TAXIFOLIN FROM DAHURIAN LARCH - APPLICATION FOR THE APPROVAL AS NOVEL FOOD

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

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ADMINISTRATIVE DATA

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GENERAL DESCRIPTION

Introduction

Ametis JSC intends to market a natural flavanone, taxifolin (dihydroquercetin) from larch wood as the dietary ingredient.

In the following text, the name taxifolin will be used, even if publications refer to the compound with its other synonyms.

The trade name Lavitol will be reserved for taxifolin derived from the wood of the Dahurian Larch tree, *Larix gmelinii* (Rupr.) Rupr. (Pinaceae family) with a water-ethanol extraction method.
Ametis JSC intends to register taxifolin from larch wood as a Novel Food ingredient in accordance with Regulation EC 258/97 (food ingredient derived from plant) or Council Directive 89/107/EEC.

In this Dossier, we present our case that taxifolin from larch wood, extracted from Dahurian Larch, is a safe ingredient for the use in food. Additionally, taxifolin provides a health benefit as a dietary supplement, or as a food ingredient of various different foods, mainly by acting as a valuable antioxidant.

Taxifolin has been sold as a dietary supplement in the Russian Federation, Switzerland, and the United States since the early 1990s. In addition, taxifolin has been used as a food additive for over a decade in the Russian Federation in the dairy, meat, and confectionery products, in oil and fats, and in both alcoholic- and non-alcoholic beverages.

As required by the novel food regulation (EC) Nr. 258/97, we present information regarding the several stated criteria: the product's identity, manufacturing process, specifications, stability, intended use, consumer exposure, metabolism, and safety. Taxifolin is a substance having a very low order of toxicity. It does not affect the odor, flavor, or appearance of the finished food products. It is an effective antioxidant at low concentrations under normal conditions of processing and storage of the

finished food products. Taxifolin is also stable at high temperatures, which are common to technological processing of some foods.

Therefore, altogether, taxifolin is well suited as a food ingredient, and will provide a significant benefit to consumer health.

IDENTIFICATION OF ESSENTIAL INFORMATION REQUIREMENTS

The structured schemes outlined for the assessment of a class 1.2 novel food ingredient, are listed below and discussed in detail in subsequent sections (Sections I through XIII).

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

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.
Sections IV to VIII were ommited, since they are not applicable.

I SPECIFICATIONS OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

• "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
• "Is the information representative of the novel food when produced on a commercial scale?"
• "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in the following subsections.

I.1 Common or Usual Names

Dihydroquercetin
(2R,3R)-trans-dihydroquercetin
Taxifolin
Diquertin (private label name)

I.2 Chemical Name

(2R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one
3,5,7,3' 4'-pentahydroxyflavanone

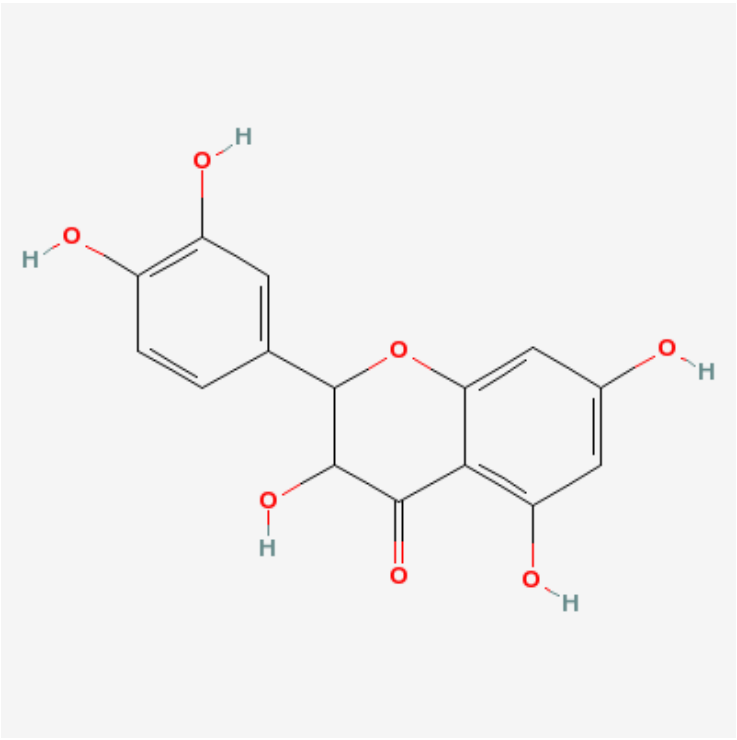
I.3 Trade Name

Lavitol

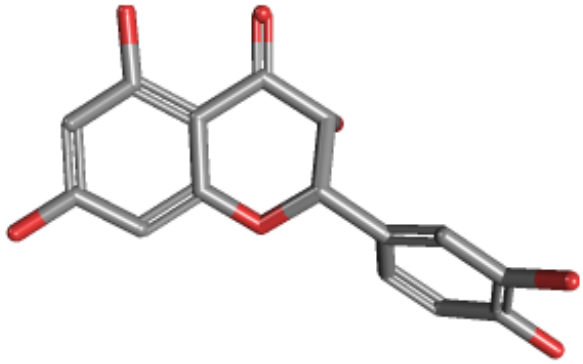
I.4 Chemical Abstract Service (CAS) Numbers

24198-97-8
480-18-2
98006-93-0

I.5 Chemical Structure

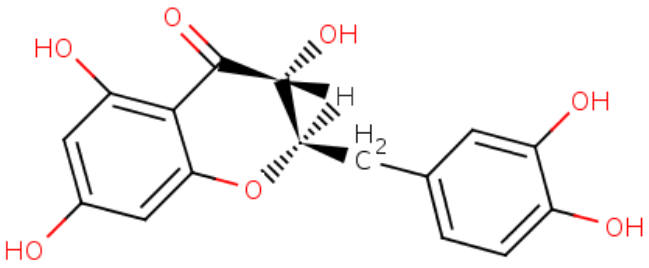


(from PubChem)



(from PubChem)

(2R,3R)-Dihydroquercitin



(from : http://www.brenda-enzymes.info/)

I.6 Molecular Formula and Molecular Mass

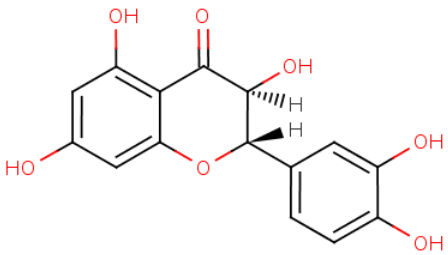
$C_{15}H_{12}O_7$
304.25158 g/mol

I.7 Chemical and Physical Properties

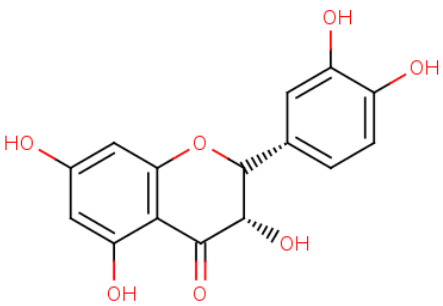
Taxifolin is a white to pale-yellow powder that crystallizes from hot aqueous solutions with water of crystallization being lost upon heating at approximately 125°C (Kurth and Chan, 1951).

There are 2 different diastereomers of taxifolin, 2R3R-trans and 2R3S-cis. Also chemically, + and – enantiomers are possible.

trans-dihydroquercitin



cis-dihydroquercitin



(from : http://www.brenda-enzymes.info/)

Taxifolin is defined as the + trans form, which is prevalent in nature. However, the cis form has also been detected (Seredin, et al. 2007). It seems that a trans form is more

stable than a cis form of taxifolin, thus suggesting a possibility of conversion of the cis form into the trans at an equilibrium favoring the trans form.
The melting point of taxifolin is 234-236°C (Sigma).

Solubility of taxifolin in water at different temperatures has an exponential character: at room temperature, the solubility is 0.1%; at 40°C- 0.3%; at 60°C- 1.0%; at 90°C- 3-5.3% (Radaeva, et al. 1997; Chernukha and Kononenko, 2009); and the solubility in boiling water is 9.3% (Kurth and Chan, 1951). Taxifolin is especially soluble in a 96% ethanol solution. Its solubility in a water-ethanol solution increases from 0.1% to 18% as the rate mass of alcohol increases from 30% to 90% (Radaeva, et al. 1997; Chernukha and Kononenko, 2009).
Taxifolin is soluble in ethyl acetate. The solubility is directly correlated to the temperature of the ethyl acetate solution. Thus, at a temperature of 20°C, the solubility of taxifolin is 1.90%; at 40°C, it is close 9 to 12%; and at 70°C, it is approximately 28% (Chernukha and Kononenko, 2009).
Taxifolin is insoluble in chloroform, ether, and benzene (Radaeva, et al. 1997; Tyukavkina, et al. 1967).

The λmax and λmin of the visible absorption spectra are at 290 nm and 330 nm, respectively.

I.8 Product Specifications and Analysis

I.8.1 Methods of Analysis

Testing facilities

The proximate composition and microbiological, heavy metal and residue contamination levels in taxifolin from larch woodare analyzed by the following facilities:

Public Health Federal State Establishment
Center of Hygiene and Epidemiology
Russian Federation

Advanced Botanical Consulting & Testing, Inc.
(ABC Testing)
Tustin, CA, USA

PhytoLab GmbH & Co. KG
Dutendorfer Strabe 5-7
D- 91487 Vestenbersgreuth
Germany

The methods of analysis, which were used to obtain a specific result, are generally stated on the certificates of analysis. In addition, the specifications within this dossier list the methods, which will be used for the final release in the future.
Please note that each batch is tested to assure the conformity to the specifications. Although some tests done by the different testing facilities may be redundant, only the results of the tests specified for the final release (done with the corresponding pre-defined methods) are relevant.
Although several methods are methods commonly used within the Russian Federation or the USA (e.g. USP microbio), they correspond to comparable methods typically used in the EU in terms of sensitivity and accuracy. This is also true for those methods, which were specifically adapted the analysis of this product (e.g. ICP/MS, Prop. 65).

All methods are included within the Appendix B (appendix-b-analytical-methods).
The method for determining the taxifolin content is an HPLC method, performed on a sample that is previously dried (MVI 72-08, file: mvi72-08.pdf).
Certificates of analysis of the reference standards are included in Appendix G.

I.8.2 Product Specifications

Taxifolin from larch wood adheres to strict limits for levels of microorganisms, heavy metals, pesticides, and radionucleotides. Also several important product characteristics are checked for each batch, including the content of taxifolin.

Directly after manufacturing, product is usually already tested following Russian guidelines and methodology, detailed in Appendix D (appendix-d-manuf-sales-CONFIDENTIAL) in the document about the technical conditions (technical-conditions-CONFIDENTIAL). Later, some of the tests are repeated with different methods in a different laboratory.

The complete final release specifications for taxifolin are presented in Table I.8.2-1
The microbial specifications are repeated in Section XII.1.

Table I.8.2-1 Complete Final Release Specifications of the Product

Specification Parameter		**Method of Analysis***	**Specification**
Physical parameters	Outward appearance	Organoleptic, same as reference standard	white or straw-colored powder
	Moisture	GOST 16483.7-71	NMT 10%
Compound analysis	Taxifolin (m/m)	MVI 72-08 (HPLC)	NLT 88.0% of dried sample
Heavy Metals, Pesticide	Lead	ICP/MS, Prop. 65	NMT 0.5 mg/kg
	Arsenic	ICP/MS, Prop. 65	NMT 0.02 mg/kg
	Cadmium	ICP/MS, Prop. 65	NMT 0.5 mg/kg
	Mercury	ICP/MS, Prop. 65	NMT 0.1 mg/kg
	Dichlorodiphenyldichloro	Method 2142-80	NMT 0.05 mg/kg

	ethane (DDT) and its metabolites**		
Residual solvents	Ethanol	USP 32/NF27 <467>	< 5000 mg/kg
	Solvent residues, Class I	USP 32/NF27 <467>	not detected
	Solvent residues, Class II	USP 32/NF27 <467>	not detected
Microbial Parameters (equivalent to Category 3B, Ph.Eur.) repeated in Section XII.1	Total Plate Count, TPC	USP microbio	NMT 10^4 CFU/g
	Enterobaceria + div. gram-neg. bacteria	USP microbio	≤ 100/g
	Yeast and Mold	USP microbio	NMT 100 CFU/g
	Escherichia coli	USP microbio	Negative/1 g
	Salmonella spp.	USP microbio	Negative/10 g
	Staphylococcus aureus	USP microbio	Negative/1 g
	Pseudomonas spp.	USP microbio	Negative/1 g

NMT = No more than NLT = No less than

*See Appendix B (appendix-b-methods) for analytical methods:

Files: gost-methods.pdf, icp-ms-prop65-abc.pdf, mvi72-08.pdf, usp-microbio.pdf, usp32-solvent-abc.pdf

**Pesticides: The Larch trees are grown in ecologically clean areas, without the application of pesticides. The reason for testing for DTT is that this is a Russian Federation requirement.

I.8.3 Product Analysis

Several lots of the manufactured product were analyzed to verify that the manufacturing process produced a consistent product within the product specifications. A summary of the chemical product analysis for 5 batches is presented in Table I.8.3-1. The microbial analysis is presented in Section XII.1.

Table I.8.3-1 Batch Analysis Results of the Food Ingredient (see also Appendix C (batch analysis), file ametis2009-coa-5batches

Specification Parameter	**Specification**	Batch 2	Batch 27a	Batch 29b	Batch 66a	Batch 950
Outward appearance	white or straw-colored powder	conforms	conforms	conforms	conforms	conforms
Moisture	NMT 10%	7.85%	7.85%	4.55%	9.6%	7.85
Taxifolin (m/m)	NLT 88.0%	92.20%	92.43%	92.36%	92.58	92.20
Lead (mg/kg)	NMT 0.5 mg/kg	0.001	0.067	0.001	0.043	0.040
Arsenic (mg/kg)	NMT 0.02 mg/kg	<0.001	0.002	<0.001	0.004	0.003
Cadmium (mg/kg)	NMT 0.5 mg/kg	0.001	0.028	<0.001	0.028	0.040
Mercury (mg/kg)	NMT 0.1 mg/kg	<0.001	0.010	<0.001	0.006	0.011
Dichlorodiphenyldichloroethane (DDT) and its metabolites (mg/kg)	NMT 0.05 mg/kg	<0.005	<0.005	<0.005	<0.005	<0.005
Ethanol (mg/kg)	< 5000 mg/kg	197.2	147.1	121.8	177.4	31.5
Solvent residues, Class I	not detected (ND)	ND	ND	ND	ND	ND
Solvent residues, Class II	not detected (ND)	ND	ND	ND	ND	ND

Detailed analysis sheets are also included in Appendix C: Files:

batch-analysis-abc-metals

batch-analysis-abc-microbio

batch-analysis-abc-solvents

batch-analysis-taxifolin

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

• “Does the novel food undergo a production process?”

• “Is there a history of use of the production process for the food?”

• “Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?”

These questions have been addressed collectively in the following subsections.

II.1 Raw Materials and Chemicals – Specifications

II.1.1 Raw material - Source Plant *Larix gmelinii* (Rupr.) Rupr.

The wood of the tree *Larix gmelinii* (Rupr.) Rupr. is harvested by experienced collectors. They are trained to recognize the tree *Larix gmelinii* (Rupr.) Rupr. without doubt. Since sawdust is the original raw material, it is tested by the domestic governmental organizations in the Russian Federation. Therefore, only methods specified by the government of the Russian Federation are applied for it. The corresponding methods can be found within the Appendix B.

The identity of the sawdust derived from the wood of this tree is confirmed with the following tests:

Table II.1.1-1 Raw material Specifications – Sawdust

Specification Parameter		**Method of Analysis***	**Specification**
Physical parameters	Outward appearance	Organoleptic	Sawdust free from mold; conforms to the required size
	Moisture Content (m/m)	GOST 16483.7-71	13-25%
Heavy Metals, mg/kg	Lead	GOST 26932-86	NMT 0.5 mg/kg
	Arsenic	GOST 26930-86	NMT 0.02 mg/kg
	Cadmium	GOST 26933-86	NMT 0.5 mg/kg
	Mercury	GOST 26927-86	NMT 0.1 mg/kg
Pesticides, mg/kg**	Hexachlorocyclohexane	Method 2142-80	NMT 0.05 mg/kg
	Dichlorodiphenyldichloroethane (DDT) and its metabolites	Method 2142-80	NMT 0.05 mg/kg
	Heptachlor	Method 2142-80	Not permitted < 0.002 mg/kg
	Aldrin	Method 2142-80	Not permitted < 0.002 mg/kg

Radionuclides, Bq/kg**	Caesium-137	Method 2.6.1.1194-03	NMT 200 Bq/kg
	Strontium-90	Method 2.6.1.1194-03	NMT 100 Bq/kg
Compound analysis	Dihydroquercetin (m/m)	MVI 72-08 (HPLC)	NLT 3.3%
Microbial Parameters	Mesophilic aerobic microorganisms and facultative anaerobic microorganisms	GOST 10444.15-94	Not more than 5×10^4 CFU/g
	Yeast and mold	GOST 10444.12-88	NMT 100 CFU/g
	Pathogenic bacteria including Salmonella	GOST P 50480-93	Absent in 10 g
	Coliform bacteria	GOST 50474-93	Absent in 0.1 g
	Escherichia coli (cfu/g)	GOST 30726-2001	Absent in 1 g

*See Appendix B (appendix-b-methods) for analytical methods:
File: gost-methods.pdf
**Pesticides and radionuclides: The Larch trees are grown in ecologically clean areas, without the application of pesticides. The reason for testing for pesticides and radionuclides is that this is a Russian Federation requirement.

II.1.2 Raw material ethanol – part of extraction solvent

The quality of the 96% ethanol used in the extraction process is required to conform to the specifications listed in Appendix B (methods) – file: specifications-methods-ethanol. Accordingly, the ethanol used for extraction puropses is complient with the EU Directive 2009/32/EC “on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients”.
The results of analysis reports concerning ethanol are summarized in Appendix C (batch analysis). File: analysis-ethanol
The results of the batch analyses conform to the specifications and to the Directive 2009/32/EC.

II.1.3 Raw material water - Extraction solvent

Deionized water is produced with an application of a purified water system, Sharya M-500-01 (Biotechrogress JSC, manufacturer). Deionized water is considered a human food ingredient and is used as part of the extraction solvent.
The water used during manufacturing fulfils the requirements as in the EU directive 98/83/EC of 3 November 1998, as can be seen from corresponding Certificates of analysis (Appendix C - File: analysis-water-summary)
The specifications and methods for determining the water quality are detailed in Appendix B - file: specifications-methods-water.pdf.

II.2 Description of the Manufacturing Process

II.2.1 Manufacturer

The processing of Larch wood to obtain taxifolin is carried out by:

Ametis JSC: 68
Naberezhnaya St.
Blagoveshchensk
Amur District
Russia 675000.

Roughly, 100% of this facility is devoted to the taxifolin production. Ametis JSC has passed the State registration for the manufacturing of taxifolin from larch wood, which is used as an antioxidant in the food industry in the Russian Federation. According to the State Institution of Research Institute of Nutrition (Moscow, the Russian Federation), the documentation-project of Technical Specifications for taxifolin from larch wood corresponds to the acting legislative act and normative regulations for the quality and safety of the population (Appendix A: a1 and a2).
Ametis JCS is ISO 9001:2000 certified for the production of food and biologically active additives and substances for biologically active additives (appendix a: a3).
Ametis JSC is the FDA registered facility (Appendix A: a4).

II.2.2 Extraction

See also Appendix D (d1-manufact-instruct-CONFIDENTIAL and d2-technical-conditions-CONFIDENTIAL).

Overview of manufacturing
The following manufacturing steps take place:
- Preparation of the Water-Ethanol Solution
- Extraction of Ground Wood Mass
- Discharge of the Extracted Solution
- Pressing of the Extracted Wood Mass

- Evaporation and Distillation of the Solvents
- Removal of Larch Oil
- Crystallization

The general manufacturing process for the production of taxifolin from larch wood is aimed at the extraction of soluble substances from Larch wood. The wood mass is extracted, followed by a separation of the liquid phase from the solid residue. The liquid phase is then submitted to an absorption stage using a saturated aqueous solution of water-ethanol extract. Taxifolin is extracted from the alcohol solution via crystallization. This process is similar to those utilized by other taxifolin manufacturers, such as those discussed in numerous patent papers published in the Russian Federation and a process discussed in the United States Patent #5,756,098 (Price, et al. 1998).

Detailed description

Stumps of Larch wood are debarked, cleaved and dried at 40-50ºC to obtain residual moisture of 23-27%. Dried stumps are chopped and ground to a particle size (sawdust). Soluble substances are extracted with a 75-85% ethanol aqueous solution at a temperature of 45-50ºC from the sawdust in a ratio of the raw material to the extracting agent as 1 to (7-10). Afterwards, the extracting agent is distilled off, and sawdust is supplied to a press machine for an additional alcohol return. The aqueous part of the extract is cooled down to 20-25ºC for 20-30 minutes to isolate resinous compounds accompanying taxifolin. An aqueous extract, free from resinous compounds, is evaporated and crystallized. The rate of recovery of taxifolin from Larch sawdust depends on the size of the chips, the extraction temperature, and the level of moisture of the sawdust; while the amount of taxifolin recovered depends on the amount of taxifolin in the wood chips. The crystallized product is transferred to the drying chamber where drying takes place. The finished product taxifolin from larch wood, with a moisture level of not more than 10% and taxifolin content of not less than 88%, is sorted and packaged.

Recordkeeping throughout the manufacturing process

The “Combined technological record chart” contains the following information:

- Date, name of a shift supervisor,

- Extraction: number of the batch, number of the rector, raw material (kg), alcohol (liters, %), temperature, date and time of beginning and of the extraction and its completion;
- The press: date and time of the beginning, date of completion, Volume of solution (liters), name of operator;
- Distillation of solution: Volume of extract, time (beginning and completion), return of alcohol on manufacturing process (volume (liters), concentrates (in %), volume of water (liters), volume of concentrate (liters), temperature on vacuum evaporator;
- Protocol of quantitative chemical analysis: sample number, concentration of taxifolin (%), moisture (%).

II.2.3 Quality control

Several in-process controls are done and assure reliable quality - see also Appendix D (d2-technical conditions-CONFIDENTIAL).

Samples of the final product taxifolin from larch wood are collected in sterilized bags or bottles, labeled, and transferred to the Public Health Federal State Establishment, the Center of Hygiene and Epidemiology of the region for microbiological assays, taxifolin content assays by HPLC, and other quality control assessments. The results of these analyses are logged into worksheets and the distributor's database. Batches failing to pass the quality control criteria are quarantined to prevent them from distribution.

Several Russian testing facilities are authorized to do specification testing:

Table II.2.3-1 Testing Facilities, their Responsibilities and Accreditations

Facility	Qualification for testing	Accreditiation included in	
		Appendix number	file name
Ametis JSC production laboratory, Amur region, Blagoveschensk	Raw Lavitol; Lavitol	a5	a5-ametis-jsc-accred

Production anlaytical laboratory, Amur region, Blagoveschensk	Mass rate of dihydroquercitin	a6	a6-ametis-pl-accred
Testing Laboratory “1403 Center of the State Sanitary Supervision...”	Organoleptic, physiochemical and Microbiological characteristics; Water analysis	a7	a7-belogorsk-accred
Technological laboratory of OAO, “Blagoveschenskiy Spirtzavod”	Ethanol analysis	a8	a8-spirtzavod-accred
Testing Laboratory,”Centre of Hygiene and Epidemiology in the Amur Region”	Pesticides, heavy metas, mircobial contaminants, radionuclides; Water analysis	a9	a9-blagoveschensk-accred

The quantitative composition of taxifolin from larch wood is performed on a contractual basis by:

Advanced Botanical Consulting
& Testing, Inc.
Tustin,
CA, USA

and

PhytoLab GmbH
& Co. KG
Dutendorfer Strabe 5-7
91487 Vestenbersgreuth

Germany

Both companies are also accredited laboratories:
ABC & Testing, Inc.: holds an FDA Certificate Registration # 2032554.
PhytoLab: Please See Appendix A10, file a10-plv-accred.

Both companies are also periodically contracted to perform tests for heavy metal, pesticide, solvent residues, and/or microbiological contamination of the product. Please refer to the section on the product specifications (I Specifications of the novel food) for the test parameters and the corresponding methods.
All reasonable precautions are taken to assure that the production environment does not contribute to contamination, such as with filth, harmful chemicals, undesirable microorganisms or other objectionable material, to the manufactured product. No chemical pesticides are used in the area where Larch trees are grown. Raw materials and ingredients are inspected and segregated, as necessary, to assure that they are wholesome and fit the processing. The raw materials are stored under conditions that protect against contamination and minimize deterioration. Packaging materials do not transmit contaminants or objectionable substances to the product, and provide adequate protection against contamination. They are of food-grade material (see Appendix G).
All operations in receiving, inspecting, segregating, preparing, processing, and storing of the raw material and the finished product, are conducted in accord with the adequate sanitation principles and are regulated by the quality control unit of the manufacturing facilities.

II.3 Potential Impurities Resulting from the Production Process

II.3.1 General Considerations

Taxifolin is the most common member of the dihydroflavonol family of flavonoids. Taxifolin occurs in nature as free phenol, as glycoside, and in the form of free and glycosylated phenol ethers or esters (Kiehlmann and Slade, 2003).

II.3.2 Residual Solvents

During the extraction process, a 75-85% ethanol aqueous solution is used. In the final product, ethanol and water are evaporated. In the final product, it is assured that the residual ethanol levels are below the specification limit. Also no more than 10% of water is allowed in the final product.

II.3.3 Side products

The larch wood (*Larix gmelinii* (Rupr.) Rupr. contains up to 3.5% flavonoids with taxifolin being the dominant flavonoid (over 80%). As measured by the photocolorimetric method with thin-layer chromatography, the flavonoids of the heartwood of Dahurian larch contain 69% of taxifolin (Tyukavkina, et al. 1967).

The finished product taxifolin from larch wood, with a moisture level of not more than 10%, has a taxifolin content of not less than 88% (of the dried matter). Only small amounts of other molecules besides taxifolin and water are present in the final product.
These other components could be identified in an HPLC analysis by using reference standards. The certificates of analysis of the reference standards are included in Appendix G.
The following compounds (besides an average of 7.54% water - average of batches 2, 27a, 29b, 66a, 950) can be found within taxifolin in small amounts (usual amounts within taxifolin are indicated):

Table II.3.3-1 Composition of taxifolin (very minor contaminants omitted)

	Average amount (5 batches*)	Specification limit	Method of Analysis
Taxifolin	92.36%	NLT 88%	MVI 72-08 (HPLC)**
Aromadendrin (Dihydrokaempferol)	2.99%	Not included in specification. Please see chromatograms for each batch in Appendix C, batch-analysis-taxifolin	MVI 72-08 (HPLC)
Eriodictyol	0.198%		MVI 72-08 (HPLC)
Quercetin	0.436%		MVI 72-08 (HPLC)
Naringenin	0.26%		MVI 72-08 (HPLC)
Kaempferol	0.06%		MVI 72-08 (HPLC)
Pinocembrin	0.088%		MVI 72-08 (HPLC)
Total***	**96.392**		

* average of batches 2, 27a, 29b, 66a, 950:

Detailes here:

	Batch 2	Batch 27a	Batch 29b	Batch 66a	Batch 950
Taxifolin (%)	92.23	92.43	92.36	92.58	92.20
Aromadendrin (%) (Dihydrokaempferol)	3.22	3.17	2.54	3.21	2.81
Eriodictyol (%)	0.21	0.24	0.12	0.16	0.26
Quercetin (%)	0.48	0.47	0.49	0.48	0.28
Naringenin (%)	0.26	0.27	0.31	0.19	0.27
Kaempferol (%)	0.05	0.07	0.11	0.02	0.05
Pinocembrin (%)	0.16	0.02	0.09	0.05	0.12
Total***	96.61	96.67	96.02	96.69	95.99

**Samples are dried in a drying cabinet for 1 hr at 105°before HPLC analysis, reducing the pre-analysis moisture content to very low levels.
*** The internal standard caffeine (0.8%) is part of this calculation. The missing 2.808% are other minor unidentified impurities.

Accordingly, the taxifolin content within our product is generally around 92-93%. Minor components are other flavonoids like the flavonoids aromadendrin (dihydrokaempferol) and naringenin, as well as eriodictyol, quercetin, kaempferol, and pinocembrin. These compounds are chemically very similar to taxifolin, in their structure, as well as in their physical and chemical properties.
All of the identified flavonoids (including taxifolin) can be found in various plants, including many regular foods.

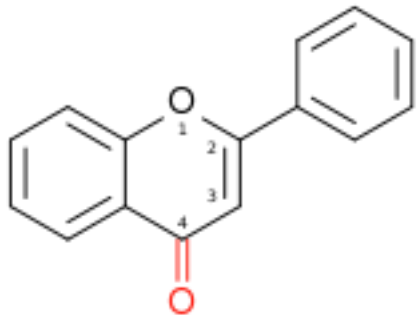
Other components are very minor. They include trace amounts of ethanol (even in the dried sample), metals, inorganic salts, and saponins.

Triterpene glycosides (saponins) are terpenes with attached sugar moieties. The sugar moieties are usually cleaved off in the intestine during digestion, allowing the terpenes to be absorbed. Saponins/ triterpene glycosides can be found in many plants in nature. Most natural triterpene glycosides are beneficial to humans, many are proven to function as adaptogens or antioxidants. These saponins are present in the taxifolin at a very low level (<0.5% - see specifications in Table I.8.1-1 Complete Final Release Specifications of the Product).

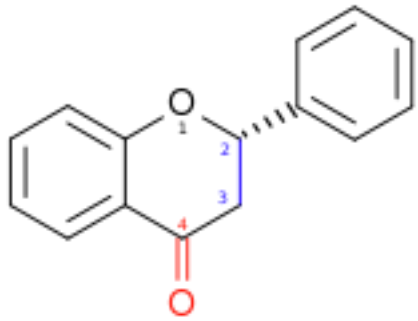
There are no toxic effects associated with the minor amounts of additional flavonoids and saponins or other trace constituents, included in taxifolin. This also clear from many toxicological experiments performed with taxifolin (from larch wood). Please refer to section XIII.1 “Toxicological Evaluation of taxifolin/ Dihydroquercetin”

Figure II.3.3-1. Structures of selected flavonoids

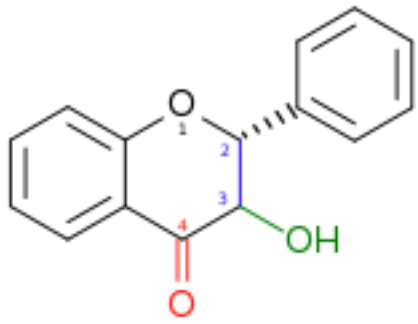
Flavone (http://en.wikipedia.org/wiki/Flavonoid)



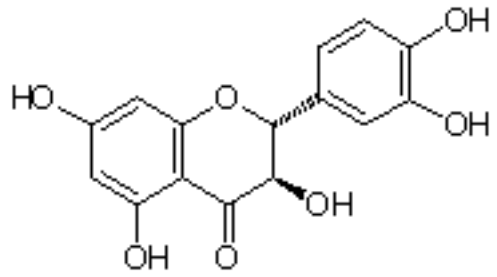
Flavanone (http://en.wikipedia.org/wiki/Flavonoid)



Flavanonol (http://en.wikipedia.org/wiki/Flavonoid)

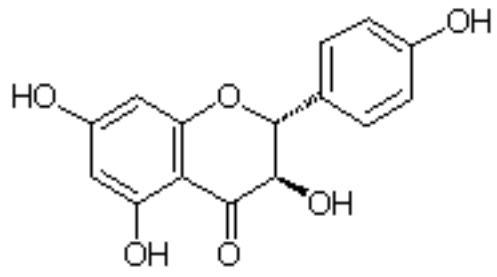


Taxifolin (http://www.3dmet.dna.affrc.go.jp/index.html)



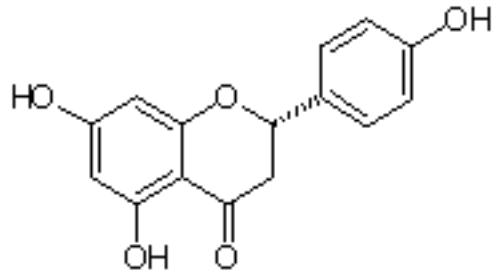
C01617

Dihydrokaempferol
(http://www.3dmet.dna.affrc.go.jp/bin2/show_data.e?acc=B01364)



C00974

Naringenin (http://www.3dmet.dna.affrc.go.jp/index.html)



C00509

II.4 Stability of the food ingredient

Generally, taxifolin from larch wood should be stored in dry, clean, well-ventilated places without strange odors. It should be kept away from moisture and sunrays. It should be stored at a temperature above 4°C and humidity in the range of 40% to 60%.

A stability study was performed with taxifolin. Batch 63 was used to conduct the stability test.

The conditions were accelerated conditions: 40°C/75% relative humidity (RH).
The storage containers for the stability study were of food-grade material (Appendix G). The following table details the result of the stability study:

Table II.4-1 Stability of the food ingredient at 40C/75% RH: Comparison of Analysis Results

Weeks at 40C/75% RH	Extrapo-lation for normal conditions	Taxifolin (HPLC)	Polyphenols (Photometric method, in stabi-12-week)		Saponins (method in stabi-12-week)	Loss on drying
			Standard 1 (Gallic acid monohydrate)	Standard 2 (Taxifolin)		
0		94.0%*	Not determined	Not determined	Not determined	3.7%****
1		94.50%**	121.62%	98.45%	< 0.5%	3.66%****
2		93.50%**	127.78%	98.20%	< 0.5%	3.87%****
4		95.20%**	126.72%	98.02%	< 0.5%	4.01%****
8		98.70%**	125.76%	97.74%	< 0.5%	4.06%****
12	2 years	95.30%**	125.67%	97.45%	< 0.5%	4.21%****
18	3 years	97.70%**	113.23%	94.58%	< 0.5%	4.57%****
30	5 years	97.50%**	108.18%	90.36%	< 0.5%	4.57%****
Stability specification		NMT+/- 5% of original value	NLT 105%	NLT 88%	< 0.5% (not detectable)	NMT 10%

* MVI 72-08

** ABC Testing HPLC method, comparable to MVI 72-08 (method in stabi-12-week of appendix-e-stability)

*** Ph. Eur. 5.0 2.5.32

**** Loss on Drying, USP (method in stabi-12-week of appendix-e-stability)

Accordingly, taxifolin was stable at accelerated conditions, 40C/75% RH, for 30 weeks. This corresponds to shelf-life stability at normal conditions of at least 5 years. Please note that over this time the taxifolin content (measured by HPLC) did not decrease, and the value stayed within +/- 5% of the baseline value. Although the

numbers indicate slightly increased moisture over time, the moisture content (determined by loss on drying) remained well below the specifications.

III HISTORY OF THE ORGANISM USED AS THE SOURCE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

• “Is the novel food obtained from a biological source (*i.e*., a plant, animal or microorganism)?”

This question has been addressed in the following subsections.

III.1 Taxonomic Classification

Taxifolin is a flavonoid derived from Larix gmelinii (Rupr.) Rupr. by a water-ethanol extraction method. This tree species is classified as the following (http://en.wikipedia.org/wiki/Larch):

Kingdom: Plantae
Division: Pinophyta
Class: Pinopsida
Order: Pinales
Family: Pinaceae
Genus: *Larix*
Species: *gmelinii*

Synonyms

Larix gmelinii (Rupr.) Rupr. Is also known as Dahurian Larch or Gmelin Larch. It is also called Listvennitsa daurskaya [Russian], 落叶松 luo ye song [Chinese] and Gui-natsu [Japanese].

Although there are certain geographic variations among the trees of this species (Oreshkova, et al. 2006), it is one single species.
The most common synonyms are (Gymnosperm database 2009):
Larix gmelinii (Rupr.) Kuzen
Larix dahurica Turcz
Larix olgensis
Larix gmelinii var. olgensis (Henry) Ostenf. & Syrach Larsen
L. sibirica Maxim. non Ledeb.

Habitat

Larix gmelinii (Rupr.) Rupr. grows in Siberia and the Russian Far East, which are recognized worldwide to be of very high environmental and ecological importance. The vast geographical area of *Larix gmelinii* (Rupr.) Rupr. shows the large ecological plasticity of this species and its high adaptability to different natural conditions found

in the boreal Eurasian zone as well as in transition to typical temperate forests. Larch trees establish both the southern and the northern timberlines and carry out water- and soil-conservation functions in mountain regions. In addition, *Larix* species are regarded as a large carbon sink. Due to their unique seed dispersion patterns and very high adaptability to the fires that often affect Siberian forests, these Larch species occupy post-fire habitats successfully (Zyryanova, et al. 2007).

III.2 Dietary Exposure to the Herb

Generally, the larch is not considered human food. However, arabinogalactan derived from trees of the genus *Larix* (*Larch*), also known as *larch gum*, is similar to *gum* arabic. Especially *Larix occidentalis* (western *larch*) is used to produce this larch gum, a sweet *gum* that hardens when exposed. Indigenous peoples used to chew gum produced from larch trees, as well as eat the cambium and sap (Turner, 1997) In folk medicine, infusions and decoctions from the larch bark (*Larix rossiea*) have been widely used in cases of colds and respiratory infections, while an extract made of needles was used in cases of hypermenorrhea (Lavrenov and Lavrenova, 1999). Bark of *Larix lacricina* has been used traditionally to treat symptoms of non-insulin-dependent diabetes by the healers in Northern Quebec, Canada (Fraser, et al. 2007).

III.3 Safety of the Herb

There are no known safety concerns about the consumption of *Larix gmelinii* (Rupr.) Rupr..

IX ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

• “Is there information on the anticipated uses of the novel food based on its properties?”

• “Is there information to show anticipated intakes for groups predicted to be at risk?”

• “Will introduction of the novel food be restricted geographically?”

• “Will the novel food replace other foods in the diet?”

• “Are any of the replaced foods significant nutritional sources?”

• “Does the probable level of substitution have a nutritional significance for any population groups?”

These questions have been addressed collectively in the following subsections.

IX.1 Intended Uses in Food

Acceptable daily intake (ADI)

The acceptable daily intake is usually calculated from the NOAEL. The NOAEL is scaled by a safety factor, conventionally 100, to account for the differences between test animals and humans (factor of 10) and possible differences in sensitivity between humans (another factor of 10). The ADI is usually given in mg per kg body weight per day.

For taxifolin from larch wood, an exact NOAEL was difficult to determine, since even very large amounts of taxifolin did not cause any adverse reactions (Section XIII, Toxicological Assessment). Thus, a NOAEL of >1500 mg/kg body mass could be defined for animals (e.g. an 6-month oral toxicity study in rats).

This means that, assuming a factor of 100, the taxifolin **ADI for humans should be 15 mg/kg body mass** (1050 mg/day for a 70 kg adult).

However, additional health benefits with daily intakes higher than 1.5 mg/kg body mass (100 mg/day for a 70 kg adult) are not expected, therefore excessive taxifolin consumption would be of no benefit.

IX.1.1. Food Ingredient

The food ingredient taxifolin is intended for use in conventional foods, and as food supplement or in PARNUTs. The **individual** proposed food-uses and use-levels in the E.U. are summarized in Table IX.1.1-1. **Note that not all food categories listed below will always contain taxifolin from larch wood.** There will probably be brands of the same type of food not containing taxifolin.

Altogether, the portion of foods containing taxifolin will certainly vary between food types, but will probably not exceed 10% within the next decades (this is already a high-end estimate).

In some foods, the amount of taxifolin added to a food will vary with the fat content of this food. The average estimated fat contents are listed in these cases, and the proposed use levels are also based on these numbers.

Although taxifolin also functions as preservative, the use described here is for nutritional purposes, and will be advertised as such. For this reason, taxifolin will be included in pre-packaged products, which allows corresponding labelling.

Table IX.1.1-1 Summary of the Individual Proposed Food-Uses and Use-Levels in the E.U.

Food Category	**Proposed Food-use**	**Proposed Use-Levels within these foods *as food-Ingredient (g/l –liquids or g/kg – solids)***	**Proposed Use-Levels within these foods *as food-Ingredient (%)***
Beverages	Concentrated soft drinks – not low calorie, as consumed	**0.02**	**0.002**
	Carbonated soft drinks – not low calorie	**0.02**	**0.002**
	Concentrated soft drinks – low calorie, as consumed	**0.02**	**0.002**
	Carbonated soft drinks – low calorie	**0.01**	**0.001**
	Ready to drink soft drinks not low-calorie (including sports and isotonic drinks)	**0.02**	**0.002**
Cereals and cereal & grain products	Biscuits	**0.07**	**0.007**
	Cereal Bars (NDNS “other cereals”)	**0.07**	**0.007**
	Energy and Diet Meal Bars (no NDNS category)	**2.144**	**0.2144%**
Meat	Ground meat (no NDNS category) (0.2 g/kg fats – ca 30%)	**1.389**	**0.1389**
	Ground chicken (no NDNS category) (0.2 g/kg fats – ca. 10%)	**2.616**	**0.2616**
	Poultry sausage (no NDNS category) (0.2 g/kg fats – ca. 10%)	**1.390**	**0.1390**
	Cutlets (no NDNS category) (0.2 g/kg fats- – ca. 10%)	**0.545**	**0.0545**
	Dumplings (no NDNS category) (0.2 g/kg fats– ca. 10%)	**0.149**	**0.0149**
	Coated and/or fried white fish (0.5 g/1 kg lipid mass - ca.10%)	**0.161**	**0.0161**
Milk products	Dry milk, 15% fat (no NDNS category)	**0.161***	**0.0161***
	Dry milk, 20% fat (no NDNS category)	**0.2***	**0.02***
	Dry milk, 25% fat (no NDNS category)	**0.2***	**0.02***
	Dry soy milk concentrate (no	**0.2***	**0.02***

	NDNS category) (0.2 g/kg fat content – ca 18% fat)		
	Condensed milk (no NDNS category) (0.2 g/kg fat content – ca. 10% fat)	**0.2***	**0.02***
	Sour milk products (no NDNS category) (0.02% per mass of fat – ca. 3% fat)	**0.2***	**0.02***
	Sour cream with 15% fats (no NDNS category) (0.025% taxifolin in the product)	**0.200**	**0.0200**
	Sterilized creams (NDNS category cream) (0.025% per mass of fat – ca. 30% fat)	**0.12***	**0.012***
	Cottage cheese (ca. 5.5% fats) (0.025% taxifolin in the product)	**0.150***	**0.0150***
	Curd desserts (NDNS category other dairy desserts) (0.025% per mass fat - ca. 4%)	**0.13***	**0.013***
	Yogurt, ca. 7.5% fat (0.02% by fat mass of milk - 4% fat in the milk)	**0.050***	**0.0050***
Sugar, Preserves, Confectionery	Chocolate confectionery (0.02% per mass of fat – ca. 35% fat)	**0.030***	**0.0030***
Fats and oils	Butter (0.05% per mass of fat. - ca 82% fat)	**0.030***	**0.0030***

The actual individual intake will depend on the average daily intake, which in turn can be determined from dietary surveys of the corresponding food categories.

IX.1.2. Food Supplements

Taxifolin will be used as dietary supplement in appropriate oral dosage forms, e.g. tablets or capsules. The recommended daily dosage will be 100 mg of taxifolin for an adult or children above 12 years, and 25 mg for children from 6-12 years.

IX.1.3 PARNUTS Products

Taxifolin from Larch is also intended for use as a food for particular nutritional uses (PARNUTS) (Council Directive 89/398/EEC) (Council of the European Communities, 1989), for example, as a food for special medical purposes (Commission Directive 1999/21/EC) (Commission of the European Communities, 1999) and as a food intended to meet the expenditure of intense muscular effort, especially for sportsmen. The proposed daily intake is 100 mg taxifolin/day. The rationale for the proposed use of taxifolin around 100 mg per day in PARNUTS products is based on the available efficacy data.

IX.2 Estimated Consumption of Taxifolin from Proposed Food Uses in the E.U.

IX.2.1 Estimated Daily Intake of Taxifolin from Proposed Food-Uses

Adults

Estimates for the intake of taxifolin in the E.U. for adults (18-64 years) were based on the proposed use-levels for taxifolin summarized in Table IX.2.1-1 and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's Dietary Survey Programme – the National Diet & Nutrition Survey (Henderson, et al. 2002: NDNS-survey).

"Worst-case" taxifolin consumption

As can bee seen from the table IX.2.1-1, there is little difference beween mean daily users-only consumtion of men and women for the intended food categories (men : 64.88 mg; women 63.45 mg): Accordingly, the mean all-user (male /female) daily consumption of taxifolin due to the intake of taxifolin in various food categories is likely to be around to 65 mg taxifolin per day, if 100% of the indicated foods will incorporate the proposed levels of taxifolin.

There are always individuals who, for some reason, consume more than the average of certain food types. From the literature evaluation of dietary surveys like the NDNS Survey (Henderson et. al., 2002) if can be seen that the 95-percentile lies around a factor 1.5 higher than the mean, and the 97.5-percentile can be as high as twice the mean.

Following this assumption, in a worst-case scenario an adult consumer who would exclusively choose taxifolin-containing products, and consume extreme amounts of all categories, would have an intake of around 130 mg of taxifolin per day. Although this value is slightly above the recommended daily dosage, it is still safe:
The ADI (acceptable daily intake) of taxifolin is at least as high as 1050 mg of taxifolin per day (at least 15 mg/kg body mass) – see above.
130 mg in an average 70 kg individual would result in 1.9 mg/kg body mass.

Realistic taxifolin consumption

Probably only 10% of all products will contain taxifolin – most likely even less. Accordingly, the the mean exposure will be around 6.5 mg taxifolin/day (all-users), or even lower considering that there are non-users of certain food categories.
Only few people will be regular consumers of all 27 indicated categories, and even if they are, they would rarely choose taxifolin-fortified foods only. Realistically, every consumer chooses certain products again and again. However there is a low chance that these favourite products all contain taxifolin, since comparable products without taxifolin are in the majority, unfortunately.
In a long-term view it would be desirable to raise the public exposure to taxifolin, to get more close to the “worst-case” consumption, which is actially the best case in terms of health benefit.
To get more Lavitol, additional supplementation with Lavitol is recommended.

(Table - see next page)

Table IX.2.1-1 Estimated Daily Intake of Taxifolin from Proposed Food-Uses (Consumers only) – Adults 18-64 Years

Food Category	**Proposed Food-use**	**Proposed Use-Levels within these foods *as food-Ingredient (g/l –liquids or g/kg –solids)***	***Weekly adult food consumption, consumers only (NDNS Data; Henderson, et al. 2002) (kg)***		**Taxifolin consumption per day for a normal adult, if ALL such foods would contain this much taxifolin (g): Use level*Weekly food consumption/7**	
			Male	***Female***	***Male***	***Female***
Beverages	Concentrated soft drinks – not low calorie, as consumed	**0.02**	**2.144**	**1.516**	0.00613	0.00433
	Carbonated soft drinks – not low calorie	**0.02**	**1.389**	**1.012**	0.00397	0.00289
	Concentrated soft drinks – low calorie, as consumed	**0.02**	**2.616**	**1.885**	0.00747	0.00539
	Carbonated soft drinks – low calorie	**0.01**	**1.390**	**1.521**	0.00199	0.00217
	Ready to drink soft drinks not low-calorie (including sports and isotonic drinks)	**0.02**	**0.545**	**0.644**	0.00156	0.00184
Cereals and Cereal Products, grain products	Biscuits	**0.07**	**0.149**	**0.106**	0.00149	0.00106
	Cereal Bars (NDNS “other cereals”)	**0.07**	**0.161**	**0.093**	0.00161	0.00093
	Energy and Diet Meal Bars (no NDNS category)	**0.07**	**0.161***	**0.093***	0.00161	0.00093
Meat	Ground meat (no NDNS category) (0.2 g/kg fats – ca 30%)	**0.06**	**0.2***	**0.162***	0.00171	0.00139
	Ground chicken (no NDNS category) (0.2 g/kg fats – ca. 10%)	**0.02**	**0.2***	**0.162***	0.00057	0.00046
	Poultry sausage (no NDNS category) (0.2 g/kg fats – ca. 10%)	**0.02**	**0.2***	**0.162***	0.00057	0.00046

	Cutlets (no NDNS category) (0.2 g/kg fats- – ca. 10%)	**0.02**	**0.2***	**0.162***	0.00057	0.00046
	Dumplings (no NDNS category) (0.2 g/kg fats– ca. 10%)	**0.02**	**0.2***	**0.162***	0.00057	0.00046
	Coated and/or fried white fish (0.5 g/1 kg lipid mass - ca.10%)	**0.05**	**0.200**	**0.162**	0.00143	0.00116
Milk products	Dry milk, 15% fat (no NDNS category)	**0.035**	**0.12***	**0.12***	0.00060	0.00060
	Dry milk, 20% fat (no NDNS category)	**0.046**	**0.150***	**0.150***	0.00099	0.00099
	Dry milk, 25% fat (no NDNS category)	**0.056**	**0.13***	**0.13***	0.00104	0.00104
	Dry soy milk concentrate (no NDNS category) (0.2 g/kg fat content – ca 18% fat)	**0.036**	**0.050***	**0.050***	0.00026	0.00026
	Condensed milk (no NDNS category) (0.2 g/kg fat content – ca. 10% fat)	**0.02**	**0.030***	**0.030***	0.00009	0.00009
	Sour milk products (no NDNS category) (0.02% per mass of fat – ca. 3% fat)	**0.006**	**0.030***	**0.030***	0.00003	0.00003
	Sour cream with 15% fats (no NDNS category) (0.025% taxifolin in the product)	**0.25**	**0.030***	**0.030***	0.00107	0.00107
	Sterilized creams (NDNS category cream) (0.025% per mass of fat – ca. 30% fat)	**0.076**	**0.055**	**0.060**	0.00060	0.00065
	Cottage cheese (ca. 5.5% fats) (0.025% taxifolin in the product)	**0.25**	**minimal**	**0.172**	0.00000	0.00614
	Curd desserts (NDNS category other dairy desserts) (0.025% per mass fat - ca. 4%)	**0.01**	**0.144**	**0.130**	0.00021	0.00019
	Yogurt, ca. 7.5% fat (0.02% by fat mass of milk - 4% fat in the milk)	**0.008**	**0.404**	**0.404**	0.00046	0.00046
Sugar, Preserves,	Chocolate confectionery	**0.07**	**0.134**	**0.106**	0.00134	0.00106

Confectionery	(0.02% per mass of fat – ca. 35% fat)					
Fats and oils	Butter (0.05% per mass of fat. - ca 82% fat)	**0.41**	**0.46**	**0.46**	0.02694	0.02694
TOTAL with 100% of foods containing taxifolin at the indicated level (unrealistic "worst case" scenario)					**0,06488**	**0,06345**
TOTAL with 10% of foods containing taxifolin at the indicated level					**0.006488**	**0.006345**

*** estimation from related category**

Chidren

"Worst-case" taxifolin consumption in children

Children will consume less food, and thus will be less exposed in absolute terms. However, relative to their body mass, the exposure in small children is generally the greatest. A good estimate is that, children consumers up to 41/2 years will on average consume up to 50% of the mean adult intake. This would be around 33 mg taxifolin from larch wood per day (2.2 mg/kg for a 15 kg child), if only taxifolin fortified foods (in all categories) would be consumed. As a "worst case scenario" (97.5%tile), such children could consume as much as the mean level in aduts, resulting in a still safe taxifolin exposure of up to 65 mg/day (4.3 mg/kg for a 15 kg child).
Derived from animal experiments, exposures of up to 15 mg/kg body mass can be regarded as completely safe even for small children (See ADI calculation above).

Realistic taxifolin consumption in children

Only 10% of all products will contain taxifolin – most likely even less. Accordingly, the child mean exposure will be around 3.3 mg taxifolin/day (all-users) or 0.22 mg/kg body mass. Most likely, it would be even lower, considering that there are non-users of certain food categories (only few children will be regular consumers of all 27 indicated categories).

Young people and

Young people (4-10 years) on average consume about 2/3 of adults (as much as 43 mg/day of taxifolin, 1.4 mg/kg body mass in a 30 kg youth). Regarding the extraordinary safety of taxifolin from larch wood, there also are no safety concerns in these age groups, even if the 97.5%tile for young people could be as high as twice the mean intake: 86 mg/day of taxifolin or 2.9 mg/kg for a 30 kg youth.
In a realistic scenario with only 10% of all products containing taxifolin, the mean exposure in young people would be only around 4.3 mg/day of taxifolin,

Teenagers

Teenagers (11-18 years) often consume adult amounts already. Due to their less than adult body mass, they may however have a slightly higher intake per body mass, resulting in a mean intake of 1.2 mg/kg taxifolin (exclusive use of taxifolin-

fortified foods), and 2.4 mg/kg taxifolin (exclusive use of taxifolin-fortified foods and 97.5%tile consumption).
Realistically, only 6.5 mg/day and 0.12 mg/kg body mass would be consumed (with 10% of all products containing taxifolin).

Summary and conclusion

The following table summarizes the estimated consumer intake of taxifolin from larch wood:

Table IX.2.1-2 "Worst Case" and Expected Use Levels in Consumers of Taxifolin-fortified Foods

Age years (kg)	ADI for the indicated age group***	"Worst case": Exclusive use of taxifolin-fortified foods				10% of products with taxifolin	
		Mean daily intake:		97.5%tile daily intake:		Realistic mean daily intake	
		mg	mg/kg	mg	mg/kg	mg	mg/kg
1.5-4.5 (15 kg)	225 mg	33	2.2	65	4.3	3.3	0.22
4-10 (30 kg)	450 mg	43	1.4	86	2.9	4.3	0.14
10-18 (55 kg)	825 mg	65	1.2	130	2.4	6.5	0.12
Adult* (70 kg)	1050 mg	65**	0.9	130	1.9	6.5	0.09

* Men and women were found to consume similar amounts regarding the proposed categories
** Value derived from Consumer data of the NDNS Data; Henderson, et al. 2002, see Table IX.2.1-1.
*** ADI=15mg/kg body mass

Since no risks are involved with the proposed dietary levels of taxifolin, the proposed consumption can be considered absolutely safe. On the contrary, the health benefits associated with taxifolin support using this food ingredient in many different foods in order to provide this benefit to a broad population of consumers. Several small

doses, e.g. as food ingredient within different foods, is optimal, since taxifolin has been shown to be metabolized within a few hours after intake. By using taxifolin within different foods, the intake is distributed over the day, thus providing its benefits for an extended time.

IX.3 Dietary Supplement and PARNUT Use

Taxifolin will be used as dietary supplement in appropriate oral dosage forms, e.g. tablets or capsules. The recommended daily dosage will be 100 mg of taxifolin for an adult or children above 12 years, and 25 mg for children from 6-12 years. Exceeding dosages will not result in any health risks. However, no additional benefit is expected.
Taxifolin may also be included in PARNUTs. The recommended daily dosage will be 100 mg of taxifolin for an adult or children above 12 years, and 25 mg for children from 6-12 years.
Taxifolin used as PARNUT products would typically be taken as an alternative to supplementation with taxifolin, and at last partially as alternative to convential food fortified with taxifolin (foods listed in Table IX.1.1-1). Therefore, the total estimated intake of taxifolin will not relevantly increase as a result of the use of taxifolin in PARNUTS products.
However, even a 97.5%tile exposure (130 mg/day) from conventional foods + 100 mg from PARNUT use + 100 mg from additional supplementation (total= 330 mg) would be below the ADI of 1050 mg/day for an adult.

IX.4 Food Product Labeling Information

Taxifolin shall be displayed on the labeling of the food product or in the list of ingredients of foodstuffs containing it as “taxifolin from larch wood”.

IX.5 Conclusion

Although taxifolin also has beneficial technical properties for enhancing product stability, it will be added to the indicated foods mainly to obtain a health benefit.
The food ingredient taxifolin is intended for use in conventional foods, and as food supplement.

An ADI of 15 mg/kg body mass was calculated, derived from the excellent safety profile in many different animal experiments.
Up to 10% of several food types will contain possibly contain taxifolin within the next decades. From the proposed food types, the exposure of the consumer adult population to taxifolin would then be around to 6.5 mg of taxifolin per day per adult. However even an excessive exposure of all indicated food types (all fortified with taxifolin) can still be regarded as safe, also for small and larger children and teenagers.
As a dietary supplement or PARNUT, the recommended daily dosage will be 100 mg for teenagers above 12 years and adults and 25 mg for children 6-12 years. Even the use of taxifolin as supplement and/or PARNUT together with foods containing taxifolin will not be harmful, since there are no health risks involved. On the contrary, the supplementation with taxifolin, together with the consumption of taxifolin-fortified foods can be highly recommended, since the compound has clear health benefits.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

• “Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”

• “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

These questions have been addressed collectively in the following subsections.

X.1 Natural Occurrence of the Food Ingredient in the Diet

X.1.1 Intake of the Food Ingredient

Taxifolin is sold as dietary antioxidant and incorporated into many foods (Sigma 2007).
Ametis JSC is the major manufacturer of taxifolin in the world. It produces 70% of all taxifolin in the Russian Federation. Several companies have taken larch-derived taxifolin produced by Ametis JSC, to use within their dietary supplements. The following table indicates the involved companies and product details:

(next page)

Table X.1.1.-1 Examples of Dietary Supplements Containing Taxifolin Derived from Larch

No.	Company	Product	DHQ (TAXIFOLIN) content stuff	Year of product launching
1	EKSPOPHARM Ltd., Sarov city	BIOFOLIN	Food supplement DHQ = 18mg per capsule	2005
2	Irwin Naturals, USA	URITROL	Food supplement DHQ = 8.3 mg per tablet	2004
3	Irwin Naturals, USA	NORMOLIV	Food supplement DHQ = 11.5 mg per tablet	2004
4	Scientific research institute of biomedicine chemistry (Russian Academy of Medicine Sciences)	LIVALON No.1	Food supplement DHQ = 15 mg per capsule	2004
5	ROSBIOPROM, Sarov city	OFTALVIT	Food supplement DHQ = 15 mg per capsule	2004
6	ROSBIOPROM, Sarov city	GINEKOVIT	Food supplement DHQ = 15 mg per capsule	2004
7	Medicine & Health Ltd., Moscow	NORMALIZER	Food Supplement DHQ = 5 mg per capsule	2004
8	YALMA Ltd., Moscow	FLAVANON 100	Food Supplement DHQ = 9.5 mg per tablet	2003
9	JSC BALZAM, Bijsk city	ARONODIQUERTIN	Food Supplement DHQ = 7.5 mg per tablet	2003
10	Laboratory NIZAR, Moscow	DE KARRO Stress Resistance	Emulsion, liquid food supplement DHQ = 50 mg per 250 ml	2003
11	JSC Russian Bread, Moscow	Gullets " MAJERIC ULTRA"	Gullets (wheat) DHQ = 20 mg per 100gr of RTE product	2007
12	State Scientific Center of Practical Medicine "PHARMA"	ANAVITA+	Food Supplement DHQ = 2.5 mg per tablet Stuff: Inulin + taxifolin	2007
13	YALMA Ltd., Moscow	ELQUERTIN	Food Supplement DHQ = 7.5 mg per tablet	2007
14	JSC REAL Caps, Moscow region	LUTEIN ACTIV	Food Supplement DHQ = 8 mg per tablet	2007
15	FAKEL Design Ltd., Moscow	VITAGEN Bilberry +	Bars DHQ = 25 mg per bar	2007
16	VITAPROM Ltd., Moscow	Heart Friend	Food Supplement DHQ = 8 mg per capsule	2007
17	State Scientific Center of Practical Medicine "PHARMA"	ASKLEZAN A	Food Supplement	2007
18	ROSBIOPROM Ltd., Sarov city	BIOSCAN +	Food Supplement DHQ = 80%	2006
19	PHARMAVID Ltd., Moscow	LADOL	Food Supplement DHQ = 88%	2006
20	Natural PHARM Ltd., Tomsk	ANTOXID	Food Supplement DHQ = 20 mg per tablet	2006
21	Natural PHARM Ltd., Tomsk	LIPOVERTIN	Food Supplement DHQ = 10 mg per tablet	2006
22	ARTLIFE Ltd., Tomsk	Discovery Best	Food Supplement DHQ = 2.5 mg per tablet	2006

23	ARTLIFE Ltd., Tomsk	Discovery Force	Food Supplement DHQ = 2.5 mg per tablet	2006
24	ARTLIFE Ltd., Tomsk	VIZUS	Food Supplement DHQ = .5 mg per tablet	2006
25	ARTLIFE Ltd., Tomsk	Discovery Beauty	Food Supplement DHQ = .2..5 mg per tablet	2006
26	UVIKS PHARM Ltd., Krasnodar city	LECETIN & DHQ	Food Supplement DHQ = .5 mg per tablet	2006
27	JSC MAXPHARM, Ryazan city	MAXIFLORUM MAXINORM	Food Supplement DHQ = .90 mg per capsule	2006
28	JSC BIOFIT, N.Novgorod city	FITOQUERTIN No.3 (with raspberry)	Food Supplement DHQ = 10 mg per capsule	2006
29	JSC BIOFIT, N.Novgorod city	FITOQUERTIN No.2 (with grapefruit)	Food Supplement DHQ = 10 mg per capsule	2006
30	JSC BIOFIT, N.Novgorod city	FITOQUERTIN No.1 (with bilberry)	Food Supplement DHQ = 10 mg per capsule	2006
31	PHARMAVID Ltd., Moscow	REMODAL	Food Supplement DHQ = 15 mg per tablet	2006
32	Scientific production company BIOTICA C Ltd. , Moscow	VITADOL	Food Supplement DHQ = 10% from capsules content	2006
33	SAPHRON Ltd., Moscow	Tiger eye CAPINORM	Food Supplement DHQ = 2.5 mg min per tablet	2006
34	ARTLIFE Ltd., Tomsk	Essential oil with DHQ and selenium	Food Supplement DHQ = 3.75 mg per capsule	2006
35	Scientific production Co BIOTICA C Ltd.	TAURINOV	Food Supplement DHQ = 3.75 mg per capsule	2006
36	JSC AMETIS	LAVIOCARD	Food Supplement DHQ = 30 mg per tablet Stuff: Vitamin C – 30 mg Ratio: 1:1	2006
37	PHARM Product Ltd., Saint-Petersburg	BIOLAN LIFE	Food Supplement DHQ = 3 mg per tablet	2006
38	Grissant Pharma Industries LTD., UK	OXYGrissant	Food Supplement DHQ = 2.5 mg per capsule	2006
39	Accurex Health Care Manufacturing Inc., Canada	Anti Aging Skin	Food Supplement DHQ = 0.8 mg per capsule Total flavonoid content = 9 mg per tablet	2006
40	ARTLIFE Ltd., Tomsk	SOPHIA	Food Supplement DHQ = 5 mg per tablet	2006
41	State Scientific Center of Practical Medicine “PHARMA”, Moscow	DIHYROQUERCETIN with grape seed extract	Food supplement DHQ = 15 mg per capsule Stuff: Caffeine, EGCG, Catehienes	2006
43	ECOMOTOR ltd., Moscow region	VEZALARIX	DHQ – 94%	2006
44	MEDBIOPHARM Ltd., Obninsk city	FLAVOCEN	DHQ – 90-94%	2006
45	FLAVIT Ltd., Moscow region	FLAVIT	DHQ – 92-98%	2007
46	JSC BALZAM, BIJSK city	ANTIORVIN	Food Supplement DHQ = 1.5 mg per tablet	2006
47	JSC MIRRA-M, Moscow	MIRRA-DIOVIT	Food Supplement DHQ = 5.5 mg per tablet	2006
48	JSC DIOD, Moscow	CAPILAR	Food Supplement DHQ = 10 mg per tablet	2006 (re-registration

				from 2003)
49	JSC DIOD, Moscow	OKULIST	Food Supplement DHQ = 5 mg per capsule	2006
50	ARTLIFE Ltd., Tomsk	CORDIS	Food Supplement DHQ = 5 mg per capsule	2006
51	ARTLIFE Ltd., Tomsk	ENERGIA	Food Supplement DHQ = 10 mg per capsule	2006
52	ARTLIFE Ltd., Tomsk	CIFROL 5 (antioxidant complex)	Food Supplement DHQ = 5 mg per capsule	2005
53	Life Extension Foundation	Vitamin C with Dihydroquercetin	Food Supplement DHQ = 10 mg/capsule	2006 [1st appearance in the Life Extensioin Magazine]
54	Country Life	Buffer-C pH controlled	Food Supplement The amount is not specified	Unknown
55	PepsiCo, USA	Aqua Minerale Beauty	Food Supplement DHQ = 2 mg/100 ml	2005
56	Newman nutrition AG, Switzerland	Unknown	Food Supplement Unknown	Unknown
57	Nutra-Ingredients, USA (SIBLAREX Group)	CAPILAR®	Food Supplement DHQ = 10 mg/ tablet	Unknown

By April 2009, over 250 products featuring taxifolin were registered with the regulatory organs of the Russian Federation. Among these products, 142 were food supplements, over 40 were food products, and over 70 were cosmetic products. Recommended adult dosages range from 5 mg to 100 mg of taxifolin per day.

The company Ametis JSC has extensive and growing sales of taxifolin from larch wood (Lavitol; dihydroquercitin). The sales between 2006-2009 can be viewed in the tables contained within the confidential Appendix D; file: d3-postmarketing2006-09-CONFIDENTIAL.pdf.
Altogether, over 18 tons of taxifolin (from larch wood) were sold by Ametis JSC to be used within dietary supplements.

Both Ametis JSC and the companies that use Lavitol (taxifolin from larch wood) in their dietary supplements, maintain an internal databases on the return products, in which they incorporate incoded reasons for return. To our knowledge, there were no side effects reported to either companies and no product, either in its finished form or as a raw material was returned to the distributor and/or manufacturer.

Taxifolin is part of the maritime pine bark extract Pycnogenol®, used as dietary supplement. According to the quantitative analysis conducted on the supplement,

each tablet of 50 mg of Pycnogenol® contains at least 14.35 µg of taxifolin per one mg of the capsule content. This amounts to 717.5 µg of taxifolin per capsule (Grimm, et al. 2006). With a daily dose of up to 2 tablets per day, the daily taxifolin intake with Pycnogenol amounts to up to 1.4 mg/day

X.1.2 Regulatory Status of the Food Ingredient

Taxifolin has been approved in the Russian Federation as both a dietary supplement and a food additive with antioxidant properties, used in a variety of food articles as a shelf-life prolongation ingredient (Sanitary-Epidemiological Conclusion 2.3.2. 1078-01 (index 1.10.5). The Sanitary-Epidemiological Conclusion 2.3.2.1293-03 (3.4.9.) recommends using 0.02% taxifolin per fat mass (200 mg per 1 kg of fat) in dry milk and cream condensate.

According to the regulations of the Russian Federation, taxifolin can be consumed as a dietary supplement when administered at levels from 25 mg to 100 mg a day (Sanitary-Epidemiological Regulation MP 2.3.1.1915-04). We are not aware of reports of adverse reactions to taxifolin supplements after years of regular human consumption of such products at the recommended taxifolin dosage of 25 to 100 mg a day.

Storage

Taxifolin (taxifolin) must be stored in a dry, clean, ventilated place, where there is no strange smell; the temperature must not be less than 4^0C and the relative air humidity must be between 40-60%. The containers used for storage should be composed of food-grade material (Appendix G)

X.2 Dietary Exposure to the Food Ingredient from Other Sources

Taxifolin is a minor component in the human diet. It is found in olive oil, vegetables (i.e., onions), fruits (especially citrus), berries, nuts, grains, and spices.

As one of more than 230 minor phytochemicals found in olive oil, taxifolin was found in several Spanish olive oils: Arbequina, Lechin of Sevilla, Picual, and Lechin of Granada *(*Carrasco-Pancorbo, et al. 2005).
In particular, in Arbequina olive oil, taxifolin was found in a concentration of 129.42±5.47 µg/l of the oil and in Picual olive oil in a concentration of 107.69±4.89 µg/l of the oil (Pancorbo, et al. 2004).

The total flavonoid concentration in red onions, *Allium cepa* L. var. Tropea, was found to be 232.12 mg/kg of onions (Corea, et al. 2005*)*. The dihydroflavonols identified in onions are all based on taxifolin (3,5,7,3′,4′-pentahydroxyflavanone). From bulbs of the cv. “Tropea”, which is cultivated in Southern Italy, 98.1 mg of taxifolin have been isolated per kilogram of fresh onions (Slimestad, et al. 2007), while the Red Baron onion variety was shown to contain minor amounts of taxifolin-4-O-glucoside (Lanzotti, 2006).

Taxifolin and its glucosides were identified in sorghum grain (Awika, et al. 2004). Taxifolin and other dihydroflavonols were also found in citrus fruits, such as grapefruit and citrus. An HPLC method showed the following taxifolin contents in different citrus fruits (dry sample content): 16.9 µg /100 mg in *Citrus panuban,* 8.1 µg /100 mg in *Citrus natsudaidai,* 8.1 µg /100 g in *Citrus grandis, and* to 3.8 µg /100 g in *Citrus paradisi* (Kawaii, et al. 1999). Analytical reversed-phase HPLC of the methanolic extract of Tamarind pericarp revealed the presence of twelve major components, including taxifolin. The amounts of phenolic compounds in Tamarind pericarp and seeds were quantitated by analytical HPLC. Tamarind pericarp contained 2.82 g/kg total phenolics represented (%) by 7.4% taxifolin of total phenols (or 209 mg taxifolin/kg of pericarp) (Sudjaroen, et al. 2005). A recently conducted study indicates that taxifolin is one of the major dihydroflavonols in tangerine juice (Abad-García, et al. 2009).

Several cultivars of white grapes were found to be rich in their flavonoid contents. In particular, Albari˜no (75.34%) and Lado (74.59%) cultivars were found to be the richest cultivars in total flavonoids due to a high content of dihydroflavonols (38.22%) and flavonols (51.22%), respectively (Masa, et al. 2007). Taxifolin was also identified in blackcurrant seeds (Lu and Foo, 2003) and strawberries (Ishimaru, et al. 1995).

The analyses of Euterpe oleracea and Euterpe precatoria showed that the levels of taxifolin derivatives in these acai species are 7.89 ± 0.57 mg/kg and 9.20 ± 0.72 mg/kg, respectively (Pacheco-Palencia, et al. 2009); yet another derivative of taxifolin, taxifolin-3-rhamnoside, is found to be at 30.3±0.4 mg/100 g in Euterpe oleracea (Ribeiro, et al. 2009).

Skin samples of *Malus* × *domestica* (apple) were shown by the HPLC method to contain higher quantities of taxifolin enantiomers when compared with flesh, 740.01 and 130.02 mg/100 g, respectively. The most abundant taxifolin enantiomers in apple skin corresponded to the (2*R*3*S*)-(−) glycoside (41.12% of the total taxifolin) and the (2*S*3*R*)-(+) aglycone (16.99% of the total taxifolin) (Vega-Villa, et al. 2009). Accordingly, the consumption of one apple (including the apple skin) will result in the intake of 300-1000 mg of Taxifolin (depending on the size of the apple).

Taxifolin and taxifolin-hexoside were found in different cultivars of mulberry (Morus alba L.) frown in Chinese provinces. Specifically, Hongguo cultivar was shown to contain 21.42 ± 1.66 µg /g FW and 9.06 ± 1.30 µg /g FW of taxifolin-hexoside, while Da-10 cultivar was shown to contain 6.53 ± 0.29 µg /g FW of taxifolin (Zhang, et al. 2008.)

Taxifolin was found to be present in Spanish peanuts in the amount of 3.4×10^{-4} mol/kg peanuts (Pratt and Miller, 1984). The seeds (nuts) of *Pinus sibirica* Du Tour (Siberian pine) were found to contain taxifolin at 172±3.1 mg/100 g (Lantto, et al. 2009). Trace amounts of dihydroquercetin (taxifolin), dihydroquercetin-3-O-glucoside and dihydroquercetin-3-O-rutinoside are found in almond (*Prunus dulcis*) skins (seed coat) (Monagas, et al. 2007).

Taxifolin was described as one of the biologically active ingredients derived from the hydrophilic extracts of dried aerial parts of thyme (*Thymus vulgaris* L*)* (Dapkevicius, et al. 2002; Fecka and Turek, 2008). An analysis of 71 wild populations of Thymus from different putative hybrid swarm areas in Andalusia, Spain showed that taxifolin was the principal dihydroflavonol in all populations (Horwath, et al. 2008).

Taxifolin was identified at concentrations similar to thymol and rosmarinic acid in *Origanum dictamnus* extracts (Kouri, et al. 2007). The fresh leaves of a popular Japanese tea drink, Kolhi tea, *Engelhardtia chrysolepis*, contain 2.8-4.6% astilbin (a glycoside of taxifolin) along with taxifolin (Haraguchi, et al. 1996). Taxifolin was also present in small amounts within an extract of *Wendita calysina,* also known as Burrito tea (Piccinelli, et al. 2004).

Taxifolin, together with related compounds (2R,3R-Dihydroquercetin 3-*O*-*â*-D-glucoside; Dihydroquercetin 3-*O*-D-glucoside; 2*R*,3*R*-dihydroquercetin 3-*O*-R-L-rhamnoside; and Dihydroquercetin 3-*O*-D-xyloside) were identified a German Riesling wine, 1992 vintage (Baderschneider, et al. 2001).
In addition, dihydroquercetin 3-*O*-R-rhamnoside also known as astilbin was found in all wine types tested, and the levels varied between 1.19 and 15.13 mg/l for red wines, between 0.77 and 9.3 mg/l for dry white wines, and between 0.84 and 5.86 mg/l for botrytized sweet white wines. Average levels of astilbin are quite similar between rose´ and white dry wines, but the level of this compound in the Chardonnay wine enriched in phenolics by the special wine-making technique is greater than the average red wine value (Landrault, et al. 2002).

Taxifolin was also found in the amount of 1 mg/l in beer (Gerhäuser, 2005).

X.3 Conclusion

Taxifolin is a minor component in the human diet, found in various fruits and vegetables. In addition, taxifolin (including taxifolin isolated from the Dahurian larch wood) is sold as dietary antioxidant and dietary supplement and incorporated into many foods all over the world.
According to the regulations of the Russian Federation, taxifolin can be consumed as a dietary supplement when administered at levels from 25 mg to 100 mg per day. There are no health concerns associated with taxifolin, - instead, taxifolin is valuable for the human nutrition as antioxidant with anti-inflammatory action.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

• “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”

This question has been addressed in the following subsections.

XI.1 Nutritional Equivalence to Existing Foods

It is expected that the taxifolin extracted from Larch wood is nutritionally equivalent to naturally occurring taxifolin.

XI.2 Nutritional Benefits of the Food Ingredient

The following main beneficial effects can be observed:

Antioxidant effects

Anti-inflammatory and anti-allergic properties

Cardiovascular protection

In animal models, clear effects occurred at an oral dosage of 20-100 mg/kg body mass. The applied doses for the physiological studies were also safe and led to no adverse effects. (Safety studies are presented in the section XIII TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD)

The following tables give an overview of the *in vitro* and animal studies supporting a nutritional benefit of taxifolin. More details regarding these studies can be found in the report on physiological studies with taxifolin (Moré, 2010).

Table XI.2-1 Summary of in vitro Studies Demonstrating Physiological Effects

Test material (source)	Test system	Methods	Results Concerning Taxifolin	Reference
Antioxidant Effects				
Taxifolin etc.	Solution	- acidity constants - formation constants of copper (II) complexes - partition coefficients in a biomimetic system (micelles) - oxidation of 2'-deoxyguanosine	No prooxidant activity of taxifolin	Teixeira et al. 2005
Taxifolin etc.	Solution	- Radical scavenging activities - Rates of superoxide-dependent oxidation of flavonoids and flavonoid-metal-complexes	- scavenger potencies of flavonoid metal complexes were higher than those of the parent flavonoids - flavonoids bound to metal ions were less subjected to oxidation	Kostyuk et al. 2004
Taxifolin etc.	Solution	- NADPH- and CCl4-dependent microsomal lipid peroxidation - antiradical activity of flavonoids to O_2^-* - Competitive replacement	- inhibition of NADPH- and CCl4-dependent microsomal lipid peroxidation - rate constant with O_2^-* determined for taxifolin: more effective scavenger of oxygen anion-radicals than ascorbic acid - chelating agents capable of producing stable complexes with transition metal ions	Potapovich and Kostyuk, 2003
Taxifolin etc.	Cell culture	- asbestos-induced damage to macrophages	Some cytoprotective effect of taxifolin, not as strong as most other flavonoids	
Taxifolin etc.		- asbestos-induced damage to macrophages / influence of metal ions	- Metals increased the capacity of taxifolin to protect peritoneal macrophages against chrysotile asbestos-induced injury. - flavonoid metal complexes are more effective radical scavengers than uncomplexed flavonoids	Kostyuk et al. 2001
Taxifolin	Solution;	- superoxide anion production in	- Inhibition of superoxide anion production	Haraguchi, et

Test material (source)	Test system	Methods	Results Concerning Taxifolin	Reference
etc.	rat liver microsomes; erythrocytes from healthy humans	the xanthine/xanthine oxidase system - Microsomal lipid peroxidation induced by NADPH-cytochrome P-450 reductase - peroxy radical-damaged mitochondria - oxidative hemolysis of red cells	- Inhibition of microsomal lipid peroxidation - Inhibition of the lysis of human red cells subjected to peroxy radical attack	al. 1996
Taxifolin etc.	mouse liver and heart microsomes, solution	doxorubicin (enzymatically)-induced and Fe^{2+}/ascorbate (nonenzymatically)-induced microsomal lipid peroxidation and Fe^{2+} chelation	- Moderate inhibition of microsomal lipid peroxidation by taxifolin - Fe^{2+}-chelation - correlation to the ability to scavenge radicals and lipophilicity	van Acker, et al. 1996
Taxifolin etc.	isolated rat liver mitochondria; solution	- $Fe^{2+/}$citrate-mediated membrane lipid peroxidation in isolated rat liver mitochondria - antioxidant activity: Effects of on DPPH, and superoxide radicals scavenging and iron chelation	- Taxifolin caused weak to moderate inhibition of lipid peroxidation in isolated rat liver mitochondria - Taxifolin displayed antioxidant activity in non-organelle systems	Dorta, et al. 2008
Taxifolin etc.	Solution	- redox potentials	-taxifolin reduced the ascorbyl radical, resulting in an "ascorbate-protective" function	Bors, et al. 1995
Taxifolin etc.	CHL cells	5, 25 and 50 µM taxifolin tested; CHL cells induced with cytokines to simulate oxidative stress. Measurement of: - GSSG/GSH ratio - intracellular superoxide anion generation, SOD protein and RNA levels	- The absolute GSSG concentration and the GSSG/GSH ratio (oxidative stress marker) were reduced by taxifolin. - Taxifolin reduced the intracellular superoxide anion generation, SOD protein and RNA levels – Intracellular ROS/RNS and nitric oxide generation was slightly reduced compared to the control at low concentrations, but increased with	Crespo, et al. 2008

Test material (source)	Test system	Methods	Results Concerning Taxifolin	Reference
		- Intracellular ROS/RNS and nitric oxide	50 µM taxifolin.	
Taxifolin etc.	polymorphonuclear neutrophils from patients with non-insulin-dependent diabetes (NIDDM)	- PMA stimulated (oxidative stress) - induced with FeSO4/ascorbic acid (lipid peroxidation)	- Suppression of generation of anion radicals and hypochlorous acid - Reduced production of malonic dialdehyde - Decrease of activities of protein kinase C and myeloperoxidase - Inhibition of lipid peroxidation - Functional activity of PMN from NIDDM patients	Fedosova, et al. 2004
Taxifolin etc.	retinal ganglion cell line, RGC-5	- glutathione (GSH) depletion - t-butyl peroxide (t-BOOH) treatment - hydrogen peroxide (H_2O_2) treatment	- Half-maximal protection (EC_{50}) using taxifolin: 30 µM in the glutathione (GSH) depletion; 60 µM with the t-BOOH treatment. No EC_{50}-value in the H_2O_2 treatment. - The LD_{50} of taxifolin was >100 µM. - Taxifolin was identified as ROS-scavenger.	Maher and Hanneken, 2005
Taxifolin etc.	cultured embryonic (day8) check retinal cells	- ascorbate/Fe^{2+}-induced oxidative stress - 30–60 mM taxifolin - oxidative stress determined by the thiobarbituric acid method or change of 2',7'-dichlorodihydrofluorescein fluorescence	- Taxifolin is a moderate antioxidant - reduction of lipid peroxidation	Areias, et al. 2001
Taxifolin etc.	Rat C6 glial (astrocyte) cell lysate	-induced oxidative deamination by MAO - H_2O_2 radical trapping assay	Taxifolin inhibited C6 glial cell monoamine oxidase (MAO) enzyme activity and scavenges peroxide products	Mazzio, et al. 1998
Taxifolin	mouse EAC	- in vitro cell response to	Taxifolin virtually failed to increase the damage	Budagova, et

Test material (source)	Test system	Methods	Results Concerning Taxifolin	Reference
etc.	cells; human umbilical vein endothelial cells	hyperthermal and chemical stress	and death of the stress-exposed cells which displayed typical induction of HSP70.	al. 2003
Taxifolin etc.	Murine melanoma B16F10 cells	- Melanoma B16F10 cells activated by α-melanocyte stimulating hormone (α-MSH) - cell pigmentation induced by expression of exogenous human tyrosinase - cellular melanogenesis	- Taxifolin inhibited the cellular melanogenesis. - It attenuated cell pigmentation induced by expression of exogenous human tyrosinase.	An, et al. 2008
Taxifolin	In plant vacuoles	- examination of active and passive transport systems of the vacuolar membrane	Taxifolin acted in a membrane stabilizing manner	Nurminskij, et al. 2009
Taxifolin etc.	Solution, rat liver slices and mash	- effect on three radical-producing reactions	- 0.8 μM taxifolin reduced peroxidase activity of the complex of cytochrome c with dioleyl cardiolipin by 50%. - 10 μM taxifolin decreased lipid radical production by 50%. - 100 μM taxifolin had low inhibitory effect on lucigenin-dependent chemiluminescence in liver tissue	Vladimirov, et al. 2009
Anti-inflammatory and anti-allergic properties				
Taxifolin etc.	peritoneal rat mast cells	- induced histamine release from peritoneal rat mast cells	- Preincubation of cells with taxifolin decreased the potency of cromoglycate to further inhibit the ionophore-induced histamine release.	Bronner and Laundry, 1985
Taxifolin etc.	C6 glial cells (astrocytes)	- NO release of LPS-and IFN - gamma stimulated C6 glial cells	- Taxifolin substantially reduced the NO production of the stimulated glial cells	Soliman, et al. 1998
Taxifolin glycoside	dendritic cells isolated from	- Dendritic cells exposed to lipopolysaccharide, lipoteichoic	- Taxifolin glycoside inhibited inflammatory responses: increased production of IL-12 p70	Kim, et al. 2008

Test material (source)	Test system	Methods	Results Concerning Taxifolin	Reference
	mouse bone marrow and spleen	acid or interleukin (IL)-1beta to induce inflammatory responses	and tumour necrosis factor alpha, increased formation of reactive oxygen species (ROS) and nitric oxide (NO), and elevation of intracellular Ca2+ levels	
Cardiovascular effects (including more antioxidant effects)				
Taxifolin	HepG2 cells	- apolipoprotein B secretion and triglyceride availability under basal and lipid-rich conditions	- taxifolin reduced apolipoprotein B secretion by limiting triglyceride availability via inhibition of diacylglycerol acyltransferase and of microsomal triglyceride transfer protein	Casaschi, et al. 2004
Taxifolin etc.	low-density lipoprotein (LDL), from human blood serum	lipid peroxidation by H_2O_2	- taxifolin (and other flavonoids) inhibited lipid peroxidation by its own oxidation - oxidation of taxifolin was accelerated in the presence of nitrite and was catalyzed by myeloperoxidase	Kostyuk, et al. 2003
Taxifolin	HepG2 cells	- Lipid, apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I) synthesis and secretion - pulse-chase experiments	- Pretreatment of cells with (+/-)-taxifolin led to a weak inhibition of inhibition of cholesterol synthesis, and concomitant decrease and increase in apoB and apoA-I secretion.	Theriault, et al. 2000
Taxifolin etc.	Solution	- Inhibition of rabbit heart carbonyl reductase; 4-benzoylpyridine as substrate	- Taxifolin moderately inhibited the rabbit heart carbonyl reductase with an IC_{50}-value of 79.3 µM, thereby helping to prevent formation of superoxide anion radicals.	Imamura, et al. 2000
Taxifolin etc.	Human red blood cells	- Hemolysis of human red blood cells by AAPH or phospholipase C	- Pre-incubation of red blood cells with taxifolin reduced the AAPH-induced or phospholipase C-induced hemolysis.	Chen, et al. 2009
Antifungal properties				
Taxifolin	White-rot fungi and other fungi	- Growth with different amounts of taxifolin - Degradation analysis	- Growth reduction of white-rot fungi by taxifolin - Degradation of taxifolin by white-rot fungi - Degradation of taxifolin by other taxifolin-tolerant fungi	Cserjesi, 1968

Table XI.2-2 Summary of Animal Studies Demonstrating Physiological Effects

Test material (source)	**Species /test system**	**method**	**Duration of Dosing; Route of Admin.**	**Daily Dose (m**g/kg body mass)	**Results Concerning Taxifolin**	**Reference**
Antioxidant Effects						
Taxifolin	rat	CCl_4-induced toxicity	4 days pre-treatment, continuing treatment after CCl_4 challenge; oral	100 mg/kg	Lipid peroxidation in serum and liver, induced by CCl_4, was significantly reduced by taxifolin.	Teselkin, et al. 2000
Ascorbic acid plus Taxifolin	rat	focal injuries in the retina due to high-intensity light exposure	for 5 days, treatment started 2 days before photoexposure	20 mg/kg taxifolin, 50 mg/kg ascorbic acid	Ascorbic acid plus taxifolin led to: - disappearance of foci of injuries - reduction of retinal blood supply disorders - protection of neurosensory and glial cells	Logvinov, et al. 2005
Taxifolin	BALB/c mice	blood plasma and liver lipid peroxidation after a single 4 Gy dose of gamma – irradiation; thiobarbituric acid method or Fe^{2+}-induced chemilumines	First 40 days: Remaining 115 days:	100 mg/kg 5 mg/kg	- Taxifolin limits lipid peroxidation after irradiation - Fe^{2+}-induced chemiluminescence of liver homogenates (taxifolin treated animals) was 25-30% less compared with control animals	Teselkin, et al. 1998 and 1998a

Test material (source)	**Species /test system**	**method**	**Duration of Dosing; Route of Admin.**	**Daily Dose (m**g/kg body mass)	**Results Concerning Taxifolin**	**Reference**
		cence				
Taxifolin, etc.	male out bred albino rats	Peroxidation processes (content of water-soluble nonenzymatic antioxidants and products of lipid peroxidation); 1, 2 and 3 months after administratio n period	90 days	86 mg/kg 860 mg/kg 3000 mg/kg	- Taxifolin in a dose of 86 mg/kg was as potent as rutin in modulating the process of peroxidation - The changes were most pronounced 1 month after 90-day administration of the test compounds	Chernyak and Shchukina, 2009
Taxifolin etc.	nemato de *C. elegans*	examination of thermal stress resistance and lifespan	oral and dermal (nematode culture)	different concentratio ns	- Taxifolin (657 µM) conferred therotolerance in 2 of 3 trials. - Taxifolin (820 µM) increased median lifespan on average by 51% in all three trails and also increased median lifespan in two other trials at lower concentrations.	Benedetti, et al. 2008
Taxifolin etc.						
Anti-inflammatory and anti-allergic properties						
Taxifolin	rat	-Carrageenin-induced paw oedema - Cotton-pellet	Single dose i.p.	40 mg/kg	- Taxifolin reduced inflammation in all model systems, but not as strong as 10 mg/kg hydrocortisone - Taxifolin prevented increase in serum aminotransferase activity	Gupta, et al. 1971

Test material (source)	**Species /test system**	**method**	**Duration of Dosing; Route of Admin.**	**Daily Dose (m**g/kg body mass)	**Results Concerning Taxifolin**	**Reference**
		implantation -induced arthritis				
Cardiovascular effects (including more antioxidant effects)						
Taxifolin + ascorbic acid (50 mg/kg)	rat	model of the high blood viscosity syndrome, developed after myocardial infarction	6 days	20 mg/kg	- improvement of haemorheological indices - improved deformability of erythrocytes, some decrease in the content of plasma fibrinogen and erythrocyte aggregation	Plotnikov, et al. 2003
Taxifolin in 1.5% starch	rat	Induced inflammation in legs (formalin/histamine)	i.g.	100 mg/kg 300 mg/kg	Taxifolin in both concentrations significantly reduced the swelling	Kolhir, et al. 1996
Taxifolin	mouse	i.p. injection of trypan blue/ inflammation by topical xylol application	Injected, single dose	100 mg/kg	- Taxifolin increased the time of trypan blue's penetration into the inflamed area by 53%	
				300 mg/kg	- Taxifolin increased the time of trypan blue's penetration into the inflamed area by 63%	
Taxifolin	rat	Induced gastric ulcers		10 - 300 mg/kg	Taxifolin reduced the area of ulceration in all ulcer models.	
Taxifolin	rat	atherogenic high-	i.g., 52 days	250 mg/kg	30%-40% decrease of the serum concentrations of beta-lipoproteins and triglycerides compared	

Test material (source)	**Species /test system**	**method**	**Duration of Dosing; Route of Admin.**	**Daily Dose (m**g/kg body mass)	**Results Concerning Taxifolin**	**Reference**
		cholesterol diet			to control animals	
Taxifolin and ascorbic acid in 1% starch	rat	experimental cerebral ischemia	5 days, i.g.	20 mg/kg 50 mg/kg	Repeated administration of taxifolin and ascorbic acid significantly attenuated ischemic damage induced by circulatory disturbances	Plotnikov et al. 2000
Taxifolin	rat	middle cerebral arterial occlusion (MCAO), followed by reperfusion: model of cerebral infarction (causing increased oxidative damage and inflammation)	i.v.	0.1 µg/kg 1.0 µg/kg	After MCAO and taxifolin injection: - Reduction of infarction rate by 42% and 62% - Reduction in malondialdehyde and nitrotyrosine adduct formation - Inhibition of leukocyte infiltration, and COX-2 and iNOS expression - Reduced Mac-1 and ICAM-1 expression - NF-kappaB inhibition - Reduction of ROS and NO production by leukocytes and microglial cells	Wang, et al. 2006
Taxifolin; or Tetramethylated taxifolin	rat	paw oedema caused by carrageenan	i.p oral	Different dosages, not indicated	- anti-oedematogenic effect	Cechinel-Filho, et al. 2000
Taxifolin	albino	ocular	Topical	0.5 µg total	Taxifolin caused a significant increase of ocular	Park, et al.

Test material (source)	**Species /test system**	**method**	**Duration of Dosing; Route of Admin.**	**Daily Dose (m**g/kg body mass)	**Results Concerning Taxifolin**	**Reference**
etc.	rabbits	hypertensive model with reduced ocular blood flow - colored microsphere technique; ERG	instillation to left eye		blood flow and in retinal function recovery	2004
Analgesic properties						
Taxifolin; or Tetramethylated taxifolin	mouse	writhing test and formalin test	i.p oral	Different dosages, not indicated	- Dose-dependent antinociceptive action - Attenuating second phase of the formalin-induced licking	Cechinel-Filho, et al. 2000

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

• “Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?”

• “Is there information to show that the NF is unlikely to contain microorganisms and/or their metabolites of adverse public health significance?”

This question has been addressed collectively below.

XII.1 Microbiological Specifications and Analyses of the Food Ingredient

As outlined below, typical food borne microbes (e.g., moulds, yeasts, Salmonella, Escherichia coli) do not appear in the final product.
The final product is tested to confirm the absence to harmful microbial contamination according to the limits of Ph.Eur. 5.1.4 Cat 3B:

Table XII.1-1 Microbial Product Specifications

Specification Parameter	**Method of Analysis**	**Specification**
Total Plate Count, TPC	USP 32/NF27, FDA/BAM Methods-file name in appendix B: usp-microbio.pdf	NMT 10^4 CFU/g
Enterobaceria + div. gram-neg. bacteria*		≤ 100/g
Yeast and Mold		NMT 100 CFU/g
Escherichia coli		Negative/1 g
Salmonella spp.		Negative/10 g
Staphylococcus aureus		Negative/1 g
Pseudomonas spp.		Negative/1 g

* Enterobaceria + div. gram-neg. bacteria are only tested in case that the TPC exceeds 100 CFU/g.
See Appendix B for analytical methods and Appendix C for certificates of analysis.

Microbiological contaminants in taxifolin are within the regulated safety limits (Please also refer to Appendix C: ametis2009-coa-5batches and batch-analysis-abc-microbio):

Table XII.1-2 Batch Analysis of Taxifolin Concerning Microbial Specifications

Specification Parameter	**Specification**	Batch 2	Batch 27a	Batch 29b	Batch 66a	Batch 950
Total Plate Count, TPC* (CFU/g)	< 10^4	< 10	< 10	< 10	< 10	< 10
Yeast and	< 100	< 10	< 10	< 10	< 10	< 10

Mold* (CFU/g)						
Escherichia coli*	Negative/1 g	negative	negative	negative	negative	negative
Salmonella spp.*	Negative/10 g	negative	negative	negative	negative	negative
Staphylococcus aureus*	Negative/1 g	negative	negative	negative	negative	negative
Pseudomonas spp.*	Negative/1 g	negative	negative	negative	negative	negative

* USP and FDA-BAM Methods

XIII TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

• “Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

• “Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”

• “Is there information which suggests that the novel food might pose an allergenic risk to humans?”

These questions have been addressed collectively in the following subsections.

XIII.1 Toxicological Evaluation of *Taxifolin/ Dihydroquercetin*

The following tables give an overview of the performed toxicological studies.
The first table contains pivotal studies, performed with taxifolin from larch wood.
The taxifolin used for these studies was manufactured using the same or a very similar procedure as for the intended product. Therefore if can be assumed that the purity of taxifolin was high and there only were only low levels of contaminant substances, similar to the intended product.
The second table contains studies performed with other sources of taxifolin.
Altogether the toxicological studies indicate that taxifolin (also taxifolin from larch wood) is extremely well tolreated, and can be regarded as safe. In all cases, the maximum oral dose (vaying among the studies, but up to 15 000 mg/kg body mass per day) did not cause any toxic effects or organ damage. For a 70 kg average human, such a dose would be equivalent to the daily intake of more than 1 kg of taxifolin per day!

In a 6-month rat chronic toxicity study the NOEL was also above >1500 mg/kg body mass, the highest dose administered chronically. Accordingly, the ADI (acceptable daily intake) for humans was calculated to be at least 15 mg/kg body mass (factor of 100).

Table XIII.1-1 Summary of Pivotal Animal Toxicity Studies – Performed with Taxifolin from Larch Wood

Test material (source)	**Species**	**No. of Animals per Group**	**Duration of Dosing; Route of Admin.**	**Daily Dose (**g/kg body mass)	**Results/Comments**	**NOAEL (mg/kg bw/day)**	**Reference**
Acute Toxicology							
Taxifolin in 1% starch solution (from larch wood)	adult albino rats (160-175 g) and adult albino mice (18-20 g)	60 80	Single dose, i.g.	4 dosing groups, Doses up to 12000 mg/kg	No deaths. shortness of breath, languor, and cyanosis of skin integuments of auricles and limbs, unstable equilibrium in some mice at highest dose. These symptoms disappeared within 1 hour	Not indicated	Shkarenkov, et al. 1998
5% Taxifolin alcoholic solution (from larch wood)	adult albino rats (160-175 g) and adult albino mice (18-20 g)	60 80	Single dose, i.p.	4 dosing groups	LD_{50} 560-600 mg/kg LD_{50} 630-680 mg/kg	Not indicated	Shkarenkov, et al. 1998
Subchronic Toxicology							
Taxifolin (from larch wood)	adult rats (150-200 g)	20	7 days oral	10 g/kg (1.5-2 g /rat)	No lethal cases. No effects of behavior or physiological parameters, no allergic reactions, no pathological changes to organ histology	>10 g/kg	Dorovskikh and Celuyko, 2008
Taxifolin (from larch wood)	adult rats (150-200 g)	20	7 days oral	15 g/kg (2.25-3 g /rat)	No lethal cases. No effects of behavior or physiological parameters, no pathological changes to organ histology	>15 g/kg	Dorovskikh and Celuyko, 2008
Taxifolin (from larch wood)	adult rats (150-200 g)	20	20 days oral	0.05 g/kg (0.05-0.075 g /rat)	No lethal cases. No effects of behavior or physiological parameters, no allergic reactions, no pathological changes to organ histology. Accumulation in the	(> 0.05 g/kg)	Dorovskikh and Celuyko, 2008

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
					blood plasma below the accumulation factor 3.		
Chronic Toxicology							
1% starch (control) or taxifolin (2 concs.) (from larch wood)	48 white male rats (150-165 g)	16	6 months, oral	0 150 mg/kg 1500 mg/kg	No intoxication symptoms. No effects of behavior or physiological parameters. Lack of visible changes in the central nervous system. Normal hematopoiesis. Slight changes in leukocyte and thrombocyte levels within the limits of the physiological norm. No differences in the body weight dynamics compared to control group	>1500 mg/kg	Shkarenkov, et al. 1998
taxifolin tablets (from larch wood)	healthy mongrel dogs (10-16 kg)		6 months, oral	190 mg/kg with food or control	No intoxication symptoms. No effects of behavior or physiological parameters. Normal hematopoiesis. Normal elimination of bromsulphalein, leukopoiesis and thrombocytopoiesis. Peripheral blood indices within the physiological norm limits Levels of glucose, urea, triglyceride, total protein, and total cholesterol and the activities of alanine- and aspartate-transaminases, alkaline phosphatase, and lactate dehydrogenase did not show significant differences compared to the control group.	> 190 mg/kg	Shkarenkov, et al. 1998
Developmental Toxicology							
Taxifolin	Pregnant rats	20	90 days	0.5 g/kg	No changes in general condition. No	> 0.5	Dorovskikh

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
(from larch wood)				(0.005-0.0075 g)	toxicosis or pathological reactions. No lethal cases or deviations from the body weight. All newborns showed normal development. No pathological changes in organ histology.	g/kg	and Celuyko, 2008
Taxifolin (from larch wood)	Rats (190-210 g)	75	Injection, 1^{st} to 19^{th} day of pregnancy	75 mg/kg; 1500 mg/kg	No increase in embryonic death, no changes in body mass or fetus size. Normal organ abnormalities in fetuses. No effect on the number of newborn. Normal increase in offspring body weight during the first 4 weeks. Normal sensorimotor reflexes, emotional-motor reactions, and coordination of the offspring. Hemapathological indices within the normal ranges. No effect on fecundity, or the antenatal and postnatal development of the rats' offspring	> 1500 mg/kg	Shkarenkov, et al. 1998
Taxifolin (from larch wood)	Male and female rats	150	Injection, 60 days (male) 15 days (female)	75 mg/kg		> 75 mg/kg	Shkarenkov, et al. 1998
Mutagenicity and Genotoxicity							
Taxifolin (from larch wood)	mouse bone marrow cells from (CBA x $C_{57}BL)F_1$ males/ females (19-21 g)	Not indicated	single dose, i.g.	1500 mg/kg	Chromosomal aberration assay: lack of mutagenic properties	>1500 mg/kg	Shkarenkov, et al. 1998
Taxifolin (Flavit Company, from larch	8-12-week-old male/female C57Bl/6 mice (18-20 g)	10-12 animals	5 days, oral	1.5 and 150 mg/kg	In vivo chromosome aberration/ DNQ-comet assay: no DNA damage in mouse bone marrow, blood ,liver, or rectal cells	>2000 mg/kg	Zhanataev, et al. 2008
			Single dose, oral	15, 150, and 2000 mg/kg			

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
wood) or control							

i.p.=intraperitoneal administration; i.g.=intragastric administration

Table XIII.1-2 Summary Other Animal Toxicity Studies – Performed with Taxifolin from Other Sources

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
Acute Toxicology							
Taxifolin (from *Madhuca butyracea*)	adult albino rats (60-65 g)	2 per dosing group	Single dose, i.p.	Different doses, not indicated in paper	LD_{50} 1200 mg/kg	Not indicated	Gupta, et al. 1971
Chronic Toxicology							
Taxifolin (source not indicated)	Weanling albino rats	5	226 days	0% 0.125% 0.25% 0.5% 1% taxifolin in basal diet	No toxic symptoms in any of the treatment animals. Normal body mass gain in all groups. No abnormalities.	>1% in the diet	Booth and De Eds, 1957
Taxifolin (source not indicated)	Weanling albino rats	5	249 days	0% 0.125% 0.25% 0.5% 1% taxifolin in basal diet	No toxic symptoms in any of the treatment animals. Normal body mass gain in all groups. No abnormalities.	>1% in the diet	
Developmental Toxicology							

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
Taxifolin (Sigma, St. Louis-MO, further source not stated) etc.	Female CD-1 mice, bred to males of the same strain; BG1Luc4E2 cell line	Not stated	Injection, 3 days	500 mg/kg	No increase uterine weight in the immature mouse's uterotrophic assay, very low measure of estrogenicity (slight uterine epithelial cell height increase at low but not high concentrations, slight uterine gland number increase); No transcriptional activation of estrogen receptor in cell culture.	> 500 mg/kg	Jefferson, et al. 2002
Taxifolin (Sigma)	rat uterine cytosol	rat uterine cytosol from 10-12 rats	*in vitro* Titration with different amounts	Not applicable	taxifolin does not bind to the rat uterine cytosolic estrogen receptor	Not applicable	Branham, et al. 2002
Mutagenicity and Genotoxicity							
Taxifolin (isolated from *Astilbe thunbergii*)	*Salmonella typhimurium* TA100 and TA98	Mutation assay with bacteria – Ames test			Taxifolin produced less than 1 revertant/nmol in TA100. Taxifolin was direct-acting, addition of S9 mix diminished mutagenicity even further. Taxifolin was non-mutagenic in TA98.		Nagao, et al. 1981
Taxifolin (Sigma)	*Salmonella typhimurium*	Mutation assay with bacteria – Ames test			Taxifolin was shown to be non-mutagenic	Not applicable	Jurado, et al. 1991
Taxifolin (Sigma, St. Louis-MO)	*Salmonella* TA102 tester strain	Mutation assay in the presence of rat liver S9 mix (Ames test): inhibition of benzidin induced mutations			Taxifolin was non-mutagenic in the Ames test. What is more, it could strongly reduce mutagenicity caused by benzidin. Taxifolin also inhibited benzidin- or Fe/ascorbate mediated lipid peroxidation.	Not applicable	Makena and Chung, 2007

Test material (source)	Species	No. of Animals per Group	Duration of Dosing; Route of Admin.	Daily Dose (g/kg body mass)	Results/Comments	NOAEL (mg/kg bw/day)	Reference
Taxifolin (Sigma, St. Louis-MO)	*Salmonella typhimurium* TA102 /*E. coli* WP-2 uvrA tester strains	Mutation assay in the presence or absence of rat liver S9 mix (Ames test)			Taxifolin was not mutagenic in the presence or absence of S9 mix in both TA102 and WP-2 uvrA 2, regardless of the presence of iron or an NADPH generating system.	Not applicable	Makena, et al. 2009
Taxifolin Extrasynthese S. A. (Genay, France)	human lung embryonic fibroblasts (TIG-1); human umbilical vein endothelial (HUVE) cells	*In vitro*	In vitro, 24 h incubation	Doses up to 300 µM	Taxifolin was only weakly toxic at very high concentrations: 80% of the human lung embryonic fibroblasts survived at concentrations of 300 µM taxifolin. 75% of the human umbilical vein endothelial cells survived at concentrations of 200 µM taxifolin. The intracellular level of reactive oxygen species in human lung embryonic fibroblasts was very low at all taxifolin concentrations.	Not applicable	Matsuo, et al. 2005
Taxifolin (Sigma-Aldrich)	Rat hepatocytes	*In vitro*	tryptan blue exclusion method	Different concentrations, not indicated	Taxifolin displayed an LD50 (2 h) of 6,000±249 µM. The log LD50 for hepatocytes was 3.78 µM. In HeLa tumor cells (after 3 days incubation) the log LD50 was 2.21 µM.	Not applicable	Moridani, et al. 2002

i.p.=intraperitoneal administration; i.g.=intragastric administration

XIII.1.1 Acute Studies

Studies with taxifolin from larch wood

Taxifolin toxicity after a single intragastric and a single intraperitoneal administration was studied on 60 white rats of both sexes with a body weight of 160-175 g and on 80 white mice of both sexes with a body weight of 18-20 g. Taxifolin was mixed with 1% starch solution for intragastric administration, and 5% taxifolin alcoholic solution was prepared for intraperitoneal injections. The Litchfield and Wilcoxon probit method was used to determine acute toxicity parameters.

The LD_{50} of taxifolin (a single intraperitoneal administration to mice and rats of both sexes) was within the limits of 560-680 mg/kg (Table XIII.1.1-1). The data obtained during the experiment supports the absence of sexual differences of the animals` taxifolin sensitivity.

Table XIII.1.1-1 Taxifolin Toxicity Indices (mg/kg) at Single Intraperitoneal Injection (Shkarenkov, et al. 1998)

Animals	LD_{10}	LD_{16}	LD_{50} (± SD)	LD_{84}
Rats, males	416	433	566 ± 15	725
Rats, females	430	465	600 ± 18	800
Mice, males	466	540	680 ± 65	850
Mice, females	472	536	635 ± 50	828

No animal death was observed at a single administration of taxifolin into the stomach of mice and rats even in the maximal (volumetric and concentration) amounts that were able to administer (≈12000 mg/kg). Few mice and rats demonstrated shortness of breath, languor, and cyanosis of skin integuments of auricles and limbs. Unstable equilibrium was observed in some mice. These symptoms disappeared within 1 hour. Thus, the results of the acute toxicity experiments suggest that taxifolin is a practically nontoxic product (Shkarenkov, et al. 1998).

Studies with taxifolin from other sources

Intraperitoneal administration of different doses of taxifolin to adult albino rats weighing between 60 and 65 gram (2 animals per dosing group) showed an LD_{50} (dose lethal to 50% of test animals) of 1200 mg/kg (Gupta, et al. 1971).

XIII.1.2 Subchronic Studies

Studies with taxifolin from larch wood

Taxifolin from larch wood was administered per orally to 20 adult rats of both genders, body weight 150-200 g, at 10 g per 1000 g body weight a day (1.5-2.0 g/day) for seven days. There were no changes in general condition of the animals during this period. The animal remained active, without changes in the sleep pattern, activity or appetite. There were no changes in rectal temperature or diuresis during the period. The observation showed no allergic reactions. There were no lethal cases. No pathological changes in the heart, lungs, liver, spleen, kidneys, stomach, small and large intestine, and cortex and spinal cord were observed during the histological examination (Dorovskikh and Celuyko, 2008).

During the next stage of the experiment, taxifolin was administered at 15 g/1000 g body weight a day (2.25-3.0 g/day) to 20 adult rats of both genders with body weight of 150-200 g for 7 days. During this stage of the experiment, there were no lethal cases among the animals. No pathological changes in the heart, lungs, liver, spleen, kidneys, stomach, small and large intestine, and cortex and spinal cord were observed during the histological examination (Dorovskikh and Celuyko, 2008).

Taxifolin from larch wood was administered per orally at 0.05 g/1000 g body weight a day (0.005-0.0075 grams a day) to 20 adult rats of both genders with body mass 150-200 g for 20 days. There were no changes in general condition of the animals during this period. The animal remained active, without changes in the sleep pattern, activity or appetite. There were no changes in rectal temperature or diuresis during the period. The observation showed no allergic reactions. There were no lethal cases. During the experiment, the blood analysis was conducted to determine the coefficient of accumulation of taxifolin. This coefficient was found to be less than 3.

No pathological changes in the heart, lungs, liver, spleen, kidneys, stomach, small and large intestine, and cortex and spinal cord were observed during the histological examination (Dorovskikh and Celuyko, 2008).

XIII.1.3 Chronic Studies

Studies with taxifolin from larch wood

The chronic 6-months experiment on rats has demonstrated that taxifolin caused no intoxication symptoms in laboratory animals. The systemic condition of the rats did not change at visual examination. The animals remained active and not aggressive. There were no changes in the smooth fur, muscular tone, or appetite of the experimental animals. No differences in the body weight dynamics were observed in the experimental animals as compared to the control animals during the six months.

There were slight changes in the peripheral blood indices in the both groups supplemented with taxifolin. Some fluctuations in the leukocyte and thrombocyte levels were noted in various observation intervals. Thus, one month after the beginning of the experiment, the leukocyte counts in the first, second, and third groups of rats were: 9.4±0.6 ($x10^9$/l), 7.5±0.5 ($x10^9$/l) ($p < 0.05$), and 7.9±0.6 ($x10^9$/l), respectively. After four months, these values were 7.7±0.4 ($x10^9$/l), 9.2±0.3 ($x10^9$/l) ($p < 0.05$), and 8.8±0.5 ($x10^9$/l), respectively. The thrombocyte counts at the same research intervals were ($x10^9$/l): 625±32, 718±22 ($p < 0.05$), and 702±36, for the respective groups (the first month), and 519+40, 604±33, and 682± 39 ($p < 0.05$) (the fourth month). The changes were within the limits of the physiological norm. The myelogram indices (the 6^{th} month) indicated the normal hematopoiesis in the laboratory animals, despite the long-term administration of taxifolin.

The analyses of the biochemical blood serum indices and of the functional state of the liver, kidneys, and cardiovascular system have shown no toxicity of taxifolin. The results of tests on the orientation responses and the summation-subliminal index suggest a lack of visible changes in the central nervous system of the experimental animals (Shkarenkov, et al. 1998).

The safety of taxifolin preparation (tablets containing 0.02g of taxifolin) was studied in a 6-month experiment on healthy mongrel dogs with a body weight of 10-16 kg. The

peripheral blood indices (the total count of leukocytes, erythrocytes, reticulocytes, thrombocytes, the hemoglobin content, the hematocrit, and hemograms) were within the physiological norm limits for the given species of animals at the beginning of the experiment. The animals were divided into 2 groups. Group 1 received taxifolin at 190 mg/kg with their food (a 5-fold therapeutic dose with the consideration of the species resistance coefficient), while group 2 was used as the control. The hematologic analysis was conducted by the universally accepted methods at the 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th}, and 24^{th} weeks of the experiment using an automatic cell counter "Picoscale", a photoelectric colorimeter PEC-56, a microscope "Ampleval", and diamond cresyl dark blue and azure-II-eosin dyes. In addition, the levels of total protein, albumins, globulins, total cholesterol, triglycerides, urea, glucose, and the activity of some enzymes, such as alanine and aspartate, transaminases, alkaline phosphatase, and lactate dehydrogenase, were determined by a biochemical analyzer "Baeckman" (USA). The bromsulphalein test was carried out at the end of the test. The functioning of the excretory system was evaluated by a water test. The electrocardiograms were recorded on a surgical polarograph “Salute” at the II standard. The functional activity of the central nervous system was tested by the Boissier method (orientation responses) and with the summation-subliminal index. The animals were killed at the end of the experiment; their bone marrow was used to determine the total count of myelocarcites and myelograms, and the pathomorphologic research of their viscera was carried out. The obtained data were processed statistically by the Student`s Criterion t.

No indications of taxifolin’s toxic effects were shown in the experiment on dogs. The experimental and control animals had their usual appearances, and they adequately reacted on environmental irritants for the observation period. The analysis of hematologic indices revealed no significant changes in erythropoiesis, leukopoiesis or thrombocytopoiesis. The dynamics of blood serum biochemical indices of the experimental and control dogs had no indication of toxicity of the preparation. The levels of glucose, urea, triglyceride, total protein, and total cholesterol and the activities of alanine- and aspartate-transaminases, alkaline phosphatase, and lactate dehydrogenase did not show significant differences between the experimental and the control groups of dogs. The results of the bromsulphalein test, which was conducted at the end of the experiment, indicated that the preparation does not

inhibit elimination of bromsulphalein. The bromsulphalein retention coefficient [factor] was equal to 37.2±2.5 and 34.8±4.6 ($p > 0.05$) for the test and control animals, respectively (Shkarenkov, et al. 1998).

Studies with taxifolin from other sources

For 226 or 249 days, weanling albino rats were fed with taxifolin, included in their diet at concentrations up to 1%. No toxic signs or abnormalities were observed (Booth and De Eds, 1957).

Chronic taxifolin toxicity was studied in a 6-month experiment on healthy 48 white male rats with their initial weights of 150-165 g. The peripheral blood indices (the total count of leukocytes, erythrocytes, reticulocytes, thrombocytes, the hemoglobin content, the hematocrit, hemograms) were within the physiological norm limits for the given species of animals at the beginning of the experiment. The animals were divided into 3 groups of 16 rats in each: the control group (group 1) was administered a 1% starch solution, the tested groups (group 2 and group 3) were intragastrically administered taxifolin at 150 and 1500 mg/kg, respectively. The maximum administered dose was 100 times greater than the one recommended for human consumption. The hematology analysis was carried out by the universally accepted methods at the 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th}, and 24^{th} weeks of the experiment using an automatic cell counter "Picoscale", a photoelectric colorimeter PEC-56, a microscope "Ampleval", and dyes — diamond cresyl dark blue, azure-II-eosin. In addition, the levels of total [crude] protein, albumins, globulins, total cholesterol, triglycerides, urea, glucose, and the activity of such enzymes as alanine and aspartate, transaminases, alkaline phosphatase, and lactate dehydrogenase were determined by a biochemical analyzer "Baeckman" (USA) on the animals` blood during the same intervals. The bromsulphalein test was carried out at the end of the experiment. The functioning of the excretory system was evaluated by a water test. The electrocardiograms were recorded on a surgical polarograph “Salute” at the II standard leads. The functional activity of the central nervous system was tested by the Boissier method (orientation responses) and with the summation-subliminal index. The animals were killed at the end of the experiment; their bone marrow was used to determine the total count of myelocariocytes and myelograms, and the

pathomorphologic research of their viscera was carried out. The obtained data were processed statistically by the Student`s Criterion t.

XIII.1.4 Developmental Studies

Studies with taxifolin from larch wood

Taxifolin from larch woodwas administered at 0.5 g/1000 g body weight (0.005-0.0075 g/day) for 90 days to 20 pregnant rats with body weight of 150-200 g. There were no changes in general condition of the animals during this period. The animal remained active, without changes in the sleep pattern, activity or appetite. There were no changes in rectal temperature or diuresis during the period. There was no toxicosis or pathological reactions in the experimental animals. The experimental animals gave birth on time, without any pathological reactions. No lethal cases or deviations from the body weight were observed among the newborns. All newborns had no deviations in the developmental and growth stages. No pathological changes in the heart, lungs, liver, spleen, kidneys, stomach, small and large intestine, and cortex and spinal cord were observed during the histological examination (Amur State Medical Academy 2008).

The embryotoxicity and teratogenicity of taxifolin were studied on 75 rats with the body weight of 190-210 g. The animals were injected with taxifolin at 75 and 1500 mg/kg from the 1st to the 19th day their pregnancies. Additionally, the effect of taxifolin influence on the reproductive function of rats was studied. Mature male and female rats (150 animals) were administered taxifolin at 75 mg/kg for 60 days (male rats) and for 15 days (female rats).

Taxifolin did not produce in an increase in embryonic death or changes in body mass or size of the fetus. The administration of taxifolin did not increase the rate of development of hydronephrosis, of intra-abdominal hemorrhage or hemorrhage in the thoracic cavity of the fetuses, or of skeleton bone anomalies. Taxifolin had no effect on the number of newborn rats or on the increase in their body weight during the first 4 weeks of their lives. The examination of sensorimotor reflexes, emotional-motor reactions, and coordination of the offspring of rats that were administered taxifolin during pregnancy revealed no differences with the control animals. Certain hemapathological indices of the newborn rats, such as erythrocyte count, leukocyte

count, thrombocyte count, and hemoglobin contents, remained within the normal ranges. The weight coefficients of the internal organs of rats killed at the age of 4 weeks remained normal. The results of experiments on the reproductive function of the rats showed that the administration of taxifolin at 75 mg/kg had no effect on fecundity of the laboratory rats, or the antenatal and postnatal development of the rats' offspring (Shkarenkov, et al. 1998).

Studies with taxifolin from other sources

In cell culture, a transcriptional activation assay showed no effect of taxifolin on the estrogen receptor. Taxifolin administered at a dose of 500 mg/kg failed to increase uterine weight in the immature mouse's uterotrophic assay. A very low measure of estrogenicity could be observed in sensitive morphological and biochemical assays (slight uterine epithelial cell height increase at low but not high concentrations, slight uterine gland number increase at 500 mg/kg) There was no significant effect the induction of the estrogen-responsive protein lactoferrin. (Jefferson, et al. 2002). Another study with rat uterine cytosol showed that taxifolin does not bind to the rat uterine cytosolic estrogen receptor (Branham, et al. 2002).

XIII.1.5 Mutagenicity and Genotoxicity Studies

Studies with taxifolin from larch wood

The mutagen properties of taxifolin from larch wood were evaluated by the analysis of chromosomal aberrations of mice` bone marrow cells and by the registration test of dominant lethal mutations induced by taxifolin in mouse germinal cells. Mouse-hybrids (CBA x C_{57}BL)F_1 of both sexes with a body weight of 19-21 g were used in the experiment. A single intragastric administration of taxifolin at 1500 mg/kg had no impact on the mitotic index and chromosomal aberrations of bone marrow cells of white out bred mice. The test results on the dominant lethal mutations in animals support the lack of mutagenic properties of taxifolin (Shkarenkov, et al. 1998).

Genotoxic properties of taxifolin were studied *in vivo* by the method of chromosome aberration counting and DNQ-comet assay. Taxifolin was orally administered to mice five times at 0.15 and 1.5 mg/kg or once at 15, 150, and 2000 mg/kg. Spontaneous level of DNA damage in bone marrow cells of control animals was 4.5±1.1%. In animals repeatedly treated with taxifolin in doses of 0.15 and 1.5 ng/kg,

this parameter was 4.9±1.1 and 3.6±0.5%, respectively, i.e. did not significantly differ from the control. Similarly, taxifolin induced no DNA damage in blood, liver, or rectal cells.
In experiments with single administration of taxifolin, the spontaneous level of DNA damage in bone marrow cells was 5.7±1.2%. After treatment with taxifolin in doses of 15, 150 and 2000 mg/kg it corresponded to 5.5±1.0, 6.1±1.0 and 7.3±2.3%, respectively. No significant differences between these values were revealed. Similarly, taxifolin induced no DNA damage in blood, liver, or rectal cells.
The authors conclude that taxifolin is not genotoxic (Zhanataev, et al. 2008).

Studies with taxifolin from other sources

The mutagenicity of taxifolin and other flavonoids was investigated using the standard Ames Test in *S. typhimurium* (strains TA100 and TA98). Taxifolin produced less than 1 revertant/nmol in TA100. Taxifolin was direct-acting, the addition of S9 mix diminished the already extremely low mutagenicity even further. Taxifolin was non-mutagenic in TA98 (Nagao, et al. 1981).

The mutagenicity of taxifolin and other flavonoids was investigated using a bacterial assay, the L-arabinose-resistance test of *S. typhimurium*. Taxifolin was found to be the weakest mutagen of all tested compounds in the L-arabinose-resistance test, in the presence or absence of mammalian metabolic activation (S9 mixture). Therefore the non-mutagenicity of taxifolin can be confirmed (Jurado, et al. 1991).

A study tested the impact of taxifolin on the benzidin-induced mutations in Salmonella TA102 tester strain in the Ames Salmonella in the presence of rat liver S9 mix. Taxifolin was non-mutagenic in the Ames test (with or without the S9 mix). At 50 µg per plate, the numbers of revertants with S9 and without S9 were 373±43 and 325±8, respectively, which is similar to the negative control. What is more, taxifolin could strongly and dose-dependently reduce mutagenicity caused by benzidin. In the presence of benzidin the number of revertants were 1049 ± 12, but only 529±91 if 50 µg/plate taxifolin was added, 451±39, if 100 µg/plate taxifolin was added, and 288±48, if 200 µg/plate taxifolin was added.
Taxifolin also inhibited benzidin- or Fe/ascorbate mediated lipid peroxidation in a time-dependent manner: Within 3 hours after incubation, there was 55.9% inhibition

of benzidin-mediated lipid peroxidation by taxifolin, while 6 hours after incubation, the percentage of inhibition was 76.5%. The Fe/ascorbate-mediated lipid peroxidation was inhibited by 70.7% after 3 hours and by 83.2% 6 hours after incubation with taxifolin (Makena and Chung, 2007).

The mutagenic effects of quercetin and taxifolin were evaluated using Salmonella typhimurium TA102 and Escherichia coli WP-2 uvrA tester strains. Taxifolin was not mutagenic in the presence or absence of S9 mix in both TA102 and WP-2 uvrA 2, regardless of the presence of iron or NGS (Makena, et al. 2009).

XIII.1.6 Cytotoxicity

Studies with taxifolin from other sources

Incubation with taxifolin in a culture medium for 24 hours was used to examine potential toxicity to human lung embryonic fibroblasts (TIG-1) and human umbilical vein endothelial (HUVE) cells. Taxifolin was only weakly toxic at very high concentrations: 80% of the human lung embryonic fibroblasts survived at concentrations of 300 µM taxifolin. 75% of the human umbilical vein endothelial cells survived at concentrations of 200 µM taxifolin.
The intracellular level of reactive oxygen species in human lung embryonic fibroblasts was very low at all taxifolin concentrations (Matsuo, et al. 2005).

In another study the toxicity of flavonoids was evaluated in isolated rat hepatocytes and HeLa tumor cells. The concentration, at which a compound caused 50% cell death (LD_{50}) in the isolated rat hepatocytes after incubation for 2 h was determined by tryptan blue exclusion method. Taxifolin displayed an LD50 (2 h) of 6,000±249 µM. The log LD50 for hepatocytes was 3.78 µM. In HeLa tumor cells (after 3 days incubation) the log LD50 was 2.21 µM. These values demonstrate the relatively low toxicity of taxifolin (Moridani, et al. 2002).

XIII.2 Absorption, Distribution, Metabolism, and Excretion (ADME)

Abbreviations

C_{max}	- maximal plasma concentration
t_{max}	- time of maximal plasma concentration
$t_{1/2\ el}$	- time of 50% elimination
AUC	- area under curve
$AUC_{0\rightarrow\infty}$	- total exposure to test substance
$AUC_{0\rightarrow6}$	- exposure to test substance within first 6 h
MRT	- Mean retention time

XIII.2.1 Absorption

The following table gives an overview of different absorption studies performed with dihydroquercetin (taxifolin).

Table XIII.2-1 Summary of Absorption Studies with Dihydroquercetin

Species	Route of admin	Single dose of dihydro quercetin (mg/kg)	C_{max} (µg/ml)	t_{max} (h)	$t_{1/2\ el}$ (h)	$AUC_{0\rightarrow\infty}$ (h*µg/ml)	$AUC_{0\rightarrow t}$ (h*µg/ml)	MRT	$C_{max}/AUC_{0\rightarrow\infty}$ (h^{-1})	Absolute bio-availability	Reference
Rabbit	oral	8	0.1081	0.50	2.22	1.16	0.361 (t=6h)	n.i.	0.0931	36%	Pozharitskaya, et al. 2009
	oral	80	0.5567	0.25	1.69	2.18	0.888 (t=6h)	n.i.	0.255		
	i.v.	8	-	-	0.56	3.21	1.004 (t=6h)	-	-		
Rat	oral	10	0.0911	6.0	16.6	1.575	1.445 (t=5h)	16.3	0.0578	"0.17%" (probably 17% ??)	Wang, et al. 2009
	oral	50	1.4388	9.5	48.0	29.257	28.19 (t=5h)	21.4	0.049		
	oral	100	4.3524	10.2	58.7	93.869	93.55 (t=5h)	27.6	0.0464		
	i.v.	10	45.2119	-	25.5	1037.486	888.5 (t=5h)	-	-		
	i.v.	1	n.i.	n.i.	n.i.	0.08	n.i.	0.04	n.i.	n.i.	Voskoboinikova, et al. 1993
	i.v.	3	n.i.	n.i.	n.i.	2.0	n.i.	0.31	n.i.	n.i.	
	i.v.	10	n.i.	n.i.	n.i.	5.7	n.i.	0.21	n.i.	n.i.	
	i.v.	30	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	
	oral	50	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	
	oral	100	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	
Rat	oral	12.5	2.046	0.5	1.25	6.17	5.95 (t=6h)	2.56	0.399	23.79%	Seredinin, et al. 2007
	oral	25	4.290	0.5	1.20	10.66	10.33 (t=6h)	2.55	0.402		
	oral	50	7.320	0.5	1.24	17.02	16.54 (t=6h)	2.57	0.430		
	i.v.	50	1137.0	-	1.15	71.53	-	-	-		
Human	oral	4.305 mg* /human	0.03334	8.2	8.89	0.39914	0.23111 (t=14h)	n.i.	0.0835	n.i.	Grimm, et al. 2006

n.i.= not indicated

* contained within 300 mg of pine bark extract

Animal Studies on Absorption

The absolute bioavailability of taxifolin after oral administration of a lipid solution to rabbits was found to be 36%, as was analyzed by HPLC with UV detection. The researchers analyzed the blood samples after enzymatic hydrolysis with beta-glucuronidase and sulfatase. General pharmacokinetic parameters were determined and are summarized in table XIII.2-1. The results of this study suggested that the bioavailability of taxifolin from lipid solution for oral administration was higher than that by oral administration of taxifolin tablets, which may result from the more efficient absorption of lipid solution due to the improved permeability (Pozharitskaya, et al. 2009).

Following intravenous and oral administration to rats, the absolute bioavailability of taxifolin in rats was found to be equal to 0.17%, as was analyzed by the ultra performance liquid chromatography-mass spectrometry method. For oral administration, the taxifolin concentration was below the lower limit of quantification or the limit of detection for the samples collected after 60 min in the 10 mg/kg group or after 150 min in the 50 mg/kg. T_{max} and $t_{½}$ were found to be dependent upon the dose with a coefficient correlated to 0.9084 for T_{max} and 0.9425 for $t_{½}$. Please also refer to Table XIII.2-1 for pharmacokinetic parameters (Wang, et al. 2009).

The pharmacokinetics of taxifolin was studied by HPLC in rats after intravenous injection of the compound in single doses of 1,3, 10 and 30 mg/kg and after oral administration in single doses of 50 and 500 mg/kg. Nonlinear pharmacokinetic behaviour was demonstrated for taxifolin when administered intravenously to rats; after oral administration taxifolin can be detected in blood plasma only in trace amounts. Model independent parameters for intravenous administration of taxifolin were calculated, and the description of the dynamics of the change in concentration of taxifolin as a two-compartmental model were given for blood plasma (Voskoboinikova, et al. 1993).

The pharmacokinetics of a single administration of taxifolin was studied in male rats with 200±20 g body weight. Taxifolin was administered orally at 12.5 mg/kg, 25.0 mg/kg, and 50.0 mg/kg and intravenously at 50 mg/kg (in 0.25% tween-80 in water) (Seredin, et al. 2007).

Oral administration of taxifolin to eight rats resulted in a dose-dependent increase in its blood plasma concentration. After the oral intake of taxifolin, the flavonoid was rapidly absorbed from the gastrointestinal tract and reached its maximum concentration in the blood plasma after 30 minutes. It was still present in the blood plasma after 6 hours, but practically non-traceable 8 h after ingestion.
After a single intravenous administration of at 50 mg/kg, the level of taxifolin in blood plasma reached its maximum value within 0.1 hour, followed by a gradual decrease, indicating faster elimination than after its oral administration (Seredin, et al. 2007). Refer to Table XIII.2-2 for the mean plasma concentrations of taxifolin at different times after application.

Table XIII.2-2 Mean Plasma Concentrations of Taxifolin (µg/ml) after Oral or i.v. Application in Rats.

Oral								
Dose	0.083 h	0.25 h	0.5 h	1.0 h	1.5 h	2.0 h	4.0 h	6.0 h
12.5 mg/kg	0.44	2.11	2.46	2.00	1.67	1.21	0.55	0.11
25 mg/kg	0.80	3.87	4.29	3.50	2.71	2.13	0.93	0.19
50 mg/kg	1.50	6.55	7.32	4.92	4.21	3.41	1.57	0.27

i.v.								
Dose	0.05 h	0.1 h	0.17 h	0.25 h	0.5 h	1.0 h	2.0 h	4.0 h	6.0 h
50 mg/kg	413.10	188.60	43.61	13.50	3.80	1.87	1.26	0.33	0.10

General pharmacokinetic parameters were determined and are summarized in table XIII.2-1. Taking into consideration that the period of half-elimination of taxifolin did not exceed 1.25 hrs, taxifolin could be regarded as a “short-living” product. A linear correlation between the dose and the area under the pharmacokinetic curve was observed. After a single intravenous administration of 50 mg/kg taxifolin reached a maximum concentration of 1,137.0 µg/ml in the blood plasma. The absolute value of taxifolin’s bioavailability was found to be equal to 23.79% (Seredin, et al. 2007).

Human Studies on Absorption

Two hours after orally administrating 300 mg of Pycnogenol® in a single dose to eleven healthy volunteers, taxifolin was detected in plasma levels, reaching the maximum concentrations within 8 hours. Thereafter, taxifolin levels dropped again, however taxifolin could still be detected in the plasma after 14 h. Repeated intakes of Pycnogenol® at 200 mg/day over 5 days did not result in steady state concentrations due to due to metabolic degradation processes (Grimm, et al. 2006).

Neither 3,4-dihydroxyphemylacetic acid nor phloroglucin, the bacterial metabolites of taxifolin, were detected in the volunteers' plasma levels.

In-vitro Studies on Absorption

A study was designed to understand the transport profiles of astilbin and taxifolin in cultured Caco-2 cells and their effects on the function and expression of P-glycoprotein. Their effects on the function and expression of P-glycoprotein were detected using Western Blot and RT-PCR. The transport was concentration- and temperature-dependent. The apparent permeability (Papp) of these two compounds in the secretory direction was larger than that in the absorptive direction in the concentration range of 10–1000 μM. Caco-2 cells exposed to astilbin or taxifolin for 36 h exhibited higher P-glycoprotein activity through up-regulating P-glycoprotein expression at protein and mRNA levels. These results indicated that P-glycoprotein and Multidrug Resistance Protein 2 might play important roles in limiting the bioavailability of those compounds (Wang, et al. 2009a).

XIII.2.2 Distribution

Animal Studies on Distribution

The taxifolin distribution after a single administration of taxifolin was studied in male rats with 200±20 g body weight. Taxifolin was administered orally at 12.5 mg/kg, 25.0 mg/kg, and 50.0 mg/kg and intravenously at 50 mg/kg (in 0.25% tween-80 in water). The levels of taxifolin were determined in the blood plasma, liver, heart, spleen, brain, skeletal muscles, lungs, and kidneys at 0, 0.083, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 12.0, and 24 hours after oral administration of taxifolin and 0, 0.05, 0.1, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, and 6 hours after its intravenous injection. Samples of the blood, tissues and organs were collected after the animals were killed. The samples of blood, homogenates of the organs and excrements of rats were analyzed by HPLC (Seredin, et al. 2007). The results are indicated below.

Table XIII.2.2-1 Taxifolin Distribution in Different Organs after Single Oral Administration of 50 mg/kg

Organ	0.083 hr	0.25 hr	0.5 hr	1.0 hr	1.5 hr	2.0 hr	4.0 hr	6.0 hr
Kidneys	0.15	2.54	7.68	5.54	2.36	1.58	0.77	0.17
Heart	0.02	0.20	0.25	0.15	0.08	0.05	0.02	0.00

Brain	0.01	0.16	0.22	0.06	0.06	0.03	0.00	0.00
Skeletal muscle	0.01	0.13	0.17	0.07	0.05	0.02	0.00	0.00

As indicated in Table XIII.2.2-1 taxifolin was still identified in the blood plasma and kidneys after 6 hours, in the heart muscle still 4 hours after administration, and in the brain and skeletal muscles still 2 hours after administration.

Table XIII.2.2-1 Pharmacokinetic Parameters of Taxifolin (50 mg/kg, p.o.) in Blood Plasma and Organs

Organ	$t_{1/2el}$ hr	T_{max} hr	C_{max} µg/ml	$C_{max}/AUC_{0\rightarrow\infty}$ hr	tissue availability %
Plasma	1.24	0.5	7.32	17.02	-
Kidneys	1.06	0.5	7.68	11.33	0.667
Heart	0.58	0.5	0.22	0.21	0.010
Brain	0.99	0.5	0.25	0.36	0.021
Skeletal muscle	0.51	0.5	0.17	0.18	0.011

Within 0.5 hours after its administration, taxifolin reached its maximum concentrations in the blood plasma (7.32 µg/ml) and organs (from 7.68 µg/ml in the kidneys to 0.17 µg/ml in the skeletal muscle). Low concentrations of taxifolin in the moderately vascularized organs indicate low permeability and distribution of taxifolin in these organs. On the other hand, the high concentration of taxifolin in the kidneys can be explained by the fact that the kidneys its major organ of elimination (Seredinin, et al. 2007).

XIII.2.3 Metabolism

Animal Studies on Metabolism

Some studies indicate that taxifolin may be subjected to metabolic degradation processes. An anaerobic bacterium (*Clostridium orbiscindens*) found in human feces, has been shown to degrade taxifolin to 3,4-dihydroxyphenylacetic acid and phloroglucin. The authors of this study demonstrated the presence of *C. orbiscindens* in 8 out of the 10 human fecal samples (Schoefer, et al. 2003).

A bacterium isolated from rat feces was also shown to produce taxifolin when grown on +-catechin. The same organism hydroxylated taxifolin to dihydrogossypetin (Jeffrey, et al. 1969).

Following the i.p. administration of taxifolin (10 mg) to 3 bile duct cannulated rats, 13 metabolites were detected in bile, all of which were absent from control bile collected for 3 h prior to dosing. Following the oral administration of taxifolin, the same metabolites were detected in bile but in reduced amounts.
The metabolites were hydrolyzed by a mixed glucuronidase/aryl sulphatase preparation to give 1 of 3 aglycone products. Separation of these aglycones was effected by TLC and the eluted compounds were submitted to mass spectrometry and UV-spectroscopy. The analysis results indicate that metabolism of taxifolin includes a methylation reaction in the ring B, resulting in 3' or 4'-O-methyltaxifolin (Brown and Griffiths, 1983).

Chromatographic examination of the urine of two human volunteers before and after the ingestion of 2 grams of taxifolin demonstrated the conversion of the compound to 3,4-dihydroxyphenylacetic, m-hydroxyphenylacetic, and 3-methoxy-4-hydroxyphenylacetic acids. These same metabolites are excreted following oral administration of quercetin, or DOPA (3,4-dihydroxyphenylalanin), to rats, rabbits, or humans (Booth and De Eds, 1957).

To study the biotransformation of taxifolin, 250 mg/kg of the compound was administered to rats. The metabolic products of taxifolin were studied in the daily urine of the rats by the HPLC and mass-spectrometry (Seredin, et al. 2007).

Two unchanged diastereomers of taxifolin, 2R3R-trans and 2R3S-cis, and two metabolites of these compounds were detected in the urine. Mass spectrometry indicated that the two metabolites were methylated derivatives of the diastereomers of taxifolin.
In addition, four more compounds of the biotransformation were detected in the urine: glucuronconjugate of diastereomer 2R3R-trans and glucuronconjugate of diastereomer 2R3S-cis and their respective methylated metabolites.

Table XIII.2.3-1 Relative Amounts of Cis- and Trans-Diasteromers in the Excretion of Non-conjugated and Conjugated Fractions of Taxifolin (DHQ) and its Metabolites with Daily Urine (mean values).

Fraction	DHQ 2R3R-trans	DHQ 2R3S-cis	DHQ 2R3R-trans, 3'-OCH3	DHQ 2R3S-cis, 3'-OCH3
Non-conjugated	65.68 %	38.29 %	61.64 %	37.06 %
Glucuronconjugated	34.14 %	61.71 %	38.36 %	62.94 %

As seen from the above table, the non-conjugated molecules are primarily found as trans forms, whereas the glucuronconjugated molecules are primarily found as cis forms (Seredin, et al. 2007).

Taxifolin has a low bathochromic effect in the presence of human albumin (compared to a relatively high bathochromic effect of quercetin). The presence of human albumin causes a shift of the absorption maximum of taxifolin from 326 nm to 330 nm. This shift can be explained by some binding of taxifolin to albumin (Manach, et al. 1996).

XIII.2.4 Excretion

Animal Studies on Excretion

Taxifolin was orally administered at 50 mg/kg to nine male rats (1 control and 8 tested) with the body weight of 190-220 g. Urine and faeces were collected from each animal within 24 and 48 hours after the administration of taxifolin. HPLC analysis of the urine of rats with oral administration of 50 mg/kg taxifolin reveals additional peaks which are absent in the analysis of the control urine, corresponding to taxifolin and its metabolites (see metabolism).
8.30±2.09% of the administered dose of the initial compound was identified in the daily urine. In daily faeces, as well as in urine and faeces collected between 24 and 48 hours, no taxifolin was detected, indicating a complete absorption into the blood system from the gastrointestinal tract (Seredin, et al. 2007).

To determine the contribution of excretion through the kidneys to the elimination of taxifolin, a study examined its disappearance through the 24 h urine. The cumulative excretion of unchanged taxifolin in the urine does not exceed 6% of the administered dose. This indicates the presence of a major pathway, other than the kidney, for the

elimination of taxifolin. Analysis of the relative cumulative excretion for 24 h (MJD) shows that it increases almost linearly with dose increase. The contribution of the kidney clearance to the overall clearance is insignificant and does not represent more than 5.7%. One can therefore assume that the nonlinearity of the pharmacokinetic behaviour of taxifolin is not due to the saturation of the process of excretion by the kidneys, but is most probably a reflection of the saturation processes of metabolism and binding of taxifolin (Voskoboinikova, et al. 1993).

Human Studies on Excretion

After oral administration of 5.28 g and 1.06 g of French maritime pine bark extract to a human volunteer, ferulic acid and taxifolin (taxifolin), conjugated as glucuronides/sulphate, were excreted within 18 hours. The peak urinary excretion was observed approximately 2-3 hours after intake. Recovery of taxifolin in urine was 7-8% (Duweler and Rohdewald, 2000).

A different study describes the normal presence of taxifolin in human fecal water in very low amounts, indicating that at least a small quantity of the taxifolin, consumed with the regular diet, passes the intestine without getting absorbed (Jenner, et al. 2005).

XIII.3 Human Studies

Studies with patients

Numerous human studies with taxifolin from larch wood have been conducted on people with different pathological conditions. Many of these studies were performed in the Russian Federation and are only available in Russian, published in various Russian professional publications and in a book by Plotnikov, et al. (2005). A translation of the relevant sections of this publication may be available upon request. The taxifolin used for these studies was manufactured using the same or a very similar procedure as for the intended product. Therefore if can be assumed that the purity of taxifolin was high and there only were only low levels of contaminant substances, similar to the intended product.

In the following, the human studies are briefly described- please refer to Table XIII.2.7-1 Summary of Human Studies for details on dosage and study population.

Randomized, double-blind, placebo-control study was conducted on **100 hypertensive patients with atherosclerosis.** Average age of patients was 61.6±1.18 years (50-70 years old). The study evaluated the effect of Taxifolin (Capilar- a trade name for Lavitol) on haemodynamic and biochemical parameters, endothelial function, and neurological status. 68 patients received Capillar (80 mg/day) + standard therapy, while the remaining patients received placebo + standard therapy for 12 weeks. At the end of the study, there was a significant derease in the frequency of headaches by 52% in the group receiving Capilar, while the decrease reached 25%. Additionally, there was a 41% decrease in the number of complaints on disturbance in coordination, while no changes were observed in the placebo group. Positive effect on lipid metabolism and improved cerebral microcircualtion were observed in the group on Capillar.
There were no side effects in the group on Capillar (Britov & Aparina, 2006).

42 patients (50-76 years old) with chronic microcircualtory disturbances due to arterial hypertension, atherosclerosis and ischemic heart disease, diabetes, etc. received basic therapy + Capillar (28 patients) at 0.75 g/day or basic therapy + placebo (14 patients) for 3 months. The treatment with Capillar resulted in positive changes in the blood microcirculation, improved rheological indices, increased the rate of blood circulation, and strenthened the capillary walls.
There were no side effects in the group on Capillar (Kozlov, et al. 2006).

60 patients (39-75 years old) with atherosclerosis of lower extremities received either basic therapy + placebo (20 patients), or basic therapy +Capillar (60 mg/d)- 20 patients, or basic therapy + Capillar (60 mg/d) + Capillar-based cream (20 patients) for 2 months. In both groups receiving Capillar tablets there was a significant improvement in ability to walk longer distances without pain, decrease in the ischemic pain in the damaged extremety, and improved microcirculation.
There were no side effects in the groups on Capillar (Koshkin & Nastavweva, 2008).

30 patients (32-68 years old) with ischemic heart disease after aorta-coronary shunting surgery received either basic rehabilitation therapy (10 patients) or basic rehabilitation therapy + Capilar (60 mg/day). Administration of Capilar as an adjunct

therapy significantly improved microcirculation, central and peripheral hemodynamic, improved blood oxygenation, and improved psychoemotional conditions.
There were no side effects in the group on Capillar (Shakula, et al. 2007).

15 patients with chronic venous insufficiency [52.4±2.5 years and 56.3±4.3 years, median age of men and women, respectively] **and 15 patients with atherosclerosis** [61.3±4.4 years and 64.5±2.3 years, meadian age of men and women, respecviely] received basic therapy + Laviocard+ (Lavitol, 30 mg + Ascorbic acid, 70 mg) [1 tab/day], while 20 patients with chronic venous insufficiency and 10 patients with atherosclerosis received basic therapy for 30 days. Administration of Laviocard+ resulted in positive changes in hemodynamic indices, rheological blood parameters, and normalization of parameters of cholesterol metabolism.
There were no side effects in the group on Laviocard+ (Tikhonov, 2008).

48 patients with arterial hypertension of the II & III degree received basic therapy + Ascovertin, or basic therapy only. 10 healthy controls were also evaluated. The treatment with Ascovertin resulted in a significant decrease in blood viscosity, an increase in the time of erythrocyte aggregation, an increase in the index of erythrocyte deformability, an increase in the erythrocyte aggregation period, decrease in blood pressure, increase in the systolic index and significant decrease in the levels of the primary and secondary products of lipid peroxidation.

Safety Result:

There were no side effects in the group on Ascovertin (Plotnikov, et al. 2005).

51 patients w/ischemic heart disease received basic therapy + Ascovertin, or basic therapy only. 10 healthy controls were also evaluated. The treatment with Ascovertin resulted in an increase in deformation of erythrocytes, decrease in the level of fibrinogen, decrease in the number of episodes of stenocardia, decrease in the number of administered nitroglyceride, increase in tolerability to physical exercise, and a decrease in the levels of the primary and secondary products of lipid peroxidation.

Safety Result:

There were no side effects in the group on Ascovertin (Plotnikov, et al. 2005).

40 patients with ischemic heart disease, received basic therapy + Ascovertin (1 tab x 3/day), or basic therapy+placebo [20 patients] for 3 months. The treatment with Ascovertin has positive effects on the hemorheological status and significantly decreased anginal episodes/week.
There were no side effects in the group on Ascovertin (Tyukavkina, et al. 2001).

29 patients 56-78 years old with discirculatory encephalopathy received basic therapy + Capilar (80 mg/d) for 18-21 days. Administration of Capilar showed significant improvements in psychoemotional conditions of the patients.
There were no side effects observed (Zavolokov, Ilyuhina, 2001).

31 patients with cerebral atherosclerosis received basic therapy + Ascovertin (Taxifolin (Diquertin) 20 mg, Ascorbic acid, 50 mg), or basic therapy only. 10 healthy controls were also evaluated. The treatment with Ascovertin resulted in a significant decrease in blood viscosity; increase in the time of erythrocyte aggregation, improvement in short-term memory and ability to concentrate, and in a significant decrease in the primary and secondary products of lipid peroxidation.
Safety Result:
There were no side effects in the group on Ascovertin (Plotnikov, et al. 2005).

40 patients, median age 56.2±8.5 years old, with diabetes mellitus received basic therapy + Diquertin (120 mg/day) or basic therapy + placebo for 12 weeks. 20 healthy volunteers comprised the control group. Administration of Diquertin resulted in a significant decrease in HbA1x levels and improved sensitivity to insulin.
There were no side effects in the group on Diquertin (Nedosugova, 2006).

37 patients 30-68 years old with diabetes-related onychomycosis of feet and hands received basic therapy (20 patients) or basic therapy+ Diquertin (120 mg/day) for 12-16 weeks. The treatment with Diquertin significantly decreased MDA levels and coefficient of intoxication, as determined by the level and value of olygopeptides.
There were no side effects in the group on Diquertin (Davudova & Zoloeva, 2009).

29 patients with NIDDM received basic therapy + Ascovertin, or basic therapy only. 10 healthy controls were also evaluated. A significant decrease in blood viscosity; an

increase in the time of erythrocyte aggregation and in the index of their deformability, a decrease in blood pressure, an increase in the systolic index and a significant decrease in the levels of the primary and secondary products of lipid peroxidation were observed. There was an improvement in the subjective evaluation by the patients.

Safety Result:

There were no side effects in the group on Ascovertin (Plotnikov, et al. 2005).

43 women with Lyme disease were treated with a basic therapy + taxifolin (Diquertin) + other vitamins, or as a control only with the basic therapy. There was an increase in levels of the endogenous antioxidants, and a decrease in the levels of the primary products of lipid peroxidation, as well as a normalization of the menstrual cycle in the taxifolin group.

Safety Result:

There were no side effects in the group on taxifolin (Plotnikov, et al. 2005).

48 women that would undergo an operation on the ovaries were treated with a basic therapy + taxifolin (Diquertin)+ vitamin C, or as a control only with the basic therapy. The MDA level was much lower in the taxifolin treated group than in the group on basic therapy only, while the levels of catalase and SOD were significantly higher. The amplitude of the uterus muscle contractions 1 month after the operation in the taxifolin-supplemented group was similar to that of the control group.

Safety Result:

There were no side effects in the group on taxifolin (Plotnikov, et al. 2005).

Forty male patients with chronic pulmonary obstructive disease, aged 30 to 65 years old (mean age 50.4±2.5), received standard therapy (the control group) or standard therapy plus Capilar at 80 mg/day (20 patients in each group) for 18-21 days. At the end of the study, patients in the Taxifolin group showed normalization of the indicies of tissue and organs' microcirculation, increased blood oxygenatioin, improved rheological blood parameters, increased tolerance to physical exercise, and improved functioning of the respiratory and cardiovascular systems.

There were no side effects in the group on Capilar (Shakula, et al. 2008).

56 patients with out-of-hospital-acquired pneumonia received standard therapy or standard therapy+Derinat or standard therapy + Diquertin, 160 mg/d (n=14 patients) or standard therapy + Derinat + Diquertin, 160 mg/d (n=14 patients). 12 healthy subjects comprosed the control group. Diquertin rendered the correcting effects on oxygen activity of granulocytes, the concentration of TNF-alpha, IL-1beta, IL-8, GSF-Gi, IL-10, while Derinat- on the content of lymphocytes examined, products of lipid peroxidation, and catalyse activity. The maximal positive effects on the immune respones and oxidant staus parameters were found in the application of both preparations.
There were no side effects in the groups on Diquertin (Serikova, et al.).

The effectiveness supplemental taxifolin in **patients with acute pneumonia** was investigated. 112 male patients were divided into three groups: the first control group (n=50), which received standard therapy without antioxidants; the second comparative group (n=32) received composite therapy including the standard therapy plus the antioxidative complex of a -tocopherol acetate, 100 mg in pills four times a day, and intravenous administration of 10 ml of 10% solution of sodium thiosulfate two times a day; the third experimental group (n=30), which in addition to the standard therapy received 40-60 mg of taxifolin four times a day. The antioxidant therapy was administered for 2 weeks following hospitalization. The observation period lasted until day 25.
It was established that the patients of the third group, receiving taxifolin, had a faster recovery from symptoms of pneumonitis and lower the content of TBARS [thiobarbituric acid reactive substances] in blood plasma compared with patients of the first group. Also, patients in the third group showed a more complete X-ray restoration of lung tissue (1.8 times) and a decrease of pulmonary fibrosis (3.6 times) ($p<0.05$). There was no essential difference between the clinical effectiveness of taxifolin and the antioxidant complex.

Safety Result:
There were no side effects in the group on taxifolin (Teselkin, et al. 1998 and 1998a; Kolhir, 1998).

Human study summary

Altogether, 507 patients were treated with taxifolin from larch wood (40-120 mg/day) for 2 weeks – 3 months. ***No side effects were connected with the administration of taxifolin during any of the studies.*** The results of the human studies, as well as details on the study population and the dosage are summarized in the following table XIII.2.7-1.

It needs to be considered that there is, next to these studies, a considerable and growing amount of human taxifolin consumption, including taxifolin from larch wood, mainly as dietary supplement. Please refer to section X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE for details

(next page)

Table XIII.2.7-1 Summary of Human Studies with Taxifolin from Larch Wood

Total study population	**Study groups and number of subjects exposed**	**Duration**	**Daily dose of taxifolin from larch wood**	**End-points**	**Results of the verum group (Efficacy/ Safety)**	**Reference**
100 hypertensive patients with atherosclerosis (50-70 y.o.)	Capillar (taxifolin) + standard therapy **68 patients** placebo + standard therapy 32 patients	12 wks	80 mg taxifolin/day	The effect of taxifolin on haemodynamic and biochemical parameters, endothelial function, and neurological status	Significant decrease in headache frequency (52%) and strength (25%); 41% decrease coordination disturbance; positive effect on lipid metabolism and improved cerebral microcircualtion. There were **no side effects** in the group on Capillar.	Britov & Aparina, 2006
42 patients with chronic microcircualtory disturbances (50-76 y.o.)	Capillar (taxifolin) + basic therapy **28 patients** placebo + basic therapy 14 patients	3 mo	75 mg taxifolin/day	Blood circulation parameters	Positive changes in the blood microcirculation, improved rheological indices, increased the rate of blood circulation, and strenthened the capillary walls. There were **no side effects** in the group on Capillar.	Kozlov, et al. 2006
60 patients with atherosclerosis of lower extremities (39-75 y.o.)	Capillar (taxifolin) + basic therapy **20 patients** Capillar (taxifolin) + Capillar-cream + basic therapy **20 patients** placebo + basic	2 mo	60 mg taxifolin/day	Painfree walking distance, ischemic pain, microcirculation	Improvement in ability to walk longer distances without pain, decrease in the ischemic pain in the damaged extremety, and improved microcirculation. There were **no side effects** in the group on Capillar.	Koshkin & Nastavweva, 2008

	therapy 20 patients					
30 patients with ischemic heart disease after aorta-coronary shunting surgery (32-68 y.o.)	Capillar (taxifolin) + basic rehabilitation **20 patients** + basic rehabilitation 10 patients	12-17 ds	60 mg taxifolin/day	Circulation parameters and psychoemotions	Significant improvement in microcirculation, central and peripheral hemodynamic, improvement in blood oxygenation, and psychoemotional conditions. There were **no side effects** in the group on Capillar.	Shakula, et al. 2007
35 patients with chronic venous insufficiency; **25** patients with atherosclerosis	basic therapy + Laviocard+ (Lavitol, 30 mg + Ascorbic acid, 70 mg) [1 tab/day] **15+15 = 30 patients** basic therapy + 10+20 = 30 patients	30 days	30 mg taxifolin/day	Blood and circulation parameters	Positive changes in hemodynamic indices, rheological blood parameters; normalization of cholesterol metabolism. There were **no side effects** in the group on Capillar.	Tikhonov, 2008
31 patients w/cerebral atherosclerosis (median age = 60.4±4.8 y.o. y) (41 total)	Basic therapy + Ascovertin*: **21 patients** Basic therapy: 10 patients Healthy controls: 10 subjects	3 wks	1st wk: 2 tab x 3/day [120 mg taxifolin/day + 300 mg ascorbic acid/day]; 2nd-3rd wks: 1 tab x 3/day [60 mg taxifolin/day + 150 mg ascorbic acid/day]	The effect of Ascovertin on haemorheological parameters and subjective symptomology	Significant decrease in blood viscosity; Increase in the time of erythrocyte aggregation; Improvement in short-term memory improvement in ability to concentrate; Significant decrease in the levels of the primary and secondary products of lipid peroxidation; There were **no side effects** in the group on Ascovertin.	Plotnikov, et al. 2005

48 patients w/arterial hypertension of the II & III degree (median age = 60±5 y.o.) (58 total)	Basic therapy + Ascovertin*: **38 patients** Basic therapy: 10 patients Healthy controls: 10 subjects	3 wks	1st wk: 2 tab x 3/day [120 mg taxifolin/day + 300 mg ascorbic acid/day]; 2nd-3rd wks: 1 tab x 3/day [60 mg taxifolin/day + 150 mg ascorbic acid/day]	The effect of Ascovertin on haemorheological parameters	Significant decrease in blood viscosity; Increase in the time of erythrocyte aggregation; Decrease in blood pressure, increase in the systolic index; Significant decrease in the levels of the primary and secondary products of lipid peroxidation; There were **no side effects** in the group on Ascovertin.	Plotnikov, et al. 2005
29 patients with NIDDM (median age = 56±4 y.o.; diagnosed w/NIDDM for 8.5±3.6 years [median]; the level of glycemia w/fasting blood glucose level of 7.8±1.8 mmol/L) (39 total)	Basic therapy + Ascovertin±÷ **19 patients** Basic therapy: 10 patients Healthy controls: 10 patients	3 wks	1st wk: 2 tab x 3/day [120 mg taxifolin/day + 300 mg ascorbic acid/day]; 2nd-3rd wks: 1 tab x 3/day [60 mg taxifolin/day + 150 mg ascorbic acid/day]	The effect of Ascovertin on haemorheological parameters	Significant decrease in blood viscosity; Increase in the time of erythrocyte aggregation; Decrease in blood pressure, increase in the systolic index; Significant decrease in the levels of the primary and secondary products of lipid peroxidation; Improvement in subjective evaluation There were **no side effects** in the group on Ascovertin.	Plotnikov, et al. 2005
51 patients w/ischemic heart disease (not older than 65 y.o.)	Basic therapy + Ascovertin*: 17 pts w/IHD without prior MI + 14 pts w/IHD w/prior MI = **31** vs. Basic therapy	3 mo	1st mo: 1 tab x 4/day after meal [80 mg taxifolin/day + 200 mg ascorbic acid/day] 2nd-3rd mo: 1 tab	Number of episodes of stenocardia; Number of nitroglycerin taken; Tolerability to	Increase in the index of deformability of erythrocytes; Decrease in the level of fibrinogen; Decrease in the number of episodes of stenocardia; Decrease in the number of administered nitroglycerine;	Plotnikov, et al. 2005

(61 total)	+ placebo: 10 pts w/IHD without prior MI; 10 pts w/IHD w/prior MI=20 healthy controls: 10 subjects		x 3/day [60 mg taxifolin/day + 150 mg ascorbic acid/day]	physical exercise; Haemorheological parameters; Antioxidant parameters	Increase in tolerability to physical exercise; Decrease in the levels of the primary and secondary products of lipid peroxidation; There were **no side effects** in the group on Ascovertin.	
40 patients with ischemic heart disease	basic therapy + Ascovertin* (1 tab x 3/day) **(20 patients)** basic therapy+placebo (20 patients)	3 mo		hemorheological status; number of anginal episodes/week	Positive effects on the hemorheological status and significantly decreased anginal episodes/week.	Tyukavkina, et al. 2001
29 patients with discirculatory encephalopathy (56-78 y.o.)	Capillar (taxifolin) + basic therapy **(29 patients)**	18-21 days	80 mg taxifolin/day	psychoemotional conditions	Significant improvements in psychoemotional conditions of the patients. There were **no side effects** observed.	Zavolokov, Ilyuhina, 2001
40 patients with diabetes mellitus, 56.2±8.5 years (60 total)	basic therapy + Diquertin* (**40 patients**) basic therapy + placebo (20 healthy volunteers)	12 wks	120 mg taxifolin/day	sensitivity to insulin; HbA1x levels	Significant decrease in HbA1x levels and improved sensitivity to insulin. There were **no side effects** in the group on Diquertin.	Nedosugova, 2006
37 patients 30-68 years old with diabetes-	basic therapy + Diquertin* (**20 patients**)	12 wks	120 mg taxifolin/day	MDA levels, intoxination parameters	Significantly decreased MDA levels and coefficient of intoxication, as determined by the level and value of olygopeptides.	Davudova & Zoloeva,

related onychomycosis of feet and hands	basic therapy (17 patients)				There were **no side effects** in the group on Diquertin.	2009
43 women w/Lyme disease (19-28 y.o.)	Basic therapy + taxifolin (Diquertin*), etc: **20 patients** Basic therapy: 23 patients	2 mo	1st mo: [taxifolin (60 mg/day) +Ascorbic acid (150 mg/day)] x 14 days + Folic acid (300 mg/d) 2nd mo: alpha-tocopherol acetate (300 mg/d)	Levels of superoxide dismutase, catalase, glutathione peroxidase. Level of the primary products of lipid peroxidation Changes in the menstrual cycle	Increase in levels of the endogenous antioxidants; Decrease in the levels of the primary products of lipid peroxidation; Normalization of the menstrual cycle; There were **no side effects** in the group on taxifolin.	Plotnikov, et al. 2005
48 women that would undergo an operation on the ovaries (20-34 y.o.)	Basic therapy + Taxifolin (Diquertin*), + ascorbic acid: **25 patients** Basic therapy: 23 patients	14 days	Taxifolin (120 mg/d) + ascorbic acid (300 mg/d) x 4 ds prior to operation and x 10 ds after the operation	The level of endogenous antioxidant system; Commissures; The level of the lipid peroxidation products Pregnancy	The MDA level was much lower in the taxifolin-treated group than in the group on basic therapy only, while the levels of catalase and SOD were significantly higher; The amplitude of the uterus muscle contractions 1 month after the operation in the taxifolin-supplemented group was similar to that of the control group; 73% of the women in taxifolin-treated group were pregnant six months after the procedure (vs. 50% of the women on basic therapy); There were **no side effects** in the group	Plotnikov, et al. 2005

					on taxifolin.	
40 male patients with chronic pulmonary obstructive disease (30-65 y.o.)	standard therapy plus Capilar **(20 patients)** standard therapy (20 patients)	18-21 days	80 mg/day taxifolin	Circulation, respiratory and cardiovascular parameters	Normalization of the indicies of tissue and organs' microcirculation, increased blood oxygenatioin, improved rheological blood parameters, increased tolerance to physical exercise, and improved functioning of the respiratory and cardiovascular systems. There were **no side effects** in the group on Capilar.	Shakula, et al. 2008
56 patients with out-of-hospital-acquired pneumonia (68 total)	standard therapy Diquertin* **(14 patients)** standard therapy + Diquertin* + Derinat **(14 patients)** standard therapy + Derinat (14 patients) standard therapy (14 patients) healthy subject control (12 volunteers)	20 ds	160 mg/day taxifolin	Inflammatory and lipid peroxidation parameters	Rendered the correcting effects on oxygen activity of granulocytes, the concentration of TNF-alpha, IL-1beta, IL-8, GSF-Gi, IL-10; administration of Derinat rendered the correcting effects on the content of lymphocytes examined, products of lipid peroxidation, and catalyse activity. The maximal positive effects on the immune respones and oxidant staus parameters were found in the application of both preparations. There were **no side effects** in the groups on Diquertin.	Serikova, et al.
112 patients with acute pneumonia (19-	Active 1 (basic therapy + vit. E): 32 patients	2 wks	40-60 mg/day taxifolin	Restoration of lung tissue	More rapid restoration of lung tissue in the group supplemented w/taxifolin Faster recovery	Kolhir, et al. 1998

40 y.o.) 30 healthy volunteers (142 total)	Active 2 (basic therapy + taxifolin (Diquertin*): **30 patients** Control (basic therapy): 50 patients				Higher levels of endogenous antioxidants There were **no side effects** in the group on taxifolin	
TOTAL patients: 896 **TOTAL subjects (patients + healthy volunteers): 988**	**Total subjects exposed to taxifolin from larch wood: 507**					

* Ascovertin: Taxifolin (Diquertin) 20 mg, Ascorbic acid, 50 mg

XIII.4 Other Studies

Immunotoxicity – studies with taxifolin from larch wood

The immunomodulatory properties of taxifolin were studied on the CBA line of mice. The hemagglutinin titer in blood serum and the spleen antibody-forming cell count were determined after a 4-time intraperitoneal administration of taxifolin from larch wood at 10 and 100 mg/kg (the local hemolysis method). The administration of taxifolin had no effect on the activity of antibody-forming cells of a spleen and the hemagglutinin titer in blood serum (Shkarenkov, et al. 1998).

The effect of taxifolin from larch wood on the cellular immunity was studied by the graft-versus-host reaction and delayed-type hypersensitivity in mice-hybrids (CBA x C_{57}BL)F_1. Taxifolin was administered intraperitoneally five times at 10 and 100 mg/kg. No effect of taxifolin on the studied reactions was observed during the experiment (Shkarenkov, et al. 1998).

XIII.5 Potential Allergenicity Concerns

Studies with taxifolin from larch wood

The allergenicity was studied on 48 guinea pigs with a body weight of 220 – 250 g. To evaluate allergenic properties of taxifolin, the following tests were conducted: development of the general anaphylaxis, taxifolin's effect on anaphylactic contracture of an isolated segment of the large intestine and taxifolin's impact on the development of delayed-type hypersensitivity. The results of the tests indicated that taxifolin did not cause sensibilizing responses in any of the applied tests, indicating the absence of allergenic properties in taxifolin (Shkarenkov, et al. 1998).

There has been substantial human exposure to taxifolin as supplement and food additive for several decades, mostly in Russia, Switzerland, USA and Canada (Please refer to Section X.1.1 "Intake of the Food Ingredient" for details). – During this exposure, allergenicity to taxifolin extracted from larch never posed a problem.

There are no reports of allergic reactions to the compound reported in the literature, or to the manufacturer.

It should also be considred that taxifolin from larch wood is an almost pure chemical, and the product does not contain protein (Please refer to section II.3 Potential Impurities Resulting from the Production Process).

Even though it is considered possible that there are people who react allergic to larch pollen, there is no pollen contained within the taxifolin extracted from larch wood. Accidental contamination of the wood source with larch pollen is theoretically possible during harvest, however the wood is thoroughly washed prior to chopping and extraction, so there should be vitrually no larch (or other) pollen within the material that is extracted. In addition it should be noted that the extraction process would denature any such pollen allergens. For example it is known that commercially available pollen extract (e.g. the Swedish product Femal Balans) has a very low allergenicity.

XIII.6 Consideration of Possible Physiological Effects of degradation products

HPLC analysis of urine samples reveals a certain degradation of taxifolin to methylated derivatives of taxifolin (See ADME section, XIII.2.3 Metabolism), which have similar properties to taxifolin.
Chromatographic examination of the urine of two human volunteers before and after the ingestion of 2 grams of taxifolin demonstrated the conversion of the compound to 3,4-dihydroxyphenylacetic, m-hydroxyphenylacetic, and 3-methoxy-4-hydroxyphenylacetic acids. These same metabolites are excreted following oral administration of quercetin, or DOPA (3,4-dihydroxyphenylalanin), to rats, rabbits, or humans (Booth and De Eds, 1957). The same metabolism of these compounds is one explanation of non-toxicity of both quercetin and dihydroquercetin (taxifolin).

Also, the anaerobic bacterium *Clostridium orbiscindens,* found in human feces, has been shown to degrade taxifolin to 3,4-dihydroxyphenylacetic acid and phloroglucin (Schoefer, et al. 2003).
3,4-dihydroxyphenylacetic acid possess significant reducing power and free radical scavenging activity (Jaganath, et al. 2009). Phloroglucin is also known as a radical scavenger (Nenadis, et al. 2004). Both catabolites may play a role in the overall antioxidant capacity of the colonic lumen.

XIII.7 Supporting Studies Justifying Consumption

Please refer to the section XI.b Nutritional Benefits of the Food Ingredient.

EVALUATIONS AND CONCLUSIONS

Taxifolin from larch wood is a beneficial, safe and well-tolerated novel food ingredient. Taxifolin is a natural compound, present in a variety of different foods. Accordingly, the novelty about taxifolin concerns mainly its origin from the wood of Dahurian larch trees, which has not been sufficiently consumed as food within the EU before.

Taxifolin will be mainly added to pre-packed foods to cause a health benefit as antioxidant and anti-inflammatory agent. This health benefit is supported by numerous in vitro and in vivo studies.

Most importantly, the **safety** of taxifolin is proven in many animal and human studies, and further supported by the prevalent human food use of taxifolin in countries outside of the EU, e.g. in Russia and the USA.
Taxifolin from larch wood is absorbed, metabolized and excreted in the urine within a few hours after consumption.

The manufacturing takes place according to the hygienic requirements for safety and nutritional value of foodstuffs. The raw materials and the final product are tested according to strict specifications. The accelerated stability over 30 weeks, corresponding to 5 years of normal shelf life, has been confirmed in a stability study.

The data presented within this dossier strongly supports the use of taxifolin as a novel food ingredient within the EU.

APPENDICES

The following table gives an overview of the appended documents.

Table of Appendices

Appendix		File name (.pdf omitted)	Content
A Manufacturer	A1	a1-registration-russia-lavitol2007	Registration of taxifolin in Russia (2007)
	A2	a2-sanitary-epidemiological-conclusion2007	Sanitary Epidemiological Conclusion (2007)
	A3	a3-iso9001-2000-certification2009	ISO 9001-2000 certification (2009)
	A4	a4-fda-registration2009	FDA registration (2009)
	A5	a5-ametis-jsc-accred	Accreditation Ametis JSC
	A6	a6-ametis-pl-accred	Accreditation Ametis PL
	A7	a7-belogorsk-accred	Accreditation Belogorsk
	A8	a8-spirtzavod-accred	Accreditation Spirtzavod
	A9	a9-blagoveschensk-accred	Accreditation Blagoveschensk
	A10	a10-plv-accred	PhytoLab accreditation
B Methods	B1	gost-methods	Various GOST methods for quality assurance of the sawdust and the final product
	B2	icp-ms-prop65-abc	Heavy metals per proposition 65, ABC Testing
	B3	mvi72-08	Methodology of Dihydroquercetin Assay by HPLC
	B4	specifications-methods-ethanol	specifications and methods for quality assurance of ethanol

	B5	specifications-methods-water	specifications and methods for quality assurance of water
	B6	usp-microbio	Microbiology screening, ABC Testing
	B7	usp32-solvent-abc	Organic volatile impurities/solvent residues
C Batch analysis	C1	ametis2009-coa-5batches	Certificates of analysis of 5 different batches of taxifolin
	C2	analysis-ethanol	Certificates of analysis concerning ethanol
	C3	analysis-water-summary	Summary of certificates of analysis concerning water
	C4	batch-analysis-abc-metals	Certificates of analysis concerning heavy metals
	C5	batch-analysis-abc-microbio	Certificates of analysis concerning microbial parameters
	C6	batch-analysis-abc-solvents	Certificates of analysis concerning solvent residues
	C7	batch-analysis-taxifolin	Certificates of analysis concerning the content of taxifolin and other polyphenols
D Manufacturing Sales CONFIDENTIAL	D1	d1-manufact-instruct-CONFIDENTIAL	Manufacturing Instructions TY 9325-001-70692152-07 (2009) CONFIDENTIAL!!!
	D2	d2-technical-conditions-CONFIDENTIAL	Technical Conditions TY 9325-001-70692152-07 including specifications (2007) CONFIDENTIAL!!!

	D3	d3-postmarketing2006-09-CONFIDENTIAL	Postmarketing data of taxifolin of the company Ametis JSC CONFIDENTIAL!!!
E Stability	E2	ametis2009-coa-batch63	Certificate of Analysis of batch 63
	E2	batch63-metal-solvent-microbio	Analysis results of batch 63: heavy metals, solvents, microbial parameters
	E3	stabi-12-week	Accelerated stability results from 1 to 12 weeks of batch 63
	E4	stabi-18-30-week	Accelerated stability results at 18 and 30 weeks of batch 63
F Containers	F1	cardboard-winding-drums	Cardboard Winding Drums
	F2	cardboard	Cardboard
	F3	polyethylene-film	Polyethylene Film
	F4	polypropylene-bags	Polypropylene Bags
G Reference standards	G1	eridictyol	Certificates of analysis of reference standards used for HPLC analysis of the taxifolin content (method mvi72-08), and for the identification of the minor components
	G2	eridictyol	
	G3	naringenin	
	G4	pinocembrin	
	G5	quercetin	
	G6	taxifolin	
H References		Sorted by first author and date of publication. Please see below.	

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Please see Appendix H for the complete publications.
References in blue are in Russian. Translations are available upon request.

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