SCIENTIFIC OPINION

Scientific Opinion on the safety of hemp (*Cannabis genus*) for use as animal feed[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

Four different types of feed materials derived from the hemp plant were identified: hemp seed, hemp seed meal/cake, hemp seed oil and whole hemp plant (including hemp flour). The hemp varieties allowed for cultivation in Europe need not to exceed 0.2 % THC (in dry matter; average of 2151 samples collected in Europe between 2006 and 2008: 0.075 %). Hemp seeds are practically free of THC (maximum 12 mg THC/kg). The THC lethal dose in acute toxicity studies in rats, mice and dogs is approximately 1000 times higher than the lowest doses known to reproduce typical THC-related symptoms in animals. Both the THC and metabolites with psychoactive properties may be distributed to the different tissues and organs, fat being the target tissue. They are excreted via milk; the transfer rate of oral THC to milk from dairy cows is likely 0.15 %. Studies in humans identified psychotropic effects at a LOEL of 0.04 mg THC/kg bw. By applying an uncertainty factor of 100, a PMTDI of 0.0004 mg/kg bw was derived. Since the PMTDI is based on acute pharmacological effects, the consumer exposure considered the single high consumption record derived from the EFSA Comprehensive European Food Consumption Database (P95 values of consumers only: 2 L milk equivalents for adults, 1.5 L for children). In all scenarios (varying intake of hemp plant derived feed material and milk yields), consumer exposure to THC was considerably above the PMTDI for adults and for children; applying the same exposure calculations to hemp seed-derived feed materials results were below the PMTDI. The FEEDAP Panel recommended to put whole hemp plant-derived feed materials list of materials whose placing on the market or use for animal nutritional purposes is restricted or prohibited and to introduce a maximum THC content of 10 mg/kg to hemp seed-derived feed materials.

KEY WORDS

Animal feed, safety, hemp, *Cannabis genus*, tetrahydrocannabinol (THC), PMTDI, safety

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SUMMARY

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety of hemp (*Cannabis genus*) for use as animal feed.

Four essentially different types of feed materials may be derived from the hemp plant: hemp seed (26 to 37.5 % lipids, 25 % crude protein, 28 % fibre), hemp seed meal/cake (about 11 % lipids, 33 % crude protein, 43 % fibre), hemp seed oil (about 56 % linoleic, 22 % alpha-linolenic acid) and whole hemp plant (including hemp hurds, fresh or dried). Further products are hemp flour (ground dried hemp leaves) and hemp protein isolate from seeds.

Hemp seed and hemp seed cake could be used as feed materials for all animal species. The maximum incorporation rates in the complete feed could be 3 % in poultry for fattening, 5–7 % in laying poultry and 2–5 % in pigs for hemp seed and hemp seed cake, 5 % in ruminants for hemp seed cake and 5 % in fish for hemp seed.

The whole hemp plant (including stalk and leaves) would be, due to its high fibre content, a suitable feed material for ruminants (and horses), and daily amounts of 0.5 to 1.5 kg whole hemp plant dry matter (DM) could likely be incorporated in the daily ration of dairy cows.

The hemp varieties allowed for cultivation in Europe must contain < 0.2 % THC (in dry matter basis). In conduct of the official control, 2151 samples were collected in Europe between 2006 and 2008 showing a mean THC content of 0.075 %, 2.6 % of the samples exceeding the maximum content (average: 0.33 % THC). In the absence of further data, the FEEDAP Panel considered data from the official control as conservative surrogates of the THC-content of the whole hemp plant-derived feed materials.

Hemp seeds have a low content of THC, mainly found on the outside of the seeds, which is mainly the result from physical contamination by the plant leaves. The maximum value found in un-treated seeds was 12 mg THC/kg.

No studies concerning tolerance or effects of graded levels of THC in food-producing animals have been found in literature. However, several case reports describing accidental poisoning are available: if poisoned animals are subjected to proper treatment, the prognosis for full recovery is excellent.

Based on a very limited number of studies performed in laboratory animals, farm animals and humans, following essentially single intravenous administration, oral or inhalation exposure to THC, it may be assumed that both the parent compound and its metabolites with psychoactive properties (especially 11-OH-THC) are distributed in the different tissues and organs, and excreted in milk. However, there is a lack of specific studies performed in food-producing species fed hemp products.

No data are available concerning the likely transfer of THC and its lipophilic metabolites to animal tissues and eggs following repeated administration. Fat can be considered as a target tissue for THC exposure. Based on two studies (with squirrel monkeys and dairy cows), the FEEDAP Panel adopted 0.15 % as the transfer rate of oral THC to milk from dairy cows.

Studies in humans, either after single or repeated exposure, identified psychotropic effects as a follow up of a single administration at the same lowest effective dose (the lowest dose tested) of 0.04 mg THC/kg bw, which is deemed by the FEEDAP Panel to be a realistic approximation of the LOEL. The FEEDAP Panel considers that a total uncertainty factor of 100 applied to the LOEL would be sufficient to take account of all sources of uncertainty.

The provisional maximum tolerable daily intake (PMTDI) would amount to 0.0004 mg/kg bw (corresponding to 0.024 mg for a 60-kg adult and 0.0048 mg for a 12-kg child).

Considering the results of a rat study with intra-peritoneal administration of THC (neuroendocrine effects at the lowest effective dose tested 0.001 mg/kg bw), the FEEDAP Panel cannot exclude the possibility that the provisional risk assessment underestimates potential adverse effects in particular for foetuses and new-borns.

The psychotropic effects of THC, the basis for establishing the PMTDI, were considered as acute pharmacological effects. Therefore, the consumer exposure calculation was based on a single high consumption records for milk (adjusted for other dairy products), derived from the EFSA Comprehensive European Food Consumption Database and expressed as P95 values of consumers only. In the exposure scenario, 2 L and 1.5 L milk equivalents were used for adults (60 kg bw) and children of one to three years old (12 kg bw), respectively.

Different exposure scenarios were considered: (i) daily intake rates per cow of 0.5, 1.0 and 1.5 kg hemp plant-derived feed material with the maximum permitted THC content of 0.20 % or the mean THC content observed in 2008 (0.08 %), (ii) three different milk yields (15, 25 and 35 L/day) assuming a constant transfer rate of THC regardless of the milk yield. In all scenarios calculated with the maximum permitted THC content, the exposure to THC was considerably above the PMTDI (4 to 25 times higher in adults, 13 to 90 times higher in children). Considering the mean THC content (0.08 %) of hemp plants grown in the EU, the consumer exposure would be reduced by a factor of 2.5 (0.2/0.08); however, the PMTDI would still be exceeded in all scenarios. By applying the same exposure calculations to hemp seed-derived feed materials containing as a worst case estimate a maximum of 0.0012 % THC, the resulting exposure of adults and children (one to three years old) was below the PMTDI in all scenarios.

Although no data is available for edible tissues, the lipophylic properties of THC would suggest that the conclusions drawn from milk consumption would in principle apply to other animal products. Consequently, the FEEDAP Panel does not see any option for the use of whole hemp plant-derived feed materials in animal nutrition. In contrast, feeding hemp seed was considered safe for the consumer.

Feed materials do not require an assessment of their environmental impact.

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 4
Background as provided by European Commission 5
Terms of reference as provided by European Commission 5
Assessment 6
1. Introduction 6
2. The hemp plant (*Cannabis sativa* L.) 6
2.1. Characterisation of hemp-derived feed materials 6
2.2. Cannabinoids in the hemp plant and in hemp-derived products 7
2.3. Use of hemp products in animal nutrition 8
3. THC and related cannabinoids in mammals 9
3.1. Kinetics 9
3.2. Distribution and carry over in animal tissues/products 9
3.3. Pharmacological properties 11
4. Safety of THC related to hemp feeding 11
4.1. Safety for target animals 11
4.2. Safety for the consumer 11
4.3. Safety for the environment 15
Conclusions and Recommendations 15
Documentation provided to EFSA 17
Appendices 18
References 36

BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Article 15 of Regulation (EC) No 178/2002 requires that feed shall not be placed on the market or fed to any food-producing animal if it is considered to have an adverse effect on human or animal health. For feed materials no general pre-market authorisation is required and the feed business operator placing the feed material on the market has the first responsibility to assure its safety.

However, based on scientific evidence or technological developments the Commission shall, as appropriate, amend the list of materials whose placing on the market or use for animal nutritional purposes is restricted or prohibited or set a maximum level for undesirable substances in feed.

In Europe, hemp products like hemp straw or hemp oil seed cakes are used for feeding of livestock. In the EU the hemp area increased from 10.500 hectares in 2008 to 16.800 hectares in 2009. Varieties of hemp that are cultivated and used for feed must be listed in the EU's official catalogue of seeds. A maximum content of Tetrahydrocannabinol (THC) applies to each variety.

The Commission services received a dossier from the Swiss authorities concerning the prohibition of hemp as feed (will be sent as electronic version). The expert opinion states that the feeding of hemp products, including those from approved varieties, results in milk with a high concentration of THC. It is concluded that the tolerable daily intake can be exceeded for certain consumer groups. THC contamination can also occur in other animal food products.

Based on the agricultural legislation (Article 33 of Regulation 796/2004) the Member States have to monitor the THC level in the hemp cultivated on their territory. The results of the years 2005 to 2008 will be sent as electronic version.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, the Commission asks the European Food Safety Authority to issue an opinion on the safety for the animals, the consumer and the environment of feeding products of EU-authorised hemp varieties taking into account amongst others the background and the information submitted by the Swiss Authorities.

This scientific opinion should:

- Based on the THC-levels in different feed material derived from hemp and considering maximum incorporation rates into the feed rations determine the potential carry over into animals products, in particular milk.
- Determine the potential human exposure after consumption of such animal products
- Identify maximum daily intake[4] of THC and, if appropriate, maximum contents of THC in feed to comply with these maximum levels.

[4] The "maximum daily intake" corresponds in terms of risk assessment to "maximum tolerable intake".

ASSESSMENT

1. Introduction

The hemp plant *Cannabis sativa* L. has a long history of cultivation. In China and in other hemp growing areas in Asia, hemp seeds are used as traditional foods. In Europe and North America, hemp seeds for food were rediscovered in the mid 1990s – currently with the reintroduction of hemp as a source of technical fibre (Lachenmeier and Walch, 2005). Hemp is cultivated in Europe at a limited extent to produce fibre but also seeds and derived oil. Hemp varieties allowed to be cultivated for those purposes must be listed in the European Union (EU) common catalogue of varieties of agricultural plant species; the maximum content of tetrahydrocannabinol (THC), which is the main psychoactive substance, is limited to 0.2 % (w/w).[5]

The whole hemp plant, its seeds and derived seed meal following oil extraction, can be – and are to a certain extent – used as feed materials in the EU countries and European Free Trade Association countries. A review on the conditions of use of hemp in some other countries can be found in Appendix A. The Swiss authorities have recently prohibited the use of hemp-derived products as feed materials[6] because of safety concerns for children consuming high amounts of milk from dairy cows fed hemp.

EFSA received a request from European Commission to issue an opinion on the safety for the animals, the consumer and the environment of feeding products of EU-authorised hemp varieties.

2. The hemp plant (*Cannabis sativa* L.)

Since relevant statistics (DG Agri, Eurostat, FAO and EIHA) considerably differ in figures, approximations on the yearly hemp production in Europe (2002 to 2010) would be as follows: ~ 15 000 ha cultivated, ~ 25 000 t of fiber, ~ 40 000 t hemp hurds and ~ 6000 t hemp seed. Details are given in Appendix B.

Fibre (80–83 % cellulose, 17–20 % lignin), the stem tissues outside the vascular cambium, is used for the production of cigarette paper and biocomposites. Hemp hurds, the wooden inner part of the plant (50–60 % of stalk of the whole plant), contain 35 % cellulose, 18 % hemicellulose, 21 % lignin and are used as animal bedding (Carus et al., 2008). Hemp seeds are used predominantly (95 %) in animal nutrition, mainly for non-food producing birds, the remaining 5 % being used as food. Hemp seed oil (also called 'hemp oil'), produced by cold pressing the seeds, is used in cosmetic formulations for body care and as food; it should not be confused with the hemp oil that is produced by the distillation of buds and leaves, which contains much higher amounts of cannabinoids than the hemp seed oil and is usually marketed as a component of health products.

2.1. Characterisation of hemp-derived feed materials

Four essentially different types of feed materials may be derived from the hemp plant: hemp seed (full-fat), hemp seed meal/cake (after lipid removal, mainly cake from mechanical pressing), hemp seed oil and whole hemp plant (which may include hemp hurds, fresh or dried). Further products are hemp flour (ground dried hemp leaves) and hemp protein isolate (from seeds).

Hemp hurds (hemp straw, 96.3 % dry matter basis (DM)) is characterised by its high fiber content (90 % neutral detergent fiber, 78.9 % acid detergent fiber, in DM), whereas the content of crude protein (3.2 % in DM) and ether extract (0.8 % in DM) are negligible low.[7]

The hemp seed is characterised by its high content of oil (26–37.5 %), protein (25 %) and fiber (28 %, with a digestibility of about 20 %). The apparent metabolisable energy for hemp seeds for pigeons is

[5] OJ, L 30, 31.1.2009, p. 16.
[6] Ordonnance du DFE 916.307.1, Annex 4.
[7] Information provided by Friedrich Schöne, Thüringer Landesanstalt für Landwirtschaft.

given with 18 MJ/kg (Hullar et al., 1999) and for hemp seed cake for chickens with 10.1 MJ/kg (Kalmendal, 2008). The hemp seed meal (cake, in which oil is removed partially at 45 °C to 11 %) contains about 33 % protein and 43 % fibre (with a digestibility of about 40 %). The protein fraction of seeds is characterised by a medium content of lysine (~ 4 g/16 g N) and a high level of S-containing amino acids (~ 4 g/16 g N). Hemp seed oil contains about 84 % PUFAs (56 % linoleic (C18:2, n-6), 22 % alpha-linolenic acid (C18:3, n-3), 4 % gamma-linolenic (C18:3, n-6), 2 % stearidonic acid (C18:4, n-3)) (Callaway, 2004).

2.2. Cannabinoids in the hemp plant and in hemp-derived products

The hemp plant, *Cannabis sativa,* produces cannabinoids in glandular organs (trichomes) spread out on the whole surface of the plant with the exception of the seeds and roots. Trichomes are densily present on the side of the leaves, along the leave veins and in the area of influorescence. They contain resin consisting of 80 to 90 % cannabinoids as well as essential oils, high polymeric phenols, terpenes and waxes. The main psychoactive compound, delta-9-tetrahydrocannabinol (THC), is mostly present under a precursor form, devoid of activity, delta-9-tetrahydrocannabinol acid (THC-A), that may represent up to 90 % of the total cannabinoids in hemp plants grown in Europe (Grotenhermen, 2003). Among sixty other identified cannabinoids, cannabidiol (CBD) and cannabinol (CBN) are the other main active components. The phenotypes of *Cannabis sativa* are characterised by the ratio THC + CBN/CBD. The hemp varieties grown for fibre production exhibit a ratio < 1, whereas a ratio > 1 is measured in varieties cultivated for cannabinoid production (Lachenmeier and Walch, 2005). The cannabinoid content of the plant varies also according to cultivation conditions (temperature, humidity) and the vegetative state of development of the plant.

The hemp varieties allowed for fibre cultivation in Europe must contain < 0.2 % THC (in DM). The sampling conditions, i.e. the upper 30 cm part of the plant (including inflorescence) and the defined period of development of the plant, are set in Regulation (EC) No 796/2004.[8] Table 1 presents a summary of the analytical results on the THC content of hemp varieties[9] derived from the Member States notifications to the European Commission on hemp varieties for which direct aid has been claimed.[10]

THC-A can be transformed by decarboxylation into THC at high temperatures or very slowly at room temperature. Therefore, free THC content could increase in heat-processed hemp feed products and also during the analysis phase (e.g. gas chromatography with injection port > 200 °C). Consequently, a conservative approach has been retained where 'total THC content', including THC and THC-A-derived THC (denoted as THC below), is determined in hemp-derived feedingstuffs. The methods of analysis of THC and related cannabinoids in hemp products and biological samples are described in Appendix C.

Table 1: THC content of hemp varieties cultivated in Europe in 2006–2008[a,b]

	2006	2007	2008
Countries (n)	12	18	19
Samples (n)	758	819	574
Mean THC content (%)	0.079	0.066	0.080
Standard deviation	0.051	0.051	0.089
Percentage of samples > 0.2 % THC	2.50	1.59	3.66
Mean THC content (%) of samples > 0.2 %	0.27	0.30	0.41

[a] Data provided by the European Commission and derived from the Member States notifications. .

[b] Measurement of 'total THC' as described in Regulation (EC) No 796/2004.

[8] OJ, L 141, 30.4.2004, p. 8.
[9] Varieties listed in the 'Common Catalogue of Varieties of Agricultural Plant Species'.
[10] OJ, L 30, 31.1.2009, p. 16.

When the hemp plant is used as roughage (e.g. for bovines) in whole or in part, the exposure of the animals to THC could be at the highest equal to that resulting from the consumption of the upper part of the same variety defined and analysed for control according to the same Regulation. As far as hemp seeds are concerned, it has been shown (Ross et al., 2000) that the bulk of THC was found on the outside of the seeds due to the contamination with plant debris, possibly as the result of physical interaction with the plant leaves during processing. The analysis of seeds from European varieties showed that only small amounts of THC were present in the seed coat (testa) or the kernel itself (< 0.5 mg/kg) of hemp seeds previously washed with a solvent, whereas the maximum value found in the un-treated seeds was 12 mg/kg. Hemp oil, due to the lipophilic nature of THC, could be expected to contain more THC than the seed. However, analytical data showed THC levels in both type of samples, hemp seed (n = 9) and hemp oil (n = 4), in the same range (below 1 mg THC/kg).[11]

Feed materials derived from the whole hemp plant (which may include hemp hurds, fresh or dried) as well as further products (hemp flour (ground dried hemp leaves)) are not subjected to any processing, which would increase the natural THC content. In the absence of data, the FEEDAP Panel considers that (i) those feed materials would not contain more than the maximum legal THC concentration in defined samples, and (ii) data from the official control of hemp varieties in the EU should be taken as conservative surrogates of the THC-content of whole hemp plant-derived feed materials.

2.3. Use of hemp products in animal nutrition

The abstracts of the studies in which hemp seed was fed to poultry, ruminants and fish are listed in Appendix D. The following summary contains the main findings.

Up to 20 % hemp seed or hemp seed cake were used in laying hens diets without adverse effects on laying performance and egg sensory characteristics, whereas linoleic acid and alpha-linolenic-acid increased in the egg yolk (Gakhar et al., 2010; Goldberg et al., 2010; Silversides and Lefrançois, 2005). No data is available for pig feeding. Hemp meal is a good source of rumen undegraded protein, with high post-ruminal availability, as concluded from studies with fistulated cows and growing lambs (Mustafa et al., 1999). Hemp meal could be used in growing sheep up to 20 % of the diet (Mustafa et al., 1999) with no detrimental effects on nutrient utilisation. Diets containing 14 % hemp seed could be fed to yearling steers for 166 days without negative effects on gain, gain to feed ratio and carcass traits; conjugated linoleic acid and n-3 fatty acids were increased in tissues (Gibb et al., 2005). In calves and in steers hemp seed cake (1 to 1.4 kg/day) compared to a mixture of soybean-meal and barley as a protein feed resulted in similar production and improved rumen function (Hessle et al., 2008). In a ten week feeding study on juvenile sunshine bass (*Morone chrysops x M. saxatilis*) a mixture of 30 % fish meal, 30 % soy bean meal and 15 % corn could be replaced by a mixture of 27 % of soy bean meal, 27 % meat and bone meal and 20 % hemp seed meal without negative effects on performance (Webster et al., 2000).

Hemp oil could be used up to 12 % in laying hens diets without exerting adverse effects on performance parameters, flavour and aroma profiles of cooked eggs (Gakhar et al., 2010; Goldberg et al., 2010).

The *in vitro* digestibility of hemp protein isolate was determined to be 88–91 % (Wang et al., 2008b), which is higher than that of soybean protein isolate (71 %). No trypsin inhibitor was found in hemp protein.

No data is available on feeding animals with whole hemp plant or other parts of the plant other than the seeds.

2.3.1. Conclusions on the potential use of hemp products in animal nutrition

The following conclusions consider only the nutritional properties of the different hemp-derived feed materials without taking into account potential adverse effects related to THC. The whole hemp plant

[11] Data provided by Hempro International.

(including stalk and leaves) is considered, due to its high fibre content, as a suitable feed material for ruminants (and horses). Hemp seed and hemp seed cake can be used as feed materials for all species. Several species-specific restrictions (fibre for poultry, polyunsaturated fatty acids for pigs) may be considered when incorporating such products into the complete feed. The proportion of rumen undegradable protein in hemp seed is considered advantageous for ruminants.

Data from feeding trials indicate that hemp seed cake could be used up to 20 % in laying hens diets; it is concluded therefore that not more than 10 % can be used in diets for chickens for fattening. No data is available for pigs; however, it is expected that 10 % hemp seed cake and 5 % hemp seed could be used in complete feed for pigs. Data indicate that 14 % of hemp seed cake can be used in a total mixed ration for dairy cows. Comparable data for rearing calves and cattle for fattening showed that a daily amount of 1 to 1.4 kg of hemp seed cake could be fed.

The maximum incorporation rates in formulating compound feedingstuffs are likely lower than the above values due to the very limited availability of hemp products (amount and price); therefore, they are difficult to estimate. If significant amounts of hemp products are locally available, the following maximum incorporation rates in feed could be expected in routine production: poultry for fattening 3 %, laying poultry 5–7 % hemp seed/hemp seed cake; pigs 2–5 % hemp seed/hemp seed cake; ruminants 5 % hemp seed cake in the daily ration; fish 5 % hemp seed. It should be noted that these figures cannot be considered additive because the simultaneous use of hemp products would considerably exceed the available resources.

The whole plant (or parts of it, e.g. leaves) may be consumed as part of the roughage in feeds for ruminants. Since no data is available, it is considered likely that daily amounts of 0.5 to 1.5 kg DM could be incorporated in the daily ration of dairy cows.

3. THC and related cannabinoids in mammals

3.1. Kinetics

The kinetics of cannabinoids, mainly THC, is summarised below. Further details are presented in Appendix E.

After oral exposure, THC bioavailability is in the range of 6–30 %, with wide inter-individual variation (Ashton, 2001). In mammalian species, THC undergoes mainly hepatic CYP 2C9-mediated oxidation, yielding the primary metabolite 11-hydroxy-delta-9-THC (11-OH-THC); this metabolite displays a psychotropic activity greater than the parent compound and is further oxidised by the same enzyme to the inactive 11-nor-9-carboxy-delta-9-THC (THC-COOH). THC and its metabolites are then subjected to glucuronidation (Yamamoto et al., 1987). Both THC and 11-OH-THC are characterised by a high degree of lipophilicity; therefore, they accumulate in fat tissues, where they reach the peak concentrations after four to five days of a single exposure. They may be released back to other compartments, including brain tissue, for several days (Ashton, 2001). This behaviour together with the intense enterohepatic recycling support the long tissue half-life (about seven days) and the slow excretion of THC and its metabolites via the urinary and faecal route (Maykut, 1985).

According to a recent study performed in rats (Jung et al., 2009), the main THC precursor in plant materials (THC-A, see Section 2.2) is not metabolised to THC and follows a specific metabolic pathway. However, this observation cannot be extrapolated to other animal species in general, and in particular to ruminants in which decarboxylation of THC-A by the ruminal micro-organisms may occur. Moreover, the psychoactive potential of THC-A metabolites has not been established.

3.2. Distribution and carry over in animal tissues/products

Based on a very limited number of studies performed in laboratory animals, farm animals and humans, following essentially single intravenous administration, oral or inhalation exposure to THC, it may be assumed that both the parent compound and its metabolites with psychoactive properties (especially

11-OH-THC) are distributed in the different tissues and organs, and also excreted in milk. However, there is a lack of specific studies performed in food-producing species fed hemp products.

3.2.1. Animal tissues

No data are available concerning the likely transfer of THC and its lipophilic metabolites to animal tissues and eggs following repeated administration.

One study on the distribution of THC in pig tissues following intravenous administration has been published (Brunet et al., 2006). Eight male pigs (29 to 44 kg) received a single intravenous injection of 200 µg/kg body weight (bw); two animals were sacrificed after 0.5, 2, 6 and 24 hours; blood and tissues were sampled and THC and its metabolites were measured using GC/MS analysis. THC was eliminated rapidly from the liver (155 µg/kg after 0.5 hour, not detectable after 6 hours). The slowest elimination occurred in the fat (91 µg/kg after 0.5 hour, 32 µg/kg after 24 hours). THC-elimination kinetics noted in kidney and muscle was comparable to that observed in blood. 11-OH-THC was found at high levels only in liver (39 µg/kg after 0.5 h and 24 µg/kg after 2 hours), whereas THC-COOH was less than 5 µg/kg in all edible tissues. A transfer rate from feed to edible tissues cannot be derived from these data. In addition, the extrapolation of a tissue deposition established after a single intravenous administration of THC to that resulting from oral exposure is of limited practical value. The only conclusion drawn from these data is that the fat can be considered as a target tissue for THC exposure.

3.2.2. Milk

Several reports indicate that milk represents an important route of excretion in humans (Perez-Reyes and Wall, 1982), squirrel monkeys (Chao et al., 1976) and ruminants, such as sheep (Jakubovic et al., 1974), buffaloes (Ahmad and Ahmad, 1990) and cows (Guidon and Zoller, 1999). The bioavailability of THC derivatives excreted by the mammary route is supported by the finding of the marker metabolite THC-COOH in the urine of children consuming milk from buffaloes fed *Cannabis*-contaminated fodder (Ahmad and Ahmad, 1990).

One published study on the quantitative transfer of THC orally administered to squirrel monkeys is available (Chao et al., 1976). A field experiment on the quantitative transfer of THC from hemp pellets (whole plant) to milk of dairy cows was made available by the Swiss Authorities (unpublished study).

The study performed in squirrel monkeys considered two groups of animals that were administered 2 mg THC/kg bw twice and five times a week, for 20 weeks. In weeks 8 and 20, a tracer dose of ^{14}C-THC combined with unlabelled THC was administered, achieving a total dose of 2 mg THC/kg bw. Milk samples were taken hourly for five consecutive hours (week 8) and for 24 hours (week 20). Total radioactivity was measured and the identification of THC and its metabolites was attempted (by thin layer chromatography). As the specific radioactivity of THC in plasma and milk was not calculated, the measurement of total radioactivity only reflects the kinetics of the single dose of labelled THC administered. The carry-over of THC-related radioactivity in milk amounted to 0.2 % of the administered dose over the 24-hour observation period. About 7 % of the total radioactivity in milk was tentatively identified as THC, the major part being distributed between many compounds that could correspond to mono and dihydroxy-metabolites, among others.

In a preliminary experiment with one cow (Guidon and Zoller, 1999), a single oral dose of 625 mg THC in gelatine capsules was administered the day before sampling started. THC and its metabolite 11-OH-THC were measured in blood (GC-MS analysis after hydrolysis), sampled for the first 48 hours (every two to six hours) following administration and after two weeks, and in milk, collected twice a day for two weeks. Based on figures derived from graphs, THC peaked after 10–12 hours in serum/plasma (5 ng/mL) and after 23 hours in milk (20 ng/mL). The corresponding figures for peak values of 11-OH-THC were 1 ng/mL and < 0.3 ng/mL. The half-life of THC in milk was shown to be 29 hours. These data confirm that orally administered THC (i) is excreted in milk by dairy cows, the

same as in humans, monkeys, buffalos, and (ii) results in concentrations of THC (and 11-OH-THC) considerably higher in milk than in blood (Perez-Reyes and Wall, 1982).

In a second experiment performed in 2005 (unpublished study) at farm level, eighty cows were fed for six consecutive days 0.5 kg/day pellets prepared from the whole hemp plant. The THC content of the pellets, measured as total THC (based on GC-MS analysis), was 6500 mg/kg (0.65 %); therefore, the daily dose was 3250 mg. Milk was collected twice a day and THC measured using LC/MS analysis with deuterated THC as internal standard. The THC content of sample 1, consisting of bulk milk from days 4 and 5, was 0.241 mg/L; the THC content of sample 2, consisting of bulk milk collected in the morning of day 6, was 0.233 mg/L. Those results indicate that THC mammary excretion had reached a steady state after four to five days. The transfer rate of 'total THC' from feed to THC in milk, calculated assuming a daily milk production of 20 L per cow, amounted to 0.15 %.

Both calculated transfer rates, 0.2 % in squirrel monkeys and 0.15 % in dairy cows, are of the same order of magnitude. Considering both (i) the weaknesses of the analytical measurements of THC in the study performed in squirrel monkeys (Chao et al., 1976) and (ii) the availability of target specific data (see the Swiss experiment), the FEEDAP Panel adopted 0.15 % as the transfer rate of oral THC to milk from dairy cows for the subsequent evaluation.

3.3. Pharmacological properties

Most of the biological effects ensuing the exposure to THC and its active metabolite(s) are due to the binding to specific G-protein coupled receptors, named cannabinoid receptors (CB_1 in the brain and CB_2 in many other tissues, including lymphoid and genital tissues), which have been identified in rats, guinea pigs, dogs, monkeys, pigs and humans. In recent years, endogenous ligands structurally related to arachidonic acid, referred to as 'endocannabinoids', have also been uncovered.

Cannabinoids, including THC, have been studied for many therapeutical applications (e.g. analgesia and pain management, muscle relaxation, immunosuppression, stimulation of appetite) (see Wang et al, 2008a and Gerra et al., 2010).

4. Safety of THC related to hemp feeding

4.1. Safety for target animals

No studies concerning tolerance or the effects of (graded levels of) THC in food-producing animals have been found in literature.

Several case reports describing accidental poisoning are available but do not allow the establishment of a dose-effect relationship. A wide variety of clinical signs have been reported in poisoned dogs, including nervous symptoms (depression, ataxia, hyperstesia, recumbency and, less commonly, stupor, tremors or seizures) and mild gastrointestinal upset. Tremors, mydriasis, hypersalivation and the lack of coordination were noted in cattle 20 hours after ingestion of about 35 kg of dried *Cannabis* material (Driemeier, 1997). Provided that poisoned animals are subjected to proper treatment, the prognosis for full recovery is excellent (Bischoff et al., 2007).

4.2. Safety for the consumer

A detailed description of the toxicological profile of THC and related cannabinoids is presented in Appendix F and summarised below.

Despite the availability of a considerable wealth of information that might be useful to establish a threshold for THC effects, it should be noted that most of the published studies have been designed to gain insight into THC mechanisms of action rather than determining the threshold for the effects under investigation. A further source of information relies in a number of published clinical trials illustrating the adverse effects of synthetic cannabinoids in humans in view of their potential therapeutic application. Psychotropic and (neuro-)endocrine effects have been the most investigated endpoints.

4.2.1. Psychotropic and central nervous system effects

4.2.1.1. Single exposure

A single oral exposure to 7.5 mg THC elicits a statistically significant increase in heart rate (~7 beats/min) in both infrequent and frequent *Cannabis* users, with peaks after 2.5–3.5 hours following drug administration (Kirk and De Wit, 1999). However, the most sensitive parameters to a single exposure to THC are by far the effects on the central nervous system, including mild euphoria, relaxation, increased sociability, enhanced sensory perception and increased appetite. In addition, cannabinoid intake is reported to affect mood and is associated with impaired function of a variety of cognitive tasks and short-term memory, including driving or operation of intricate machinery (WHO, 1997).

Experimental studies on the effects of cannabinoids on isolated cognitive functions and psychomotor skills related to driving performance indicate that THC at doses between 0.04 and 0.30 mg/kg bw causes a dose-dependant reduction in performance, as observed in laboratory tasks measuring memory function, divided and sustained attention, reaction time, tracking or motor control (see Ramaekers et al., 2004).

Chesher et al. (1990) performed a study aimed at investigating the effect of oral THC when administered in capsules, dissolved in sesame oil, at doses of 0, 5, 10, 15 or 20 mg/person in a total of 80 students of both sexes, with a body weight range of 58 to 84 kg (groups of 16 volunteers each). The authors concluded that an effect on skill performances (standing steadiness, hand-eye coordination, reaction time, etc) can occur with a single oral dose of 5 mg THC/person, corresponding to 0.06 mg/kg bw calculated for the highest individual body weight.

4.2.1.2. Repeated exposure

Fewer reports are available on the effects of a repeated exposure to THC in humans. In a multi-center, double-blind, placebo controlled study performed by Beal et al. (1995), in which HIV patients of either sex were orally administered Dronabinol© (THC) for several days, psychotropic effects (euphoria, dizziness, thinking abnormalities, somnolence) were elicited in 25/72 (~ 35 %) patients at the lowest tested dose (twice x 2.5 mg/person/day for 42 days). In a further multi-center, open-label study published by the same research team (Beal et al., 1997), comparable effects were described for a repeated daily dose of 2.5 mg THC (administered for 12 months).

In 1997, the German Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV), predecessor institute of the Federal Institute for Risk Assessment, performed a risk assessment on hemp food based on the information available at that time. The outcome of that risk assessment was published in two press releases (BgVV, 1997 and 2000). The recommendation of limiting the daily intake to 0.001–0.002 mg THC/kg bw was derived by applying an uncertainty factor in the range of 20–40 to the lowest oral dose (2.5 mg THC/day) associated with central nervous effects that was found in an unpublished human study.

4.2.2. Neuroendocrine effects

Animal models have been developed in which, as a consequence of the binding to the endogenous cannabinoid receptors, the administration of THC and related compounds has been found to acutely affect multiple hormonal systems, including gonadal steroids, prolactin, growth and thyroid hormones, and to activate the hypothalamic-pituitary-adrenal axis. Despite these findings in animals, studies in humans have given inconsistent results, partly due to the possible development of tolerance, and have been mostly conducted in marijuana smokers (Brown and Dobs, 2002). In addition, only a very limited number of the experimental studies performed in people did address those effects.

Healthy individuals with previous *Cannabis* exposure but without abuse disorders were administered 2 or 5 mg THC by a single intravenous injection (D´Souza et al., 2004). The resulting THC blood levels were within the range achieved by smoking a standard cigarette (70–163 ng/mL) containing 1–2.5 %

THC (16–34 mg). A dose-related increase in blood cortisol and a wide array of psychotropic symptoms were noticed.

The repeated intraperitoneal administration of THC at a dose of 0.001 mg/kg bw/day in rats, starting on day 22 postnatal until the expected day of vaginal opening, induced a two-day delay in vaginal opening. The number of ova on the day of the first oestrus was significantly lower in treated rats than in controls. In animals treated in the same way but kept under observation until adulthood, oestrous cycles were irregular and serum luteinising hormone was decreased in all the cycle phases (Wenger et al., 1988).

Considering (i) the absence of a dose-response approach in the protocol, (ii) the limited time of administration and (iii) uncertainties related to the intraperitoneal route of administration, an oral NOAEL based on neuroendocrine effects cannot be derived. Consequently, (i) in view of the lack of conclusive data for neuroendocrine effects in humans and (ii) the possible greater sensitivity of rats to the endocrine effects, a current risk assessment could only be provisional and based on psychotropic effects observed in humans. At present, the FEEDAP Panel cannot exclude that the provisional risk assessment underestimates potential adverse effects, in particular for foetuses and newborns (see below).

4.2.3. Risk factors

Increased sensitivity of neonates and infants, genetic polymorphisms, interaction with other drugs and body mass index should be considered as risk factors in deriving threshold limits for THC in humans.

THC and its metabolites can easily cross both the placental (Little and Van Bevren, 1996) and the mammary barrier (Perez-Reyes and Wall, 1982). According to Glass et al. (1997), the foetal and neonatal human brains show patterns of cannabinoid receptor distribution similar to those observed in the adult human brain; the density of receptor binding, however, is generally markedly higher, especially in the basal ganglia and *substantia nigra*, thus pointing to an increased magnitude of the central nervous and possibly neuroendocrine effects of the exogenous cannabinoids. In addition, foetal and newborn drug metabolising enzymes are not fully developed (until three to four weeks of age), including phase I (CYPs) and phase II enzymes (UGTs) involved in the generation of inactive metabolites (i.e. THC-COOH and glucuronides). This conclusion is supported by the absence of THC-COOH in an infant exposed to THC through breast milk from a marijuana-using mother (Perez-Reyes and Wall, 1982).

Both cannabinoid receptors so far identified (CB_1 and CB_2) are encoded by specific genes (*CNR1* and *CNR2*) displaying several identified polymorphisms (Onaivi, 2009), which may alter the overall THC-mediated response. CYP2C9, which is responsible for the main oxidative biotransformation pathways of THC, is also subject to polymorphisms in Caucasian populations which have been implicated in marked differences (almost 20 fold) in both the maximum peak concentrations and total clearance of the orally administered cannabinoid to human volunteers (Sachse-Seeboth et al., 2009).

The interactions with ethanol and other drugs of abuse are well documented (Ramaekers et al., 2000) and may potentiate the overall THC adverse effects in foetuses, newborns and adults. Moreover, cannabinoids have been found to interact with other drugs like hexobarbital (Benowitz et al., 1980) and phenytoin (Bland et al., 2005).

Finally, a significant correlation was found between Body Mass Index and C_{max} values for both THC and its active derivative 11-OH-THC, suggesting a greater deposition in adipose tissue and a subsequent prolonged release to plasma in obese individuals (Goodwin et al., 2006).

4.2.4. Maximum Tolerable THC intake

Studies in humans, either after single or repeated exposure, identified psychotropic effects as a follow up of a single administration at the same lowest effective dose (lowest dose tested) of 0.04 mg THC/kg

bw (Beal et al. 1997; BgVV, 1997 and 2000; see also the review of Ramaekers et al., 2004). The FEEDAP Panel considered this dose as a realistic approximation of the LOEL.

In deriving a provisional maximum tolerable THC intake from the above LOEL, the BgVV applied an uncertainty factor between 20 and 40 taking into account inter alia the lack of knowledge concerning the onset and exact dose-effect relationship of the psychomotor effects of orally administered THC, the inter-individual differences in sensitivity to THC and possible interactions with other active substances in the botanical source or with alcoholic beverages or drugs taken concomitant with the hemp food. The BgVV recommended that the daily intake of THC with hemp food should not exceed 0.001–0.002 mg/kg bw.

The FEEDAP Panel considers it necessary to introduce in addition to the safety factors applied by the BgVV a further safety factor to take into account that the basis for deriving a provisional maximum tolerable THC intake is regarded as a LOEL. A total uncertainty factor of 100 applied to the LOEL would be sufficient to take account of all sources of uncertainty.

The provisional maximum tolerable daily intake (PMTDI) would amount to 0.0004 mg/kg bw (corresponding to 0.024 mg for a 60-kg adult and 0.0048 mg for a 12-kg child).

4.2.5. Consumer exposure calculation

As the available data allow a reliable exposure calculation via milk only, other potential sources of THC exposure (fat and other tissues and products) could not be considered further.

The psychotropic effects of THC, the basis for establishing the PMTDI, were considered as acute pharmacological effects. Therefore, the consumer exposure calculation was based on the maximum daily intake of milk. Data for single high consumption records for milk (adjusted for other dairy products) were derived from the EFSA Comprehensive European Food Consumption Database and expressed as P95 values (consumers only). In the exposure scenario, 2 L and 1.5 L milk equivalents were used for adults (60 kg bw) and children of one to three years old (12 kg bw), respectively.

The THC content in milk has been calculated by applying a transfer rate to milk of 0.15 %.

Different exposure scenarios were considered: (i) daily intake rates per cow of 0.5, 1.0 and 1.5 kg hemp plant-derived feed material with the maximum permitted THC content of 0.20 % or the mean THC content observed in 2008 (0.08 %) and (ii) three different milk yields (15, 25 and 35 L/day), assuming a constant transfer rate of THC regardless of the milk yield.

The results are summarised in Table 2. It appears that all scenarios estimate an exposure to THC considerably above the PMTDI (4 to 25 times higher in adults, 13 to 90 times higher in children). Considering the mean THC content (0.08 %) of the hemp plants grown in the EU, the consumer exposure would be reduced by a factor of 2.5 (0.2/0.08); however, the PMTDI would still be exceeded in all scenarios.

By applying the same exposure calculations with hemp seed-derived feed materials containing as a worst case estimate a maximum of 0.0012 % THC (Ross et al., 2000), the resulting exposure of adults and children (one to three years old) would in all scenarios appear below the PMTDI (Table 3).

Table 2: Exposure of adults and children (one to three years of age) to THC via milk from dairy cows ingesting different levels of whole hemp plant-derived feed materials with 0.2 % THC (maximum legal content) and with different milk yields

	THC intake (mg)					
	Adults from 2.0 L milk			Children from 1.5 L milk		
Milk yield (L/day)	*15*	*25*	*35*	*15*	*25*	*35*
Cow daily intake (kg DM)						
1.5	0.60	0.36	0.26	0.45	0.27	0.19
1.0	0.40	0.24	0.17	0.30	0.18	0.13
0.5	0.20	0.12	0.09	0.15	0.09	0.06

Table 3: Exposure of adults and children (one to three years of age) to THC via milk from dairy cows ingesting different levels of hemp seed-derived feed materials with 0.0012 % THC and with different milk yields

	THC intake (mg)					
	Adults from 2.0 L milk			Children from 1.5 L milk		
Milk yield (L/day)	*15*	*25*	*35*	*15*	*25*	*35*
Cow daily intake (kg DM)						
1.5	0.0036	0.0022	0.0015	0.0027	0.0016	0.0012
1.0	0.0024	0.0014	0.0010	0.0018	0.0011	0.0008
0.5	0.0012	0.0007	0.0005	0.0009	0.0005	0.0004

The FEEDAP Panel back calculated also (see Appendix G) the maximum THC content in hemp-derived feed materials which would result in milk concentrations corresponding to a THC exposure of adults and children (one to three years of age) in accordance with the PMTDI. The data demonstrated that the use of hemp-derived feed materials should not exceed 0.002 % (20 mg/kg) to ensure consumer safety.

Consequently, the FEEDAP Panel does not see any option for the further use of whole hemp plant-derived feed materials in feeding dairy cows. Although no data is available for edible tissues, the lipophylic properties of THC would suggest that the results obtained from milk would in principle apply to other animal products.

4.3. Safety for the environment

Feed materials do not require an assessment of their environmental impact.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Hemp seed and hemp seed cake could be used as feed materials for all animal species. Several species-specific restrictions (fibre for poultry, polyunsaturated fatty acids for pigs) may limit the incorporation rate into the complete feed. The maximum incorporation rates in the complete feed could be 3 % in poultry for fattening, 5–7 % in laying poultry and 2–5 % in pigs for hemp seed and hemp seed cake, 5 % in ruminants for hemp seed cake and 5 % in fish for hemp seed.

The whole hemp plant (including stalk and leaves) would be, due to its high fibre content, a suitable feed material for ruminants (and horses), and daily amounts of 0.5 to 1.5 kg whole hemp plant DM could likely be incorporated in the daily ration of dairy cows.

No studies concerning tolerance or effects of graded levels of THC in food producing animals have been found in literature.

Based on a very limited number of studies performed in laboratory animals, farm animals and humans, following essentially single intravenous administration, oral or inhalation exposure to THC, it may be assumed that both the parent compound and its metabolites with psychoactive properties (especially 11-OH-THC) are distributed in the different tissues and organs, and excreted in milk. However, there is a lack of specific studies performed in food-producing species fed hemp products. Fat can be considered as a target tissue for THC exposure. Based on two studies (squirrel monkeys and dairy cows), the FEEDAP Panel adopted 0.15 % as transfer rate of oral THC to milk from dairy cows.

Studies in humans, either after single or repeated exposure, identified psychotropic effects as a follow up of a single administration at the same lowest effective dose (the lowest dose tested) of 0.04 mg THC/kg bw, which is deemed by the FEEDAP Panel to be a realistic approximation of the LOEL. The FEEDAP Panel considers that a total uncertainty factor of 100 applied to the LOEL would be sufficient to take account of all sources of uncertainty.

The provisional maximum tolerable daily intake (PMTDI) would amount to 0.0004 mg/kg bw (corresponding to 0.024 mg for a 60-kg adult and 0.0048 mg for a 12-kg child).

Considering the results of a rat study with intra-peritoneal administration of THC (neuroendocrine effects at the lowest effective dose tested 0.001 mg/kg bw), the FEEDAP Panel cannot exclude the possibility that the provisional risk assessment underestimates potential adverse effects in particular for foetuses and new-borns.

The psychotropic effects of THC, the basis for establishing the PMTDI, were considered as acute pharmacological effects. Therefore, the consumer exposure calculation was based on a single high consumption records for milk (adjusted for other dairy products), derived from the EFSA Comprehensive European Food Consumption Database and expressed as P95 values of consumers only. In the exposure scenario, 2 L and 1.5 L milk equivalents were used for adults (60 kg bw) and children of one to three years old (12 kg bw), respectively.

Different exposure scenarios were considered: (i) daily intake rates per cow of 0.5, 1.0 and 1.5 kg hemp plant-derived feed material with the maximum permitted THC content of 0.20 % or the mean THC content observed in 2008 (0.08 %), and (ii) three different milk yields (15, 25 and 35 L/day) assuming a constant transfer rate of THC regardless of the milk yield. In all scenarios calculated with the maximum permitted THC content, the exposure to THC was considerably above the PMTDI (4 to 25 times higher in adults, 13 to 90 times higher in children). Considering the mean THC content (0.08 %) of hemp plants grown in the EU, the PMTDI would still be exceeded in all scenarios. By applying the same exposure calculations to hemp seed-derived feed materials containing as a worst case estimate a maximum of 0.0012 % THC, the resulting exposure of adults and children (one to three years old) was below the PMTDI in all scenarios.

Although no data is available for edible tissues, the lipophylic properties of THC would suggest that the conclusions drawn from milk consumption would in principle apply to other animal products.

The FEEDAP Panel does not see any option for the use of whole hemp plant-derived feed materials in animal nutrition. In contrast, feeding hemp seed was considered safe for the consumer exposed to milk from dairy cows fed the feed material.

RECOMMENDATIONS

The FEEDAP Panel recommends the introduction of an upper level of THC for hemp seed-derived feed materials of 10 mg/kg. Hemp seed-derived feed materials are hemp seed with hulls (properly processed), dehulled hemp seed, defatted hemp seed, hemp oil and hemp protein concentrate.

All other hemp-derived feed materials (whole hemp plant, hemp hurds, hemp flour (ground dried hemp leaves)) should be placed on the list of materials whose placing on the market or use for animal nutritional purposes is restricted or prohibited as referred to in Article 6 of Regulation (EC) No 767/2009.[12]

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on the evaluation of the safety of Hemp as animal feed. December 2009. Provided by European Commission.
2. Information provided by the Federal Office for Agriculture, Switzerland.
3. Information provided by the Focal Points in Europe.
4. Information provided by the BgVV (German Federal Institute for Consumer Health Protection and Veterinary Medicine).
5. Information provided by the following International Organisations: Canadian Food Inspection Agency and Health Canada, Food Standards Australia New Zealand, New Zealand Food Safety Authority, U.S. Food and Drug Administration.
6. Information provided by the European Industrial Hemp Association.

[12] OJ, L 229, 1.9.2009, p. 1.

APPENDICES

Appendix A	Cultivation and use of hemp in other countries	Page 19
Appendix B	Cultivation of hemp	Page 20
Appendix C	Analysis of THC and cannabinoids in hemp products and biological samples	Page 21
Appendix D	Use of hemp products in animal nutrition	Page 23
Appendix E	Kinetics and dynamics of THC and main related cannabinoids	Page 26
Appendix F	Toxicological profile of THC and related cannabinoids	Page 29
Appendix G	Extrapolation of the maximum THC content in hemp derived products	Page 35

APPENDIX A

Cultivation and use of Hemp in other countries

In the USA, the cultivation of hemp has been forbidden since 1970. However, the Drug Enforcement Administration has adopted an interim rule exempting from Controlled Substances Act certain items derived from the *Cannabis* plant and containing tetrahydrocannabinols (THC). Specifically, the interim rule exempted […] processed plant materials used to make […] animal feed mixtures, provided [they] are made from those portions of the *Cannabis* plant that are excluded from the definition of marijuana [and] are not used, or intended for use, for human consumption and therefore cannot cause THC to enter the human body.[13] In practical terms: i) hemp products (hemp stalks, hemp seed, hemp seed oil and hemp seed meal) can be imported in the US as far as US Customs verify that their THC contents is below 0.3 % and that seeds are sterilised; ii) these products can be used as feed materials without restriction for non-food producing animals, iii) feedingstuffs prepared with such products must not give rise to the presence of THC in human food, i.e. either the hemp products are devoid of THC or a clear demonstration of the non-transfer to animal products is made and assessed by the U.S. Food and Drug Administration.

In Canada, all feed ingredients must be approved by the Federal Feeds Act and Regulations which regulates the manufacture and sale of feed. According to the Canadian Food Inspection Agency, industrial hemp and hemp derivatives are currently not approved for use as livestock feed. To date, in the absence of THC analytical data, no maximum limits have been established for THC content in industrial hemp or hemp derivatives intended for livestock feed. The Animal Feed Division at the mentioned Agency has provided the industry with a guidance document to help preparing a product submission in the event that an ingredient approval is sought for industrial hemp and hemp derivatives.[14]

Australia's regulation of stock feeds is managed at the Australian State and Territory level by relevant agencies.[15] Only licensed or authorised persons (Register of the Industrial Hemp Act) are able to possess industrial *Cannabis* plants and seed and to produce industrial *Cannabis* plants from certified *Cannabis* seed. An 'industrial *Cannabis* plant' has been defined to mean a *Cannabis* plant with a THC concentration in its leaves and flowering heads of not more than 0.35 %. Similar rules apply in New Zealand. Hemp products are not allowed to be used for feed and food applications in Australia. There is no restriction on the use in animal fodder of hemp products in compliance with the licensed condition (i.e. for standing crops opened to animal grazing and oral nutritional compounds such as traded feed) in New Zealand. There is no specific standard that establishes maximum permissible limits for THC in food products in Australia and New Zealand, but hemp seed oil can be used in food products in the latter.[16]

[13] Federal Register, Volume 68, No 55. 21.03.2003, pages 14114 – 14126.
[14] Available at: http://www.inspection.gc.ca/english/anima/feebet/regdir/sect3_10e.shtml
[15] Available at: http://www.agric.wa.gov.au/objtwr/imported_assets/aboutus/as/information_paper_2008.pdf
[16] Available at: http://www.legislation.govt.nz/regulation/public/2002/0396/latest/DLM174564.html

APPENDIX B

Cultivation of hemp

Recent data on the cultivation areas in the EU (27 countries) under the processing aid scheme for hemp fibre (and flax fibre)[17] were made available by the European Commission.

Table B.1: Hemp cultivation areas (in hectares) in the European Union

Country	2006-2007	2007-2008	2008-2009	2009-2010
Austria	546	-	52	40
Check Republic	1086	1396	518	142
Denmark	1	44	-	58
Deutschland	1233	824	896	1203
Finland	75	5	-	-
France	8083	7350	6187	11 326
Hungary	198	-	-	-
Italy	236	404	263	-
Lithuania	-	-	5	136
Netherlands	16	117	274	886
Poland	762	1081	987	452
Romania	-	73	-	-
Spain	3	-	-	-
United Kingdom	1671	643	1362	307
Total production	13 911	11 936	10 545	14 550

The total production in the EU, as presented in the table above, does not include hemp areas which are outside the processing aid scheme because the hemp plants are not used to produce fibre or for other reasons. Therefore, the real production values are underestimated.

The European production is only a small part of the worldwide production, estimated by the FAO (FAOSTAT) in 2005 to be 360 000 ha, Asia being the main contributor with 80 000 ha, followed by Europe and Canada.

[17] Council Regulation (EC) No 1234/2007 and Commission Regulation (EC) No 507/2008.

APPENDIX C

Analysis of THC and cannabinoids in hemp products and biological samples

C.1. Analysis of cannabinoids in feedingstuffs

C.1.1. THC

Besides THC, its precursor in the hemp plant, delta-9-tetrahydrocannabinolcarboxylic acid (THC-A) may represent up to 90 % of total cannabinoids. This compound can be transformed by decarboxylation into THC under certain circumstances. The phenomenon occurs very slowly at room temperature but rapidly at high temperatures. Therefore, THC content in hemp products could increase if those products are heat-processed (extrusion, pelleting). Consequently, a conservative approach has been retained where 'total THC content', including THC and THC-A-derived THC, is determined in feedingstuffs. This is the case for the 'Community method for the quantitative determination of delta-9-tetrahydrocannabinol' enforced at the EU level (Regulation (EC) No 796/2004, Annex I)[18], but also for the 'Gas chromatographic determination of tetrahydrocannabinol in cannabis' enforced in Canada (Bureau of Drug Research, Health Protection Branch, 1992).[19] The THC and THC-A in hemp plant materials are extracted simultaneously from the plant matrix by a non-polar solvent (e.g. toluene, dichloromethane-methanol) and the extract is analysed by gas chromatography with flame ionisation detection. THC-A, if present in the extract, is decarboxylated quantitatively to THC in the injector (> 200 °C) of the gas chromatograph and detected/quantified as THC.

Other methods, based on more recent GC/MS developments or using different analytical approaches, such as HPLC or LC/MS with prior conversion (thermal or enzymatic) of THC-A to THC, have been developed (Lachenmeier and Walch, 2005).

The simultaneous and specific analysis of THC-A and THC in feedingstuffs has been achieved using either a gas chromatographic separation of THC and THC-A after a pre-analytical derivatisation of both compounds (Lehmann and Brenneisen, 1995) or an HPLC with UV detection (Zoller et al., 2000).

C.1.2. Other cannabinoids

Among 60 other known cannabinoids, cannabidiol (CBD) and cannabinol (CBN) are the next main components. In reference to the content of THC, it is possible to distinguish between fibre hemp and drug hemp. The phenotypes of *Cannabis sativa* are characterised by the ratio of (THC+CBN)/CBD (drug hemp > 1; fibre hemp < 1).

Gas chromatography coupled with mass spectrometry (GC/MS) is the method of choice for the identification and the determination of cannabinoids in hemp food products (Lachenmeier and Walch, 2005; Pellegrini et al., 2005). A totally automated headspace solid-phase micro extraction method coupled to GC/MS determination of THC, CBD and CBN in all kinds of hemp food products has been proposed (Lachenmeier et al., 2004).

C.2. Analysis of cannabinoids in biological samples

Analytical methods have been developed to measure THC in biological fluids: blood and urine (Jung et al., 2007), oral fluid (Laloup et al., 2005; Teixeira et al., 2005). Those methods, based on LC/MS or LC/MS/MS analysis, are specific and very sensitive (limit of quantification between 0.2 and 1 ng/ml). The simultaneous identification and quantification of THC-A, THC, CBN and CBD in oral fluid has been proposed (Moore et al., 2007), based on GC/MS analysis.

The LC/MS/MS identification and quantification of THC and its metabolites 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in blood has been proposed (del Mar Ramirez Fernandez et al., 2008), with limits of

[18] OJ L 141, 30.04.2004, p.18.
[19] Available at: http://www.hc-sc.gc.ca/hc-ps/pubs/precurs/hempthc-eng.php

quantification of 0.5, 1 and 2 ng/mL. A 2D-GC/MS method has been carried out to measure the same compounds plus CBN in plasma, offering performances of the same order of magnitude (Karschner et al., 2011). The GC/MS analysis of THC and THC-COOH in the blood and urine has been achieved (Schroeder et al., 2008), with limits of quantification of 1 and 2 ng/mL for THC and THC-COOH in blood, 3 ng/mL for THC-COOH in urine.

No specific method has been published to quantify THC in milk and tissues.

APPENDIX D

Use of hemp products in animal nutrition

Safety of industrial hemp as feed ingredient in the diets of laying hens and its impact on their performance (Gakhar et al., 2010)

'A total of sixteen 19-wk-old individually housed Bovan White laying hens were fed one of the 2 diets containing 10 and 20% of hemp seed (HS). Concurrently, a total of twenty-four 19-wk-old individually housed Bovan White laying hens were fed one of the 3 diets containing 4, 8 and 12% of hemp oil (HO). Eight birds fed wheat, soy and corn oil based diets served as control. The diets were fed over a period of 12 weeks. All the diets were formulated to be isonitrogenous and isoenergetic. Daily egg weights, egg production, average daily feed intake (ADFI), feed defficiency (FE) and weekly body weights were recorded for the entire 12 weeks. Shell thickness and Haugh units (HU) were recorded from the eggs collected in wk 4, 8 and 12. Data were subjected to statistical analysis using Proc Mixed procedure of SAS. Daily egg weights (55.13 vs. 51.49 ± 1.2 g), FE (1.74 vs. 1.88 ± 0.04) and body weights (1.47 vs. 1.43 ± 0.02 kg) were higher ($P < 0.05$) for the birds fed 20% HS in comparison to the control. ADFI was lower ($P < 0.05$) in all HO treatments as compared with the control. Hen day egg production (91.12 vs. 96.84 ± 0.07%) and HU (83.8 vs. 86.8 ± 1.53 HU) were lower ($P < 0.05$) in 4% HO group whereas HU increased ($P < 0.05$) in 8% HO group as compared with the control. FE was higher ($P < 0.05$) in 12% HO group (1.70 vs. 1.85 ± 0.04) as compared with the control. In conclusion, this study allays concerns over the safety of feeding industrial hemp to the laying hens and demonstrates the positive impact of feeding HS on their performance.'

Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle (Gibb et al., 2005)

'Sixty individually penned steers (380±39 kg) were fed barley-based finishing diets containing 0 (control), 9 or 14% full-fat hemp seed (HS) and effects on performance and tissue fatty acid profiles were assessed. At harvest, samples of pars costalis diaphragmatis (PCD) and brisket fat were collected from each carcass. Feeding HS did not affect ($P > 0.25$) dry matter intake (DMI), average daily gain (ADG), or gain feed^{-1}. Carcass traits were also unaffected ($P > 0.35$) by treatment. Feeding HS linearly increased ($P < 0.001$) proportions of C18:0, C18:3 and C18:1 *trans*-9 in PCD, and 18:2 *trans*, *trans* in both PCD and brisket fat. As well, HS linearly increased cis-9 *trans*-11 CLA ($P < 0.001$), total saturates ($P = 0.002$) and polyunsaturated fatty acids (PUFA) ($P = 0.01$) in PCD. The presence of C20:4, C20:5 and C22:5 was detected only in tissues of cattle supplemented with HS ($P < 0.06$). Linear reductions ($P < 0.002$) in C16:1 *cis*, C17:1, C18:1 *cis*-9, C20:1, and total unsaturates in PCD, as well as linear decreases in C17:0 ($P = 0.04$) and C17:1 ($P < 0.001$) in brisket fat were observed when HS was fed. Levels of HS up to 14% of dietary DM exerted no detrimental effect on the growth or feed efficiency of cattle as compared to cattle fed a standard barley-based finishing diet. Including HS in the diet had both positive (increased CLA content) and negative (increased trans and saturated fats) effects on fatty acid profiles of beef tissues.'

Sensory characteristics of table eggs from laying hens fed diets containing hemp oil or hemp seed (Goldberg et al., 2010)

'The current study was designed to assess the sensory attributes of eggs procured from hens consuming diets containing hemp seed products. Forty-eight individually caged Bovan hens received 1 of 6 isonitrogenous and isoenergetic diets containing 0, 4, 8, 12% hemp oil or 10, 20% hemp seed for a 12 week period. Trained panelists (n = 8) evaluated 6 aroma and 7 flavor attributes of cooked eggs. Attributes that were measured included "egg," "salty," "sour," "milky," "creamy" and "buttery," with "sweet" as the additional flavor attribute. No significant differences in aroma or flavor ($P > 0.05$) were found between eggs from different dietary treatments. For yolk color, L*, a* and b* values (mean ± SD) for control (0%) eggs were 61.0 ± 0.3, 1.0 ± 0.1, and 43.2 ± 0.4, respectively. Addition of either hemp seed or hemp oil led to significant ($P < 0.05$) reductions in L*, and significant ($P < 0.05$) increases in a* and b*, with the largest changes observed in the 20% hemp seed treatment (L* = 58.7

± 0.1; a*= 5.3 ± 0.1; b* = 60.0 ± 0.3). The results provide evidence that hemp oil or seed use in poultry diet formulations leads to increased yolk color intensity, but does not have adverse effects on flavor and aroma profiles of the cooked eggs.'

Cold-pressed hempseed cake as protein feed for growing cattle (Hessle et al., 2008)

'Cold-pressed hempseed cake was investigated as a protein feed for young calves and finishing steers. Half of the animals were fed cold-pressed hempseed cake, whereas the other half were fed a mixture of soybean meal and barley. Effects on feed intake, liveweight gain (LWG), faecal traits and carcass traits (steers only) were studied. Neutral detergent fibre intake was higher for animals fed hempseed cake than for those fed soybean meal ($P< 0.05$). In addition, the number of long particles in faeces was lower ($P< 0.05$) and faecal dry matter content and consistency were higher from animals which were fed hempseed cake ($P < 0.05$; steers only). Higher feed intakes in calves fed hempseed cake ($P < 0.05$) combined with similar LWG resulted in lower feed efficiency in hemp-fed calves ($P < 0.05$). In conclusion, hempseed cake compared to soybean meal as a protein feed for intensively fed growing cattle results in similar production and improved rumen function.'

The nutritive value of hemp meal for ruminants (Mustafa et al., 1999)

'Hemp meal (HM) is derived from the processing of hemp (*Cannabis sativa* L.) seeds. The objective of this study was to determine the nutritive value of HM for ruminants. Two ruminally fistulated cows were used in a randomized complete block design to estimate in situ ruminal dry matter (DM) and crude protein (CP) degradability of HM relative to canola meal (CM), heated canola meal (HCM) and borage meal (BM) meal. Intestinal availability of rumen undegraded CP was estimated using a pepsin–pancreatin in vitro assay. Twenty growing lambs were utilized in a completely randomized design to determine total–tract nutrient digestibility coefficients of diets in which HM replaced CM at 0, 25, 50, 75 and 100% as a protein source. Results of the in situ study showed that the soluble-CP fraction of HM was similar to that of HCM and lower ($P < 0.05$) than those of CM and BM. Rate of degradation of the potentially degradable CP fraction and effective CP degradability of HM was higher ($P < 0.05$) than HCM and lower ($P < 0.05$) than CM and BM. Rumen undegraded CP and intestinal digestibility of RUP were highest ($P < 0.05$) for HM and HCM (average 782.5 and 644.5 g kg^{-1} of CP, respectively), intermediate for CM (473.9 and 342.9 g kg^{-1} of CP, respectively) and lowest for BM (401.5 and 242.3 g kg^{-1} of CP, respectively). However, total available CP was similar for the four protein sources (average 857.8 g kg^{-1} of CP). Feeding up to 200 g kg^{-1} HM did not affect voluntary intake or total-tract nutrient digestibility coefficients for sheep fed a barley-based diets. Hemp meal is an excellent source of RUP, with high post-ruminal availability, and may be used to replace CM with no detrimental effects on nutrient utilization by sheep.'

The effect of feeding hemp seed meal to laying hens (Silversides and Lefrançois, 2005)

'1. Seed of the hemp cultivar Unika-b was cold-pressed to obtain hemp seed meal (HSM) containing 307 g/kg crude protein and 164 g/kg ether extract (60 g/kg linoleic acid, 120 g/kg α -linolenic acid, 160 g/kg oleic acid, lesser amounts of palmitic, stearic, and α -linolenic acids).

2. For 4 weeks, 102 43-week-old DeKalb Sigma hens were fed on isonitrogenous and isoenergetic diets containing 0, 50, 100 or 200 g/kg HSM. Eggs were collected for fatty acid analysis during the fourth week of feeding these diets.

3. No significant differences were found between feed treatments for egg production, feed consumption, feed efficiency, body weight change or egg quality.

4. Increasing dietary inclusion of HSM produced eggs with lower concentrations of palmitic acid and higher concentrations of linoleic and α-linolenic acids.'

Characterization, amino acid composition and *in vitro* digestibility of hemp (Cannabis sativa L.) proteins (Wang et al., 2008b)

'The protein constituents and thermal properties of hemp (Cannabis sativa L.) protein isolate (HPI) as well as 11S- and 7S-rich HPIs (HPI-11S and HPI-7S) were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and different scanning calorimetry (DSC), and their amino acid composition and in vitro digestibility were also evaluated, as compared to soy protein isolate (SPI). SDS-PAGE analysis showed that the edestin (consisting of acidic and basic subunits, AS and BS) was the main protein component for HPI and HPI-11S, while HPI-7S was composed of the BS of edestin and a subunit of about 4.8 kDa. DSC analysis characterized thermal transition of the edestin component and the possible present form of different subunits. Except lysine and sulfur-containing amino acids, the essential amino acids of various HPIs met the suggested requirements of FAO/WHO for 2–5 year old infants. The proportion of essential amino acids to the total amino acids (E/T) for HPI (as well as HPI-11S) was significantly higher than that of SPI. In an in vitro digestion model, various protein constituents of various HPIs were much easily digested by pepsin plus trypsin, to release oligo-peptides with molecular weight less than 10.0 kDa (under reduced condition). Only after pepsin digestion, in vitro digestibility of HPIs was comparable to that of SPI, however after pepsin plus trypsin digestion, the digestibility (88–91%) was significantly higher than that (71%) of SPI ($P < 0.05$). These results suggest that the protein isolates from hempseed are much more nutritional in amino acid nutrition and easily digestible than SPI, and can be utilized as a good source of protein nutrition for human consumption.'

Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (Webster et al., 2000)

'In an effort to reduce fish meal (FM) use in diets for sunshine bass, a feeding trial was conducted. Four practical floating diets were formulated to contain 40% protein, similar energy levels, and without FM. A fifth diet was formulated to contain 30% FM and served as the control diet. Ten fish were stocked into each of 20 110-l aquaria and were fed twice daily 0730 and 1600 h amounts of diet similar to that of the aquarium consuming the most diet at that feeding. Diets were formulated to contain as major protein sources: Diet 1, 35% soybean meal (SBM) and 35% meat-and-bone meal (MBM); Diet 2, 27% SBM + 27% MBM + 20% hempseed meal (HSM); Diet 3, 30% SBM and 30% poultry by-product meal (PBM); Diet 4, 27% SBM + 27% MBM + 20% canola meal (CM). The control diet (Diet 5) had 30% SBM and 30% FM.'

'At the conclusion of the feeding trial, percentage weight gain of sunshine bass fed Diet 1 was significantly ($P < 0.05$) higher (299%) compared to fish fed Diet 3 (197%) and Diet 4 (226%), but not different from fish fed Diets 2 and 5. Specific growth rate (SGR) of fish fed Diet 1 was significantly higher (1.97%/day) compared to fish fed Diet 3 (1.52%/day), but not different compared to fish fed all other diets. Percentage survival and the amount of diet fed were not significantly different among all treatments and averaged 95% and 111 g diet/fish, respectively. Feed conversion ratios (FCRs) of fish fed Diets 3 and 4 were significantly higher (2.71 and 2.88, respectively) compared to fish fed the other diets. Percentage fillet weight and hepatosomatic index (HSI) were not significantly different among treatments and averaged 22.7% and 2.04%, respectively. Proximate compositions of fillets were not different among fish fed all diets and averaged 23.9%, 19.6%, and 2.0% for moisture, protein (wet weight basis), and lipid (wet weight basis), respectively.'

'Results from the present study indicate that diets without FM can be fed to juvenile sunshine bass without adverse effects on growth, survival, and body composition. Further research needs to be conducted in ponds on the diet formulations used in the present study to verify results.'

APPENDIX E

Kinetics and dynamics of THC and main related cannabinoids

Some 60 cannabinoids have been isolated in hemp. Besides the psychoactive delta-9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) are the next main components. Although apparently lacking of any cognitive and psychoactive effects, CBD is characterised by noteworthy interactions with THC and other effects which deserve attention.

Most of the available information concerning the kinetics and the toxic effects of cannabinoids are derived from studies conducted in humans, so that, in line with the aim of this opinion, animal studies will be referred to only when dealing with target species or with specific toxic effects potentially occurring in the consumer. In general, studies published in peer-reviewed journals have been considered.

E.1. Kinetics

Due to its lipophilic nature, THC is rapidly absorbed upon smoke inhalation, reaching a bioavailability of up to 50 %; by contrast, a slower absorption rate and a lower bioavailability (6 to 30 %) are reported through oral ingestion with wide inter-individual variation (Ashton, 2001). The difference between the two exposure routes may be due to partial degradation under gastric acidic conditions and the first pass effect mainly occurring in the liver (Maykut, 1985).

The hepatic and possibly extrahepatic cannabinoid biotransformations have been reviewed (Yamamoto et al., 2003). In mammalian species, THC undergoes mainly a CYP2C-mediated oxidation of the allylic methyl group, yielding the primary metabolite 11-hydroxy-delta-9-THC (11-OH-THC), which is further oxidated (very likely by the same enzymes) to 11-nor-9-carboxy-delta-9-THC (THC-COOH) (Figure E1); both THC and its metabolites are then subjected to glucuronidation. It is worth noting that 11-OH-THC, a more potent derivative than THC which may be responsible for some of the effects of *Cannabis*, reaches higher plasma concentrations after oral than inhalation exposure (Wall and Perez-Reyes, 1981). In contrast, COOH-THC represents an inactive derivative whose presence in biological fluids is routinely used to monitor the exposure to THC-containing products (Ahmad and Ahmad, 1990).

The occurrence of 11-hydroxylation as a key metabolic step has also been demonstrated for CBN, yielding a pharmacologically active OH-metabolite (Yamamoto et al., 1987). Cannabielsoin, the ultimate oxidised derivative of CBD, is considered almost devoid of significant biological effects (Yamamoto et al., 2003).

Of a single oral dose in humans only 10 to 25 % is excreted as the parent compound, metabolites and conjugated derivatives in the urine, whereas between 65 and 90 % may be recovered in the gut, mainly as the result of biliary excretion, with a significant enterohepatic cycling prolonging the drug action (Ashton, 2001).

The remarkable lipid solubility results in both a high degree of THC binding to plasma proteins (up to 99 % in humans) and a large volume of distribution (> 3 L/kg). Circulating THC and its metabolites still maintaining a relative degree of lipophilicity (11-OH-THC and possibly other metabolites) are rapidly distributed to all tissues at rates dependent on the blood flow, with a tendency to accumulate in fatty tissues, where they reach peak concentrations four to five days after a single exposure and may be released back to other compartments, including brain tissue, for several days (Ashton, 2001). Accordingly, a tissue distribution study performed in Large White pigs intravenously administered the cannabinoid (200 μg/kg) revealed that THC builds up mostly in lungs, fat and brain (Brunet et al., 2006), where, unlike liver, the drug was still detectable 6 hours or 24 hours (lungs and fat) after dosing. Such results were confirmed by a more recent investigation performed in pigs using the same experimental protocol (Brunet et al., 2010).

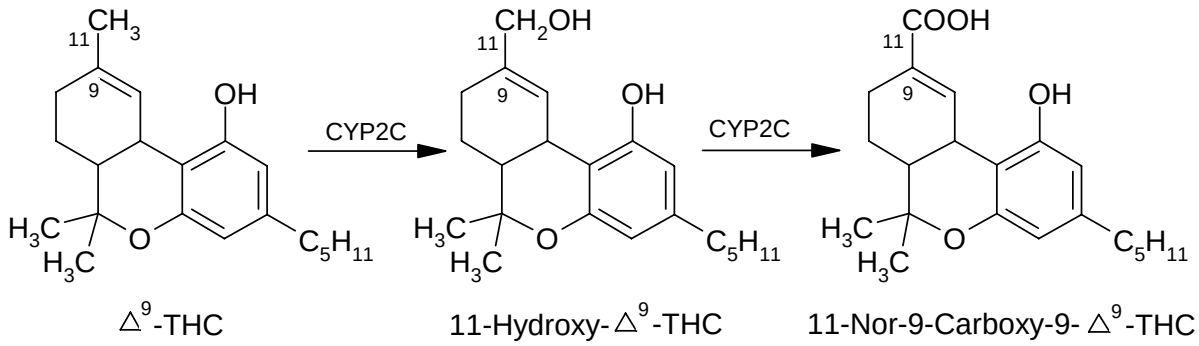

Figure E.1: Main THC biotransformation pathways

According to Mason and McBay (1985), plasma levels after a single oral exposure peaked within two to three hours, but in a more recent paper (Goodwin et al., 2006) much longer times were reported for THC, 11-OH-THC and THC-COOH (between nine and 107 hours) in human volunteers assuming hemp oil for five days; interestingly, a positive correlation was found between Body Mass Index and C_{max} values for both THC and its active derivative 11-OH-THC, suggesting a greater deposition in adipose tissue and a subsequent prolonged release to plasma in obese individuals. The enterohepatic recycling and the sequestration in adipose tissue support the relatively long tissue half-life of THC and its derivatives, amounting to about seven days; a complete elimination of a single dose is expected to take 30 days (Maykut, 1985). Body stores of THC increase with increasing frequency and chronicity of *Cannabis* use, and the half-life values of THC have been reported to be higher in chronic marijuana users (Johansson et al., 1988). The slow release of THC from fat back into blood was demonstrated to be the rate-limiting step in cannabinoid elimination from the body (Hunt and Jones, 1980).

Several reports indicate that milk represents an important route of excretion in humans (Perez-Reyes and Wall, 1982), squirrel monkeys (Chao et al., 1976) and ruminants, such as sheep (Jakubovic et al., 1974), buffaloes (Ahmad and Ahmad, 1990) and cows (Guidon and Zoller, 1999). The bioavailability of THC derivatives excreted by the mammary route is supported by the finding of the marker metabolite THC-COOH in the urine of children assuming milk from buffaloes fed *Cannabis*-contaminated fodder (Ahmad and Ahmad, 1990).

The placental transfer of THC has been documented in both humans and non-human primates (Little and Van Bevren, 1996). According to the kinetic profile described above, it is expected that such event may occur also for other cannabinoids and their metabolites.

Although no data were available in the open literature, considering the lypophilicity of THC and some of its metabolites, their transfer to eggs is also expected to occur.

The bioavailability, the main pharmacokinetics parameters as well as the biotransformation and the distribution pattern of CBD do not substantially differ from those of THC (Grotenhermen, 2003; Huestis, 2007).

E.2. Dynamics

For obvious reasons, considerable attention has been paid to THC, but information is also available for the other prominent cannabinoids CBN and CBD.

Most of the biological effects ensuing the exposure to THC and its active metabolite(s) are due to the binding to specific G-protein coupled receptors, named cannabinoid receptors (CB_1 and CB_2), which have been identified in rats, guinea pigs, dogs, monkeys, pigs and humans. In recent years, endogenous ligands structurally related to arachidonic acid, referred to as 'endocannabinoids', have been also uncovered; among them, the most representative are anandamide and 2-arachydonoylglycerol (Izzo et al., 2009).

CB_1 receptors are widely distributed in certain areas of the brain: in the cerebral cortex they regulate cognitive function, while those located in the hippocampus and amygdala are important in emotional status. Cerebellar CB_1 receptors are involved in dopaminergic signalling, movement and postural reflexes; CB_1 receptors are also expressed in the basal ganglia, brain stem and in the autonomic nervous system, where they participate in the regulation of pain perception and cardiovascular and gastrointestinal functions (Ashton, 2001). Upon binding with agonists, CB_1 receptors entail the inhibition of cAmp and the stimulation of *Mitogen Activated Protein Kinases* to modulate control of ion channels, particularly voltage-activated calcium channels and potassium channels, resulting, in turn, in the inhibition of the release of neurotransmitters, both excitatory and inhibitory (Di Marzo and De Petrocellis, 2006)

A second cannabinoid receptor, named CB_2, was first identified in spleen macrophages and is more abundant in the immune system, where they are expressed in B and T lymphocytes (Schatz et al., 1997).

As regards the respective receptorial targets and affinities, it is relevant to note that THC and 11-OH-THC are agonists of both CB receptor types with the highest affinity among all cannabinoids. CBN is a weak CB_1 and CB_2 agonist, retaining only 10 % of THC potency, while CBD does not interact with CBs, but exerts a plethora of pharmacological effects mediated by different mechanisms; for example, it can acts both as an antagonist of CB1/CB2 agonists and as a CB2 inverse agonist (Izzo et al., 2009).

The unravelling of the complex interactions between CBs and endocannabinoids and their ability to modulate a variety of physiological and pathophysiological processes (i.e. neurotransmitter release in the central and peripheral nervous systems as well as pain perception and cardiovascular and intestinal functions), have prompted several research group to suggest the use of THC and/or non-psychotropic cannabinoids, including synthetic ones (e.g. dronabinol, nabilone), for a wide array of therapeutic purposes, including analgesia and pain management, muscle relaxation, immunosuppression, stimulation of appetite (Di Marzo and De Petrocellis, 2006; Wang et al., 2008a; Gerra et al., 2010).

Both CB_1 and CB_2 are encoded by specific genes (*CNR1* and *CNR2*) displaying several identified polymorphisms which are associated with a number of (physio)pathologic conditions (e.g. obesity, osteoporosis, myocardial infarction, autoimmune disorders) and psychiatric disorders, including *Cannabis* and other drugs dependence, schizophrenia, depression and anxiety (Onaivi, 2009).

APPENDIX F

Toxicological profile of THC and related cannabinoids

Most of the available information is related to the exposure to marijuana. As far as humans are concerned, published literature is mainly focused on damage subsequent to smoking dried *Cannabis* leaves. In recent years, an increasing body of literature has addressed the adverse effects of cannabinoids used for therapeutic purposes. Little is reported about the toxicity of hemp-based foods. The main relevant studies for assessing consumer safety are summarised in Table F.1.

Table F.1: Main THC-related endpoints for deriving a threshold limit for consumers

ACUTE EXPOSURE						
				Least effective THC dose		
Species	**Exp. schedule**[1]	**Route of exposure**	**Effects/endpoints**	**(mg/person)**[1]	**mg/kg bw**	**Ref**
Humans	1 x 0-5-10-15-20	oral	Mood (self-reported intoxication scale) Skills performances (standing steadiness, hand-eye coordination, reaction time, etc.)	Mood: 10 Skills performance:5	0.17* 0.060†	a
Humans	1 x 7.5	oral	Increase in heart rate	7.5 mg/person	0.12*	b
Humans	NA	oral	Isolated cognitive functions and psychomotor skills related to driving performances	NA	0.04 -0.30	c
Humans	1 x 2.5 - 5	i.v.	Rise in blood cortisol levels	2.5 mg/person	0.04*	d
REPEATED EXPOSURE						
				Least effective THC dose		
Species	**Exp. Schedule Dose (days)**	**Route of exposure**	**Effects/endpoints**	**(mg/person)**[1]	**mg/kg bw**	**Ref**
Humans	2.5	oral	Psychotropyc effects	2.5 mg/person/day	0.04*	e
Humans	Dronabinol© (delta-9-THC) 2.5 mg twice a day for 42 days	oral	General health status and adverse effects monitoring	Psychotropic effects : (euphoria, dizziness, thinking abnormalities, somnolence) 2.5 mg x 2/day	0.08*	f
Humans	Dronabinol© (delta-9-THC) 2.5 mg twice a day or once a day for a mean duration of 5.8 months	oral	General health status and adverse effects monitoring	Psychotropic effects : (anxiety, confusion, depersonalization, dizziness, euphoria, somnolence) 2.5 mg x day	0.04*	g
Rats (♀)	1 µg/kg b.w. from postnatal day 22 to the day of vaginal open	i.p.	Onset of puberty (delayed) Number of ova (reduced)	NA	0.001	h
Rats (♀)	1 µg/kg b.w. from postnatal day 22 to the day of vaginal open; sacrifice 35-40 days after vaginal opening	i.p.	Serum gonadotropins levels in the different phases of cycle → decrease in LH (all phases)	NA	0.001	h

[1] mg/subject in the acute exposure trials.
† Calculated on the highest weight of individual enrolled in the study.
* Calculated on a 60 Kg bw basis.
[a] Chesher et al., 1990: Subjects were young adults of either sex in the weight range 58 – 84 Kg. - median 64.5.
[b] Kirk and De Wit, 1999.
[c] Ramaekers et al., 2004, Review.
[d] D´Souza et al., 2004: Subjects were healthy individuals with prior exposure to *Cannabis* but without abuse disorders.
[e] BgVV, 1997 and 2000.
[f] Beal et al., 1995: Human immunodeficiency virus affected patients, 67 males and 5 females.
[g] Beal et al., 1997: Human immunodeficiency virus affected patients, 87 males and 7 females.
[h] Wenger et al., 1988.

F.1. Acute toxicity in laboratory and domestic animals

Marijuana has a very wide safety margin, with the lethal dose being approximately 1000 times the effective one. Grotenhermen (2003) reports that the LD50 for oral marijuana exposure in rats is in the range of 800–1900 mg/kg, while in mice it amounts to 21 600 mg/kg for *Cannabis* extracts (IPCS-INCHEM 1989). The survival of a dog ingesting 26 800 mg marijuana per kg bw has been documented (Janczyk et al., 2004); deaths were recorded in four out of five debilitated cattle after the group ingested 35 Kg of plant material (Driemeier, 1997). A wide variety of clinical signs have been reported in poisoned dogs, including nervous symptoms (depression, ataxia, hyperstesia, recumbency and, less commonly, stupor, tremors or seizures) and mild gastrointestinal upset; tremors, mydriasis, hypersalivation and lack of coordination were noted in cattle 20 hours after ingesting dried *Cannabis* material. Provided that poisoned animals are subjected to proper treatment, the prognosis for full recovery is excellent (Bischoff et al., 2007).

F.2. Acute toxicity in human beings

Almost every system in the body is affected by *Cannabis*, which combines many of the properties of alcohol, tranquillisers, opiates and hallucinogens. It is relevant to note that the severity of the adverse effects is greater for non habitual ('naïve') *Cannabis* consumers. According to Ashton et al. (2001), the effects of high doses of *Cannabis* on the central nervous system consist in disphoria, including severe anxiety and panics, paranoia and psychosis as well as hallucinations. In addition, several experimental studies demonstrated deleterious THC effects on isolated cognitive functions and psychomotor skills related to driving performances (Ramaekers et al., 2004).

Other effects include tachycardia (up to 160 beats/min. or more), postural hypotension and fainting, widespread vasodilatation and reddening of the conjunctivae. Damage of the respiratory system is well characterised and occurs almost exclusively following chronic exposure to *Cannabis* smoke.

In 1996/1997, some cases of accidental intoxications were reported in Switzerland after the consumption of a salad prepared with hemp oil found to contain 1500 mg THC/kg, which is significantly over the Swiss limits. Gastrointestinal and perception disturbances were the prominent signs (Meier and Vonesch, 1997).

Toxicity from unintentional ingestion of cannabinoids-containing drugs has been reported. Although in most cases symptoms are short-lasting and not severe, the exposure to high amounts of THC may lead to coma (Macnab et al., 1989; Carstairs et al., in press).

F.3. Minimal toxic (effective) dose upon single exposure

In a systematic review reporting details of nine randomised controlled trials on the efficacy of cannabinoids for the management of pain (Campbell et al., 2001), dose-related adverse effects were observed for single oral doses of 5, 10, 15 or 20 mg THC, consisting in mental cloudness, ataxia, dizziness, numbness, disorientation, blurred vision, impaired mecampbell carroll reynomory and dry mouth. The number of adverse reactions per ten patients in individuals assuming 5 mg THC were more than twice that recorded in those receiving a placebo. According to IPCS-INCHEM (1989), the minimal effective dose of THC is 5 mg and the minimum plasma concentration of THC which produces psychotropic effects is 25 ng/mL. However, Ramaekers et al. (2004) report that THC in doses between 40 and 300 μg/kg (corresponding to 2.4 to 18 mg for a 60-kg individual) causes a dose-dependent impairment of cognitive and psychomotor performances, including driving or piloting, even after oral intake. Moreover, a 35 % decrease in psychomotor performances in exposed people was already observed at plasma THC concentrations of about 5 ng/mL, whilst maximal performance decrement of all psychomotor tests (- 70–80 %) was seen at concentrations between 14 and 60 ng/mL.

F.4. Toxicity after repeated exposure

As recently reviewed by Gonzàlez et al. (2005), it is now accepted that most of the central and peripheral (i.e. those not involving the central nervous system, see below) effects of cannabinoids generates tolerance in laboratory animals when administration prolongs for several days, more as the result of down-regulation/desensitisation of CBs than of an increase in the rate of the metabolic fate. These observations are consistent with the human situation, where a variable degree of tolerance has been reported for most of the effects of *Cannabis*, although such phenomenon is expected to fully develop only in heavy social abusers or in patients regularly assuming cannabinoids for therapeutic purposes (Hart et al., 2002). In line with the above concepts, a decreased CB_1 concentration was measured in brains from chronic marijuana smokers (Villares, 2007). Interestingly, there is evidence that CB_1 down-regulation may be associated with severe neurological diseases (e.g. epilepsy and possibly other syndromes), as it would limit the endocannabinoids role in suppressing pathological neuronal excitability (Ludány et al., 2008). It has not yet been established whether the same biochemical and molecular events responsible for the onset of tolerance could also be involved in the genesis of the *Cannabis*-dependence syndrome, which has been unequivocally documented in humans and in non-human primates (Clapper et al., 2009).

The adverse effects following the prolonged exposure to *Cannabis* smoking or to oral cannabinoids have been the subject of several reviews (Ashton, 2001; Smith, 2005; Wang et al., 2008a; Reece, 2009; Hall and Degenhardt, 2009). Effects clearly related to the exposure through the inhalatory route will not be mentioned. The features associated with chronic *Cannabis* use may be summarised as follows :

a) Cognitive dysfunctions consisting in the impairment of short-term memory and visual information processing, attention disturbances, increased reaction times, possibly subjected to the development of tolerance and sometimes subtle with the notable exception of exposure starting before the age of 17 years (permanent brain changes with lower IQ).

b) Psychiatric and social disorders: elevated risk of psychosis in many studies with odds ratio ranging from 2.3 to 2.1, with prevalence of bipolar disorders and depression accompanied by psychomotor agitation with interpersonal violence and suicide attempts. According to Smith (2005), the main action of chronic *Cannabis* use consists in the exacerbation of pre-existing psychotic disorders, with young adolescents (under 18 years) being again at higher risk.

c) Cardiovascular effects: a significant association between *Cannabis* use and myocardial infarction has been demonstrated, with hazard ratios of 2.5 and 4.2 for less than weekly and weekly use, respectively (Mukamal et al., 2008). A positive correlation has also been found between *Cannabis* use and infarctions in several other organs (brain, kidney, digits) and a severe inflammatory angitis resembling Bürger syndrome (Ducasse et al, 2004). Finally, *Cannabis* exposure has been linked with elevated rates of cardiac arrhythmias (mostly supraventricular, in few cases ventricular with lethal outcomes).

d) Bone loss: recorded in heavy users, and involving CB_1 (and possibly CB_2) stimulation, with loss of alveolar bone from the jaws.

e) Adverse effects on the foetus (prenatal exposure): cannabinoids easily cross the placental barrier and a wide array of adverse effects have been linked to maternal use during pregnancy, although the role of possible confounding factors (e.g. poor nutrition, smoking, exposure to alcohol or other drugs of abuse) is not always easy to evaluate. They are:

   - reduced body weight at birth;

   - birth defects mostly involving the brain such as encephalocele, hydrocephaly, microcephaly, but also affecting cardiovascular system (tetralogy of Fallot, septal defects) and limbs (polydactily, syndactily, deformities);

- psychiatric disorders (depression, anxiety);
- deficits in attention, visual analysis and hypothesis testing, reading comprehension.

The enhancement of the susceptibility of immature brains to the apoptotic effects of ethanol on neurons has been demonstrated in rats (Hansen et al., 2008)

f) Adverse effects in children due to breastfeeding (perinatal exposure)

As mentioned above, THC is excreted in human breast milk cannabinoids and the ratio milk to plasma has been found to rate 8 in heavy users (Perez-Reyes and Wall, 1982). Again, although the role of other factors (e.g. association with nicotine or other drugs, or the quality of mother-infant interactions) must be considered, a wide array of adverse effects emerging as early as the first weeks of age and persisting in the school age have been attributed to maternal use during lactation (for a review see Garry et al., 2009),

- signs of sedation, reduced muscular and poor sucking in infants;
- decrease in infant motor development at one year of age (Astley and Little, 1990);
- reduction in intellectual performances, executive function, sustained attention and verbal ability;
- low IQ, with hyperactivity, impulsivity.

Of great concern is the evidence of inheritable tumours, like childhood neuroblastoma, rhabdomyosarcoma and leukemia (particularly of the non-lymphoblastic type) (Hashibe, 2005), in spite of the fact that a number of studies failed to demonstrate an unequivocal link with the maternal use of *Cannabis* (Reece, 2009).

In conclusion, though a link between the use of marijuana and the development of adverse effects in the offspring could not be established in all the studies in this field, the picture that has emerged is that there are a number of neonatal neurobehavioral variables that are correlated with marijuana exposure and persist after adjusting for other confounding factors, such as socioeconomic status and nutritional (including caffeine) intake (Fried, 1989). Accordingly, *Cannabis* consumption during breastfeeding is contraindicated and addicted mothers who want to breastfeed their infants must be sustained by a medical team (Garry et al., 2009).

F.5. Mutagenicity and carcinogenicity

Cannabinoids are reported to generate reactive oxidative species and nitroxide with both receptor-dependent and receptor-independent mechanisms, resulting in the oxidation of the DNA base guanosine (a normal event in endocannabinoid signaling); deficits in the repair mechanisms may result in the fixation of the mutagenic event. A further mechanism may be the stimulation of the oncogenic MAPkinase pathway (Reece, 2009).

According to the National Toxicology Program (1996), THC (10 to 10 000 µg/plate) was not mutagenic in a number of *S. tiphymurium* strains, with or without metabolic activation, and did induce SCEs in CHO cells only at the highest tested concentration (12.5 µg/plate) and in the presence of S9 fraction. No evidence of induced chromosomal damage was provided by the only *in vivo* test performed, i.e. mouse peripheral blood micronucleus test. Taken together, those results point to a weak mutagenic activity of THC both under *in vitro* and *in vivo* conditions.

Although an increased risk for a certain types of cancer (e.g. prostate, cervix or childhood leukemia, astrocytoma and rhabdomyosarcoma) is emerging from epidemiological surveys conducted in marijuana smokers or in their offspring, the available data are still not sufficient to adequately evaluate the impact of marijuana on cancer risk (Hashibe et al., 2005).

On the other hand, there is a general consensus that cannabinoids, the active components of the hemp plant *Cannabis sativa*, along with their endogenous counterparts and synthetic derivatives, have elicited anti-cancer effects in many different *in vitro* and *in vivo* models of cancer (see Alexander et al., 2009). For example, in a breast cancer murine model, CBD has been found effective in down-regulating the expression of gene Id-1, shown to be a key regulator of the metastatic potential of breast tumor and of other cancers (McAllister et al., 2007). This notwhistanding, only very few clinical trials with cannabinoids have been reported so far, and some studies indicate that THC has a biphasic, dose-related effect in cancer cells (Alexander et al., 2009). In this respect, THC concentrations in the nanomolar range increase the proliferation rate of certain tumor cell lines, while only concentrations in the micromolar range elicit the opposite effect (Hart et al., 2004).

F.6. Other potential adverse effects of cannabinoids

F.6.1. Effects on neuroendocrine functions and on reproduction

Cannabinoids have been demonstrated to disrupt the hypothalamus-pituitary-gonadal axis and/or to affect related neurotransmitters in monkeys and rodents with potential long-term consequences on brain development (see above) and the reproductive and immunological systems. Evidence for permanent cannabinoid-mediated effects on reproduction and on behaviour in animals is supported by studies in which monkeys or rodents were exposed in utero and/or during lactation, then kept under observation until adulthood. Changes in the density of brain opioid receptors and catecholamine levels, increased corticosterone release in response to hypothalamus-pituitary-adrenal axis stimulation, reduction of copulatory behaviour and inhibition of testosterone release in response to a receptive female, and changes in gonadotropin secretion in females are among the observed adverse effects (see many references in Brown and Dobs, 2002). Rosenkrantz and Esber (1980) reported that the repeated oral exposure of rats to THC (10 mg/kg bw x 14 days) resulted in a lowering of both serum testosterone (- 66 %) and T3 and T4 levels (- 20–30 %); in the same study, the oral treatment of female rats during gestation (days 6 to 17) with graded THC levels (1, 5 or 12 mg/kg bw) produced variable hormone results, with LH being decreased only at the lowest level, FSH increased at all levels, and total estrogens rose in individuals exposed to the higher dosages. In women, marijuana smoking has been associated with depression in LH secretion (Mendelson et al., 1985a; Mendelson et al., 1986) or alteration of prolactin levels (Mendelson et al., 1985b). The repeated i.p. exposure of rats to doses as low as 1 μg THC/kg bw between day 22 postnatal and the day of vaginal opening induced a two-day delay in vaginal opening, and the number of ova on the day of first oestrus was significantly lower in treated rats than in controls; irregular oestrous cycles and decreased serum levels of luteinising hormone were recorded in animals treated in the same way but kept under observation until adulthood (Wenger et al., 1988).

A large amount of experimental data obtained *in vitro* have clearly demonstrated that cannabinoids negatively influence important sperm functions, including motility and acrosome reaction, two fundamental processes necessary for oocyte fertilisation; *in vivo*, it is now believed that the reported negative effects on hypothalamic–hypophyseal reproductive hormone secretion and the testicular endocrine and exocrine functions may occur through the activation of the cannabinoid receptor subtype CB_1 (Rossato et al., 2008).

F.6.2. Effects on immune system

The roles of cannabinoids in regulating both the cellular and the humoral immune networks have been recently reviewed and are believed to be mediated mainly by CB_2 receptors (Tanasescu and Constantinescu, 2010). A depression in both T cells number and functions has been associated with chronic exposure, which might explain the increased incidence of infections and of certain tumours in marijuana users (Tanasescu and Constantinescu, 2010). On the other hand, the cannabinoid-mediated modification of T helper cell subsets (Th1 and Th2) affecting the synthesis of many cytokines (such as TNF-a, IL-1, IL-2,IL-6,IL-12), provide promising therapeutic implications in a variety of conditions, such as certain neurodegenerative diseases and/or several autoimmune or inflammatory disorders (Massi et al., 2006).

While it is suggested that endocannabinoids play a positive role in mobilising B cells during the immune response, phytocannabinoids and synthetic cannabinoids are generally believed to negatively affect the humoral immunity; for example, B cells, IgG and IgM, and some complement proteins have been found to decrease in high-school and university students ingesting a particular form of marijuana called 'bhang' (El-Gohary and Eid, 2004).

F.6.3. Drug interactions

Due to their high lipophilicity, THC, CBN and CBD, the major plant cannabinoids, are extensively metabolised mainly by CYP 2C and 3A in humans and experimental animals, thus envisaging the potential for drug interactions with drugs or toxicants metabolised by the same CYPs (Yamaori et al., 2010). A further matter of concern arises from the inhibition conveyed by certain cannabinoids (notably CBD and CBN) on CYP1A and CYP 1B (Yamaori et al., 2010) or on CYP3A (Bornheim and Grillo, 1998). The systemic clearance of hexobarbital was decreased in patients administered CBD (Benowitz et al., 1980), and *in vitro* interactions of THC with phenytoin have been demonstrated (Bland et al., 2005).

APPENDIX G

Extrapolation of the maximum THC content in hemp derived feed materials

The tables below (Tables G.1 and G.2) present the calculations to extrapolate the maximum THC content in hemp-derived feed materials which would result in milk concentrations corresponding to a THC exposure of adults and children (one to three years old) in accordance with the PMTDI of 0.0004 mg/kg bw (corresponding to 0.024 mg for a 60 kg adult and 0.0048 mg for a 12 kg child).

Table G.1: Maximum content of THC in hemp (%) in compliance with the PMTDI of 0.0004 mg/kg bw, derived from a exposure of children (12 kg bw) to 1.5 L milk/day and taking a transfer rate from oral intake to milk of 0.15 % for three different intake levels at three different milk yields each

	Maximum content THC in hemp (%)		
Milk yield (L/day)	*15*	*25*	*35*
Cow intake (kg hemp/day)			
1.5	0.002	0.004	0.005
1.0	0.003	0.005	0.008
0.5	0.006	0.011	0.015

Table G.2: Maximum content of THC in hemp (%) in compliance with the PMTDI of 0.0004 mg/kg bw, derived from a exposure of adult (60 kg bw) to 2 L milk/day and taking a transfer rate from oral intake to milk of 0.15 % for three different intake levels at three different milk yields each

	Maximum content THC in hemp (%)		
Milk yield (L/day)	*15*	*25*	*35*
Cow intake (kg hemp/day)			
1.5	0.008	0.013	0.019
1.0	0.012	0.020	0.028
0.5	0.024	0.040	0.056

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