SAFETY EVALUATIONS OF SPECIFIC FOOD ADDITIVES (OTHER THAN FLAVOURING AGENTS)

BEESWAX

First draft prepared by

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1. EXPLANATION

Beeswax was evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference 101). At that time, the Committee concluded that beeswax could be regarded as a food constituent. Although beeswax could not be evaluated in the usual manner, as the only data consisted of an LD_{50} in rats of > 5 g/kg bw per day and results showing lack of mutagenic potential in microbial assays in vitro, the Committee considered that its long history of use (as natural yellow beeswax) without apparent adverse effects provided a degree of assurance that its current functional uses (release and glazing agent in bakery products, glazing agent on fresh and frozen fruit, glazing agent on sweets, carrier for flavours and component of chewing-gum bases) did not raise any toxicological concern. The Committee also noted the possibility that beeswax is allergenic and that toxic substances present in honey in some parts of the world might also occur in beeswax. In view of an additional proposed use of beeswax in water-based, flavoured drinks, with a maximum use level of 200 mg/kg (0.02%), the Committee re-evaluated the data on this substance.

Beeswax (white and yellow) is the refined wax from honeycombs. The wax is produced in wax glands located in the abdomen of honeybees of the genus *Apis* (e.g. *A. mellifica* and *A. carnica*). It is a complex mixture of several chemical components, consisting mainly of free fatty acids with even-numbered chain lengths of C24–C36 (about 85% saturated), linear wax monoesters and hydroxymonoesters

with chain lengths of mainly 40–48 carbon atoms, complex wax esters, diesters and triesters and straight-chain hydrocarbons with odd-numbered chain lengths of C27–C33. Free fatty alcohols (C28–C34) occur in minor amounts. Terpenoids, flavonoids and volatile produce have also been detected in small amounts in crude beeswax. Depending on the analytical technique used, the main components have been reported to occur at the following concentrations: linear monoesters and hydroxy-monoesters, 35–45%; complex esters, diesters and triesters, 15–27%; odd-numbered straight-chain hydrocarbons, 15%; free fatty acids, 12–14%; and free fatty alcohols, 1% (Tulloch, 1980; Brand-Garnys & Sprenger, 1988; Brüschweiler et al., 1989; Aichholz & Lorbeer, 1999, 2000; Jiménez et al., 2004). These proportions are consistent with the composition of beeswax described by the Committee at its thirty-ninth meeting. The fractional composition, but not the chemical identity, of beeswax components varies slightly among subspecies of bees, the age of the wax and the climate at the time of its production. White beeswax is obtained from yellow beeswax by treatment with hydrogen peroxide or bleaching earths and activated carbon (American College of Toxicology, 1984; Blum et al., 1988).

Beeswax is used in foods in several forms, as a stabilizer or release agent in soft gelatine capsules and tablet formulations sold as food supplements in the European Union, in glazings and coatings, as a component of the gum base of chewing-gums and as a carrier for food additives and flavours, such as in water-based drinks.

The Committee evaluated the safety of beeswax for use as a food additive. The previously published monograph was expanded to include the newly available data and is incorporated into this monograph.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

It is generally considered that waxes, including beeswax, are not digested or absorbed to any significant extent after ingestion by most mammals, but limited information is available on beeswax per se. Biochemical data are available on the main components of beeswax—esters, hydrocarbons, free fatty acids and free fatty alcohols—after digestion and absorption.

(a) Absorption, distribution and excretion

Beeswax is assumed to be indigestible owing to its high melting-point (62–65 °C), which prevents its dissolution at body temperature (Federation of American Societies for Experimental Biology, 1975), its insolubility in water and its hydrophobic surface, which makes it difficult for digestive enzymes to hydrolyse the product and for intestinal microbiota to affect its degradation. No data are available, however, to support this assumption. Studies of its absorption, distribution, metabolism and excretion would be difficult to perform because of its highly complex composition, assuming that it can be digested or absorbed at all.

There is evidence that some solubilization of beeswax is mediated by the action of bile acids, at least in some species. Beeswax appears to be digested by larvae of *Galleria mellonella*, the wax moth, which thrives on this substrate (Opdyke, 1976). This information was considered to suggest that certain amounts of ingested

beeswax can be broken down by gut microflora and then absorbed as a source of carbon.

While it has also been reported that beeswax is used efficiently by seabirds via a bile-dependent, pancreatic esterase (EC 3.1.1.13), this appears to be the result of an evolutionary adaptation of certain species, probably because wax esters are important lipid components in many terrestrial arthropods and marine organisms, and not a general trait of the vertebrate digestive tract (Place, 1992).

Linear monoesters

Linear monoesters are the most important constituents of beeswax, representing 40–50% of the product. The esters consist of long-chain fatty alcohols (C24–C38) and fatty acids, mainly palmitic acid (C16) and oleic acid (C18). Monoesters with a total chain length of 40–48 carbon atoms are quantitatively the most important, but C38, C50, C52 and C54 monoesters are also present. Most wax esters are saturated, but unsaturated, monoenoic wax esters also exist, mainly among the esters of longer chain length (C46, C48, C50) (Tulloch, 1980; Aichholz & Lorbeer, 1999, 2000). Recently, ethyl esters of even-chain fatty acids were also detected in beeswax (Jiménez et al., 2004).

Monoesters (C40–C52) with a free hydroxyl group are formed when 15-hydroxypalmitic acid is esterified with fatty alcohols or if palmitic or oleic acid is esterified with an α,ω-2 diol. Earlier reports referred to the presence of α,ω and α,ω-1 diols (Tulloch, 1980). Ingested and solubilized waxy monoesters may be hydrolysed at least partly by pancreatic wax esterase. The long-chain fatty alcohols and fatty acids thereby released are then available for absorption and further metabolism. Metabolic use of wax esters has been observed, particularly by animals that feed on zooplankton and other marine organisms, such as certain seabirds and fish (Mankura et al., 1987; Place, 1992).

In porcine pancreatic lipase in vitro, this enzyme hydrolysed oleyl palmitate (the ester of palmitic acid and oleyl alcohol). The reaction appeared to be reversible. In vivo, absorption of liberated fatty acid, which appears to be better absorbed than the fatty alcohol, would shift the equilibrium in the direction of degradation; however, absorption of intact oleyl palmitate could not be verified. Young rats have been reported to digest about 50% of ingested oleyl palmitate, the waxy ester of palmitic acid and oleyl alcohol, indicating incomplete digestion of a wax ester (Hansen & Mead, 1965). Digestion of beeswax may, however, differ, given that the tested wax ester is liquid at body temperature (Patel et al., 2001), whereas beeswax remains solid.

The occurrence of significant amounts (in relation to triglycerides) of oleyl palmitate and oleyl alcohol in the lipids of rats fed diets containing oleyl palmitate (15%) or oleyl alcohol (4%) merely indicates that, at the high dose used, the capacity of the oxidative conversion of fatty alcohol to fatty acid in the intestinal mucosa and liver is exceeded (Hansen & Mead, 1965). Thus, the oleyl palmitate that was detected in liver might have been formed by re-esterification of absorbed oleyl alcohol. Certain waxy esters also occur naturally in animal tissues, as demonstrated by the presence of cetyl myristoleate in Swiss albino mice (Diehl & May, 1994).

Additional evidence for partial digestion of wax esters comes from a study on jojoba oil, the oil obtained from the nut-like fruit of the jojoba plant (*Simmondsia chinensis*). This oil consists of liquid wax esters: oleic acid (C18), eicosanoic acid

(C20:1) and erucic acid (C22:1) are esterified with eicosenyl alcohol (C20:1), docosenyl alcohol (C22:1) and tetracosenyl alcohol (C24:1) (Environmental Protection Agency, 1995). A diet containing 1% or 2% jojoba oil given to mice for 3 weeks was well tolerated; however, at the 2% dose, faeces were soft and weight gain was reduced. The stool softening suggests that hydrolysis and absorption of the wax esters were incomplete at this relatively low dose (Verbiscar et al., 1980). In a study of the metabolism of refined jojoba oil, in which four groups of 10 rats received a single daily dose of 0.5, 1, 2 or 3 g for 4–7 consecutive days, the observation of oily coats due to 'anal leakage', even at the lowest dose, supports the conclusion that the digestibility of the wax esters is low (Hamm, 1984). In an earlier study with jojoba oil in rats, 20% was reported to have been digested (Booth, 1972, cited by Hamm, 1984).

Complex esters

Complex esters with two, three or more ester bonds occur in beeswax. These products are formed by esterification of the hydroxyl groups of 15-hydroxy palmitic acid (acylated hydroxy esters) or the terminal hydroxyl groups of an α,ω-1 or α,ω diol (diol diesters). These diesters have chain lengths of C54–C64.

Diesters represent about 7% of beeswax (Aichholz & Lorbeer, 1999). According to earlier analyses, diesters, triesters, hydroxypolyesters and acid polyesters constitute 14%, 3%, 8% and 2% of beeswax, respectively (Tulloch, 1980).

Owing to the large size of these molecules, direct absorption appears unlikely. No direct evidence for enzymatic degradation by carboxyl ester hydrolases or lipases was found. Given the lack of specificity of carboxyl ester hydrolase, which can also cleave cholesterol esters, some digestion is, however, possible. In this event, the products of digestion would be the same as those arising from digestion of the monoesters.

Hydrocarbons

Beeswax contains about 15% (range, 12–16%) hydrocarbons. Odd-chain alkanes (C23–C33) and mono-unsaturated alkenes (C27–C39) were detected (Aichholz & Lorbeer, 1999, 2000). Major components are the C27, C29 and C31 alkanes and the C31:1 and C33:1 alkenes (Tulloch, 1980), alkadienes and alkatrienes being present at only low concentrations (Carlson et al., 1989; Giumanini et al., 1995; Jiménez et al., 2004).

The rate of absorption of linear alkanes from the gut decreases with increasing chain length. About 5% of the C28 *n*-alkane is absorbed, but essentially no compounds with a chain length greater than C32 are absorbed (Smith et al., 1996). Although the chain length of some of the hydrocarbons present in beeswax would permit partial absorption, it has not been determined whether these hydrocarbons leak from the wax particles into the chyme, thereby becoming available for potential absorption, or whether they remain bound in the solid wax particles and are thus excreted completely with the faeces.

(i) Free fatty acids

Beeswax contains 12–14% free fatty acids, most of which are saturated. In an early study, eight major and 10 minor fatty acids were detected, lignoceric acid

(C24), hexacosanoic acid (C26) and octacosanoic acid (C28) being the most prevalent species (at 6%, 1% and 1%, respectively) (Tulloch, 1980). In another investigation, fatty acids with carbon numbers between C20 and C36 were identified (Aichholz & Lorbeer, 2000). Most of these so-called 'wax(y) acids' or 'very long-chain fatty acids' have carbon numbers between C24 and C32. While most fatty acids in human foods have up to 22 carbon atoms, longer chains can be found in vegetable oils. Lignoceric acid (C24), for example, is present in small amounts in most plant oils as well as in menhaden oil (fish oil) and most fats.

The very long-chain fatty acids are metabolized like other fatty acids by β-oxidation; however, they are degraded mainly in peroxisomes and not in mitochondria. The main differences between mitochondrial and peroxisomal β-oxidation are:

- Fatty acids diffuse freely into peroxisomes and do not need to be transported by L-carnitine.
- Acyl-coenzyme A oxidation involves oxygen instead of FAD as the electron acceptor, yielding hydrogen peroxide, which is then converted to water and oxygen by catalase. If part or all of the free long-chain fatty acids are absorbed, they will be readily metabolized by β-oxidation. The chain length has no significance in this regard, except that very long-chain fatty acids can be degraded only by peroxisomes. Unsaturated or hydroxylated fatty acids are processed by the same metabolic pathway. Very long-chain fatty acids are normal components of most human and animal tissues (Poulos, 1995).

(ii) Free fatty alcohols

Beeswax contains about 1% free long-chain alcohols (fatty alcohols, waxy alcohols). Five main components were found in an early study (Tulloch, 1980), and another group reported the occurrence of two odd-chain fatty alcohols (C33 and C35), each present at a concentration of about 0.3% (Aichholz & Lorbeer, 1999, 2000). Another group detected four even-numbered fatty alcohols (C28, C30, C32 and C34) (Brüschweiler et al., 1989).

It is generally assumed that ingested aliphatic alcohols are absorbed and oxidized to the corresponding aldehydes, which are then rapidly oxidized to the corresponding acids. The acids obtained, whether even- or odd-numbered, are oxidized by β-oxidation. In view of this metabolism and the toxicological data on the common aliphatic alcohols of lower chain length (e.g. decanol), the presence of free fatty alcohols in beeswax does not raise toxicological concern (see also Annex 1, reference 131, for safety assessments of nonyl alcohol, decanol and other aliphatic alcohols used as a flavouring substances).

2.2 Toxicological studies

Few toxicological studies have been carried out on beeswax per se; however, information is available from studies in mice, rats, rabbits and dogs for some of the main components of beeswax (free fatty acids, monoesters, complex esters, hydrocarbons and free fatty alcohols).

2.2.1 Acute toxicity

The only available study on the acute oral toxicity of beeswax was one in which 10 rats of undefined sex were given undiluted beeswax (type not specified) at

a single oral dose of 5 g/kg bw. Four animals died on the second of the 14 observation days, from unknown causes. Depression and ataxia were observed in the surviving animals. The LD_{50} was > 5 g/kg bw (McGee Laboratories, 1974, cited in American College of Toxicology, 1984).

2.2.2 Short-term studies of toxicity

No information was available on the toxicity of beeswax in short-term studies.

(a) Linear monoesters

When a diet containing 15% oleyl palmitate (the ester of palmitic acid and oleyl alcohol), or about 15 000 mg/kg bw day, was fed to five weanling male Sprague-Dawley rats for 4 weeks, about 50% of the wax ester was absorbed. The unabsorbed fraction acted as a faecal lubricant, inducing some degree of diarrhoea and a corresponding reduction of weight gain; however, overt signs of toxicity were not observed (Hansen & Mead, 1965).

Further information on wax esters comes from a study in which jojoba oil (see 2.1.1 above) was administered in the diet to groups of 10 CD-1 mice of each sex for 3 weeks. Concentrations of 1% or 2% jojoba oil were well tolerated, despite the presence of some toxic cyanoglycosides in the oil. At the 2% dose, the faeces were soft and weight gain was reduced, suggesting incomplete hydrolysis and absorption of the wax esters (Verbiscar et al., 1980). Some deaths occurred at the 10% concentration, which were attributed to reduced absorption of nutrients owing to physiological effects on the gastrointestinal tract, rather than to toxicity.

(b) Complex esters

No information was available on the toxicity of complex esters, but any complex ester that is absorbed (minimal, given the large size of the molecules), would be subject to cleavage by carboxyl ester hydrolase. The products of digestion would be the same as that of the monoesters, thus, data on the monoesters would be applicable to the complex esters.

(c) Hydrocarbons

The safety of hydrocarbons with characteristics similar to those present in beeswax was tested indirectly in a 13-week study of carnauba wax in groups of 20 Fischer 344 rats of each sex. The highest dose tested (1500 mg/kg bw per day) was the NOEL (additional details not provided) (Scientific Committee on Food, 2001).

In an earlier 13-week study of carnauba wax in groups of 15 Wistar rats of each sex, no treatment-related effects on body weight, haematological parameters, urinary concentration, organ weights or histopathological appearance were observed at a dietary level of 1%, 5% or 10%. Controls were given 10% cellulose powder. The highest concentration of carnauba wax tested, 10%, corresponded to an intake of 8.8 g/kg bw per day (Rowland et al., 1982). As carnauba wax contains 1.5–3% hydrocarbons (linear, odd-numbered *n*-alkanes of C27–C31), ingestion of hydrocarbons as a component of carnauba wax at doses of 130–300 mg/kg bw per day did not have any adverse effects.

(d) Free fatty acids

The presence of free fatty acids raises no toxicological concern, as these compounds are common components of the diet and are readily metabolized by β-oxidation after absorption. The metabolism of very long-chain fatty acid is comparable, except that these compounds are degraded in peroxisomes, where they diffuse freely, rather than in mitochondria. Very long-chain fatty acids are also normal components of most human and animal tissues (Poulos, 1995).

(e) Free fatty alcohols

Free fatty alcohols are metabolized via common pathways after absorption. The ready metabolism of these compounds and the available data on aliphatic alcohols of lower chain length, which were previously evaluated by the Committee (Annex 1, reference 131) and found to be acceptable as flavouring substances, indicate that the presence of free fatty alcohols in beeswax is not of toxicological concern.

To test the safety of very long-chain fatty alcohols isolated from hydrolysed beeswax, D002, a mixture consisting of C24 (13.2%), C26 (15.3%), C28 (17.5%), C30 (26.6%), C32 (17.0%) and C34 (2.2%) fatty alcohols, was administered to groups of 12 Sprague-Dawley rats of each sex at a dose of 5, 25, 125 or 625 mg/kg bw per day for 90 days by gavage in a suspension with gum acacia. The mixture. Four males and two females died due to gavage accidents. Body-weight gains, haematological and clinical chemical parameters, organ weights and histopathological results were similar in treated groups and controls (Rodeiro et al., 1998a). The NOEL was 625 mg/kg bw per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available for beeswax.

Rats

In a 52-week study, D002 was administered to groups of 20 male and 20 female Sprague-Dawley rats by gavage at a dose of 0, 200, 500 or 1000 mg/kg bw per day. A slight, statistically nonsignificant reduction in body weight of about 8% was observed in female rats at the the two higher doses. There were no treatment-related changes in haematological or clinical chemical parameters, relative organ weights or histopathological observations (Rodeiro et al., 1998a). The NOEL was 1000 mg/kg bw, the highest dose tested.

Dogs

In a 52-week study, groups of four beagle dogs of each sex received D002 at a dose of 0, 50 or 250 mg/kg bw per day by gavage. All groups showed similar weight gains, and there were no clinical signs in response to treatment. There were no changes in haematological or clinical chemical parameters, and the weights of the main organs remained unaffected. There were no histopathological changes that could be attributed to treatment. Thus, the NOEL was the highest dose tested, 250 mg/kg bw per day (Alemán et al., 2001).

2.2.4 Genotoxicity

The results of tests for the genotoxic and mutagenic potential of white and yellow beeswax, of D003, a mixture of very long-chain fatty acids from sugar cane wax, and D002, a mixture of very long-chain fatty alcohols from beeswax, are summarized in Table 1.

Table 1. Results of tests for the genotoxicity of beeswax and components

End-point	Test system	Test substance, concentration or dose	Results	Reference
In vitro				
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538; *S. cerevisiae* D4	White beeswax; 0.5 and 1 mg/plate (0.1 ml/plate of 5000 ppm and 10 000 ppm preparations of beeswax)	Negative[a]	Federation of American Societies for Experimental Biology (1975)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2	Yellow beeswax; 0.033–10 mg/plate	Negative[a]	Prival et al. (1991)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	D003[b]; 0.005–5 mg/plate	Negative[a]	Gámez et al. (2002)
In vivo				
Micronucleus formation	NMRI mouse bone marrow	D002[c]; 2000 mg/kg bw per day orally for 5 days	Negative	Rodeiro et al. (1998b)
Dominant lethal mutation	NMRI mouse	D002[c]; 25, 125 or 625 mg/kg bw per day orally for 6 weeks to females and 8 weeks to males	Negative	Rodeiro et al. (1998b)

[a] In the presence and absence of Arochlor-induced rat liver microsomal fraction
[b] Mixture of very long-chain fatty acids from hydrolysed sugar-cane wax
[c] Mixture of very long-chain fatty alcohols from hydrolysed beeswax

Negative results were obtained in all the assays for reverse mutation in *S. typhimurium* in vitro. In addition, it was not mutagenic in *Saccharomyces cerevisiae* strain D4, in plate or suspension tests, with or without the addition of metabolic activation systems from mice, rats or monkeys (Federation of American Societies for Experimental Biology, 1975). Taken together with the results of tests for the genotoxicity of very long-chain fatty alcohols and acids, tested with the fatty alcohol mixture D002, in vitro and in vivo, the data indicate that beeswax does not have genotoxic potential.

2.2.5 Reproductive toxicity

No information was available on the reproductive or developmental toxicity of beeswax.

(a) Hydrocarbons

The reproductive toxicity of hydrocarbons with characteristics similar to those present in beeswax was tested indirectly in a study in rats. Groups of 25 Wistar rats of each sex were given diets containing 0, 0.1%, 0.3% or 1.0% (w/w) carnauba wax (equal to 0, 80, 250 and 810 mg/kg bw per day for males and 0, 90, 270 and 670 mg/kg bw per day for females on the basis of food consumption in this study) for 4 weeks before mating and throughout the mating and gestation periods and the remainder of the study, including lactation. The F_1 progeny were given the same diet after weaning for an additional 13 weeks. There were no effects on reproduction parameters (including fertility, gestation, viability and lactation indices and pup body weights) or the results of clinical pathology, ophthalmology, gross pathology or histology (Parent et al., 1983).

(b) Free fatty alcohols

The developmental toxicity of D002 isolated and purified from beeswax was evaluated in groups of 25 Sprague-Dawley rats given 0, 320 or 1000 mg/kg bw by gavage on days 6–15 of gestation. Litters were removed surgically on day 20 of gestation. There were no signs of maternal toxicity, and the body-weight gain of dams was similar in all groups. No significant differences were found between treated and control groups in the numbers of implantation sites, corpora lutea, live fetuses, dead fetuses or resorptions or in the sex ratio or fetal weight. No external, visceral or skeletal malformations or variations attributable to D002 were observed (Rodríguez et al., 1998).

D002 was also evaluated for developmental toxicity in groups of 16–20 New Zealand white rabbits given 0, 100, 320 or 1000 mg/kg bw by gavage on days 6–18 of gestation. Litters were removed surgically on gestation day 29. No adverse effects of treatment on the appearance of the dams or on body-weight gain were observed, and no treatment-related effects on reproductive indices or the occurrence of external, visceral or skeletal malformations were found. Two cases of hemivertebrae with fused ribs were observed at the intermediate dose but not at the lower or higher dose. The background incidence of fused ribs was approximately 1.29% (Rodríguez et al., 1998).

The results of the studies of reproductive toxicity indicate that D002 is not embryotoxic, fetotoxic or teratogenic.

2.3 Observations in humans

The studies in humans were limited to tests for skin sensitization after topical application, which were conducted to support the use of beeswax in cosmetic formulations (American College of Toxicology, 1984). No signs of irritation, sensitization or photosensitization were noted, except in three studies with a cleansing cream, which resulted in minimal irritation. No attempt was made to identify the ingredient in the cream formulation that caused this effect.

Because of the route of exposure, these tests are of little relevance for assessing the safety of beeswax as a food additive. Nevertheless, given the concern about the allergenic potential of beeswax or its components, the results of two tests for skin sensitization or maximization were considered. In the first test, beeswax had no allergenic potential in 22 healthy volunteers (Epstein, 1975, cited in American College of Toxicology, 1984). In the second study, performed in persons with varying degrees of sensitivity to grass pollen, application of crude or refined beeswax by the scratch method or by intracutaneous injection did not induce an allergic response (Gay, 1945, as cited in American College of Toxicology, 1984).

3. DIETARY EXPOSURE

The sponsor submitted information about the current and proposed uses of beeswax in food and the resulting exposure. In addition, the International Organization of the Flavor Industry supplied information on the poundage of beeswax sold in the food market in the European Union in 2003.

Beeswax is used in foods to achieve several effects: as a stabilizer or release agent in soft gelatine capsules and tablet formulations sold as food supplements in the European Union, in glazings and coatings, as a component of the gum base of chewing-gums and as a carrier for food additives and flavours, such as in water-based drinks. The following estimates of daily exposure to beeswax from various sources have been reported, not including possible exposure with honey sold in jars with the honeycombs: soft gelatine capsules, 50–150 mg/day; tablet formulations, 50–150 mg/day; glazings and coatings, 50 mg/day; chewing-gum (mean exposure), 4–6 mg/day; chewing-gum (90th percentile exposure), 8–12 mg/day; carrier for food additives and flavours (mean exposure), 70 mg/day; and carrier for food additives and flavours (90th percentile exposure), ~ 140 mg/day.

The sponsor stated, without supporting documentation but on the basis of estimates of consumption at the 90th percentile and assuming that consumers are unlikely to ingest the maximum amount from food supplements in both capsules and tablets, that the total exposure from all these sources would be 350 mg/day (150 mg from tablets + 50 mg from glazings + 10 mg from gum + 140 mg from carriers), or about 6 mg/kg bw per day for a 60-kg person.

Additionally, the sponsor calculated, on the basis of estimated exposure to beeswax from food supplement capsules and the poundage data supplied by International Organization of the Flavor Industry for use of beeswax in all other food applications, that the average exposure to beeswax per consumer would be 10–100 mg/day, assuming that 1–10% of the European Union population of 379×10^6 persons consumed foods or food supplements with added beeswax (European Federation of Associations of Health Product Manufacturers, 2002; European Wax Federation, 2003).

The Committee estimated 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 (Table 2). Data on food intake were taken from 7-day surveys (95th percentiles) conducted in France (Volatier, 2000), Italy (Turrini et al., 2001) and Sweden (Becker et al., 1997) and a 2-day survey in the USA (United States Department of Agriculture, 1998; 90th percentile). Information on exposure from tablets and capsules was taken from surveys in France and the

Table 2. Food uses of beeswax

Type of food	Maximum level of use (g/kg)
Food supplements	
Soft gelatine capsules	60
Tablets	34
Glazings and coatings	0.5
Chewing-gum	0.65
Water-based flavoured drinks	0.2

United Kingdom (Table 3). On the basis of the very conservative assumption that a person would consume all foods (and tablets or capsules) containing beeswax at the highest percentile in each food category and that all those foods contained beeswax, the Committee calculated that the exposure to beeswax would be < 650 mg per person per day (40 mg from coatings + 6.5 mg from gum + 340 mg from tablets + 60 mg from capsules + 200 mg from water-based drinks). The new use in water-based drinks would result in an increase of about 50% (from 450 mg to 650 mg). If it assumed that persons consume only tablets or capsules (not both) and that all foods containing beeswax are consumed at the mean reported intake, exposure to beeswax would be 460 mg per person per day (15 mg from coatings + 3.5 mg from gum + 340 mg from tablets + 100 mg from water-based drinks).

Table 3. Food intakes and resulting intakes of beeswax

Use	Food intake (g/person per day)		Beeswax intake (mg/person per day)	
	95th percentile	Mean	95th percentile	Mean
Glazings and coatings				
France	70	20	35	10
Italy	60	20	30	10
Sweden	80	30	40	15
USA	50	30	25	15
Chewing-gum				
USA	10	5	6.5	3.5
Tablets (1.5 g/tablet)				
France (7 tablets)	10.5		340	
United Kingdom (7 tablets)	10.5		340	
Capsules (0.15 g/capsule)				
France (7 capsules)	1.0		60	
United Kingdom (7 capsules)	1.0		60	
Water-based drinks (new use)				
France	410	120	82	24
Italy	350	110	70	22
Sweden	700	250	140	50
USA	1000	500	200	100

4. COMMENTS

Toxicological data

The 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 considered the use of beeswax in water-based, flavoured drinks. The toxicological studies conducted on the various components of beeswax included short-term studies with oral administration, long-term studies of toxicity and carcinogenicity and studies of reproductive toxicity. The components, which are common in other foods, were not toxic. A search of the literature did not reveal the presence of naturally occurring toxic substances in commercial beeswax. It was noted that beeswax administered topically or by intracutaneous injection did not induce an allergenic response in humans.

Assessment of dietary exposure

Information was submitted on the food uses and resulting exposures to beeswax. Dietary exposure would be about 350 mg per person per day for a person with 90th percentile exposure to foods containing beeswax, in addition to consumption as a component of food supplement tablets or capsules. The Committee received information on the poundage of beeswax sold to the food market in the European Union in 2003. If it is assumed that 1–10% of the population consumes products containing beeswax, the average dietary exposure to beeswax per consumer would be 10–100 mg per person per day.

The Committee estimated the dietary exposure to beeswax from reports of its current use and maximum levels of use in foods, including as a flavour carrier in water-based drinks. On the basis of the conservative assumption that a person would consume all foods (and food supplement tablets or capsules) containing beeswax at the 95th percentile in each food category and that all those foods would contain beeswax, exposure to beeswax would be < 650 mg per person per day. Addition of use as a carrier for flavours in water-based drinks would result in an increase in the estimated dietary exposure of 200 mg per person per day, about 50% higher than the estimated exposure from current uses (450–650 mg per person per day).

5. EVALUATION

The Committee concluded 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 expsoure 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. As the available information was very limited, the Committee was unable to reach a conclusion about the potential allergenicity of beeswax noted by the Committee at its thirty-ninth meeting.

6. REFERENCES

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