Maisons-Alfort, 16 November 2011

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the assessment of the initial assessment report by the UK authorities concerning the placing on the market of the novel food ingredient: taxifolin from Dahurian Larch wood (Larix gmelinii)

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are made public.

1. 1. REVIEW OF THE REQUEST

On 30 September 2011, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) received a request from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) for an opinion on the assessment of the initial assessment report by the UK authorities concerning the placing on the market of the novel food ingredient (NI): taxifolin from Dahurian Larch wood (*Larix gmelinii*).

2. 2. BACKGROUND AND PURPOSE OF THE REQUEST

The request concerns an extract enriched with dihydroquercetin (DHQ), also called taxifolin, a flavanone-type flavonoid, from Dahurian Larch wood (*Larix gmelinii*). The application dossier states that taxifolin is also present in other trees, such as Maritime Pine, which is used to make other products, such as pycnogenol which contains small quantities of taxifolin. According to the application dossier, the NI has been available for 15 to 20 years on the Russian and US markets as a food additive used for its antioxidant properties. The NI is intended for use as a food ingredient and the applicant claims that it has beneficial effects because of its antioxidant and anti-inflammatory properties.

The NI containing taxifolin was the subject of an application for marketing authorisation by the British Food Standards Agency (FSA) under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. The initial assessment report carried out by the UK authorities has been submitted to ANSES for their observations or possible objections.

It is the applicant's view that the NI belongs to Class 1.2 of Commission Recommendation 97/618, which covers 'single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified, the source of which has no history of food

use in the Community'. In accordance with the said Recommendation, the following information is required for NIs in Class 1.2:

I. Specification of the NI
II. Effect of the production process applied to the NI
III. History of the organism used as the source of the NI
IX. Anticipated intake/extent of use of the NI
X. Information from previous human exposure to the NI
XI. Nutritional information on the NI
XII. Microbiological information on the NI
XIII. Toxicological information on the NI

The UK authorities questioned this proposed classification, due to the fact that taxifolin has not previously been used as a foodstuff in the European Community. The UK authorities feel that it is more appropriate to place the NI in Class 2 (Complex novel food from non-GM source).

3. 3. ORGANISATION OF THE EXPERT ASSESSMENT

The expert assessment was carried out in accordance with French standard NF X 50-110 “Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)”.

The collective expert assessment was carried out by the Expert Committees (CESs) on Additives, flavourings and processing aids (AAAT) and on Human nutrition (NUT) (which were consulted by correspondence due to the short time frame), based on the initial reports written by rapporteurs from these CESs.

4. 4. ANALYSIS AND CONCLUSION OF THE CESs

I. Specification of the NI

The novel ingredient is a crystallised powder obtained by extracting soluble substances from the sawdust of wild *Larix gmelinii* stumps using an ethanol aqueous solution (80% ethanol). The NI is principally composed (over 90%) of taxifolin (dry weight). Taxifolin, a white to pale-yellow flavonoid, is described as soluble in warm water, ethanol and ethyl acetate. Its solubility depends on the temperature and concentration of the ethanol. Its chemical name is (2R,3R)-2-(3,4-dihydroxyphenyl)-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one. According to the application dossier, taxifolin is made up of two stereoisomers, the predominant of these being trans-dihydroquercetin. The Chemical Abstract Service (CAS) number given in the dossier for this compound is: 480-18-2. The following numbers for this compound are obsolete: 5117-01-1; 5323-70-6; 17654-26-1; 20254-28-8; 24198-96-7; 28929-10-4; 98006-93-0.

The applicant is fully aware that the composition of the *Larix gmelinii* extract may vary depending on habitat, climate conditions and seasons. The applicant has submitted the results of analyses performed on five batches of the NI. The levels of taxifolin in these batches ranged from 92.2% to 92.6%.

Certain flavonoids that form part of the extract have also been determined, but the method used has not been explained clearly. Only a few articles on general methods of determination have been provided. The analysis protocol does not go into much detail, particularly in regard to preparation of the sample (which solvent was used?) and the HPLC method used (for example, what proportion of solvents A and B? Gradient or isocratic elution? Was the signal emitted during the column packing recorded?). Furthermore, the chromatogram is not available. Generally speaking, there are biases in the determination method which, although acceptable from an analytical point of view, may distort the assessment of the real content of impurities. It is also noted that with this method the wavelength is 290 nm, which does not make it possible to detect compounds not absorbed at this wavelength (however, the fact that this product is crystallised

suggests that, for example, carbohydrates and/or lipids are absent), and that quantification of the flavonoids is done through internal normalisation.

The other flavonoids identified in the extract are the flavonones dihydrokaempferol or aromadendrin (2.5-3.22%), eriodictyol (0.12-0.26%), quercetin (0.28-0.49%), naringenin (0.19-0.31%), kaempferol (0.02-0.11%) and pinocembrin (0.02-0.16%).

The sum of these determined compounds is between 96.0% and 96.7% of dry material. The remaining compounds in the extract, between 3% and 4% (the applicant claims 2.8% but does not explain its calculation), has not been identified. Steps should be taken to assess the possibility that these impurities contain harmful substances, and to ensure that they are indeed safe, even if these impurities exist only in small quantities (4 mg per 100 mg sample of taxifolin). The applicant mentions that traces of saponosides were detected, which it claims are harmless. In sum, the nature of the impurities is still unknown, as is the exact level thereof.

The UK authorities have requested further clarification regarding these unidentified compounds. The applicant replied that, according to the bibliographical analysis, plant-based foodstuffs contain a wide variety of flavonoids, 'which are not usually associated with any significant toxicological effects'.

It is the opinion of the UK authorities that the specifications set out by the applicant for the product are acceptable. They have accepted the claim by the applicant that the presence of the unidentified flavonoids in the NI does not raise any public health concerns. Furthermore, the UK authorities have agreed to increase the taxifolin specification from 88% to 90% of dry matter, in accordance with the specifications set out by the Russian Federation. Finally, they have also accepted the claim by the applicant that the starting materials of the NI have not been treated with herbicides or pesticides, and as such they have stated that they do not object to bypassing the pesticide residue tests not required by the Russian Federation.

The CES NUT feels that the specifications of the finished product are satisfactory as they are and that the FSA's request for 90% taxifolin instead of 88% can be accepted.

The CES NUT feels that all of these compounds (minor flavonoids and saponins) are commonly found, in small quantities (<0.5%), in plant products that form part of our diet.

The CES NUT feels that the presence of mycotoxins is unlikely given the production procedure (particularly as there was no significant delay between grinding and extraction). The possibility of fungal contamination on the tree stumps is also unlikely since, firstly, these stumps come from felled wood which is therefore healthy, and secondly, if a fungal contamination were to appear (in the event that the stumps were not collected quickly after the trees were felled), this would essentially be an external contamination which would be eliminated when the stumps were examined before the grinding process.

However the CES NUT notes that in the applicant's dossier there is mention of radionuclide monitoring but no details regarding the corresponding action and specifications, and suggests that checks should be performed to verify that monitoring is carried out systematically for all batches.

The CES AAAT remarks that taxifolin is characterised by the absence of the 2.3-double bond and by the presence of two asymmetric centres. It is therefore theoretically possible for four taxifolin enantiomers to be present in the NI. The CES NUT adds that while four isomers are theoretically possible in the natural flavanone, almost all of the compounds known so far are 2R, 3R. As the biogenesis of these compounds is enzymatic, it favours one single diastereoisomer. The applicant describes the *trans* 2R, 3R configuration, but the CES AAAT points out that there has been no real chemical verification of the NI to identify any other taxifolin enantiomers. However, it is known that the metabolism of the enantiomers and their biological activity/toxicity may vary depending on the enantiomer in question.

The CES AAAT points out that the available literature, most of which is in Russian, does not make it possible to give an exhaustive definition of the composition of the NI extract of the *Larix gmelinii* genus. It has been reported that the extracts of *Larix gmelinii* and *Larix sibirica* contain larixidinol, larixinol, larisinol and the trimer triflarixinol[1]. However, little biological data is available on these compounds which also have similar antioxidant properties to taxifolin. The extracts of the *Larix* genus may also contain other components, such as diterpenes, triterpenes, volatile compounds and arabinogalactane polysaccharides. These compounds may be extracted with water and ethanol.

The CES AAAT also feels that the NI specifications correctly cover 96% of the components. However, the CES feels that the remaining 3-4% of compounds must be better identified and must appear in these specifications. With regard to the possible presence of herbicide or pesticide residues in the NI, the CES AAAT points out that these types of products, together with the tolerable levels of residues in food, are covered in numerous European regulations[2].

II. Effect of the production process applied to the NI.

The dossier states that the NI is produced from the bark of the *Larix gmelinii* tree. The bark is dried (at 40-50°C) to obtain residual moisture of 20-25%. It is then ground (sawdust). Soluble substances are extracted with a 75-85% ethanol aqueous solution at a temperature of 45-50ºC. Taxifolin is highly soluble in ethanol, but less so in water, although solubility in water increases when it is heated (0.3% at 40°C). The ethanol is then evaporated and the aqueous part of the extract is cooled down to 20-25ºC to isolate resinous compounds which should have been extracted. The aqueous extract is then evaporated and crystallized. According to the applicant, it contains at least 90% taxifolin and has a moisture level of not more than 10%. The solvents used (ethanol, deionised water) apparently comply with the EU directives.

It seems that some of the ethanol used had been recycled, although it is expected that the source of the recycled solvent will be traced.

The stability of the NI has been assessed in accelerated storage test conditions. The applicant concluded that the NI is stable for up to five years in 'normal' storage conditions (i.e. at a temperature above 4°C, with 40-60% humidity, in a ventilated area, shielded from ultraviolet rays).

It is the opinion of the UK authorities that the quality controls carried out on the individual batches were adequate. Nevertheless, they pointed out that the applicant has not proposed an expiry date, an upper temperature limit during storage has not been specified and the stability of the NI in the intended foodstuffs has not been tested.

The CES AAAT notes that certain flavonoid compounds (spiroflavonoids) are compounds that present a certain level of instability in alcoholic solvents such as methanol when they are slightly acidified. Spiroflavonoids are molecules that have been structurally identified since 1985 and are flavanone-flavanol dimers connected by their C3-C8 carbons. They are mainly found among Gymnospermae.

The CES AAAT agrees with the comments made by the UK authorities regarding the need to set an expiry date for the NI.

The CES NUT agrees with the comments made by the UK authorities regarding the need to specify an upper temperature limit during storage and to test the stability of the extract in food matrices (this point is very important for a food ingredient). The CES NUT would also like further clarification on the quality controls performed (it would particularly like to know what parameters are used for the

[1] Fedorova T, Ivanova S, et al. 2010. Spiroflavonoid compounds: structure elucidation and distribution in nature. Russian Journal of Bioorganic Chemistry 36: 793-801.
[2] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market; Regulation (EC) No 396/2005 of the European Parliament and the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

assessment and whether all of the batches are assessed). Furthermore, the CES NUT feels that the characteristics of the ethanol aqueous solution (% ethanol) and the temperature during the extraction process must be outlined clearly in both the dossier and the d2 technical appendix.

III. History of the organism used as the source of the NI

According to the applicant, a very limited number of species from the genus *Larix* are used as foodstuffs. *Larix occidentalis*, *Larix rossiea* and *Larix lacricinia* have been identified by the applicant as being used as sources of arabinogalactan, which is used in food supplements as a gelling agent and in traditional medicine.

The UK authorities note that there is no evidence to suggest that Larix gmelinii is used as a foodstuff. They conclude that the genus Larix is used very little as a foodstuff and that the products produced from the species of Larix described by the applicant bear no resemblance to the taxifolin subject to the request.

The CES NUT adds that the name given by the applicant is that mentioned in the ARS database[3], while in other databases it is registered under the name *Larix gmelinii* (Rupr.) Kuzen. This has no substantial impact on the assessment of the NI.

On the other hand, there are at least six varieties of this species listed. They are not synonyms, as the applicant claims in the dossier. Furthermore, the applicant mentions that the tree stumps are collected by 'experienced people', who can identify the genus 'without any doubts', which is difficult to prove. As such the risk of this species being confused with another cannot be ruled out. Given the specific biotope in question (Siberian taiga, cool continental climate), which has a characteristic flora, it is difficult to assess the risk of confusion with another species, and where relevant, with which species. Therefore, if the extract of another larch species is not significantly different from that of *Larix gmelinii,* with the method of determination used the confusion may go unnoticed. The CES NUT feels, however, that this is unlikely.

The CES AAAT and the CES NUT agree with the comments made by the UK authorities. The CES NUT adds that few larch derivatives have as yet been used in food and none of those used contain taxifolin.

IX. Anticipated intake/extent of use of the NI

As proposed by the applicant, the NI would be used in a wide range of foodstuffs. The quantities used would vary in accordance with the fat content of these foodstuffs. The UK authorities point out that the applicant does not fully explain how the levels of consumption have been estimated, but the applicant has provided calculations based on 'worst case' and 'realistic' estimates.

Although the UK authorities felt that the calculations provided were not satisfactory, they felt that as the maximum estimates of exposure given are below the acceptable daily intake (ADI) proposed by the applicant, there was no need to perform more detailed estimates.

The average consumption of the NI simulated by the applicant for the adult population is 65 mg/day (0.9 mg/kg body weight (bw)/day), while at the 97.5th percentile, consumption is 130 mg/day (1.9 mg/kg bw/day). Consumption by children, simulated depending on body weight and split into age groups (1.5 to 4.5 years – 4 to 10 years – 10 to 18 years), is higher than for adults. On average, potential consumption has been estimated at between 1.2 and 2.2 mg/kg bw/day, and 2.4 and 4.3 mg/kg bw/day at the 97.5th percentile.

The UK authorities have pointed out that these estimates did not include use of the NI as a food supplement or use in foods for particular nutritional uses (PARNUTS), but they felt that consumption of the NI at the maximum recommended levels were still below the adult ADI. Moreover, they accepted that despite the

[3] USDA ARS GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?21487)

rough estimates made by the applicant in the calculations of exposure, these were overestimates and therefore there was no need to refine the estimates to prove the safety of the NI.

The CES NUT states that the addition of the NI to foodstuffs including food supplements may mean that some people (particularly those who practise sports) consume great amounts of the NI while also consuming other medicinal products. Interaction with medicinal products has not been studied. However, taxifolin belongs to the same chemical class as naringenin, which is known to interact with medicinal substances. The CES NUT feels that this point should be looked into by studying the relevant literature.

The CES AAAT feels that the applicant must specify the data needed to calculate the level of exposure for European consumers (particularly the uses, intended foodstuffs, consumption data used and the method of calculating exposure to the NI).

X. Information from previous human exposure to the NI or its source

The UK authorities acknowledge the information submitted regarding the natural presence of taxifolin in plant products that form part of our day-to-day diet (olive oil, red onions, citrus fruits), but it is important to note that there are only very small quantities of taxifolin present in these foodstuffs.

Taxifolin is already widely used with the claim 'food antioxidant' (food supplements (25 – 100 mg/day), beverages, fruit bars) on Russian, Swiss and US markets.

The applicant states that it has not been informed of any adverse effects associated with its product.

The CES NUT has its reservations about this point due to the lack of information on a nutritional vigilance system in the Russian Federation.

XI. Nutrition information on the NI

The UK authorities feel that the nutritional benefits of taxifolin shown by the studies presented are more similar to health claims and that these claims cannot be considered under the novel foods regulation.

The CES NUT agrees with this conclusion and adds that health claims are covered by Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods.

XII. Microbiological information on the NI

The applicant states that the finished product has been tested in order to verify the absence of pathogenic microorganisms in accordance with the European Pharmacopoeia. The applicant explains the microbiological specifications for the NI and provides the results of the analyses performed on five batches which are in accordance with these specifications.

The UK authorities agree that the manufacturing process of the NI does not give rise to any microbiological problems and that the microbiological specifications of the NI make it possible to ensure that it does not contain any pathogens. The only risk mentioned is the possible presence of mycotoxins. A batch tested revealed an absence of mycotoxins. Nevertheless, the UK authorities requested that these tests be carried out throughout the production process (yeasts, mould, mycotoxins).

This point did not give rise to any particular comments from the CES AAAT or the CES NUT

XIII. Toxicological information on the NI

a. Bioavailability and metabolic fate

- In animals

The dossier mentions that in rabbits the bioavailability of taxifolin is around 36% higher in lipid solution than in tablet form.

A pharmacokinetic study has been carried out on taxifolin in rats, partly through intravenous injection at doses of 1 mg, 3 mg, 10 mg and 30 mg/kg bw, and partly after adding single doses of 50 mg and 500 mg/kg bw. HPLC analyses of animal plasma only revealed traces of taxifolin after oral administration[4]. The UK authorities state the bioavailability of taxifolin calculated from this study is around 0.2%.

According to the report by the UK authorities, another study (not available in the dossier) performed on 8 male rats shows a rapid absorption of taxifolin, reaching a maximum concentration in blood plasma after 30 minutes, and undetectable levels in plasma after 8 hours. The UK authorities cite the conclusion of the study authors who calculate the bioavailability of taxifolin to be around 23%[5].

No study with radiolabelled compounds was provided. The UK authorities, referring to the previous study, indicate that taxifolin was detectable in blood plasma, liver, kidneys, spleen, brain, muscles, lungs and heart (without specifying the concentrations).

Taxifolin is converted to methylated metabolite (3' or 4'-O-methyltaxifolin) in rats[64]. A study from the 1950s (study not available) using two human volunteers consuming 2g of taxifolin reported the presence of hydroxyphenylacetic acids (in urine or blood plasma).

The UK authorities mention that Seredin et al (2007, study not provided[5]) report that around 8% of the original dose of taxifolin was seen in the rats' urine during the first 24 hours after administration, but none was seen in the urine or faeces after 48 hours, indicating complete absorption of taxifolin into the blood system.

- In humans

The scope of the studies is limited by the fact that none take into consideration the bioavailability or metabolism of the flavonoids (Manach et al, 2004[6]). It is now known that flavonoids are essentially absorbed in the small intestine (aglycones and glucosides) and in the colon (glycosides), that they have a low level of absorption and that the flavonoids that are recognised by the organism as being xenobiotic trigger the endogenous detoxification systems. This means that, with regard to the flavonoids absorbed, there is a great deal of conjugation in the intestine and liver, which leads to the production of sulphate, glucuronide and methylated derivatives, later found in the circulation. As a result of this intense activity of conjugated systems, the aglycone forms are in the minority in the plasma, and may even be completely absent. The conjugated metabolites of the flavonoids circulate in the blood bound to albumin, reach the target tissue and are then excreted in either urine or bile depending on the molecular structure. Globally speaking, the total concentration of metabolites of a flavonoid from food in the plasma is measured in µM, or is even lower (Manach et al, 2005[7]).

b. Acute toxicity study

[4] Voskoboinikova IV et al. 1993. Experimental pharmacokinetics of biologically active plant phenolic compounds. III. Pharmacokinetics of dihydroquercetin. Phytotherapy Research 7, 208-210.

[5] Seredin SB et al. 2007. Preclinical investigation of pharmacokinetics of dihydroquercetin. Report. SU Nil. Moscow.

[6] Manach C, Scalbert A, Morand C, Rémésy C, Jimenez L. 2004. Polyphenols: food sources and bioavailability. Am J Clin Nutr 79, 727-747.

[7] Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. 2005. Bioavailability and bioefficacy of polyphenols in humans: l-A review of 97 bioavailability studies. Am J Clin Nutr 81,230S-242S.

The UK authorities point to an LD_{50} above 560 mg and 580 mg in rats and mice by intraperitoneal and intragastric administration. Other studies performed with taxifolin extracted from different sources give an LD_{50} of 1200 mg/kg by intraperitoneal administration.

c. Subchronic toxicity study

The information presented in the application dossier cites studies with a duration of 7 to 20 days, for doses of 10 to 15 g/kg bw with no adverse effects. The biological and histological examinations carried out on 9 tissues or organs did not record any changes[8].

d. Chronic toxicity study

The UK authorities mention the studies carried out over 6 months on rats and dogs with taxifolin[9] (publication not provided). The authorities report that according to these publications no effect was observed on rats receiving between 150 and 1500 mg/kg/day of taxifolin. Some changes in leukocyte and thrombocyte levels were observed, but according to the report, these remained within the normal levels of variation. The biochemical tests did not show any toxic effects on the liver, kidneys or cardiovascular system. There was no visible effect on the behaviour of dogs receiving 190 mg/kg/day of taxifolin, and the electrocardiogram and investigation of the nervous system did not indicate any adverse effects. Similarly, the haematological tests did not show any damage to the blood parameters or bone marrow.

The UK authorities mention two other studies carried out on albino rats lasting 226 and 249 days respectively. These date from 1957 and did not show any adverse effects.

e. Developmental toxicity study

The UK authorities describe a 90-day study on pregnant rats (from day 1 to day 19 of gestation) receiving 75 and 1500 mg/kg of taxifolin by intraperitoneal administration. In this study no effects were observed during either gestation or the postnatal period. It should be noted that, according to the UK authorities, this study was repeated in summarised form in the publication by Dorovskikh and Celuyiko (2008) according to the assessment report, but as mentioned earlier, this publication is not included in the dossier. There was no reported effect on the newborns (development and growth), and no effect was noted during the histological examinations (heart, liver, spleen, kidneys, stomach, intestine). Some minor effects (not specified) were reported but did not exceed the physiological variation. The conclusion is that there is no effect on development.

The UK authorities describe another study in which 75 pregnant rats received doses of 75 and 1500 mg/kg of taxifolin by intravenous injection over 19 days. In this publication, it was reported that another study on reproduction was performed in which both male and female rats were administered taxifolin. In this study, no effect on the reproductive functions was observed.

Other studies carried out using taxifolin from other sources showed that no effect on the oestrogen receptor is observed in the rat uterus or cell culture.

f. Genotoxicity study

With regard to research on possible gene mutations, the report by the UK authorities states that an Ames test on taxifolin (dihydroquercetin) extracted from other natural sources (strains not specified) produced negative results. This may be comparable to studies (not cited in the UK report) published in 1977[10] which state that dihydroquercetin did not demonstrate any toxic effects on an Ames test carried out with four strains

[8] Dorovskikn VA, Celuyko SS. 2008. Toxicity study: Dated the 10th of June 2008 Nà. 429. Amur State Medical Academy. 'Ametis' JSC.
[9] Shkarenkov AA et al. 1998. Preclinical toxicological study of diquertin. Problems of Biological, Médical and Pharmaceutical Chemistry (Voprosy Bioiogicheskoi, Meditsinskoi i Farmatsevticheskoi Khimii) 3, 36-39.
[10] Bjeldanes LF, Chang GW. Mutagenic activity of quercetin and related compounds. 1977. Science 197, 577-578.

of *Salmonella typhimurium* (TA1535, TA100, TA1538 and TA98) with doses of 50, 250, 100 and 2500 μg per box.

The UK authorities point out that taxifolin has no effect on the induction of micronuclei in mice at a dose of 1500 mg/kg bw, or on a comet assay in mice performed on the blood cells, liver and colon.

g. Assessment of the risk associated with use of the NI

The UK authorities feel that sufficient studies have been performed with the NI or comparable products to be certain that the NI does not pose any risks to consumers at the levels proposed by the applicant (15 mg/kg bw/day). The UK authorities noted that these studies were performed in accordance with good laboratory practice (GLP) in the Russian Federation.

The CES AAAT feels that the compliance with the GLP claimed by the applicant, in accordance with Russian regulations, cannot be assessed in relation to the European requirements. Therefore, the protocols of the studies performed, like those carried out over 226 and 249 days on albino rats in 1957, cannot be verified. Furthermore, the product tested was administered in addition to food up to a dose of 1% without specifying the real quantities ingested by the animal.

Generally speaking, the CES AAAT has not had access to the bibliographical references. A review frequently referred to in the application dossier which is supposed to report the results of the acute toxicity studies, chronic toxicity studies (in rats and dogs), embryotoxicity studies and genotoxicity studies has not been included in the application dossier and, it seems, is only four pages long. Under these circumstances, it is impossible for the CES AAAT to analyse the findings of these studies.

The CES AAAT feels that the applicant's report does not give sufficient details about the results of the toxicological studies cited. Often, changes or fluctuations in the parameters measured are mentioned, but it is not specified whether these are increases or decreases. There is no mention of whether these changes are linked to the treatment because they remain within the normal physiological values (as per laboratory records), but they are not compared to the physiological values of control animals used in related studies. The histological assessments carried out for these studies do not go into detail and do not include enough organs or tissues examined in accordance with international protocols (e.g. OECD). Furthermore, the real bioavailability of taxifolin is very uncertain as the values in rats mentioned in the report vary from 0.17% to 17%.

With regard to the available genotoxicity results, only a small number are mentioned in the report by the UK authorities. The CES AAAT concludes that as no details have been provided on the protocols used in the publications, the genotoxicity of the NI does not seem to have been studied in great depth. According to another publication[11], identified by the CES in the scientific literature and not cited in the UK report, taxifolin, unlike quercetin and kaempferol, does not induce genotoxicity *in vitro*. Nevertheless, the CES AAAT feels that it would be best to carry out a more in-depth investigation into the genotoxicity of the NI.

With regard to the reproductive toxicity studies, these do not make it possible to assess the risks to the consumer. Mention is made of a 90-day study (in pregnant rats) which includes the reproductive stage. The anatomopathological observations given do not correspond to those set out in international guidelines (e.g. OECD). From the information provided in the dossier, it is not possible to know what specific reproductive parameters, particularly regarding fertility, were measured. Not even the route of administration has been specified. The second embryotoxicity study performed by intraperitoneal administration did not state what criteria were followed for observation of the foetus, particularly with regard to the viscera and skeleton. The only information given is that there is no increase in hydronephrosis, or haemorrhages, but the reason these criteria in particular were looked

[11] Jurado J et al. 1991. Study on the mutagenicity activity of 13 bioflavonoids with the Salmonella Ara test. Mutagenesis 6, 289-295.

at is not given. As the route of administration in this study (intraperitoneal) does not correspond to that used by the consumer of the NI, the results of these studies cannot be extrapolated to the oral route.

The CES NUT also mentions the fact that the studies in humans available (carried out on over 500 patients), presented by the applicant have not been published in international reviews. In particular, the numerous effects associated with consumption of taxifolin are reported in the application dossier, but no details are given of the experimental design of the clinical trial, or of the conditions and measurement techniques of the assessment criteria. Furthermore, the CES NUT points out that all of the supplement studies lasted for less than 12 weeks and none of these were performed on healthy subjects. The extrapolation to the population and the long-term toxicity risks have not been assessed.

h. Allergenicity

The UK authorities add that, although there is no experimental proof that traces of protein are present in the NI, the applicant notes that it is unlikely that the production procedure would allow the presence of proteins in the finished product. However, the applicant acknowledges that as allergies have been linked to birch pollen, the risk that allergies could also be linked to larch pollen cannot be ruled out. The applicant stresses, however, that the production procedure 'seems capable' of eliminating the presence of undenatured pollen from the finished product.

The UK authorities feel that it is unlikely that taxifolin would pose a risk of allergy for the consumer.

The CES AAAT and NUT agree with the UK authorities that it is unlikely that taxifolin in itself would present a risk of allergy. Nevertheless, with regard to the extract proposed for the NI, both CESs recommend that the absence of proteins and larch pollen in the NI be ruled out experimentally in order to dispel any doubts, and if necessary, the risk of allergy must be indicated on the label.

General conclusions of the UK authorities

The UK authorities feel that use of the NI in the forms proposed is acceptable, as long as the specifications proposed by the applicant are respected and the quality controls described by the applicant in the application dossier are performed.

General conclusions of the CES on Human Nutrition

The CES NUT agrees with most of the conclusions and comments made by the UK authorities, given in the opinion, but has some reservations. The stability of the NI in food matrices has not been tested. Interaction with medicinal substances has not been studied. The CES NUT would also like more details on the quality controls (and the corresponding specifications), the characteristics of the ethanol aqueous solution and the temperature during the extraction process.

The CES NUT stresses that the risks of toxicity of the NI in humans associated with long-term consumption have not been ruled out.

General conclusions of the CES on Additives, Flavourings and Processing Aids

The CES AAAT feels that, as the main publications were not available, the results of the toxicological studies presented in the application dossier are not sufficiently detailed to reach a conclusion on the safety of the NI.

The supplementary evaluation approach proposed by the applicant in the application dossier, i.e. to individually assess the classes of compounds most abundant in the extract in order to draw a conclusion on the overall safety of an ingredient, is not acceptable for this type of compound.

The CES AAAT notes that the findings of the literature suggest that the composition of the extracts of the *Larix* species depends greatly on the source studied. The publications on the bioavailability of the components of plant extracts, such as polyphenols and flavonoids, suggest that their bioavailability is strongly influenced by both the sources and the active forms that make up the extract[12]. However, the bioavailability of the compounds in the plant extracts obviously has on influence on their toxicity in the studies performed in animals.

Due to the fact that the NI would be used in a large number of foodstuffs, and that its reactive compounds may have synergistic or antagonistic effects on each other, the CES AAAT feels that it is necessary to perform, at least, a 90-day animal-based toxicological assessment of the NI in question, in accordance with the chemical specifications set out for the ingredient as it will be marketed, and with the accepted guidelines for performance of a toxicological evaluation (e.g. OECD). The CES AAAT also notes that European legislation on novel ingredients requests conducting mutagenicity studies with the NI, if substantial equivalence cannot be established to an accepted traditional food[13].

In conclusion, for the reasons given in its opinion, the CES AAAT does not agree with the general conclusions issued by the UK authorities.

[12] Williamson G, Manach C. 2005. Bioavailability and bioefficiency of polyphenols in humans. Il; Review of 93 intervention trials. Am J Clin Nutr 81, 243S-255S ; Scalbert A, Williamson G. 2000. Dietary intake and bioavailability of polyphenols. J Nutr 130,2073S-2085S.

[13] Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council (97/618/EC)

5. 5. CONCLUSIONS AND RECOMMENDATIONS OF THE AGENCY

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) hereby adopts the conclusions of the CES on Human nutrition.

In view of the conclusions of the toxicological assessment of the NI, ANSES feels that the assessment of the risks relating to exposure to the NI cannot be performed at this stage.

ANSES also noted that if it were claimed that the ingredient had antioxidant properties[14], an assessment would have to be carried out in accordance with European provisions in force on the authorisation of food additives[15].

Director General

Marc MORTUREUX

[14] Tjukavkina NA, Ruienko IA, Kolesnik YA. 1997. Dihydroquercetin - A new antioxidant and biologically active food supplement. Voprosy Pitanîia (only the summarised version is available). 6,12-15.
[15] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354/16.

6. KEY WORDS

NOVEL FOOD INGREDIENT; TAXIFOLIN; *LARIX GMELINII*; LARCH EXTRACT