA application updated. Even if it is raining honey <b>can't leave the bait box and water can't touch honey in</b> the bait box. Honey is packaged in nylon, plastic, bio degradable plastic or bio plastic, paper, capsules of gelatin.</p>	<p>Addressed. Applicant has clarified that the basic substance cannot leave the bait box even during rainfall events.  See also 6(1) and 7(1).</p>

### 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 <b>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.</b>	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 <b>capsules in baits when it's not raining and when the ground and soil are dry.</b>	BSA application updated. <b>Even if its raining honey can't leave the bait box and water can't touch honey in the bait box.</b> 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
		<p>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) from EFSA in relation to potential indirect poisoning of predators. .</p>	<p>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.</p>	<p>nylon, plastic, bio degradable plastic or bio plastic, paper, capsules of gelatin.</p>	<p>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)</p>

### 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)		<p>DE: The applicant wrote: "The substance is not expected to</p>	<p>The applicant should correct the sentence: "The substance is</p>	<p>BSA application updated. It is not relevant since trap baits</p>	<p>Addressed</p>

**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 <b>not exist! Bees can't enter the bait box.</b>	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 <b>'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry'</b> , 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) from EFSA in relation to potential indirect poisoning of predators.	Applicant to clarify the reason why it is recommended to use <b>capsules in baits when it's not raining</b> 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 <b>that 'it is recommended to use capsules in baits when it's not raining and when the ground and soil are dry'</b> , 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 <b>capsules in baits when it's not raining</b> 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 <sup>3</sup> .		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)

<sup>3</sup> 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

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

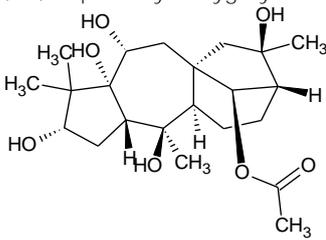
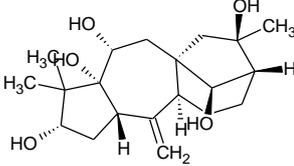
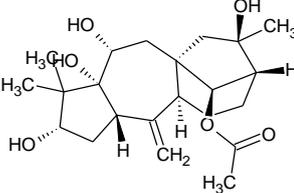
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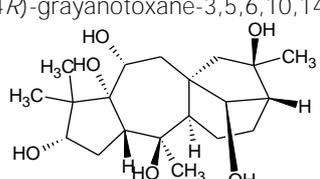
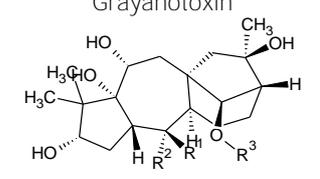
No.	Column 1 Reference to Application Template	Column 2 Comments from Member States / EFSA	Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment	Column 4 Follow up response from applicant	Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application
		<p>excitatory and inhibitory nerve terminals in rat ventromedial hypothalamic neurons. Neurotoxicology 31, 230-238</p> <p>10. Hikino H. et al. (1979): Subchronic toxicity of ericaceous toxins and rhododendron leaves. Chem. Pharm. Bull. 27, 874-879</p> <p>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</p> <p>12. Kobayashi T. et al. (1990): Developmental toxicity potential of grayanotoxin I in mice and chicks. J. Toxicol. Sci. 15, 227-234</p>			
10(4)		<p>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</p>	<p>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</p>	<p>BSA application updated</p>	<p>See 10(2)</p>

**Other comments**

<b>No.</b>	<b>Column 1 Reference to Application Template</b>	<b>Column 2 Comments from Member States / EFSA</b>	<b>Column 3 Proposal by Member States/EFSA on how the application should be updated to address the comment</b>	<b>Column 4 Follow up response from applicant</b>	<b>Column 5 EFSA's scientific views on the specific points raised in the commenting phase conducted on the application</b>
		<p>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.</p> <p>It is noted that additional references were provided by PL.</p>	<p>under Regulation (EC) No 1107/2009.</p> <p>The additional references indicated by PL should be considered further.</p>		

## Appendix B – Identity and biological properties

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

	<p>O)C1(C)C(=C)[C@@H]4CC[C@H]2[C@](C)(O)C3</p> <p>Grayanotoxin IV (3<math>\beta</math>,6<math>\beta</math>,14<i>R</i>)-grayanotoxane-3,5,6,10,14,16-hexol</p>  <p>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</p> <p>Grayanotoxin</p>  <table border="1" data-bbox="638 828 1404 985"> <thead> <tr> <th>Grayanotoxin</th> <th>R<sup>1</sup></th> <th>R<sup>2</sup></th> <th>R<sup>3</sup></th> </tr> </thead> <tbody> <tr> <td>Grayanotoxin I</td> <td>OH</td> <td>CH<sub>3</sub></td> <td>Ac</td> </tr> <tr> <td>Grayanotoxin II</td> <td></td> <td>CH<sub>2</sub></td> <td>H</td> </tr> <tr> <td>Grayanotoxin III</td> <td>OH</td> <td>CH<sub>3</sub></td> <td>H</td> </tr> <tr> <td>Grayanotoxin IV</td> <td></td> <td>CH<sub>2</sub></td> <td>Ac</td> </tr> </tbody> </table> <p>Ac =acetyl</p>	Grayanotoxin	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Grayanotoxin I	OH	CH <sub>3</sub>	Ac	Grayanotoxin II		CH <sub>2</sub>	H	Grayanotoxin III	OH	CH <sub>3</sub>	H	Grayanotoxin IV		CH <sub>2</sub>	Ac
Grayanotoxin	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>																		
Grayanotoxin I	OH	CH <sub>3</sub>	Ac																		
Grayanotoxin II		CH <sub>2</sub>	H																		
Grayanotoxin III	OH	CH <sub>3</sub>	H																		
Grayanotoxin IV		CH <sub>2</sub>	Ac																		
<b>Mode of Use</b>	Mice baits boxes																				
<b>Preparation to be used</b>	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.																				
<b>Function of plant protection</b>	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	



# Honey

## Safety Data Sheet

according to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

Revision date: 06/ 23/2017

Supersedes: 04/02/2013

Version: 1. 0

### 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](http://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.

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### 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

# Honey

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<b>Boiling point</b>	: No data available
<b>Flash Point</b>	: No data available
<b>Auto-ignition temperature</b>	: No data available
<b>Decomposition Temperature</b>	: No data available
<b>Flammability (solid, gas)</b>	: No data available
<b>Vapor pressure</b>	: No data available
<b>Relative vapor density at 20 °C</b>	: No data available
<b>Relative density</b>	: No data available
<b>Solubility</b>	: No data available
<b>Log Pow</b>	: No data available
<b>Log Kow</b>	: No data available
<b>Viscosity, kinematic</b>	: No data available
<b>Viscosity, dynamic</b>	: No data available
<b>Explosive properties</b>	: No data available
<b>Oxidizing properties</b>	: No data available
<b>Explosive limits</b>	: 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, CO<sub>2</sub>)

## 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.

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### 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