European Food Safety Authority

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# Beeswax (E 901) as a glazing agent and as carrier for flavours[1]

## Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC)

**(Question No EFSA-Q-2006-021)**

**Adopted on 27 November 2007**

**PANEL MEMBERS**[*]

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**SUMMARY**

Following a request from the Commission, the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC), was asked to update the opinion of the Scientific Committee on Food of the European Commission (SCF) on the safety in use of beeswax and to consider also in its opinion the additional use of beeswax as a carrier of flavours. Beeswax was evaluated by the SCF in 1990, which concluded that because of the paucity of experimental toxicological data it was unable to establish the full safety of this compound but considered that its use as glazing agent was temporarily acceptable.

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

Beeswax is a complex mixture of saturated and unsaturated linear and complex monoesters, hydrocarbons, free fatty acids, free fatty alcohols, and other minor substances produced by the worker honeybee.

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The Panel noted that experimental biochemical and toxicological studies carried out specifically on beeswax were still lacking and considered that the data on beeswax itself were insufficient to establish an ADI. However, the Panel concluded that the safety of beeswax could be assessed, based on the available scientific literature on the main constituents of beeswax and plant waxes showing chemical structural similarities to beeswax, published since the last SCF evaluation.

The conservative exposure estimate of 1290 mg beeswax/person per day calculated in this opinion corresponds to an exposure approaching 22 mg beeswax /kg bw/day for a 60 kg individual. The Panel considered this estimate to be very conservative as it was based on the assumptions that a person would consume all the proposed foods and tablets or capsules at the 95$^{th}$ percentile and that beeswax would be used in the proposed applications at the maximum usage level. In addition, the data on soft drink consumption used were obtained from a two day survey, which may overestimate the higher percentiles more than a more realistic seven day survey will do.

The Panel concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not of safety concern. The Panel noted that NOAELs identified in the toxicological studies on the main constituents of beeswax and plant waxes showing chemical structural similarities were 10 to 50 times higher than the very conservative exposure estimate of 22 mg/kg body weight (bw)/day and were generally the highest doses tested. The Panel considered such margins of safety to be adequate for the assessment of beeswax which consists of components poorly absorbed from the gastrointestinal tract, which if absorbed to any extent at all, would be metabolised to compounds also occurring endogenously.

The Panel examined information on the presence of varroacide residues in beeswax and found that the active varroacide substances most often found in European samples of beeswax are fluvalinate, coumaphos and bromopropylate. The Panel noted that veterinary medicine residues and contaminants found in foodstuffs of animal origin are regulated by specific European legislation.

The Panel noted that beeswax specifications for lead were set to 5 mg/kg in the European legislation and to 2 mg/kg by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Panel considers that the specification for lead levels should be set as low as possible.

**Key words:**

Beeswax, E901, food additive, glazing agent, carrier for flavour, CAS no. 8006-40-4, CAS no. 8012-89-3.

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## BACKGROUND AS PROVIDED BY THE COMMISSION

Beeswax identified as E 901 is an authorised food additive in the European Union (EC, 1995). It is permitted as a glazing agent following the *quantum satis* principle on confectionery (excluding chocolate), small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans and for the surface treatment only of certain fruits (fresh citrus fruits, melons, apples, pears, peaches and pineapples). It is also permitted in food supplements and as a carrier for colours. Specific purity criteria for beeswax are laid down in Directive 96/77/EC (as amended).

The Scientific Committee on Food of the European Commission (SCF) has previously evaluated the safety of beeswax in 1990 and found its use temporarily acceptable as a glazing agent.

## TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to provide an updated scientific opinion on the safety in use of beeswax. In addition, EFSA should issue an opinion on the use of beeswax as a carrier of flavours.

## ACKNOWLEDGEMENTS

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## ASSESSMENT

### 1. Chemistry

#### Beeswax

| | |
|---|---|
| Synonyms: | White wax, yellow wax. Others synonyms found in the literature are: comb wax, bee capping, yellow beeswax, white beeswax, beeswax bleached, bleached yellow wax. |
| CAS Registry Number: | 8006-40-4 (yellow beeswax)<br>8012-89-3 (white beeswax) |
| EINECS Number | 232-383-7 |
| EU purity criteria: | Yellow beeswax is the wax obtained by melting the walls of the honeycomb made by the honey bee, *Apis mellifera* L. with hot water and removing foreign material. White beeswax is obtained by bleaching yellow beeswax |
| Description | Yellowish white (white form) or yellowish to greyish brown (yellow form) pieces or plates with a fine-grained and non-crystalline fracture, having an agreeable, honey-like odour. |

### 2. Specifications in existing European legislation (EC 1996)

| | |
|---|---|
| Identification: | Melting range: between 62 ºC and 65 ºC |
| | Specific gravity ($D^{20}_{20}$): about 0,96 |
| | Solubility: Insoluble in water |
| | Sparingly soluble in alcohol |
| | Very soluble in chloroform and ether |
| Purity | Acid value (mg KOH/g beeswax): not less than 17 and not more than 24 |
| | Saponification (mg KOH/g beeswax): value 87-104 |
| | Peroxide value (mM $H_2O_2$/1000 g beeswax): not more than 5 |
| | Glycerol and other polyols: not more than 0,5 % (as glycerol) |
| | Ceresin, paraffins and certain other waxes: absent |
| | Fats, Japan wax, rosin and soaps: absent |
| | Arsenic: not more than 3 mg/kg |
| | Lead: not more than 5 mg/kg |
| | Mercury: not more than 1 mg/kg |

## 3. Manufacturing process

According to the petitioner, beeswax is obtained by melting the honeycombs of bees after removal of the honey by draining and filtering or centrifuging. The combs are melted in boiling water or steam. Melting in hot water, to which activated carbon and/or diatomaceous earth may be added to extract impurities, further refines the wax. The resulting wax obtained by pressure filtration is referred to as yellow beeswax (Technical dossier, 2006). Bleaching the natural constituent pigments of yellow beeswax with peroxides, by sunlight, bleaching earth, or activated carbon has been used to produce white beeswax (CFR, 2003; Technical dossier, 2006).

## 4. Biosynthesis and composition of beeswax

### 4.1. Biosynthesis

According to the EU specifications, beeswax is produced by the domestic worker bee *Apis mellifera* L. (EC, 1996). In other parts of the world beeswax may also be produced by *A. cerana* and *A. florae* as well as other honeybee species. Production takes place at wax gland complexes consisting of three cell types: epithelial cells, oenocytes and adipocytes, which act synergistically to secrete wax. The secretion of wax is a constant process and begins in 1 week old honey bee workers, peaks after 2 weeks of age and thereafter decreases (Cassier and Lensky, 1995).

### 4.2. Composition

Beeswax is a complex mixture of saturated and unsaturated linear and complex monoesters (see below), hydrocarbons, free fatty acids, free fatty alcohols and other minor exogenous substances [see section 4.7] (Aichholz and Lorbeer, 1999).

More than 300 individual components have been reported in beeswax from various species of honeybees (Tulloch, 1980). Although their concentrations may vary depending upon the honeybee species and the geographical origin, only small differences are observed in the concentration of individual components and substance classes (Aichholz and Lorbeer, 1999; Wolfmeier *et al*., 1996).

A total content of 27 to 40% monoesters, 9 to 23 % hydroxymonoesters, 7 to 16% diesters, 3.9 % hydroxydiesters, 11 to 28% hydrocarbons, 1 to 18% saturated, unbranched free fatty acids, 4 to 8% of other substances and < 0.3% free fatty alcohols have been reported in beeswaxes from different bees species (Aichholz and Lorbeer, 1999). A typical composition of beeswax from *A. mellifera* is shown in Table 1.

### 4.3. Fatty acid esters

Fatty acid monoesters constitute the most abundant compounds in beeswax with saturated alkyl palmitates (C38 – C52) and unsaturated alkyl esters of oleic acid (C46 – C54) constituting the predominant structures (Aichholz and Lorbeer, 1999; Tulloch, 1980). Hydroxymonoesters are long chain alcohols, esterified by a hydroxy acid (mainly 15-hydroxypalmitic acid) or a primary hydroxy group of a diol (mainly palmitic acid), whereas diesters and hydroxydiesters consist mainly of diol diesters and acylated hydroxyesters and of hydroxypalmitic acid esters

and palmitic acid diolesters acylated by hydroxypalmitic acid, respectively, in beeswaxes of different bees species (Aichholz and Lorbeer, 1999; Tulloch, 1980).

### 4.4. Hydrocarbons (*n*-alkanes and *n*-alkenes)

Odd chain *n*-alkanes ($C_{23}$ – $C_{31}$) constitute the predominant hydrocarbons in beeswax with heptacosane ($C_{27}$), nonacosane ($C_{29}$), hentriacontane ($C_{31}$), pentacosane ($C_{25}$) and tricosane ($C_{23}$) being reported as the most abundant in *A. mellifera* beeswax (Aichhloz and Lorbeer, 1999; Jiménez *et al.*, 2004). The most common alkenes in *A. mellifera* beeswax are odd chain alkenes ($C_{27}$-$C_{39}$) with a *cis* double bond at position $C_{10}$. However, the exact unsaturation status of hydrocarbons in beeswax is not clearly established (Aichholz and Lorbeer, 1999).

### 4.5. Free fatty acids

Free fatty acids in beeswax are unbranched saturated molecules with even carbon numbers from $C_{20}$ to $C_{36}$. Tetracosanoic acid ($C_{24}$) has been reported as the most abundant free fatty acid in *A. mellifera* beeswax (6%) (Aichholz and Lorbeer, 1999).

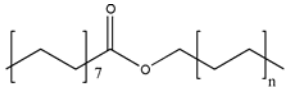
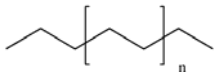
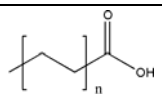
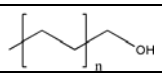
### 4.6. Free fatty alcohols

Free fatty alcohols with $C_{33}$ (0.3% - 1.8%) and $C_{35}$ (0.3%) alcohols have been identified in *A. mellifera*, *A. cerana* and *A. florea* beeswaxes (Aichholz and Lorbeer, 1999).

### 4.7. Other components and contaminants

Other components of beeswax identified are propolis (bee glue), flower and pollen pigments (Wolfmeier *et al.*, 1996). No quantitative information was found on these components.

Table 1. **Composition of *A. mellifera* beeswax (adapted from Aichholz and Lorbeer, 1999)**

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

m: only the structural formula of alkylesters of palmitic acid is shown as example

Bee products can be contaminated by compounds originating from beekeeping practices or environmental sources (Bogdanov, 2006). Beeswax is often contaminated by persistent

lipophilic acaricides (Jiménez *et al*., 2005; Bogdanov, 2006). Active synthetic acaricides such as cymiazol, fluvalinate, amitraz, flumethrin and coumaphos are ingredients of varroacides, used for long-term parasite control of *Varroa destructor* (Wallner, 1999; Bogdanov, 2006).

The active varroacide substances coumaphos, fluvanilate and bromopropylate constitute the main acaricides residues found in wax combs and commercial beeswax samples in some European countries. The potential varroacide residues accumulation in beeswax appears to be correlated with the quantities of the active ingredient used in the formulation and the number of applications during the year that can lead to higher wax residues. Acaricide levels detected in wax combs and wax cappings ranged from 0.05 to 16.40 mg/kg for coumaphos, from <0.01 to 15 mg/kg for fluvalinate and from 0.07 to 15 mg/kg for bromopropylate (Persano *et al*., 2003; Tsigouri *et al*., 2003; Jiménez *et al*., 2005; Wallner, 1999; Bogdanov ,2006).

Other contaminants reported in some samples of beeswax were the acaricides z-chlorfenvinphos (0.16 to 4.82 mg/kg) and amitraz (0.10 to 0.56 mg/kg) as well as minor amounts of lindane (0.042 to 0.29 mg/kg), and endosulfan sulphate (0.12 to 0.36 mg/kg) (Jiménez *et al*., 2005). Heavy metals contamination reported in beeswax in a few studies was summarised recently as follows: for Pb 0.06-6.2 mg/kg, for Cd 0.01-0.1 mg/kg and for Hg 0.0001-0.06 mg/kg (Bogdanov, 2006).

The Panel noted that veterinary medicine residues and contaminants found in foodstuffs of animal origin are regulated by specific European legislation. Maximum residue levels (MRLs) for some of the main active substances used in beekeeping practices have been established generally for foodstuffs of animal origin in the European Union (EC, 1990, 1986).

## 5. Existing authorisations and evaluations

Beeswax is an authorised food additive in the European Union identified as E 901 (EC, 1995). It is currently permitted as a glazing agent, according to the *quantum satis* principle, on confectionery (excluding chocolate), small products of fine bakery wares coated with chocolate, snacks, nuts and coffee beans and for the surface treatment of certain fruits (fresh citrus fruits, melons, apples, pears, peaches and pineapples). It is also permitted in food supplements and as a carrier for colours.

In the USA, beeswax (yellow and white) is affirmed as GRAS. It is used as a flavouring agent and adjuvant, as a lubricant, and as a surface-finishing agent. When used in food the levels should not exceed good manufacturing practice as a multipurpose ingredient. Current use levels in food result in concentrations of 0.065% for chewing gum, 0.005% for confectioneries and frostings, 0.04% for hard candy, 0.1% for soft candy and 0.002% or less for all other food categories (CFR, 2003).

The SCF evaluated the safety of beeswax in 1990 (SCF, 1992). The Committee reviewed acute toxicity studies, dermal toxicity studies in rats and rabbits, and local implantation studies in the cervix of mice. The Committee concluded that, *because of the paucity of experimental toxicological data* it *was unable to establish the full safety of this compound but considered the continued use as glazing agent at present levels temporarily acceptable until further toxicological data and technical data on use was provided.*

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated beeswax at its 65$^{th}$ meeting. This Committee evaluated additional biochemical and toxicological studies on the main components of beeswax (linear monoesters, complex esters, hydrocarbons, free fatty acids and free fatty alcohols) and data on the use of beeswax in water-based, flavoured drinks. The conclusion was that *current uses of beeswax, including that as a carrier for flavours and as a clouding agent in water-based drinks, would not result in dietary exposure that raised concern about safety, especially in view of the long history of use of beeswax and the absence*

*of toxicity of the main components.* The committee could not *reach a conclusion about the potential allergenicity of beeswax as the available information was very limited* (WHO, 2006a, b). The specifications for beeswax were revised (FAO, 2005).

From a chemical point of view, the beeswax hydrocarbon fraction in which chain lengths higher than $C_{25}$ account for more than 98% of total hydrocarbon content (Aichholz and Lorbeer, 1999), would correspond to class I mineral oils and high-melting point, microcrystalline waxes, characterised by a high content of $\geq C_{25}$ hydrocarbons as defined by JECFA and SCF (WHO, 2003; SCF, 1997). Therefore results of studies conducted on mineral oils are also considered in this opinion. An ADI of 10 mg/kg body weight (bw) and 20 mg/kg bw were established by JECFA for class I mineral oils and microcrystalline waxes, respectively. The SCF established a temporary Group ADI of 4 mg/kg bw for white paraffinic mineral oils derived from petroleum based hydrocarbon feed stocks and an ADI of 20 mg/kg bw for highly refined waxes derived from petroleum based or synthetic hydrocarbon feed stocks.

Carnauba wax is a complex mixture of compounds containing predominantly aliphatic, cinnamic, and hydroxycarboxylic acid esters, constituting around 80% (w/w) of the mixture (Wolfmeier *et al.*, 1996). Carnauba wax has been defined as being composed of linear-chain hydroxy acids with even–numbered carbon chains from $C_{22}$ to $C_{28}$, linear-chain acids with even–numbered carbon chains from $C_{24}$ to $C_{28}$, linear-chain monohydric alcohols with even–numbered carbon chains from $C_{24}$ to $C_{34}$ and dihydric alcohols with even-numbered carbon chains from $C_{24}$ to $C_{34}$ as well as cinnamic aliphatic diesters of p-methoxycinnamic acid and dihydric alcohols with even-numbered carbon chains from $C_{24}$ to $C_{34}$ (FAO, 1998). It also contains free linear-chain acids with even-numbered carbon chains from $C_{24}$ to $C_{28}$, free linear-chain alcohols with even-numbered carbon chains from $C_{30}$ to $C_{34}$, linear-chain hydrocarbons with odd-numbered carbon chains from $C_{27}$ to $C_{31}$ and resins, around 19% (w/w) (FAO, 1998, Wolfmeier *et al.,* 1996). From a chemical point of view, the fatty acids ester fraction in carnauba wax would correspond to beeswax fatty acid esters. Therefore, results of studies conducted on carnauba wax are also considered in this opinion. Carnauba wax has been evaluated previously by SCF (SCF, 1992, 2001) which concluded that its use as a glazing agent up to a maximum use level of 200 mg/kg of food was acceptable (SCF, 2001).

## 6. Case of need and proposed uses

Use of beeswax as a suspending agent is reported to be essential in the formulation of soft gelatine capsules for food supplements. In addition to the current permitted uses of beeswax (see below) a petitioner has also applied for its use as a carrier for flavours (intended for use in water-based, flavoured beverages).

## 7. Exposure assessment

Beeswax is consumed in some parts of the world and important constituents of beeswax like *n*-alkanes are found in other foodstuffs such as vegetables (cabbage), grain products (wheat bran and flour), edible oils (olive oil, corn oil, soybean oil, sunflower oil), meat, poultry, fish, dairy products, and other commodities (Reich *et al.,* 1997). Concentrations of naturally occurring *n*-alkanes up to 120 and 140 mg/kg have been reported in sunflower seed oil and apples, respectively (WHO, 2003). In the USA it has been estimated that dietary exposure to naturally occurring saturated *n*-alkanes, in the carbon range $C_{18}$ to $C_{32}$ including straight chain, branched chain and cyclic alkanes, is 9 mg/day (Reich *et al.,* 1997). According to the authors this value is

an underestimation of the actual exposure since there was insufficient information to include all food types known to contain naturally occurring hydrocarbons.

Beeswax is authorised in some foods following the *quantum satis* principle. This allows the use of the additive in these applications according to good manufacturing practices, at a level not higher than necessary to achieve the intended purpose.

A petitioner submitted information on the current and proposed new uses of beeswax in food (Table 2) and the exposure estimates.

Table 2. **Food uses of beeswax (from Technical dossier 2006)**

| Type of food | Maximum level of use (g/kg) |
|---|---|
| Food supplements | |
| - soft gelatine capsules | 60 |
| - tablets | 34 |
| Glazings and coatings | 0.5 |
| Water-based flavoured drinks | 0.2 |

According to a petitioner the following estimates of the daily exposure to beeswax from various sources were (Technical dossier, 2006): soft gelatine capsules, 50-450 mg/day; tablet formulations, 34-100 mg/day; glazings and coatings, 50 mg/day; chewing-gum, 4-6 mg/day (mean) and 8-12 mg/day (90th percentile); carrier for food additives and flavours, 70 mg/day (mean) and 140 mg/day (90th percentile).

The Panel estimated the exposure to beeswax from its reported current uses, from maximum levels of use in foods and from its additional new use as a flavour carrier in water-based drinks.

Data from the UK Food Standards Agency survey on the consumption of food supplements indicate that 24% of adults (Henderson *et al.*, 2002), 14% of young people (Gregory, 2000) and 17% of toddlers consumed food supplements (Gregory, 1995). The use among high consumers ($97.5^{th}$ percentile) ranged from 2 units per day (data do not discriminate between tablets or capsules) in young people to 7 units per day in adults, corresponding with 238 mg (based on tablets) to 1050 mg (based on capsules) beeswax for high consumers.

For the consumption of water-based drinks the Panel used data from the European concise food consumption database, under construction by EFSA. Currently, data referring to the adult population are available from national food consumption surveys conducted in four countries: Italy (7-day dietary record, Turrini *et al.*, 2001), Sweden (7-day dietary record, Becker *et al.*, 1998), United Kingdom (7-day dietary record, Henderson *et al.*, 2002) and the Netherlands (2-day dietary record, Anonymous, 1998).

High consumption ($95^{th}$ percentile) of soft drinks (including fruit drinks with a lower percentage fruit than nectar) varied from 347 g/day in Italy, 685 g/day in Sweden, 840 g/day in UK to 986 g/day in the Netherlands. Based on a maximum proposed use level of 200 mg/kg the exposure of beeswax from soft drinks will range from 69 to 197 mg/day.

Based on food consumption data of confectionery in Italy, France and Sweden the exposure to beeswax for high consumers was assumed not to exceed 40 mg per day (Volatier, 2000; Turrini *et al.,* 2001; Becker *et al.,* 1998).

On the basis of the very conservative assumption that a person would consume all foods and tablets or capsules containing beeswax at the $95^{th}$ percentile in the proposed applications presented in Table 1 and that all these foods would contain beeswax, the Panel calculated that

in the country with the lowest soft drink consumption (Italy), the exposure to beeswax would range from about 350 mg (238 mg from tablets, 40 mg from glazings and 69 mg from soft drinks) to 1160 mg/person per day (1050 mg from capsules, 40 mg from glazings and 69 mg from soft drinks). In the country with the highest soft drink consumption (the Netherlands) the exposure would range from 475 (238 mg from tablets, 40 mg from glazings and 197 mg from soft drinks) to ~1290 mg/person per day (1050 mg from capsules, 40 mg from glazings and 197 mg from soft drinks). The estimate of 1290 mg/person per day corresponds to a beeswax exposure approaching 22 mg/kg bw/day for a 60 kg individual.

In addition, the Panel noted that the highest soft drink consumption was derived from a survey using a 2-day dietary record. The duration of the survey is expected to affect the distribution of the exposure, particularly the upper tails. Data obtained from a two day survey may overestimate the higher percentiles more than a seven day survey will do.

## 8. Toxicological evaluation

Experimental biochemical and toxicological studies on beeswax as such are lacking. However, the Panel considered that the safety of beeswax could be evaluated on the basis of published toxicological studies on its main constituents.

### 8.1. Absorption, Distribution and Excretion

#### 8.1.1. Fatty acid esters

The most important constituents of beeswax are fatty acid monoesters (Table 1). According to the limited literature available, intact wax esters such as those found in beeswax, are poorly absorbed in mammals (Place, 1992). It has been suggested that the ester constituents in beeswax can be hydrolysed in the intestinal lumen to their corresponding alcohols and acids, which are then absorbed and incorporated into normal cellular metabolic pathways. This process involves the release of the long chain fatty acid and alcohol by lipases, such as a carboxyl ester hydrolase (E.C. 3.1.1.13), in the presence of bile salts, followed by a passive or carrier-mediated absorption by the mucosal epithelial cell. The dominant routes of metabolism of long chain alcohols in the cell are either synthesis of phospholipids or oxidation to the corresponding long chain fatty acid. The fatty acids are metabolised by β-oxidation in normal endogenous pathways (Hargrove *et al.,* 2004). Enzymes involved in the hydrolysis of wax esters are widely distributed in tissues from various mammals, including man (Hargrove *et al.,* 2004) and some hydrolysis and absorption of beeswax constituents in humans seems plausible upon ingestion. However, the rate of hydrolysis in the gastrointestinal tract appeared to be low in dogs and may well be the limiting step in the absorption of beeswax constituents by mammals (Place, 1992).

In a study, Sprague-Dawley rats were fed for 2-4 weeks on diets containing, either 40 g of oleyl palmitate /kg or 150 g of oleyl alcohol /kg. The ester was excreted as a mixture of the intact ester, free fatty acid and free alcohol in faeces of the animals, suggesting that wax esters are susceptible to some rate of hydrolysis in the intestinal tract (Hansen and Mead, 1965).

Refined jojoba oil, which contains essentially 97% (w/w) plant wax esters of monounsaturated, straight-chain acids and alcohols with high molecular weights ($C_{16}$-$C_{24-26}$) (Wolfmeier *et al.,* 1996), was administered to four groups of 10 male and 10 female Sprague-Dawley rats. It was

concluded that the oil was poorly absorbed and was resistant to digestion in the intestinal tract (EPA, 1995).

### 8.1.2. Hydrocarbons ( n-alkanes and n-alkenes)

The second major constituents of beeswax are hydrocarbons (Table 1).

Absorption and tissue distribution of radiolabelled [$^{14}$C]-nonacosane ($C_{29}$) was studied in one male Osborne-Mendel rat (Kolattukudy and Hankin, 1966). More than 75% of the total administered radioactivity was excreted through the faeces as unchanged hydrocarbon and about 4% was excreted through expired $CO_2$. Five days after the administration, radioactivity was mainly found in the liver (2 % of the total administered radioactivity mostly in the phospholipids fraction) and in other organs and tissues like the kidney, plasma, muscle, lung and brain. The absorbed long-chain hydrocarbon appeared to undergo extensive metabolism as indicated by the distribution of radioactivity in various organs and by the presence of radioactivity in $C_{15}$, $C_{16}$, $C_{17}$, $C_{18}$ and $C_{19}$ fatty acids isolated from the rat liver phospholipids and triglycerides.

*In vitro* studies of the linear, saturated hydrocarbons *n*-octadecane ($C_{18}$) and *n*-heptadecane ($C_{17}$) in liver or small intestinal microsomes prepared from Wistar, Sprague-Dawley and Fischer 344 rats, showed that, if any, metabolism of linear saturated hydrocarbons resulted in the production of their corresponding alcohol and acid metabolites, as shown by *n*-heptadecane producing heptadecanol and heptadecanoic acid metabolites (WHO, 2003).

Absorption, distribution and excretion of [$^{14}$C] radiolabelled medium- and low-viscosity mineral oils (having a high content of $\geq C_{17}$ hydrocarbons) showed that bioavailability was greater in Fisher 344 rats as compared to Sprague-Dawley rats and that faecal and urinary excretion were the major routes of elimination. Liver and mesenteric lymph nodes retained radioactivity at levels of 2.5% and 0.002% of the administered dose, respectively (WHO, 2003).

### 8.1.3. Free fatty acids and alcohols

Free long-chain fatty acids and long-chain alcohols are constituents of beeswax (Table 1). In a study conducted with radiolabelled [$^{14}$C]-octacosanol ($C_{28}$) administered to 12 Wistar rats in a single dose by *gavage* using tricaproyl glycerol as vehicle, radioactivity was found in liver and adipose tissue, especially in brown adipose tissue, perirenal adipose tissue and epididymal adipose tissue, one day after administration (Kabir and Kimura, 1993). Less radioactivity was found in spleen, kidney, heart, lung, brain, muscle and plasma of the treated animals 1 day after administration. Faeces and urine collected over a 7-day period after administration showed that radioactivity was mainly found in these excreta. According to the authors, free octacosanol in faeces and octacosanol metabolites in urine might account for the radioactivity found. The expiration of radiolabelled $CO_2$ over 7 days after administration represented about 15% of the administered dose.

In summary, based on the available information beeswax long-chain fatty acid esters and long-chain *n*-alkanes are predicted not to be significantly absorbed from the diet. However, to a limited extent long chain fatty acid esters can be hydrolysed into their corresponding long chain fatty acids and alcohols, which then may be absorbed. *N*-alkanes can be also, to a limited extent, be absorbed and converted to their corresponding long chain alcohols and long chain fatty acids. These metabolites can be further metabolised into normal cellular constituents. The same metabolic pathway is also expected for both free long-chain fatty acids and long-chain alcohols present in beeswax in small amounts in the free form.

### 8.2. Acute toxicity studies

No specific studies on beeswax were available.

#### 8.2.1. Aliphatic alcohols

A mixture of high molecular-weight aliphatic alcohols extracted from beeswax, designated as D-002, composed roughly of 27% triacontanol ($C_{30}$), 17% octacosanol ($C_{28}$), 17% dotriacontanol ($C_{32}$), 15% hexacosanol ($C_{23}$), 13% tetracosanol ($C_{24}$) and 2% tetratriacontanol ($C_{34}$), was administered as single doses by gavage to groups of 8 male and 8 female Sprague-Dawley rats (Rodeiro *et al.,* 1995). The rats were observed for up to 14 days after the administration. D-002 was administered as a suspension in a vehicle containing 10 mg acacia gum/ml of water. The administered doses were 0, 500, 1500, 2500, and 5000 mg/kg bw. No clinical signs of toxicity were observed. No histopathological changes were observed. A $LD_{50}$ > 5000 mg/kg bw (the highest dose tested) was identified.

### 8.3. Short-term and sub-chronic toxicity studies

No specific studies on beeswax were available.

#### 8.3.1. Fatty acid esters

Oleyl palmitate as well as oleyl alcohol, fed at either 40 or 150 g/kg diet to Sprague-Dawley rats for 2-4 weeks (equivalent to 2000 and 7500 mg/kg bw/day, respectively) did not produce any toxic effect except for a purgative effect at the highest oleyl palmitate dose tested (Hansen and Mead, 1965).

Refined jojoba oil, (jojoba oil is composed essentially of 97% (w/w) plant wax esters of monounsaturated, straight-chain acids and alcohols with high molecular weights [$C_{16}$-$C_{24\text{-}26}$]), was administered in the diet to four groups of 10 young male Sprague-Dawley rats fed with 0.5, 1.0, 2.0, and 3.0 grams in 5 g of basal diet (equivalent to 10000, 20000, 40000, 60000 mg/kg bw/day assuming an animal weight of 0.1 kg and food consumption of 10 g per day per animal). The first two groups were dosed for 7 days, the last two for 4 days (EPA, 1995; Hamm, 1984). No effect on food consumption was noted in any dose group. Jojoba oil was poorly absorbed and was predicted to be resistant to digestion *in vivo*. Low tolerance of the animals toward high doses of jojoba oil was observed. Low tolerance was also observed towards another non-digestible, non-absorbable oil, trialkoxytricarballyte (TATCA), tested in the same experiment. Oily coats, weakness, and depression (unspecified) were observed in animals treated with both oils at concentrations higher than 10000 mg/kg bw/day. Anal leakage of the unabsorbed oils was noted in some animals during the study. One rat died in each of the higher than 10000 mg/kg bw/day jojoba groups and two rats died in the equivalent TATCA groups. The low tolerance of animals toward the higher concentrations of jojoba and TATCA oils is attributed to secondary metabolic disturbances caused by the laxative effects of oils rather than to a direct toxicity. Animals receiving the basal diet (controls) lost weight and developed rough coats suggesting that the test animals were already under nutritional stress before starting the treatment. This nutritional stress could only be exacerbated by the laxative effects of non-absorbable oils. Furthermore, the authors suggest that doses tested in their study may have reached a threshold dose which may be a typical physiological limit for any non-digestible, non absorbable oil. None of the reported effects was observed in the 10000 mg/kg bw/day groups.

Results from a sub-chronic 90-day study in 20 male and 20 female Fischer 344 rats carried out with carnauba wax, a complex mixture of compounds containing predominantly aliphatic, cinnamic and hydroxycarboxylic acid esters, constituting around 80% (w/w) of the mixture, did not show treatment related effects and no evidence of tissue accumulation of carnauba wax at diets providing doses up to 1500 mg/kg bw/day (SCF, 2001).

Carnauba wax administered to 6 male and 6 female Beagle dogs per group, fed commercial meals containing 0.1, 0.3 or 1.0 % (w/w) of carnauba wax (equivalent to 25, 75 or 250 mg/kg bw/day[2]), for 28 weeks, did not show significant treatment related effects upon haematological, clinical chemical, organ weights or histopathological examination as reported by the authors (Parent *et al.,* 1983a). A NOAEL of 1.0 % (equivalent to 250 mg/kg bw/day - the highest dose tested) can be identified from this study.

### 8.3.2. Aliphatic alcohols

D-002 was administered by gavage to groups of 5 male and 5 female Sprague-Dawley rats for up to 14 days (Rodeiro *et al.,* 1998a). D-002 was administered in a vehicle as indicated before at doses of 0, 2000, 3000, and 5000 mg/kg bw/day. No mortality or clinical signs of toxicity were observed during the study. Haematology and blood biochemical parameters were unchanged and no treatment-related histological findings were observed. A no-observed-adverse-effect level (NOAEL) of 5000 mg/kg bw/day (the highest dose tested) can be identified from this study.

D-002 was administered by gavage to groups of 12 male and 12 female Sprague-Dawley rats in a standard 90-day study (Rodeiro *et al.,* 1998a). D-002 was administered in a vehicle as indicated before at doses of 0, 5, 25, 125, and 625 mg/kg bw/day. No clinical signs of toxicity were observed, haematology and blood biochemical parameters were unchanged and no treatment-related effects were reported on histopathology. Some non-compound related deaths were reported, probably due to gavage problems. A NOAEL of 625 mg/kg bw/day (the highest dose tested) can be derived from this study.

D-002 was administered by gavage to groups of 12 male and 12 female Beagle dogs for up to one year (Alemán *et al.,* 2001). D-002 was administered by gavage in a vehicle as indicated before at doses of 0, 50, and 250 mg/kg bw/day. No deaths occurred during the study and D-002 ingested at these doses was well tolerated. No clinical signs of toxicity were observed. No histopathological changes were observed in examined organs and tissues (40 tissue samples including brain, stomach, colon, liver, spleen). No significant differences were detected in the haematological and blood biochemical parameters. A NOAEL of 250 mg/kg bw/day (the highest dose tested) can be identified from this study.

A mixture of long chain primary aliphatic alcohols isolated from sugar cane, designated as policosanol, containing mainly $C_{28}$ octacosanol (66%) as well as $C_{30}$ tricontanol (12%), $C_{26}$ hexacosanol (7%) and other minor alcohols has been tested in a subchronic study. Policosanol administered by gavage to 20 male and 20 female Sprague-Dawley rats per group for 12 months at doses of 0, 0.5, 5, 50, and 500 mg/kg bw/day and to 4 male and 4 female Beagle dogs per group at doses of 30 and 180 mg/kg bw/day did not show any treatment related adverse effects (Alemán *et al.,* 1994a; Mesa *et al.,* 1994).

[2] assuming a dry chow diet

### 8.3.3. Hydrocarbons (*n*-alkanes and *n*-alkenes)

Class I mineral oils (medium and low viscosity) have been evaluated previously by the SCF (SCF, 1997) and most recently by JECFA (WHO, 2003). Evaluation of short and sub-chronic toxicity studies (28 and 90 day) conducted with class I mineral oils at concentrations up to 2% in the diet (equal to approximately 2600 mg/kg bw/day for the 28 days study and 2000 mg/kg bw/day for the 90 days study) indicated a greater response in the Fischer 344 rat strain than in the Sprague-Dawley strain (WHO, 2003). The effects seen were increased weights of liver, mesenteric lymph nodes and spleen, granulomatous inflammation of the liver, and reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes. The increased liver weights reversed during a 30-day recovery period. In Sprague-Dawley rats only increases in liver weight and reticuloendothelial-cell hyperplasia of the lymph nodes of minimal severity were observed (WHO, 2003).

High-melting point, microcrystalline wax, administered to groups of 20 male and 20 female F344 rats for 90 days at levels of 0.002%, 0.02%, 0.2%, and 2% in the diet (equivalent to 2, 20, 200, and 2000 mg/kg bw/day) was reported not to show the adverse effects described above for class I mineral oils (WHO, 1996). At the highest dose tested, increases in food intake, especially in females were reported, returning to normal during a reversal period. Body weights were not affected by the treatment however. Increased organ weights were reported particularly in kidney, liver, spleen, lymph node and caecum, but histopathological changes and tissue accumulation that were described following mineral oils administration were not observed for high-melting point, microcrystalline wax (WHO, 1996). Base on these studies, a group ADI of 20 mg/kg bw, including clay treated, microcrystalline wax, was set for these substances (WHO, 1996; SCF, 1997).

## 8.4. Chronic toxicity and carcinogenicity studies

No specific studies on beeswax were available.

### 8.4.1. Aliphatic alcohols

D-002 was administered by gavage to groups of 20 male and 20 female Sprague-Dawley rats per group for up to one year (Rodeiro *et al.,* 1998a). D-002 was administered as indicated before at doses of 0, 250, 500, and 1000 mg/kg bw/day. No clinical signs of toxicity were reported except for a non-significant reduction of body weight gain in the highest dose groups. Haematology and blood biochemical parameters were unchanged and no treatment-related histological findings were observed. Some non-compound related deaths were reported probably due to gavage problems (Rodeiro *et al.,* 1998a). A NOAEL of 1000 mg/kg bw/day can be identified from this study (the highest dose tested).

Carcinogenicity studies conducted on 55 male and 55 female Sprague-Dawley rats per group and on male and female Swiss mice (number of animals per group not specified) for 24 and 18 months, respectively, at doses of 0, 50, and 500 mg policosanol/kg bw/day did not show significant dose-related differences in the frequency of occurrence of non-neoplastic and neoplastic (benign and malignant) lesions between treated and control animals (Alemán *at al.,* 1994b; Alemán *et al.,* 1995). A NOAEL of 500 mg/kg bw/day (the highest dose tested) can be identified from these studies.

### 8.4.2. Hydrocarbons ( *n*-alkanes and *n*-alkenes)

Long-term toxicity studies (24 months) conducted with class I mineral oils at doses between 60 and 1200 mg/kg bw/day showed treatment related effects in groups of 50 Fischer 344 rats (WHO, 2003). According to the JECFA evaluation, the effects included dose-related increases in the mesenteric lymph nodes weights, especially in females, reticuloendothelial-cell hyperplasia and vacuolisation of periportal hepatocytes, predominantly in males. Accumulated hydrocarbons were detected in the liver, mesenteric lymph nodes and spleen of treated animals. Accumulation of hydrocarbons in the liver was reversible, with concentrations returning to control levels within 12 months after ending the treatment. Mineral oil treatment was not carcinogenic in this assay.

Class I mineral oil, as a blend of equal quantities of eight commercially available paraffinic medium-viscosity white mineral oils, was fed to groups of 50 Fischer 344 rats at concentrations of 2.5% or 5% in the diet (corresponding to 1250 and 2500 mg/kg bw/day) for 24 months (WHO, 2003). At the highest dose tested, food consumption and body weights of animals were slightly increased. The absolute weights of the liver and kidney were increased in males but this was attributed to an increased body weight of males at this concentration. Heart and spleen absolute and relative weights were unaffected by treatment. Increased severity and incidence of reticuloendothelial-cell hyperplasia of the mesenteric lymph nodes was observed in each sex of rats at both concentrations. Mineral oil treatment was not carcinogenic in this assay.

An ADI of 10 mg/kg bw was allocated to class I medium- and low-viscosity minerals oils by JECFA based on a NOEL of 1200 mg/kg bw/day from the combined long term toxicity and carcinogenicity study in rats using a safety factor of 100 (WHO, 2003).

## 8.5. Reproductive and developmental toxicity studies

No specific studies on beeswax were available.

### 8.5.1. Aliphatic alcohols

Developmental toxicity of D-002 was investigated in groups of 25 pregnant female Sprague-Dawley rats and 16-20 New Zealand White rabbits per group (Rodríguez *et al.* 1998). D-002 was administered daily by gavage at dose levels of 0, 100, 320, and 1000 mg/kg bw/day on gestation days (GD) 6-15 in rats and GD 6-18 in rabbits. No compound-related adverse effects were reported. None of the studied reproductive or fetal parameters differed significantly from controls. A NOAEL of 1000 mg/kg bw/day can be identified from this study (the highest dose tested).

Policosanol containing mainly $C_{28}$ octacosanol (66%) as well as $C_{30}$ tricontanol (12%), $C_{26}$ hexacosanol (7%) and other minor alcohols was tested in 25 female Sprague-Dawley rats per group and 15 female New Zealand White rabbits per group for developmental toxicity. The study did not show any treatment-related effects on maternal toxicity or the survival rate, postnatal growth and behaviour of offspring at levels up to 500 and 1000 mg/kg bw/day, in rats and rabbits respectively (Rodríguez and García, 1994). NOAELs of 500 and 1000 mg/kg bw/day (the highest doses tested) can be identified from these studies.

A multigeneration reproduction study of policosanol in 60 male and 120 weaned female Sprague-Dawley rats distributed randomly in four groups used as $F_0$ parental generation, did not show any adverse effects on the fertility, reproductive performance or development of the observed $F_0$, $F_{1b}$ and $F_{2b}$ generations, at levels up to 500 mg/kg bw/day (Rodríguez *et al.*,

1997). A NOAEL of 500 mg/kg bw/day (the highest dose tested) can be identified from this study.

Evaluation of fetal and neonatal developmental toxicity of policosanol in 16 mated female Sprague-Dawley rats per group treated at levels up to 500 mg/kg bw/day did not show any adverse effect in the observed $F_0$, $F_1$ and $F_2$ dams and pups (Rodríguez and García, 1998). A NOAEL of 500 mg/kg bw/day (the highest dose tested) can be identified from this study.

### 8.5.2. Long chain fatty acids

A mixture of very long chain fatty acids isolated from sugar cane wax, designated as D-003, in which octacosanoic acid ($C_{28}$) represents the major component followed by triacontanoic acid ($C_{30}$), dotriacontanoic ($C_{32}$) and tetratriacontanoic acid ($C_{34}$), has been tested in a reproductive and developmental toxicity study. D-003 did not show any reproductive or fetal and neonatal developmental toxicity when tested in 25 mated Sprague-Dawley female rats and 27 New Zealand White female rabbits per group at levels up to 1000 mg/kg bw/day (Rodríguez *et al.,* 2004, 2006). A NOAEL of 1000 mg/kg bw/day (the highest dose tested) for D-003 can be established in both studies.

### 8.5.3. Fatty acid esters

Carnauba wax administered to groups of 15 male and 15 female Wistar rats in a reproduction and subchronic toxicity study, at doses of 0.1, 0.3 or 1.0 % in the diet (w/w) (equivalent to 50, 150 or 500 mg/kg bw/day) did not show significant treatment related effects upon haematological, clinical chemical, organ weights, histopathological examination or reproductive performance of $F_0$ rats as reported by the authors (Parent *et al.,* 1983b). A NOAEL of 1.0 % (equivalent to 500 mg/kg bw/day-the highest dose tested) can be identified from this study.

## 8.6. Mutagenicity studies

### 8.6.1. Beeswax

Yellow beeswax in phosphate buffer was tested in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 and *E.coli* WP2 strains, at concentrations of up to 10 mg/plate with and without S9 activation, gave negative results (Prival *et al,.* 1991).

### 8.6.2. Long chain fatty acids

D-003 gave negative results when tested in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 strains at concentrations of up to 5 mg/plate with and without S9 activation (Gámez *et al*., 2002).

### 8.6.3. Aliphatic alcohols

D-002 tested for *in vivo* genotoxicity in the bone marrow micronucleus mutation assay and the dominant lethal mutation assay in mice did not show statistically significant effects compared

to controls (Rodeiro *et al.,* 1998b). D-002 was administered by gavage to groups of 6 male and 6 female NMR1 mice per group for up 5 days. An oral dose of 2000 mg/kg bw/day was evaluated in the bone marrow micronucleus mutation assay whereas doses of 25, 125 and 625 mg/kg bw/day were tested in the dominant lethal mutation assay (Rodeiro *et al.,* 1998b).

### 8.6.4. Fatty acid esters

Carnauba wax was considered not genotoxic *in vitro* (not clastogenic nor mutagenic) by the SCF (SCF, 2001).

## 8.7. Human data

### 8.7.1. Long chain fatty acids

Very long chain fatty acids (>$C_{22}$) are present normally in most animal tissues (Poulos, 1995). The brain contains high amounts of saturated and monoenoic fatty acids of 23, 24, 25, 26 and 27 carbons. Smaller amounts of 28 and 30 carbon fatty acids can also be found in the brain. Most other tissues and body fluids contain only small amounts of long chain saturated and monoenoic fatty acids, some of them as α–hydroxy derivatives. Polyenoic (unsaturated) fatty acids of 24, 32, 30 and 34 carbons have also been found in animal tissues, such as testis, mature spermatozoa, seminiferous tubules, retina and the brain (Poulos, 1995). In retina most of the polyenoic fatty acids are present in polyunsaturated phosphatidylcholine.

The cholesterolaemic effect of D-003, a mixture of very long chain fatty acids isolated from sugar cane wax containing octacosanoic acid ($C_{28}$), triacontanoic acid ($C_{30}$), dotriacontanoic ($C_{32}$) and tetratriacontanoic acid ($C_{34}$), was evaluated in a randomised, double-blind, placebo-controlled, parallel-group study with 18 normocholesterolaemic volunteers per group, taking 5 and 10 mg/day of the sugar cane extract (Arruzazabala *et al*., 2005). After 30 days of ingestion an inhibition effect of platelet aggregation to arachidonic acid and collagen was identified mostly at the highest dose tested. This effect was not observed with shorter exposure time. D-003 treatments reduced low-density lipoprotein-cholesterol and total cholesterol levels at both doses and at the higher dose tested increased high-density lipoprotein cholesterol levels, as compared to controls (Arruzazabala *et al.,* 2005).

### 8.7.2. Hydrocarbons (*n*-alkanes, *n*-alkenes)

Two case reports in human patients with sudden death showed the presence of abnormal compounds, notably, giant macrophagic lung granulomas of 'needle-like' inclusions at microscopic examination in one case (Salvayre *et al.,* 1988) and pulmonary granulomatosis and storage of "crystallized fatty substance" in the lymph nodes, spleen, adrenal glands and the lungs in the other case (Duboucher *et al*., 1989). Both patients were reported to eat daily about 1 kg of unpeeled apples. Duboucher *et al*. reports that the patient ate that amount of apples for the past 18 years and Salvayre *et al.* mention that this amount of apples contained around 10 mg of *n*-alkanes. Further chemical analysis of the inclusion bodies in the first case showed they were composed of long-chain *n*-alkanes ($C_{29}$, $C_{31}$, $C_{33}$), which were also found in the lumbo-aortic lymph nodes, adrenal glands and liver (Salvayre *et al.,* 1988). Lower amounts of these alkanes were detected in the myocardium and kidney, but not in brain tissues. Chemical analysis in the second case identified two straight-chain saturated hydrocarbons (*n*-alkanes)

with 29 carbons and 31 carbons as main components in the isolated crystals (Duboucher *et al.,* 1989).

The authors postulated that paraffins (n-alkanes) from plant waxes could have been partially absorbed by the intestine due to the high intake of unpeeled apples reported for the patients (1 kg unpeeled apples/day) (Duboucher *et al.,* 1989; Salvayre *et al.,* 1988). However, in the second case report a link between the deposition of "crystallized fatty substances" and the coronary atherosclerosis to which the death of the subject was attributed was discarded by the authors (Duboucher *et al.,* 1989). Mechanisms involving an abnormal intestinal absorption of plant *n*-alkanes (associated with the high food intake) or metabolic or enzymatic defects in the normal catabolism of *n*-alkanes were suggested in both cases (Salvayre *et al.,* 1988; Duboucher *et al.,* 1989).

### 8.7.3. Aliphatic alcohols

In a human double-blind placebo-controlled clinical study aimed to investigate the effects of D-002 on gastric symptoms associated with piroxicam use in 59 patients, treatment with 40 or 100 mg D-002 /day for 14 days was reported to be well tolerated by most patients (Illnait *et al.,* 2005).

Doses of 10 mg policosanol/day are reported to lower total cholesterol and low-density lipoprotein-cholesterol serum levels and to raise high-density lipoprotein-cholesterol upon ingestion. It is reported that doses of up to 20 mg policosanol/day are well tolerated by humans, as reported in studies of more than 3 years therapy, although the findings need to be confirmed (Gouni-Berthold and Berthold, 2002).

## 8.8. Allergenicity data

Although there are isolated reports of contact sensitisation related to beeswax in the literature, no information on allergic responses to beeswax following oral exposure was available.

## 8.9. Other data

D-003, a mixture of very long chain fatty acids isolated from sugar cane wax containing octacosanoic acid ($C_{28}$), triacontanoic acid ($C_{30}$), dotriacontanoic ($C_{32}$) and tetratriacontanoic acid ($C_{34}$),administered to 15 hyper- and 6 normo-cholesterolaemic New Zealand White male rabbits per group in studies aimed to demonstrate its lowering effect on serum total cholesterol and low-density lipoprotein-cholesterol and an increased effect on serum high-density lipoprotein, did not show any adverse effects at doses up to 100 and 400 mg/kg bw/day, administered for 30 and 10 days, respectively (Gámez *et al.,* 2003; Menéndez *et al.,* 2004).

# 9. Discussion

In the present evaluation, the Panel was aware that biochemical and toxicological studies carried out specifically on beeswax were still lacking.

Based on the available information, the main constituents of beeswax generally do not appear to be absorbed from the gastrointestinal tract to any significant extent. However, *n*-alkanes can be absorbed to a limited extent and some long chain fatty acid esters can be hydrolysed in the gastrointestinal tract to their corresponding long chain fatty acids and long chain alcohols,

which then are absorbed and metabolised into normal cellular constituents. The same metabolic oxidation pathway is expected for both long-chain fatty acids and long-chain fatty alcohols present in beeswax in small amounts in the free form.

Available toxicological studies including *in vitro* and *in vivo* genotoxicity as well as acute, short-term, sub-chronic and chronic toxicological assays and carcinogenicity, reproductive and developmental toxicity studies, conducted in several animal species with extracts of beeswax and plant waxes showing chemical structural similarities to beeswax, did not show adverse effects. The results of the available toxicity studies evaluated in this opinion are summarized in the following table.

Table 3. **Available toxicology studies on beeswax evaluated**

| Substance | Total of equivalent substance in *A. mellifera* beeswax (%) | Species; sex | Route | Duration | NOAEL (mg/kg bw/day) | Reference |
|---|---|---|---|---|---|---|
| ***Short-term , sub-chronic, chronic and carcinogenicity studies*** | | | | | | |
| Fatty acid esters | 57.4 | Rat; M,F | Oral | 7 d | ~10000 | Hamm, 1984 |
| | | Rat; M | Oral | 14 & 28 d | 2000 & 7500 | Hansen and Mead, 1965 |
| | | Rat; M,F | Oral | 90 d | 1500 | SCF, 2001 |
| | | Dog; M,F | Oral | 7 m | > ~ 250 | Parent *et al*., 1983a |
| Hydrocarbons | 15.7 | Rat; M,F | Oral | 28 & 90 d | 1200 | WHO, 2003 |
| | | Rat; M,F | Oral | 24 m | 1200 | |
| Long chain fatty alcohols | 0.6 | Rat; M,F | Oral | 14 d | > 5000 | Rodeiro *et al*., 1998a |
| | | Rat; M,F | Oral | 90 d | > 625 | Rodeiro *et al*., 1998a |
| | | Dog; M,F | Oral | 12 m | > 250 | Aleman *et al*., 2001 |
| | | Rat; M,F | Oral | 12 m | > 500 | Aleman *et al*., 1994a |
| | | Dog; M,F | Oral | 12 m | > 180 | Mesa *et al*., 1994 |
| | | Rat; M,F | Oral | 12 m | > 1000 | Rodeiro *et al*., 1998a |
| | | Mice; M,F | Oral | 18m | > 500 | Aleman *et al*., 1994b |
| | | Rat; M,F | Oral | 24 m | > 500 | Aleman *et al*., 1995 |
| ***Reproductive and developmental studies*** [a] | | | | | | |
| Fatty acid esters | 57.4 | Rat ; F | Oral | 21 d | > ~ 500 | Parent *et al*. 1983b |
| Long chain fatty acids | 18 | Rat; F | Oral | 21 d | > 1000 | Rodriguez *et al*., 2006 |
| | | Rabbit; F | Oral | 21 d | > 1000 | Rodriguez *et al*., 2004 |
| Long chain fatty alcohols | 0.6 | Rat; F | Oral | 20 d | > 1000 | Rodriguez *et al*. 1998 |
| | | Rabbit; F | Oral | 29 d | > 1000 | Rodriguez and Garcia, 1994 |
| | | Rat; F | Oral | 19 d | > 500 | |
| | | Rat; F | Oral | Multigenerational | > 500 | Rodriguez *et al*., 1997 |
| | | Rat; F | Oral | 21 d | > 500 | Rodriguez and Garcia, 1998 |

a: only $F_0$ duration is shown

M: male, F: female, d: days, m: months

The conservative exposure estimate of 1290 mg beeswax/person per day calculated in this opinion corresponds to an exposure approaching 22 mg beeswax /kg bw/day for a 60 kg

individual. The Panel considered this estimate to be very conservative as it was based on the assumptions that a person would consume all the proposed foods and tablets or capsules at the 95$^{th}$ percentile and that beeswax would be used in the proposed applications at the maximum usage level. In addition, the data on soft drink consumption used were obtained from a two day survey, which may overestimate the higher percentiles more than a more realistic seven day survey will do.

The Panel noted that NOAELs identified in the toxicological studies on the main constituents of beeswax and plant waxes showing chemical structural similarities were 10 to 50 times higher than the very conservative exposure estimate of 22 mg beeswax/kg bw/day and were generally the highest doses tested. The Panel considered such margins of safety to be adequate for the assessment of beeswax, which consists of components poorly absorbed from the gastrointestinal tract, and, if absorbed to any extent at all, they would be metabolised to endogenous compounds.

## CONCLUSIONS AND RECOMMENDATIONS

The Panel considered that the data on beeswax itself were insufficient to establish an ADI, but concluded that the safety of beeswax could be assessed, based on available scientific literature on the main constituents of beeswax and plant waxes showing chemical structural similarities to beeswax, published since the last SCF evaluation.

The Panel concluded that the use of beeswax as an additive for the existing food uses and the proposed new food use is not of safety concern. The Panel noted that NOAELs identified in the toxicological studies on the main constituents of beeswax and plant waxes showing chemical structural similarities were 10 to 50 times higher than the very conservative exposure estimate of 22 mg/kg bw/day and were generally the highest doses tested. The Panel considered such margins of safety to be adequate for the assessment of beeswax which consists of components poorly absorbed from the gastrointestinal tract, which if absorbed to any extent at all, would be metabolised to compounds also occurring endogenously.

The Panel examined information on the presence of varroacides residues in beeswax and found that the active varroacides substances most often found in European samples of beeswax are fluvalinate, coumaphos and bromopropylate. The Panel noted that veterinary medicine residues and contaminants found in foodstuffs of animal origin are regulated by specific European legislation.

The Panel noted that beeswax specifications for lead were set to 5 mg/kg in the European legislation and to 2 mg/kg by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Panel considers that the specification for lead should be set as low as possible.

## DOCUMENTATION PROVIDED TO EFSA

1. Level of use and financial implications of beeswax for the food supplements industry in the EU. 4th December 2002. European Federation of Associations of Health Product Manufacturers (EHPM). Brussels.
2. Technical dossier. January 2006. Beeswax (E 901) dossier and CD Rom prepared by Bioresco on behalf of the European Wax Federation.

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## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AFC | Scientific Panel on Food Additive, Flavourings, Processing Aids and Materials in Contact with Food |
| CAS | Chemical Abstract Service |
| EFSA | European Food Safety Authority |
| EINECS | European Inventory of Existing Chemical Substances |
| EU | European Union |
| FDA | Food and Drug Administration |
| FAO/WHO | Food and Agriculture Organization/World Health Organization |
| GD | Gestation Days |
| GRAS | Generally Recognized As Safe |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| MRLs | Maximum Residue Levels |
| NOAEL | No Observed Adverse Effect Level |
| SCF | Scientific Committee for Food |
| TATCA | Trialkoxytricarballyte |