Application to amend the Australian New Zealand Food Standards Code

3.1.2 Applicant Details

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[redacted]

f) Peak industry body (Not-for-profit)
g) Lallemand Australia Pty Ltd (Technical input)

3.1.3 Purpose of the application

The purpose of the application is to amend Standard 1.3.3 and Standard 4.5.1 to permit the use of fungal chitosan as a processing aid (in particular as a fining agent and for microbial stabilisation) in wine , beer and cider.

3.1.4 Justification of the application

The technical application of fungal chitosan as a processing aid has been demonstrated in a number of wine producing countries around the world and it has been approved for use in some of Australia's major competitors. In addition, under the Australia – European wine agreement, wine from Europe made using fungal chitosan can be sold in Australia, but Australian producers cannot yet use it for domestic production.

A. Regulatory impact information

1. Costs and benefits

- Consumers will benefit from better techniques to stabilise wine
- Industry will benefit from increased availability of techniques to make better quality wine, increasing quality and lowering cost of production
- There are no regulatory impacts on government and this will enhance relations with Europe by implementing in regulation the ability to use fungal chitin for wine making.

2. Impact on international trade
   This will enhance the competitiveness of Australian wine domestically and in major export markets where fungal chitin is approved for wine making. It will make Australia consistant with the standards from other major producers.

3.1.5 Information to support the application

A. Data requirements

Attached as per the requirements in Section 3.3.2

3.1.6 Assessment procedure

This is an unpaid application and we would consider it as General Level 1 procedure.

3.1.7 Confidential commercial information

There is no confidential commercial information in this application.

3.1.8 Exclusive capturable commercial benefit

The applicant is a not-for-profit organisation and has no commercial or financial interest in the application. We are not seeking approval for a proprietary product and to the best of our knowledge there is no exclusive capturable commercial benefit.

3.1.9 International and other national standards

A. Other national standards or regulations

This application is for a processing aid and therefore the Codex General Standard for Food Additives is not applicable.

Resolutions permitting the use of fungal chitosan in winemaking as a fining agent and contaminant treatment have been granted by the International Organisation of Vine and Wine (OIV/OENO 336A/2009; 337A/2009; 338A/2009; 339A/2009) (OIV, 2011) (Attachments 1-4). A monograph for fungal chitosan has been added to the International Oenological Codex by decision if the OIV general assembly dated July 2009 considering the works of the group of experts "Specifications of Oenological Products" (OIV/OENO OIV/OENO 368/2009).

B. Other national standards or regulations

The corresponding approval for use of fungal chitosan in wine products marketed within the European Union has been issued by the European Commission (EU, 2011). Since 2011, chitosan from fungal origin is therefore approved as oenological practices for clarification according to ANNEX I of REGULATION (EU) No 53/2011 and for treatment of wines.

Fungal Chitosan (from *Aspergillus niger)* is GRAS self-affirmed for use as a processing-aid in the manufacture of alcoholic beverages. This GRAS self-affirmation was notified to the offices of the U.S. Food and Drug Administration (FDA), without objection from the Agency (FDA 2011, GRAS NOTICE No. 397).

The application of fungal chitosan in the winemaking process has been approved by the National Authority of Argentina.

The European Union requested the addition of chitosan and chitin-glucan of fungal origin to the Annex of the Wine Agreement in November 2010. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement.

3.1.10 Statutory declaration

Attached

3.1.11 Checklist

Attached

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STATUTORY DECLARATION – AUSTRALIA

The information provided in Parts 1 to 3 must be attested to by a statutory declaration in some suitable form along the following lines:

STATUTORY DECLARATION

Statutory Declarations Act 1959

I, [*Name, address and occupation of person making the declaration*] ANTHONY NICHOLAS BATTAGLENE, [redacted]

make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]

[*Signature of person making the declaration*]

[redacted] ADELAIDE 18 of [~~*month*~~] [~~*year*~~] SEPTEMBER 2012

[redacted]

[~~*Signature of person before whom the declaration is made*~~]*

[*Full name, qualification and address of person before whom the declaration is made (in printed letters)*]

[redacted] Policy Officer and Commissioner for taking affidavits in the State of South Australia of 12, Queen St CROYDON SA 5008

* A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*, available online at http://www.frli.gov.au/ComLaw/Legislation/ActCompilation1.nsf/current/bytitle/7E3AE20F8329B422CA256F71004DB642?OpenDocument&mostrecent=1.

CHECKLIST FOR GENERAL REQUIREMENTS

This Checklist will assist you in determining if you have met the information requirements as detailed in Section 3.1 – General Requirements. All applications must include this Checklist.

General Requirements (3.1)

3.1.1 Form of application
- ☑ *Executive Summary*
- ☑ *Relevant sections of Part 3 identified*
- ☑ *Pages sequentially numbered*
- ☐ *Electronic + 2 hard copies*
- ☑ *Electronic and hard copies identical*
- ☑ *Hard copies capable of being laid flat*
- ☑ *All references provided*

3.1.2 Applicant details

3.1.3 Purpose of the application

3.1.4 Justification for the application

3.2.5 Information to support the application

3.1.6 Assessment procedure
- ☑ *General*
- ☐ *Major*
- ☐ *Minor*

☑ 3.1.7 Confidential Commercial Information
- ☐ *Confidential material separated in both electronic and hard copy*
- ☐ *Justification provided*

☑ 3.1.8 Exclusive Capturable Commercial Benefit

☑ 3.1.9 International and Other National standards

☑ 3.1.10 Statutory Declaration

☑ 3.1.11 Checklist/s provided with Application
- ☑ *3.1 Checklist*
- ☑ *Any other relevant checklists for Sections 3.2-3.7*

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CHECKLIST FOR STANDARDS RELATED TO SUBSTANCES ADDED TO FOOD

This Checklist is in addition to the Checklist for Section 3.1 and will assist you in determining if you have met the information requirements as specified in Sections 3.3.1-3.3.3.

Food Additives (3.3.1)

☐	A.1 Nature and technological function information	☐	B.1 Toxicokinetics and metabolism information
☐	A.2 Identification information	☐	B.2 Toxicity information
☐	A.3 Chemical and physical properties	☐	B.3 Safety assessments from international agencies
☐	A.4 Impurity profile	☐	C.1 List of foods likely to contain the food additive
☐	A.5 Manufacturing process	☐	C.2 Proposed levels in foods
☐	A.6 Specifications	☐	C.3 Likely level of consumption
☐	A.7 Food labelling	☐	C.4 Percentage of food group to contain the food additive
☐	A.8 Analytical detection method	☐	C.5 Use in other countries (if applicable)
☐	A.9 Additional functions	☐	C.6 Where consumption has changed, information on likely consumption

Processing Aids (3.3.2)

☑	A.1 Type of processing aid	☐	C.3. Allergenicity information of enzyme (enzyme only)
☑	A.2 Identification information	☑	C.4. Overseas safety Assessment Reports
☑	A.3 Chemical and physical properties	☐	D.1 Information on source organism (enzyme from microorganism only)
☑	A.4 Manufacturing process	☐	D.2 Pathogenicity and toxicity of source microorganism (enzyme from microorganism only)
☑	A.5 Specification information	☐	D.3 Genetic stability of source organism (enzyme from microorganism only)
☑	A.6 Analytical method for detection	☐	E.1 Nature of genetic modification of source organism (enzyme from GM source microorganism)
☑	B.1 Industrial use information (chemical only)	☑	F.1 List of foods likely to contain the processing aid
☑	B.2 Information on use in other countries (chemical only)	☑	F.2 Anticipated residue levels in foods

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☑	B.3 Toxicokinetics and metabolism information (chemical only)	☑	F.3 Information on likely level of consumption
☑	B.4 Toxicity information (chemical only)	☑	F.4 Percentage of food group to use processing aid
☐	B.5 Safety assessments from international agencies (chemical only)	☑	F.5 Information on residues in foods in other countries (if available)
☐	C.1 Information on enzyme use on other countries (enzyme only)	☑	F.6 Where consumption has changed, information on likely consumption
☐	C.2 Toxicity information of enzyme (enzyme only)		

Nutritive Substances (3.3.3)

☐	A.1 Identification information	☐	C.2 Proposed maximum levels in food groups or foods
☐	A.2 Chemical and physical properties	☐	C.3 Likely level of consumption
☐	A.3 Impurity profile information	☐	C.4 Percentage of food group to use nutritive substance
☐	A.4 Manufacturing process	☐	C.5 Use in other countries (if available)
☐	A.5 Specification information	☐	C.6 Where consumption has changed, information on likely consumption
☐	A.6 Analytical detection method	☐	D.1 Nutritional purpose
☐	A.7 Proposed food label	☐	E.1 Need for nutritive substance
☐	B.1 Toxicokinetics and metabolism information	☐	E.2 Demonstrated potential deficit or health benefit
☐	B.2 Animal or human toxicity studies	☐	F.1 Consumer awareness and understanding
☐	B.3 Safety assessments from international agencies	☐	F.2 Actual or potential behaviour of consumers
☐	C.1 List of food groups or foods likely to contain the nutritive substance	☐	F.3 Demonstration of no adverse effects on any population groups

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Application for a NEW PROCESSING AID:

Fungal chitosan from *Aspergillus niger*

Prepared for: Food Standards Australia New Zealand
PO Box 786
CANBERRA BC ACT 2610

Prepared by: Winemakers Federation of Australia

August, 2012

(9)

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Summary

Chitosan is a naturally occurring carbohydrate polymer that is widely distributed in nature (crustacean shells, fungal cell walls). It is a non-allergenic, biodegradable polysaccharide of glucosamine and N-acetylglucosamine that is derived from chitin. Fungal chitosan is obtained by deacetylation of chitin present in the cell walls of non-genetically modified *A. niger* mycelium.

Fungal chitosan from *A. niger* is proposed for use as a processing-aid in the manufacture of wine, beer, cider and spirits, as well as grain and beet derived food grade ethanol. They are proposed for use in stabilizing through their antimicrobial effect on a number of economically important microbial contaminants such as *Brettanomyces* as well as for flotation, clarification to reduce cloudiness and the content of unstable colloids, for use as fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, for reducing organic and mineral contaminants in wine and spirit, and for encapsulation of fermentation yeast, lactic bacteria and nutrients. Two fungal chitosans are proposed which differ slightly in their residual glucan contents as well as in their granulometry.

Fungal chitosan as a processing aid does not fall in any of the categories of processing aids listed in FSANZ standard 1.3.3.

Evidence that the form and the amount of the processing aid perform the intended function has been demonstrated through a thesis, laboratory and industrial experimentations. These assessments have been conducted in particular during two years consecutively experiments (year 2008-2009 and 2009-2010) under derogation of the DGCCRF (the French official body supervising regulations), in the frame of the Regulation EC 423/2008 (Art. 44) and Regulation EC 606/2009, respectively.

As fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, clarification, and removal of mineral and organic contaminant, fungal chitosan is added at the end of the alcoholic fermentation.

For flotation clarification from must, chitosan can be added before or during the alcoholic fermentation. In spirits, it is used before filtration and bottling to remove mineral contaminants.

For microbiological stabilization in wine, cider, and beer, chitosan can be added during all process of winemaking.

Regardless of the technological purpose, the sediments that contain the chitosan are removed from the wine, must, or spirits at the end of the treatment by physical separation processes such as racking, centrifugation and/ or filtration. Since chitosan is insoluble at slightly acidic to neutral pH levels, as well as in aqueous and ethanol solutions, it is unlikely that any residual chitosan will remain in the treated

products. High-performance liquid chromatography (HPLC) analyses for residual chitosan in wine processed with chitosan indicate that the final product is free from chitosan carry-over products up to the limit of detection of the analysis method (10 mg/L).

Therefore, the estimated intake of chitosan from all proposed technological uses can be considered as negligible. Chitosan derived from *A. niger*, was shown to be chemically and structurally equivalent to shellfish derived chitosan. The principal difference between the two chitosan preparations is the presence of small quantities of *beta*-1,3-glucans in *A. niger* sources of chitosan that are not present in shellfish chitosan. Therefore, data establishing the safety of shellfish-derived chitosan are considered relevant to the safety evaluation of fungal chitosan for the proposed food uses described herein.

Shellfish derived chitosan is widely available in the food supply through use in dietary supplement products, industrial, pharmaceutical, agricultural, and cosmetic applications, and background exposures to chitosan are therefore expected to exceed those occurring from the proposed food uses of fungal chitosan. Thus, based on the absence/trivial exposure to chitosan under the proposed food uses, calculation of estimated intakes was not deemed necessary in the assessment of the safety of the material under the proposed food uses in wine/alcoholic beverage processing for the GRAS determination.

A number of animal, human, and *in vitro* studies relevant to the safety of shellfish chitosan, which has a long history of safe use in the food supply, have been published. Published studies examining the metabolism and kinetics; acute, subchronic, and chronic toxicity; reproductive toxicity in animals; and safety in human of shellfish-derived chitosan or chitosan oligosaccharides are presented in the dossier.

Shellfish derived chitosan has a long history of safe use in the food supply. It is currently approved for use as a natural food additive for general food use in Japan and Korea (Japan Food Chemical Research Foundation, 2011; KFDA, 2011), and has widespread use as a dietary supplement product in the United States, the European Union, and other regulatory jurisdictions throughout the world. Finally, fungal chitosan (derived from *Agaricus bisporus* and *Aspergillus niger* sources) has been granted Novel Food approval by the European Commission, for use in supplement products in the European Union based on its substantial equivalence to existing shellfish derived chitosan products that are currently in the market[1].

Resolutions permitting the use of fungal chitosan in winemaking as a fining agent and contaminant treatment have been granted by the International Organisation of Vine and Wine (OIV/OENO 336A/2009; 337A/2009; 338A/2009; 339A/2009) (OIV, 2011) (Attachments 1-4).

A monograph for fungal chitosan has been added to the International Oenological Codex by decision if the OIV general assembly dated July 2009 considering the works of the group of experts "Specifications of Oenological Products" (OIV/OENO OIV/OENO 368/2009).

The corresponding approval for use of fungal chitosan in wine products marketed within the European Union has been issued by the European Commission (EU, 2011). Since 2011, chitosan from fungal origin is therefore approved as oenological practices for clarification according to ANNEX I of REGULATION (EU) No 53/2011 and for treatment of wines.

Fungal Chitosan (from *Aspergillus niger)* is GRAS self-affirmed for use as a processing-aid in the manufacture of alcoholic beverages. This GRAS self-affirmation was notified to the offices of the U.S. Food and Drug Administration (FDA), without objection from the Agency (FDA 2011, GRAS NOTICE No. 397).

The application of fungal chitosan in the winemaking process has been approved by the National Authority of Argentina.

The European Union requested the addition of chitosan and chitin-glucan of fungal origin to the Annex of the Wine Agreement in November 2010. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement.

A. Technical information on the processing aid

1. Information on the type of processing aid

1.1 Description

Chitosan is a linear polysaccharide of glucosamine and N-acetylglucosamine that is derived from chitin, a naturally occurring carbohydrate polymer that is widely distributed in nature (crustacean shells, fungal cell walls). Fungal chitosan is obtained by deacetylation of chitin present in the cell walls of non-genetically modified *A. niger* mycelium. Small quantities (10 to 15% wt/wt maximum) of *beta*-1,3-D-glucans also are present in the fungal chitosan as residuals from the manufacturing process. *beta*-1,3-D-Glucans are a major constituent of the cell walls of fungi, and also are present as structural components of many edible vegetables (Ko and Lin, 2004). *beta*-1,3-D-Glucans are composed of linear polysaccharide chains of varying molecular weight and can be linear (vegetable, and *A. niger* sources) or branched (Baker's yeast).

Fungal chitosan as a processing aid does not fall in any of the categories of processing aids listed in Standard 1.3.3. Evidence that the form and the amount of the processing aid perform the intended function has been demonstrated through laboratory and industrial experimentations. Report on industrial tests on chitosan from fungal source as a technological auxiliary on must and wine are enclosed in Appendix 1. These assessments have been conducted two years consecutively (year 2008-2009 and 2009-2010) under derogation of the DGCCRF (the French official body supervising such experiments) in France, in the frame of the Regulation EC 423/2008 (Art. 44) and Regulation EC 606/2009, respectively.

Fungal chitosan from *A. niger* is proposed for use as a processing-aid in the manufacture of wine, beer, cider, and spirits, as well as grain and beet derived food grade ethanol. Two fungal chitosans are proposed which differ slightly in their residual glucan contents as well as in their granulometry. There are proposed for use in stabilizing wine, cider and beer through their antimicrobial effect on a number of economically important microbial contaminants such as *Brettanomyces* as well as for flotation, clarification to reduce cloudiness and the content of unstable colloids, for use as fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, for reducing organic and mineral contaminants in wine and spirit, and for encapsulation of fermentation yeast, lactic bacteria and nutrients. Proposed food uses and use-levels of chitosan are detailed below in Table 3.2-1. The amount (use-levels) proposed are maximum use levels for the technological function to be achieved

Table 1.2-1 Summary of the Individual Proposed Technological Food-Uses and Use-Levels for Chitosan*

Food Category	Proposed Food-Uses	Technological Use	Use-Level (%)
Beverages, Alcoholic	Wine	Microbiological stabilization	10 g/hl
	Wine	Removal of organic contaminants	500 g/hl
	Wine	Removal of mineral contaminants	100 g/hl
	Wine	Clarification wine or in bottle (riddling)	100 g/hl
	Wine	Encapsulation of fermentation yeast lactic bacteria and nutrients	5%[2]
	Spirit	Removal of mineral contaminants	100 g/hl
	Cider	Microbiological stabilization	10 g/hl
	Beer	Microbiological stabilization	10 g/hl*
	Beer	Clarification	100 g/hl*

*the use levels are given for information purpose but chitosan is removed after treatment by physical separation process

As fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, clarification, and removal of mineral and organic contaminant, fungal chitosan is added at the end of the alcoholic fermentation.

For flotation clarification from must, chitosan can be added before or during the alcoholic fermentation. In spirits, it is used before filtration and bottling to remove mineral contaminants.

For microbiological stabilization in wine, cider, and beer, chitosan can be added during all process of winemaking. There is no analytical data available on residual chitosan after treatment of beer and cider.

[2] The 5% for encapsulation is based on data collected from the literature for food applications. The purpose is to use chitosan as an encapsule for sensitive molecules like lactic bacteria, yeast and yeast nutrients. This application has not been experimented yet and is provided as an example of other possible applications of chitosan in wine. The application is given for information only and there is not data on residual levels, although as chitosan is insoluble it will all be filtered out.

However, the product is expected to behave similarly in beer and cider than it behaves in wine. It is expected to be insoluble in beer and in cider as it is in wine and to be used at the same dose levels. The product is also removed after treatment by physical separation leaving behind negligible residual levels. Some tests have been performed on ciders showing the efficacy of the product to decrease the contamination of Brettanomyces. The product has been removed from cider by physical separation. No measurement of residual chitosan has been performed.

Regardless of the technological purpose, the sediments that contain the chitosan are removed from the wine, must, or spirits at the end of the treatment by physical separation processes such as racking, centrifugation and/ or filtration. Since chitosan is insoluble at slightly acidic to neutral pH levels, as well as in aqueous and ethanol solutions, it is unlikely that any residual chitosan will remain in the treated products. High-performance liquid chromatography (HPLC) analyses for residual chitosan in wine processed with chitosan indicate that the final product is free from chitosan carry-over products up to the limit of detection of the analysis method (10 mg/L).

Therefore, the estimated intake of chitosan from all proposed technological uses can be considered as negligible. Chitosan derived from *A. niger*, was shown to be chemically and structurally equivalent to shellfish derived chitosan. The principal difference between the two chitosan preparations is the presence of small quantities of *beta*-1,3-glucans in *A. niger* sources of chitosan that are not present in shellfish chitosan. Therefore, data establishing the safety of shellfish-derived chitosan are considered relevant to the safety evaluation of fungal chitosan for the proposed food uses described herein.

2. Information on the identity of the processing aid

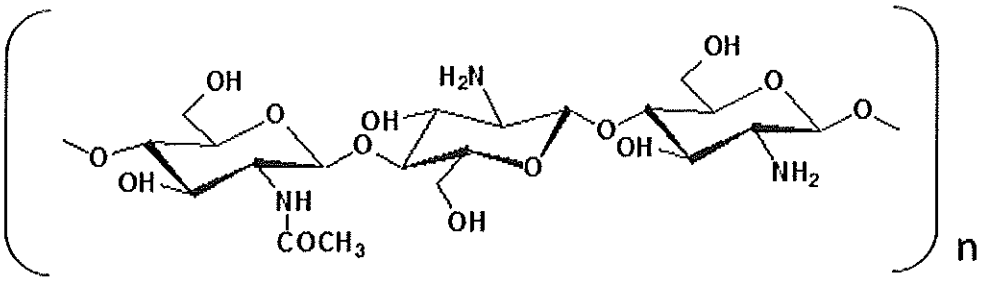
2.1 Chemical Abstracts name

2-Amino-2-deoxy-poly-D-glucosamine

2.2 International Union for Pure and Applied Chemistry name:

(2R, 3R, 4R, 5S, 6R) 3-amino-6-(hydroxymethyl) oxane-2, 4, 5-triol

2.3 Structural formula:



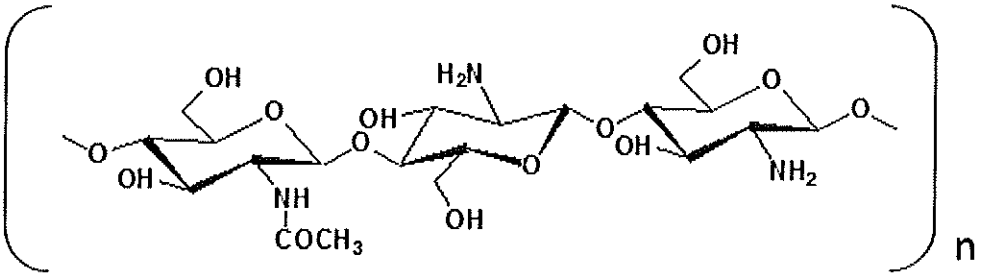
2.4 Common Name:
Fungal Chitosan

2.5 Manufacturer's code:
Tariff code: 3913900099

2.6 Marketing name:
At the moment fungal chitosan to be used in microbiological stabilization of wines is marketed under KiOfine-B, No Brett Inside but could be marketed also by third party distributors under their trade-names.
The fungal chitosan to be used in other wine applications (clarification, fining, removal of contaminants) is in final marketing development and trade-names are under final selection.

2.7 Chemical Abstract Service (CAS) Number
Chitosan: 9012-76-4

2.8 Chemical and Physical Characteristics

Molecular formula	Chitosan: $(C_6H_{11}NO_4)_n$
Chemical Structure	Chitosan 
Viscosity	Mv of 10-15K (Mv= viscosimetric molecular weight)
Appearance and Odor	Odorless, off white to slightly brownish fine free-flowing powder.

3. Information on the chemical and physical properties of the processing aid

Fungal chitosan products are provided as an odorless, off white to slightly brownish fine free-flowing powder with a tapped density >0.7g/cm^3. The two products differ in terms of their granulometry, i.e. 90% less than 50 µm and 90% less than 500 µm, in relation with their intended use. Except for the

content of residual beta-glucans that slightly differ, the two products have an overall similar chemical composition and share the same specifications (see Table 5).

Chemical properties of chitosan are as follows: linear polyamine, presence of amino and hydroxyl groups,chelating properties (metal ions). The physicochemical properties of chitosan in solution are related to the degree of deacetylation and molecular weight.

As fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, clarification, and removal of mineral and organic contaminant, fungal chitosan is added at the end of the alcoholic fermentation. For flotation clarification from must, chitosan can be added before or during the alcoholic fermentation. In spirits, it is used before filtration and bottling to remove mineral contaminants. For microbiological stabilization in wine, cider, and beer, chitosan can be added during all process of winemaking for fermentation yeast, lactic bacteria, and nutrients encapsulated within chitosan, it can be used during all process of winemaking

Regardless of the technological purpose, the sediments that contain the chitosan are removed from the wine, must, or spirits at the end of the treatment by physical separation processes such as racking, centrifugation, or filtration. Since chitosan is insoluble at slightly acidic to neutral pH levels, as well as in aqueous and ethanol solutions, it is unlikely that any residual chitosan will remain in the treated products. High-performance liquid chromatography (HPLC) analyses for residual chitosan in wine processed with chitosan indicate that the final product is free from chitosan carry-over products up to the limit of detection of the analysis method (10 mg/L) (see Appendix 2 for results).

Therefore, the estimated intake of chitosan from all proposed technological uses can be considered as negligible. For example, using a conservative estimate of chitosan carry-over into wine of 10 mg/L (detection limit of HPLC analysis), the consumption of two 750 mL bottles of wine per day would result in exposures chitosan exposures of 15 mg per person, equivalent to 214 µg/kg bodyweight for a 70 kg individual. This intake estimate represents a gross overestimate of exposure to chitosan under the proposed food uses. Moreover, shellfish derived chitosan is widely available in the food supply through use in dietary supplement products, and industrial, pharmaceutical (excipient), agricultural (pesticide), and cosmetic applications, and background exposures to chitosan are therefore expected to exceed those occurring from the proposed food uses of fungal chitosan. Thus, based on the absence/trivial exposure to chitosan under the proposed food uses described in Table 1.2-1, calculation of estimated intakes was not deemed necessary in the assessment of the safety of the material under the proposed food uses in wine/alcoholic beverage processing for the GRAS determination.

4. Manufacturing process

4.1 Raw Material

Fungal chitosan is manufactured from the non-viable post-fermentation microbial biomass of non-genetically modified *A. niger,* which is sourced from manufacturers of food grade citric acid and is permitted for use in feed applications in the European Union. Commercial sources of *A. niger* used for production of chitosan are subject to strict quality control for compliance with specifications as described in Table 4.1.1. Sources of *A. niger* used for manufacture of chitosan are authorized for sale for

use in animal feed and conform to the European legislation (EC Directives 2002/32/EC, 2003/57/EC, and 2003/100/EC) regarding contaminants (heavy metals and aflatoxins), and pesticide residues (EC, 2002, 2003a,b). All raw materials and processing aids used in the manufacture of chitosan are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations, and/or are permitted for use in food as described in Table 4.1.2 below.

Table 4.1.1 Quality Control Specifications for *A. niger* Raw Material Used for Production of Chitosan

Analysis Parameter	Specification
Water Content (%)	≤10%
Ash (% dw)	≤2%
Protein (%)	≤10%
Lipids (%)	≤1%
Density (kg/m^3)	≤670
Metals (ppm) As Hg Pb Cu Cd Zn	 ND ND ≤0.3 ≤ 0.3 - 4.5 ≤0.2 ≤17 – 20
Bacteria cfu/g	≤1000
Yeasts and Molds cfu/g	≤10

dw = dry weight; ND = not detected

Table 4.1.2 Raw Materials and Processing Aids Used in the Manufacture of Chitosan

Material	Use	Regulatory Status
Aspergillus niger	Source of chitin	Permitted for use in the production of citric acid for use in food in the United States (21 CFR §173.280 – U.S. FDA, 2011)
Processing-Aids		
Water	Solvent	N/A
Sodium Hydroxide	Processing-aid	In accordance with 21 CFR §184.1763, sodium hydroxide is permitted for use in food as a processing aid with no limitations other than cGMP (U.S. FDA, 2011).
Acetic acid	Processing-aid	In accordance with 21 CFR §184.1005, acetic acid is a direct food substance affirmed as generally recognized as safe (U.S. FDA, 2011).

N/A = Not applicable

4.2 Method of Manufacture

Fungal chitosans are obtained through the deacetylation (*i.e.,* hydrolysis of acetyl groups) of chitin present within the cell wall of *A. niger* mycelium. For the manufacture of chitosan, the manufacturer follows the Belgian legislation relative to auto-control in the food industry[3] and the auto-control guide established by the Belgian Federation for food supplements, dietary, and organic products (NAREDI) for the traceability, mandatory notification and risk analysis based on the Hazard Analysis and Critical Control Points (HACCP) method[4]. A schematic of the manufacturing process is described below (Figure 4.2-1). Briefly, *A. niger* raw material, meeting quality control standards, is introduced to a stainless steel vessel, and the material is subject to thermal alkali hydrolysis (sodium hydroxide) conditions which result in the partial deacetylation of chitin as well as the hydrolysis of native proteins and nucleic acids, and saponification of cellular lipids. Following several wash steps, the chitosan is solubilized using acetic acid, the acid insoluble constituents are removed through filtration and washing, and the chitosan is then precipitated using sodium hydroxide. The chitosan is then washed, concentrated, and dried. The dried material is held in quarantine for quality control testing prior to packaging and storage. Two chitosan are produced. During manufacturing, one is subject to an additional filtration step which results in a reduction in the amount of insoluble *beta*-(1,3)-D-glucans in the material relative, rendering the ingredient more suitable for use in microbial growth control applications; otherwise the materials are compositionally indistinguishable.

[3] Arrêté Royal 27/04/2007; Arrêté Royal 24/10/2005; Arrêté Royal 22/01/2004; Arrêté Royal 14/11/2003 (AFSCA, 2011)

[4] http://www.naredi.be/engels/home.htm (Naredi, 2011)

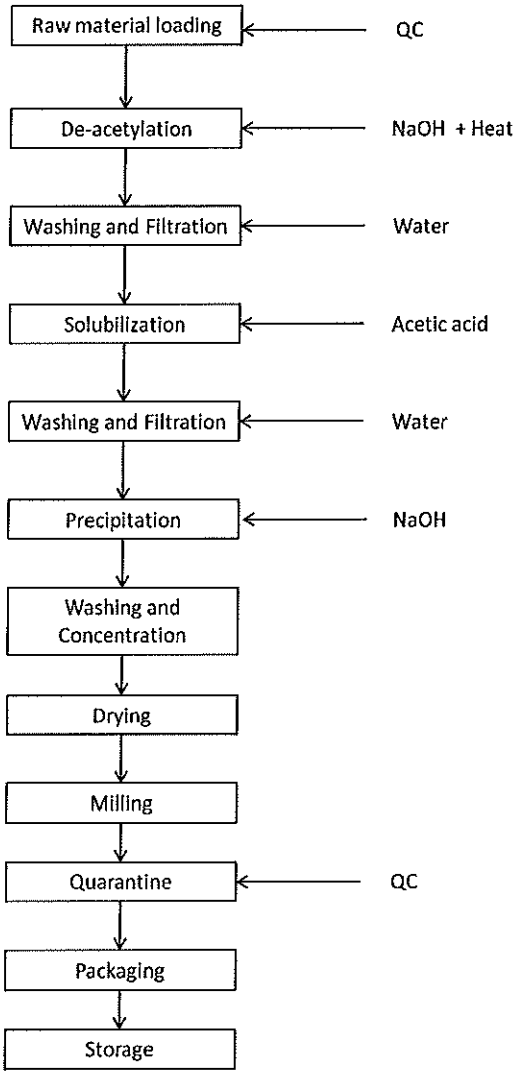


Figure 4.2.-1 Schematic Overview of the Manufacturing Process for Chitosan

The processing aid has been tested for the presence of the food allergens including sulphites, milk (including lactose, casein), eggs, peanuts, nuts, almonds, pistachio, gluten, soy, celery, mustard, sesame seeds, lupin, mollusks, crustaceans, fish. Results are negative (or at concentration below the detection limit) which confirms that the manufacturing process does not carry-over any allergens.

5. Specification for identity and purity

5.1. Product specifications

The detailed product specifications for fungal chitosan are provided in Table 5 below. Analytical methods are also described below. Some methods are internal analytical methods developed and validated by the manufacturer.

Table 5 Product Specifications for fungal chitosan

Test Parameters/Test	Specification	
Identification-Composition		
Degree of acetylation (mol%)	0 – 30	
Residual glucans (%, w/w)	≥2	
Viscosity 1% in HAc 1% (mPa.s)	1 – 15	
Taped density (g/cm^3)	≥0.7	
Loss on drying	≤10	
Ash (%, w/w)	≤3	
Soluble residues (%, w/w)	≤5	
Heavy Metals		
Mercury (mg/kg)	≤0.1	
Lead (mg/kg)	≤1	
Arsenic (mg/kg)	≤1	
Cadmium (mg/kg)	≤1	
Chromium (mg/kg)	≤10	
Zinc (mg/kg)	≤50	
Iron (mg/kg)	≤100	
Copper (mg/kg)	≤30	
Microbial		
Aerobic microbial count (cfu/g)	<1000	
Yeasts and moulds (cfu/g)	<100	
Coliforms (cfu/g)	<100	
Enterobacteriaceae (cfu/g)	<10	
Salmonella	Absence/25 g	

In addition to these specifications, information on purity and particle size can also be provided. The purity, refereed here is the chitosan content which is calculated on the basis of the sample dried weight by removing the values of glucans, proteins and ashes from the sample dried weight.

The specification on the particle size is ≥ 90% less than 50 µm for the first product and ≥90% less than 500 µm for the other one.

5.2 Analytical methods

Test methods are briefly described below.

5.2.1 Degree of acetylation

The degree of acetylation of is determined by potentiometric titration, using. The method relies on the titration of hydrochloric acid in excess to amino groups of chitosan using sodium hydroxide. Briefly, chitosan is dissolved in an excess of dilute hydrochloric acid prepared from hydrochloric R solution. The solution is then titrated with dilute sodium hydroxide prepared from sodium hydroxide R solution, using an automatic titrator (KEM, automatic potentiometric titrator, AT-500N), and the pH is measured. The titration curve shows two inflection points. The difference between these two inflection points among the abscissa corresponds to the amount of acid required to protonate the amino groups of chitosan.

5.2.2 Residual glucans

The determination of residual beta-glucan is performed by UV spectrophotometry (Thermospectronic Biomate 3. Briefly, beta-glucan is thermally decomposed by addition of hot sulphuric acid R, leading to

hydroxymethylfurfural moieties that are able to react with phenol and form a yellowish to brown colored product which absorbs at a wavelength of 420 nm. The absorption intensity of the solution is then compared to an external calibration curve established with a reference oat beta-glucan (medium viscosity, lot #60501, Megazyme) using the same method.

5.2.3 Viscosity

The apparent viscosity of the chitosan solution is measured using a calibrated rotational viscometer (Brookfield digital viscosimeter model DV-II+pro) at controlled temperature, using an appropriate spindle, spindle rotation speed and a temperature-controlled bath.

5.2.4 Loss on drying

The loss on drying of is determined thermogravimetrically. Briefly, a known quantity of sample is heated at 105°C, and the sample weight loss is continuously measured using a calibrated moisture analyser (Ohaus MB 45) until reaching a value less than 1mg per 90s. When this value is reached, the weight of dry matter is calculated by removing the loss on drying value from the total weight.

5.2.5. Ash

The total ash content is determined. The porcelain crucible is weighed. A known quantity of chitosan is placed in the porcelain crucible and heated for 10hrs at 600°C in a calibrated muffle oven (Carbolite, 201). After combustion, porcelain crucible containing the chitosan sample is weighed.

5.2.6. Soluble residues

A known quantity of chitosan is washed with water and filtered. The residual matter is then filtered and weighed.

5.2.7. Mercury, Lead, Arsenic, Cadmium, Chromium, Zinc, Iron Copper

The individual content of metals is determined by ICP-MS derived from the ISO 11885 standard.

5.2.8. Total viable aerobic microbial count

The total viable aerobic microbial count is determined according to the ISO 4833:2003 standard, an horizontal method for the enumeration of microorganisms, by counting the colonies growing in a solid medium after aerobic incubation at 30°C.

5.2.9. Total yeasts and molds count

The total yeasts and molds count is determined according to the ISO 7954 standard, a horizontal method for the enumeration of yeasts and molds, by counting the colonies growing in a solid medium after aerobic incubation.

5.2.10. Coliformes

The amount of *Escherichia coli* is determined according to the ISO7251 standard, a horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* (Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide)

5.2.11. Enterobacteriaceae

The amount of *Enterobacteriaceae* is determined according to the ISO 215-28-2 standard, a horizontal method for the detection and enumeration of *Enterobacteriaceae* (Colony-count method).

5.2.12. Salmonella

The amount of *Salmonella* is determined according to the ISO 6579 standard, a horizontal method for the detection of *Salmonella*, including *Salmonella typhi* and *Salmonella paratyphi*

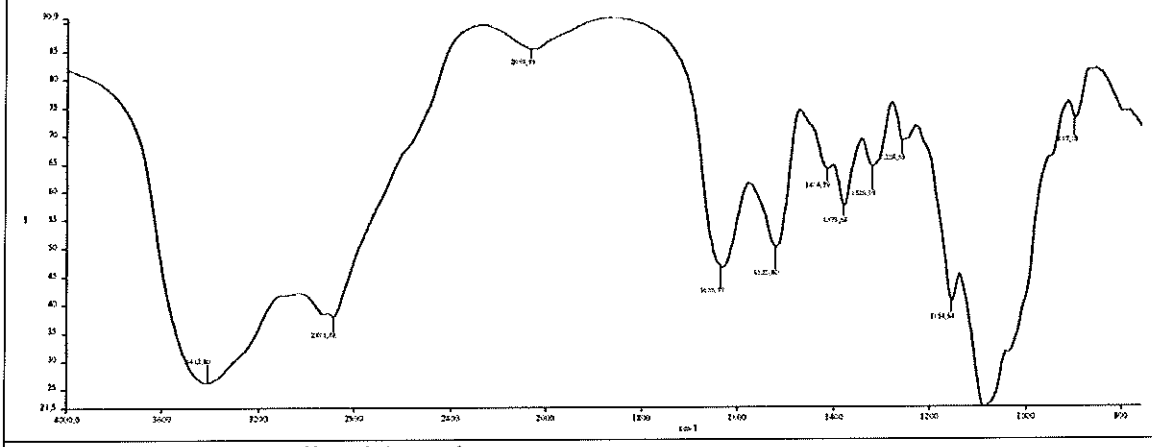
5.3 Analytical method for identification

The methods for identification of chitosan are reported below.

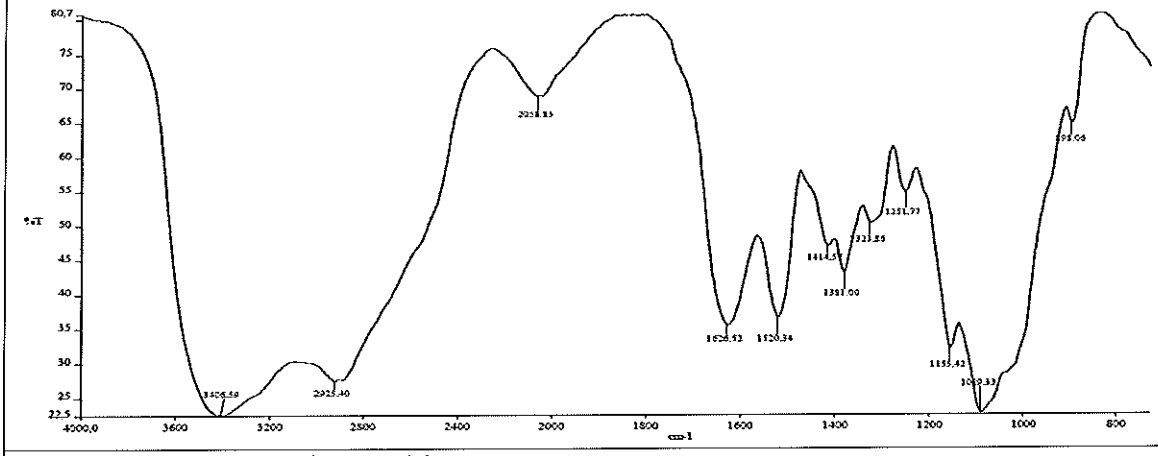
5.3.1. Infrared Spectroscopy Analysis

Infrared (IR) spectroscopy is one of the most common methods used for chemical characterization of chitosan (Kumirska *et al.*, 2010). Using Fourier Infrared Transmission (FTIR) spectroscopy chemical analysis, shellfish chitosan sources were compared to *A. niger* derived chitosan. Two shellfish sources of chitosan were used for the comparison: a commercial food grade source of chitosan currently on the market for use in food, and supplement products throughout the world; and chitosan chlorhydrate, a qualified European Pharmacopoeia chitosan standard (Ph. Eur., 2004). As shown in Figure 2.2.8-1 below, all three sources of chitosan produced comparable FTIR absorption spectral profiles, supporting the chemical equivalence of *A. niger* derived chitosan sources to shellfish sources.

A – European Pharmacopoeia Standard Chitosan Chlorhydrate



B – *A. niger* Derived Chitosan (fungal chitosan)



C – Shellfish Derived Chitosan (Food grade)

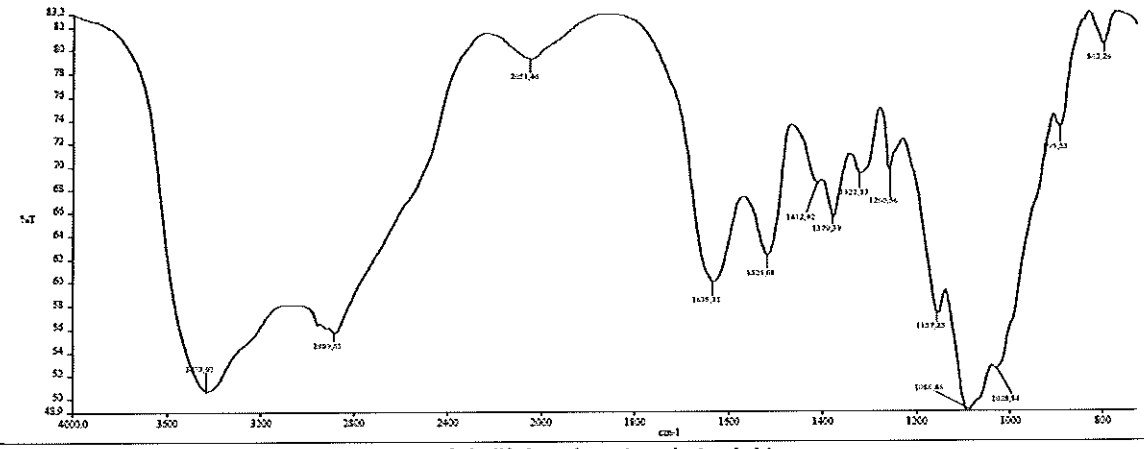


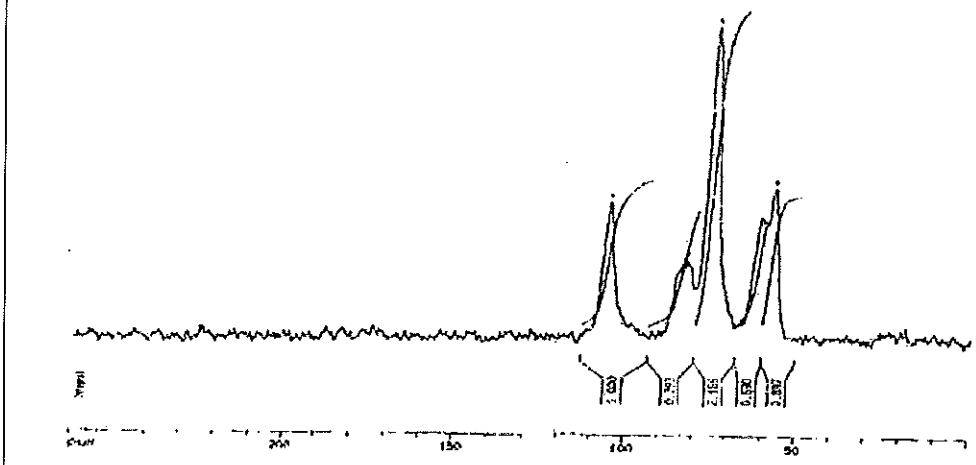
Figure 2.2.8-1 FTIR spectral comparison of shellfish and *A. niger* derived chitosan

Each peak within the absorption spectrum corresponds to the vibration frequency of a component of the molecule, which allows for quantitative differentiation of bond types within chemically similar materials. **A)** European Pharmacopoeia chitosan chlorohydrate standard (shellfish derived); **B)** commercial food grade chitosan (shellfish derived); **C)** *A. niger* derived chitosan Analysis conducted in accordance with the Ph.Eur. monograph of chitosan chlorhydrate 1774.

5.3.2 NMR Spectroscopy

A. niger derived chitosan was further characterized using Nuclear Magnetic Resonance (NMR) spectroscopy of carbon 13 in the solid state (13C-NMR), a technique that is well suited for use with polysaccharides such as chitosan (Kumirska *et al.*, 2010). As depicted in Figure 2.2.8-2, the NMR spectrum generated for *A. niger* derived chitosan was equivalent to that of shellfish derived chitosan. The two additional peaks in the NMR spectrum *A. niger* derived chitosan were identified to be a carboxyl group (173 ppm) and methyl group (23 ppm) of the N-acetylglucosamine units.

A - Shellfish Derived Chitosan (Food grade)



B – *A. niger* Derived Chitosan

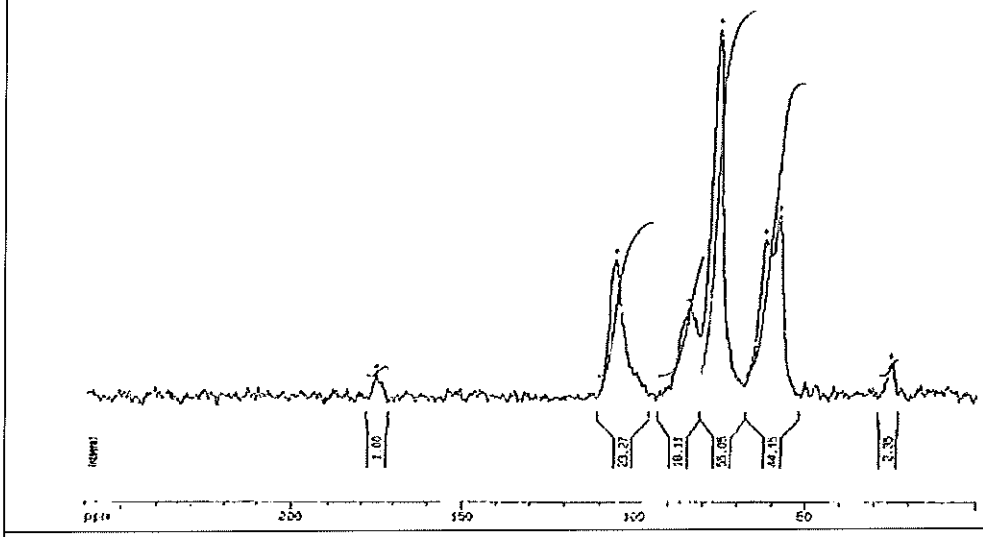


Figure 2.2.8-2 NMR Spectroscopy
A) commercial food grade chitosan (; shellfish derived); B) *A. niger* derived chitosan(fungal chitosan)

6. Analytical method for detection

Since no residues are likely to be present in the wine after treatment, there is no need to provide analytical method for detection and quantification of the amount of processing aid remaining in the wine. It should however be pointed out the HPLC (High Performance Liquid Chromatography) may be suitable method for detection of chitosan, although the limit of detection is quite high in regard with the use levels.

B/ Information related to the safety of a chemical processing aid

B1. General information on the non-food industrial use of the chemical

Due to its physical and chemical properties, chitosan is being used in a wide variety of product and applications going from pharmceutical, medical and cosmetics products to water treatment and plant protection. In pharmaceutical applications, chitosan is used as an excipient for drug delivery systems. In medical devices, it is uses as for its haemostatic, wound healing and antibacterial properties in different forms (sponges, granules, bandages, fibers, sutures...). In cosmetics, chitosan is used for hair care (in shampoos...), skin care (in body washes, creams, lotions...) and oral care (in toothpastes, mouthwashes, chew-gum...) to prevent formation of plaque. Other industrial applications of chitosan include water engineering (flocculating agent, chelating agent and heavy metal trappers), textile and paper industry; and agriculture. Considerable amount of chitosan are used in waste water treatment. For all these applications, shellfish derived chitosan has been used so far since fungal chitosan has been developed recently.

A number of animal, human, and *in vitro* studies relevant to the safety of shellfish chitosan, which has a long history of safe use in the food supply, have been published. Chitosan derived from *A. niger,* was shown to be chemically and structurally equivalent to shellfish derived chitosan (section 5.3). The principal difference between the two chitosan preparations is the presence of small quantities of *beta*-1,3-glucans in *A. niger* sources of chitosan that are not present in shellfish chitosan. Therefore, data establishing the safety of shellfish-derived chitosan are considered relevant to the safety evaluation of fungal chitosan for the proposed food uses described herein.

Published studies examining the metabolism and kinetics; acute, subchronic, and chronic toxicity; reproductive toxicity in animals; and safety in human of shellfish-derived chitosan or chitosan oligosaccharides are summarized below. Generally available studies conducted in adult subjects have evaluated the safety and tolerability of repeated consumption of chitosan, and multiple studies investigating the effects of consuming shellfish-derived chitosan on various biological parameters (*e.g.*, plasma lipid levels, mineral and vitamin absorption, weight gain, sugar metabolism) have been reported.

Shellfish derived chitosan has a long history of safe use in the food supply. It is currently approved for use as a natural food additive for general food use in Japan and Korea (Japan Food Chemical Research Foundation, 2011; KFDA, 2011), and has widespread use as a dietary supplement product in the United States, the European Union, and other regulatory jurisdictions throughout the world. Supplement products typically promote consumption of 1 to 2 g/person/ day for use in weight control, and/or maintenance of cardiovascular health. Several chitosan hydrolysate formulations also are approved for use as a plant growth enhancer, and as a substance that boosts the ability of plants to defend against fungal infections. Exemption from the requirement of a tolerance requirement for these applications has been granted by the United States Environmental Protection Agency (Proposed final rule; U.S. EPA, 2008).

Finally, fungal chitosan (derived from *Agaricus bisporus* and *Aspergillus niger* sources) has been granted Novel Food approval by the European Commission, for use in supplement products in the European Union based on its substantial equivalence to existing shellfish derived chitosan products that are currently in the market[5].

Below are general information relevant to safety of chitosan as reported in the literature for shellfishes-derived chitosan.

1.1 Metabolic Fate

A limited number of studies have examined the metabolic fate of chitosan. Similar to dietary fibers, chitosan is poorly absorbed, is not subject to digestive processes within the gastrointestinal tract, and therefore travels intact throughout the small intestine to the colon where it is then subject to microbial fermentation and excretion in the feces. These findings have been summarized in brief below.

1.2.1 Absorption and Distribution

Chitosan is poorly absorbed due its highly insoluble physico-chemical properties (reviewed in Kean and Thanou, 2010). Upon ingestion, chitosan is solubilized by hydrochloric acid in the stomach and converted into a viscous liquid that emulsifies dietary fat droplets. As this viscous chitosan gel enters the duodenum, it starts to precipitate due to the gradual increase in pH and is excreted in the feces (described in Furda 2000). *In vitro* cellular models have also provided additional evidence that chitosan is poorly absorbed. Chitosan with molecular weight of 30 kDa or higher was not taken up by intestinal epithelial Caco-2 cells (Schipper *et al.*, 1997). A subsequent study also demonstrated water-soluble chitosans of 230 kDa did not penetrate through the Caco-2 cell layer (Chae *et al.*, 2005).

Zeng *et al.* (2008a) labeled chitosan with FITC (fluorescein isothiocyanate) and administered it to female Kunming mice *via* oral gavage. Following a dose of 500 mg/kg of FITC-chitosan, blood samples were collected at 30, 60, 120, and 240 minutes. Four preparations of chitosans were used: chitosan oligomer (MW=0.99 kDa); middle molecular weight chitosan (M-chitosan, MW=32.7 kDa), water-soluble chitosan (MW=39.1 kDa); and high molecular weight chitosan (H-chitosan, MW=760 kDa). The authors reported that the extent of chitosan absorption was inversely related to its molecular weight, and occurred in the following rank order: H-chitosan < M-chitosan < chitosan oligosaccharide. Water-soluble chitosan had the greatest amount absorbed compared to all of the compounds tested despite its moderate molecular weight, which may be attributed to its greater water solubility. In another study, FITC-labeled chitosan was orally administered to Sprague-Dawley rats at a dose of 20 mg/kg (Chae *et al.*, 2005). Chitosans, ranging from 3.8 to 230 kDa, were detected in the plasma with levels peaking at 30 minutes after oral administration. However, high molecular weight chitosan (230 kDa) was not absorbed and negligible amounts were detected in the plasma. Furthermore, the area-under-the-curve (AUC) values for the 3.8 kDa chitosan was nearly 12 times higher than that for 22 kDa chitosan.

[5] Notification #108 Pursuant to Article 5 of Regulation (EC) No 258/97 of the European Parliament and of the Council (April 2007) (EC, 1997, 2007)

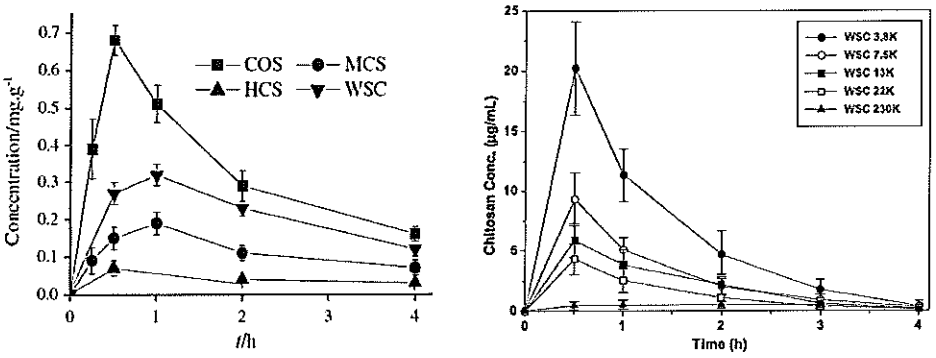


Figure 1.2.1-1 Plasma concentration following oral administration of chitosan.
Data were taken from Zeng *et al.*, 2008a (left) and Chae *et al.*, 2005 (right). COS = chitosan oligosaccharide; MCS = middle molecular weight chitosan; HCS = high molecular weight chitosan; WSC = water-soluble chitosan. WSC 3.8K to WSC 2430K refers to WSC ranging in size from 3.8 to 230 kDa.

Interpretation of these findings is complicated by several limitations in study design. For example, FITC labeling of chitosan is known to be highly variable, with coupling efficiency ranging between 24 to 91% (Ma *et al.*, 2008). This variability is due to the fact that isothiocyanate reacts optimally with neutral amine groups at pH 9.0, and most chitosan are insoluble when amine groups are neutral at pH ≥6.5, preventing homogenous labeling. Also, no control groups were used in the studies by Chae *et al.* (2005) or Zeng *et al.* (2008a), and non-specific binding of the FITC label may have occurred. Thus, the significance of the authors' findings must await confirmation using other sensitive assays.

1.2.2 Metabolism

The human digestive tract can efficiently hydrolyze glucose polymers linked by *alpha*-glycosidic linkages, such as those found in starch and glycogen, and the *beta*-glycosidic bond in lactose can be hydrolyzed by *beta*-galactosidase (Wisker *et al.*, 1985). The *beta*-glycosidic bonds in chitosan are resistant to hydrolyzation by hydrochloric acid present in the stomach; however, these bonds can be hydrolyzed by chitosanases to produce a mixture of chitosan oligomers ranging between 2 and 8 degrees of polymerization, which can be subsequently degraded further to glucosamine (reviewed in Shaikh and Deshpande, 1993; Muzzarelli *et al.*, 1997).

Chitin can also be hydrolyzed by chitinases (reviewed in Shaikh and Deshpande, 1993; Muzzarelli *et al.*, 1997). Although traditionally thought to be chitin-specific, non-specific chitinases have been shown to hydrolyze chitosan (reviewed in Aam *et al.*, 2010). At least 2 functional chitinases have been identified in humans; chitotriosidase is unlikely to be involved in the digestion of ingested chitosan as it is expressed primarily in the lymph nodes, bone marrow, and lungs. Acidic mammalian chitinase (AMCase) have been isolated in the human stomach, tears, sinus mucosa, and lungs, though its activity on chitosan has yet to be characterized.

Recent *in vitro* studies have reported that other digestive enzymes, including pepsin, amylase, and lipase, can hydrolyze chitosan at rates comparable to chitosanase (reviewed in Xia *et al.*, 2008). However, it is unknown whether these enzymes can effectively hydrolyze chitosan in the human gastrointestinal tract. Lysozyme, in addition to hydrolyzing the glycosidic linkages of bacterial cell wall peptidoglycans, can also

hydrolyze chitosan and chitin (Aam *et al.*, 2010). Varum *et al.* (1997) incubated three different chitosan with different degrees of deacetylation (42, 51, and 60%) in human serum, and measured degradation rates by changes in viscosity as a function of time. The degradation rate increased proportionally with the degree of deacetylation. Addition of lysozyme increased degradation rates, while addition of allosamidin, a chitinase inhibitor, had no effect. The authors concluded that the degradation of chitosan in human serum is mediated primarily by lysozyme and not other enzymes or depolymerizing mechanisms. In another study, chitosan with approximately 70% deacetylation was found to have the greatest susceptibility to lysozyme degradation (Sashiwa *et al.*, 1990).

1.2.3 Fermentation by Intestinal Microflora

Bacteria are known to express chitosanases (Gooday, 1989), and it is plausible that the intestinal microflora may also possess the ability to hydrolyze chitosan. Although it is unknown whether the intestinal microflora in humans expresses chitosanases or other enzymes that can degrade chitosan, rat bacterial enzymes isolated from the colon were able to degrade chitosan *in vitro* (Zhang and Neau, 2002). In another study examining the utility of chitosan as an excipient for the delivery of peptide drugs, pills containing 5(6)-carboxyfluorescein (CF) that were encapsulated by chitosan were not degraded when incubated with artificial gastric or intestinal juice, but its contents were released when incubated with rat cecal contents (Tozaki *et al.*, 1997). Moreover, oral administration of chitosan encapsulated pills containing insulin resulted in the systemic absorption of insulin, with the hypoglycemic effects observed as the capsules entered the colon.

Although it is unknown whether bacterial strain with chitosanase activity exists in the human intestines, *Clostridium paraputrificum* was isolated from human feces containing endochitinase and *beta*-N-acetylglucosaminidase activity. *In vitro* cultivation of the bacteria with colloidal chitin resulted in the production of hydrogen, carbon dioxide, acetate, and lactate, as well as minute quantities of propionate and butyrate (Simunek *et al.*, 2002).

1.2.4 Elimination

Onishi *et al.* (1999) administered FITC-labeled chitosan (50% deacetylation) intraperitoneally (29 mg/kg body weight) to 3 male ddy mice. Urine was collected at 1, 14, and 24 hours following chitosan administration; approximately 25% of the dose was excreted in the urine within 1 hour, and nearly the entire chitosan dose was accounted for in the urine by 14 hours. In addition, Richardson *et al.* (1999) administered [^{125}I]-labeled chitosan of various molecular weights (<5 kDa, between 5 to 10 kDa, and >10 kDa) intravenously to male Wistar rats. Chitosan with molecular weights >5 kDa was rapidly cleared from the plasma. At 60 minutes following injection, <10% of the administered dose recovered in the plasma while more than 50% of the administered dose was recovered in the liver. In contrast, approximately 30% of chitosan with smaller molecular weight (<5 kDa) was recovered in the plasma, and approximately 30% was found in the liver, at 60 minutes post-injection.

As a conclusion, neither chitosan nor *beta*-1,3-D-glucans are absorbed from the gastrointestinal tract, thus systemic exposure does not occur. Some evidence that small molecular weight chitosan preparations (*i.e.*, chitosan oligomers, ~1kDa) may be absorbed intact in mice was reported. However, these studies were confounded by poor study design and the use of non-qualitative analytical methods

for detection of chitosan oligosaccharides/metabolites in the plasma, limiting the reliability and usefulness of the data. Moreover, monomeric constituents of chitosan (*i.e.* glucosamine and N-acetylglucosamine) are known to be poorly absorbed in most animals species tested (Simon *et al.*, 2011), which contradicts literature reports that chitosan oligomers may be bioavailable.

Although there is no evidence presented in the literature to suggest that chitosan would be digested/hydrolyzed during gastrointestinal transit, putative hydrolysis products generated during transit would consist of compounds (chitosan oligomers, glucosamine, N-acetylglucosamine and glucose) that are known to be poorly bioavailable, and non-toxic even when consumed at high dietary concentrations in animals and humans (Lee *et al.*, 2004; Anderson *et al.*, 2005; Takahashi *et al.*, 2009). Therefore it can be concluded that neither chitosan nor *beta*-1,3-D-glucans would be chemically altered (metabolized) in the human gastrointestinal tract; limited colonic metabolism of chitosan by endogenous microflora could potentially occur. Microbial fermentation of chitosan and *beta*-1,3-glucans would produce innocuous metabolites of fermentation such as short-chain fatty acids, and H_2, CO_2, and CH_4 gases. Exposure to these metabolites occurs daily from the consumption of non-digestible dietary fiber.

B2. General information on the use of the chemical as a food processing aid in other countries:

2.1. OIV Resolution and Monograph

Resolutions permitting the use of fungal chitosan in winemaking as a fining agent and contaminant treatment have been granted by the International Organisation of Vine and Wine (OIV/OENO 336A/2009; 337A/2009; 338A/2009; 339A/2009) (OIV, 2011) (Attachments 1-4)

A monograph for fungal chitosan has been added to the International Oenological Codex by decision if the OIV general assembly dated July 2009 considering the works of the group of experts "Specifications of Oenological Products" (OIV/OENO OIV/OENO 368/2009) (Attachment 5).

In appendix the following documents are attached:

- Appendix 3 Resolution OIV oeno 336A-2009
- Appendix 4 Resolution OIV oeno 337A-2009
- Appendix 5 Resolution OIV oeno 338A-2009
- Appendix 6 Resolution OIV oeno 339A-2009
- Appendix 7 Resolution OIV oeno 368-2009

2.2. Approval in European Union

The corresponding approval for use of fungal chitosan in wine products marketed within the European Union has been issued by the European Commission (EU, 2011).

Since 2011, chitosan from fungal origin is therefore approved as oenological practices for clarification according to ANNEX I of REGULATION (EU) No 53/2011 and for treatment of wines under the conditions set up in Appendix 13. Commission Regulation CE 53/2011 (ENG) is provided in Appendix 8.

2.3 Approval in US

Fungal Chitosan (from *Aspergillus niger)* is GRAS self-affirmed for use as a processing-aid in the manufacture of alcoholic beverages. This GRAS self-affirmation was notified to the offices of the U.S. Food and Drug Administration (FDA), without objection from the Agency (FDA 2011, GRAS NOTICE No. 397, Appendix 9)

2.4 Approval in Argentina:

The application of fungal chitosan has been approved by the National Authority (Appendix 10).

3. Information on the toxicity of the chemical processing aid and if necessary its majors metabolites

Unless specified, information reported below has been collected from the literature on shellfish chitosan, or chitosan oligomers.

3.1 Acute Toxicity Studies

The acute oral toxicity of fungal chitosan, (KiOmedine-CsU[6]), was examined in female Sprague-Dawley rats (6/group). A single dose of 2,000 mg/kg body weight or vehicle control was administered by gavage and animals were followed for 14 days after administration. No clinical signs were presented and no changes were observed by macroscopic examination. The LD_{50} was determined to be greater than 2,000 mg/kg body weight in this study (KitoZyme 2008a).

The acute systemic toxicity of fungal chitosan was also examined in male Swiss mice (5/group). The polar extract of KiOmedine-CsU Chitosan was administered intravenously at a dose of 50 mL/kg body weight, and no clinical signs were observed up to 3 days after administration. As such, the LD_{50} of KiOmedine-CsU Chitosan was determined to be greater than 50 mL/kg body weight in this study (KitoZyme 2008b).

An additional study was identified in the literature that examined the acute toxicity of chitosan oligosaccharide in Kunming mice (Qin *et al.*, 2006). Mice of both sexes were administered a single dose of chitosan oligosaccharide (molecular weight of 1.86 kDa) *via* oral gavage at doses of 0, 1,000, 2,150, 4,640, or 10,000 mg/kg body weight and monitored for 7 days after treatment. Mice administered chitosan oligosaccharide did not exhibit any clinical signs of toxicity and no mortalities were recorded. The authors concluded that the oral maximum tolerant dose was greater than 10,000 mg/kg in mice.

3.2 Subacute Toxicity Study

Chitosan Oligosaccharides

Kim *et al.* (2001) evaluated the subacute toxicity of chitosan oligosaccharide in rats. The study was not conducted using GLP, and study methodologies were not reported to be consistent with recognized U.S., or International guidelines for toxicity testing of chemicals. Five-week-old male and female SPF Sprague-Dawley rats (n=9/sex/group) were administered 0, 500, 1,000, or 2,000 mg chitosan oligosaccharide/kg body weight/day by gavage for 28 consecutive days. Body weight and feed consumption were monitored weekly during the administration period. After the administration period, rats were killed by exsanguination under phenobarbitol anesthesia. No significant between-group differences at any time point were observed with respect to feed intake, body weight, clinical signs, or mortality. All urinalysis parameters measured at necropsy (*i.e.,* color, pH, and concentrations of glucose, ketone bodies, nitrites, protein, occult blood, urobilinogen, and bilirubin) were within normal ranges with the exception of increased mean leukocyte concentrations in the urine of male rats administered 500 mg chitosan oligosaccharide/kg body weight/day. However, this finding was not considered to be toxicologically relevant as it was observed only in males, and its occurrence was not dose-related.

Hematological analysis revealed significantly increased mean leukocyte concentration in male rats administered 2,000 mg chitosan oligosaccharide/kg body weight/day (compared to controls) and significantly decreased percentage of granulocytes in female rats administered 1,000 mg chitosan oligosaccharide/kg body weight/day. These changes were within normal ranges and were therefore not considered to be toxicologically relevant. A significant increase in mean platelet volume in male rats administered 1,000 mg chitosan oligosaccharide/kg body weight/day was reported, but its toxicological significance was not addressed by the study authors. However, given that increased mean platelet volume was observed in males only, and not in a dose-dependent manner, this finding is unlikely to be toxicologically significant. No significant differences between the treated and control groups were observed for the remaining hematological parameters assessed (*i.e.,* red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, platelet count, lymphocytes, and mid-range population).

Significantly decreased albumin and total blood protein concentrations were observed in male rats administered 500 mg chitosan oligosaccharide/kg body weight/day (compared to controls), although this difference was not considered to be toxicologically relevant due to a lack of a dose-response relationship. No significant between-group differences were observed for any other biochemical parameter assessed (*i.e.,* alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, lactate dehydrogenase, glucose, and triglycerides). In addition, no significant between-group differences attributable to treatment with chitosan oligosaccharide were reported with respect to organ weights or histopathological findings. The authors determined the no-observed-adverse-effect level (NOAEL) to be 2,000 mg/kg body weight/day in rats, the highest dose tested in this study.

3.3 Subchronic Toxicity Studies

Chitosan Oligosaccharides

One 90-day subchronic toxicity study was conducted in four week old female Kunming mice (10/group), weighing 20 to 26 g (Zeng *et al.*, 2008b). Four different types of chitosan with different molecular weights were prepared, including high molecular weight chitosan (H-chitosan; MW=760 kDa), middle molecular weight chitosan (M-chitosan; MW=32.7 kDa), water-soluble chitosan (MW=39.1 kDa) and chitosan oligomer (MW=0.99 kDa). Animals were fed a control diet or diet supplemented with chitosan at 1.05% provided *ad libitum* for 90 days, equivalent to intakes of approximately 500 mg/kg bw/day (U.S. FDA, 1993). Food intake was monitored daily, and animals were weighed and checked for signs of toxicity weekly. Upon termination of the study, the vital organs were removed for gross and histopathological examination. The levels of trace elements were also measured in the organs. No mortality and no significant clinical signs (*i.e.* food intake, abnormal behaviors and state of feces or fur) were observed in all treatment groups. No differences in body weight were observed among the chitosan treatment groups when compared to controls. Relative organ weights of the heart, liver, spleen, thymus, and kidney were not altered in the H-chitosan, M-chitosan, and chitosan oligomer treatment groups. However, relative thymus weight was significantly increased by 46% for animals in the water-soluble chitosan group compared to controls (0.22 *vs.* 0.15 g/100 g body weight). Gross examination of the organs did not reveal any treatment-related abnormalities, and histopathological findings were normal in all treatment groups. Trace levels of iron, zinc, and copper in the liver, heart, spleen and kidneys of animals in the H-chitosan, water-soluble chitosan, and chitosan oligomer groups did not differ from controls. However, compared to controls, animals in the M-chitosan group exhibited significantly higher iron levels in the liver (168 *vs.* 146 µg/g; $p < 0.05$) and spleen (958 *vs.* 843 µg/g; $p < 0.05$); significantly higher zinc levels in the liver (55 *vs.*32 µg/g; $p < 0.05$), spleen (45 *vs.*32 µg/g; $p < 0.05$), and heart (66 *vs.* 38 µg/g; $p < 0.05$); and copper levels in the liver (5.4 *vs.* 4.9 µg/g; $p < 0.05$). The authors attributed this finding to the greater accumulation of M-chitosan in the liver and other organs where they exert metal-chelating properties. Although oral administration of chitosan for 90-days did not produce explicit adverse effects, the use of a single dose of each test article and failure to adhere to GLP and OECD guidelines for toxicity testing present limitations to interpretation of the data.

Naito *et al.* (2007) conducted a 90-day toxicity study to determine the safety profile of oligoglucosamine (OG)[7], in rats. Six-week-old male and female F344 rats (10/sex/group) were given *ad libitum* access to food containing 0, 0.04, 0.2, or 1% OG for 90 days. The study was carried out in accordance with the Guidelines for Designation of Food Additives and for Revision of Standards of Use of Food Additives of the Japanese Ministry of Health, Labour and Welfare (Notification Eika No.29, March 22nd, 1996). No mortality was observed during the study period, and no abnormal clinical signs were observed in the control or 0.2% groups. Animals administered the 1% dose of OG developed clinical signs including swelling of the snout, auricals and forelimbs, alopecia of the forelimbs, and emaciation. All adverse clinical signs disappeared by the end of the study period, with the exception of piloerection. The observed erythema and loss of fur was considered by the study authors to be due to topical inflammation due to OG adhering to the skin and fur rather than systemic effects of OG ingestion. The study authors also proposed that OG may inhibit the absorption of vitamin E in the small intestine, potentially exacerbating any existing skin irritation.

[7] Chitosan oligosaccharide with 100% deacetylation

From Study Day 22 until the end of the study period, mean body weights of males and females in the 1% group were significantly lower than those of the control group. The study authors attributed this effect to feeding difficulties caused by the observed concomitant dermatitis. In males in the 1% group, feed consumption was significantly reduced by compared to control starting from Study Week 3 until the end of the study period ($p < 0.01$). In females in the 1% group, feed consumption was significantly reduced compared to control only on Study Days 15, 22, and 29 ($p < 0.01$).

Significantly more males in the 1% group were positive for proteinuria, ketone bodies, and bilirubin compared to the control group. Urinary volume also was significantly increased in males in the 1% group, and sodium, potassium, and chloride excretion were significantly decreased compared to controls. Males in the 0.2% group had significantly decreased urinary sodium and potassium excretion compared to controls, although concentrations remained within historical ranges. Females in the 1% group had significantly decreased levels of urinary sodium excretion, and significantly more females in the 1% group showed traces of urinary protein and ketone bodies compared to controls. No abnormalities were detected upon histological examination, and the increases in urinary protein are not likely to be indicative of renal dysfunction. In addition, the study authors suggested that the observed increases in urinary ketone bodies may have been due to increased use of body fat to compensate for reduced feed intake. No other significant differences with respect to urinary parameters (*i.e.,* color, pH, occult blood, red blood cells, crystals, casts, white blood cells, and epithelial cells) were reported.

In males in the 1% group, significant decreases in hemoglobin, and counts of red blood cells, lymphocytes, and platelets, and increases in mean corpuscular volume, mean corpuscular hemoglobin, and neutrophil count, compared to controls, were reported. In males in the 0.2% group, a significant decrease in red blood cell count (which remained within background control range), and significant increases in mean corpuscular volume and mean corpuscular hemoglobin, were reported. The only significant differences compared to controls in females were an increase in neutrophil count and a decrease in lymphocyte count. Changes in mean corpuscular volume and hemoglobin were within background control ranges, and were not considered to be toxicologically significant. The authors noted that changes in leukocyte and platelet counts may have been caused by the dermal inflammation. No other significant differences with respect to hematological parameters (*i.e.,* hematocrit, mean corpuscular hemoglobin concentration, eosinophils, basophils, and monocytes) were reported.

In males in the 1% group, significant decreases in blood levels of total protein, albumin, albumin:globulin ratio, creatinine, glucose, total cholesterol, triglycerides, calcium, and sodium compared to controls were reported. Albumin:globulin ratios were significantly reduced in dose-dependent manner in males in the 0.2 and 0.04% groups. However, these changes were within background control ranges and not accompanied by changes in albumin levels, and were therefore not considered to be toxicologically relevant. In females, significant decreases in blood levels of albumin, albumin:globulin ratio, calcium, and sodium were observed in the 1% group only. The study authors noted that the observed changes in blood biochemical parameters may have been attributable to reduced feed intake in the 1% group, and that reduced cholesterol levels may have been due to chitosan-induced increases in fecal cholesterol excretion. The toxicological relevance of the observed changes in creatinine concentration is unknown due to the absence of pathological kidney or liver abnormalities.

In males in the 1% group, significant decreases in most absolute organ weights (*i.e.,* thymus, heart, lungs, liver, spleen, kidneys, testes, prostate, and seminal vesicles), and significant increases in most organ weights relative to body weight (*i.e.,* brain, heart, lungs, kidneys, testes, and submaxillary, pituitary, thyroid, and adrenal glands) were observed. In addition, males in the 0.2% group had significantly increased absolute and relative pituitary gland weight. In females in the 1% group, significant reductions in several absolute organ weights (*i.e.,* thymus, liver, spleen, uterus, ovaries, and pituitary gland), and significant increases in several relative organ weights (*i.e.,* brain, heart, lungs, kidneys, and adrenal glands) were observed. Relative liver weights were significantly decreased in 0.04% group females. In females in the 0.2 and 1% groups, increased absolute and relative submaxillary gland weights were observed (compared to controls), although the relevance of this finding was not elaborated. The study authors attributed the decreased absolute and increased relative organ weights to malnutrition-induced suppression of body weight gain.

Significant microscopic abnormalities of the spleen, with significantly fewer male animals in the 1% group having extramedullary hematopoesis of the spleen compared to controls. Abnormal histopathology was also seen in the thymus, with animals developing degeneration or necrosis of the lymphocytes in the cortex of the thymus. No significant microscopic findings were reported for female animals. The study authors suggested that the observed effects on the spleen and thymus in the 1% group may have been secondary to malnutrition and/or decreased blood lymphocyte counts. In males, significantly more animals in the 1% group showed evidence of testicular toxicity (*i.e.,* sertoli cell vacuolization, unilaterally decreased germ cell production, and luminar cell debris). The study authors suggested that the observed testicular effects may have been due to decreased absorption of zinc and vitamins A and E, deficiencies of which have been reported to induce testicular atrophy and inhibit spermatogenesis.

Unilateral corneal opacities were observed in 1 male in each of the 0.04, 0.2, and 1% groups. Failure of mydriasis with synechia, increased light reflection by the retina, and distension of the eyeball also were observed in the animal in the 1% group that displayed corneal opacities. In addition, unilateral increases in light reflection by the retina were observed in 1 male in the 0.04% group, and 1 female in each of the control and 0.2% groups, and lens opacity was observed in 1 female in the 0.04% group. In the 0.04% group, enlargement of the left eye in 1 male, and opacity in the right eye in 1 female were observed, although these findings were considered to be incidental. Although the study authors noted that no significant differences in macroscopic eye examination were observed, the toxicological significance of the ophthalmological findings were not mentioned.

The authors concluded that "from these results, oligoglucosamine gave rise to no adverse effects in rats up to the dose level of 0.2 (w/w)%. Thus, the no-observed-adverse-effect level was determined to be 0.2(w/w) % for rats of either sex (124.0 mg/kg/day in males, 142.0 mg/kg/day in females)"

3.4 Developmental and Reproductive Toxicity Studies

Chitosan

The developmental and reproductive effects of chitosan have not been fully explored. A study was conducted where B6C3F1 female mice (15/group) that were induced to ovulate were orally administered

water-soluble chitosan (approximately 300 kDa; >90% deacetylation), at daily doses of 480 mg/kg body weight/day for 4 days (Choi *et al.*, 2002). Chitosan treatment did not have any effects on the oocyte and fertilization rates in animals fed a standard control diet. In contrast, chitosan treatment increased the number of ovulated oocytes and normal oocytes, as well as the *in vivo* and *in vitro* fertilization rates, compared to controls in animals fed a high-fat diet. The authors suggested that chitosan "might improve the functions of the ovary and the oviduct in obese mice".

3.5 Chitosan Oligosaccharide

In a study by Yoon et al., (2005), 4 generations of ICR mice ingested approximately 10 mg/kg bw/day of chitosan oligosaccharide *via* drinking water for up to 180 days. Though developmental and reproductive toxicity endpoints were not specifically examined in the study, no adverse effects were reported in all generations. Male and female ICR mice of the parental generation were provided with drinking water containing 0.1% chitosan oligosaccharide (equivalent to approximately 1 µg chitosan oligosaccharide/kg body weight/day) for 30 days. It was not indicated whether a control group was included in the parental generation. Subsequent generations (referred to as F1, F2, and F3 generations) were provided drinking water containing 0, 0.01, 0.1, or 1% chitosan oligosaccharide (equivalent to approximately 0, 0.1, 1, or 10 µg chitosan oligosaccharide/kg body weight/day) for 7, 60, or 180 days. Timing and conditions of mating and killing of animals were not specified (age of parental generation at mating was not specified, although animals were purchased at 8 to 10 weeks of age). Following the experimental periods, bone marrow was taken from the femur of each mouse and used to assess the formation of chromosomal aberrations. The authors noted that no significant differences with respect to chromosomal aberrations were observed between any of the treated groups compared to the control group. Other adverse effects or safety parameters were not assessed.

3.6 Chronic Toxicity Studies

The chronic toxicity of chitosan was evaluated by the National Toxicology Program in US (NTP, 2009). Shellfish chitosan was administered to Sprague-Dawley rats (10/sex/group) in the feed at dietary concentrations of 0, 1, 3, or 9% chitosan, equivalent to calculated intakes of 0, 500, 1,500, or 4,500 mg/kg body weight/day (U.S. FDA, 1993). Although the final study report by NTP has not been published, tabulated data/findings are publically available. The NTP reported that total body weight was reduced by approximately 10% in both male and female animals fed the highest dose of chitosan (no statistical testing described); no notable differences or trends towards chitosan related effects on body weight were observed for the other doses. In the absence of between group differences in feed consumption the observation of reduced weight gain is likely attributed to caloric dilution occurring from the addition of large dietary concentrations of non-caloric fiber to the diet. Statistically significant differences in the incidences of various non-neoplastic lesions were reported between select treatment groups and controls. The incidence of hematopoietic cell proliferation in the liver was significantly higher in males - but not females - treated with 9% chitosan compared to control animals (60% *vs.* 20% respectively). The incidence of kidney nephropathy was significantly lower in females treated with 9% chitosan compared to controls (0% *vs.* 50%), though similar incidence was observed in animals treated with 1% and 3% as the controls, and no difference were reported in males. Females treated with 9% chitosan also had significantly lower incidence of fatty changes in the periportal liver compared to controls (0% *vs.* 70%), and animals in the 1% and 3% treatment group also trended to lower incidences (40%). However, such effects were not observed in males. The differences in the incidence of these non-neoplastic lesions are unlikely to be biologically relevant given the lack of dose-response and inconsistencies between sexes.

No neoplastic lesions attributed to chitosan were reported. A NOAEL determination has not been reported by the NTP at this time.

3.7 Genotoxicity/Mutagenicity Studies

(i) In vitro: Ames

The genotoxic/mutagenic potential and the anti-genotoxic/anti-mutagenic potential of chitosan were examined in both *in vitro* and *in vivo* assays. These studies are summarized in Table IV.E-1. The Ames reverse mutation test was conducted using fungal chitosan (KiOmedine-CsU), in accordance with OECD Guideline 471 for the Testing of Chemicals (OECD, 1997). Chitosan (at doses up to 1,000 µg/plate) did not increase the number of revertent colonies in 4 strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537) and in 1 *Escherichia coli* WP2 strain (pKM101), in the presence and absence of metabolic activation by S9 fractions (KitoZyme, 2008c). In another study, chitosan oligomers (0.5, 5, 50, 500 and 5000 µg/plate) tested negative in the Ames reverse mutation test conducted in 4 *Salmonella* Typhimurium strains (TA97, TA98, TA100 and TA102), in presence and absence of metabolic activation by S9 fractions (Qin *et al.*, 2006).

(ii) In vivo: Micronucleus

It was also reported that chitosan oligosaccharide tested negative for the mouse bone marrow micronucleus test in Kunming male and female mice (5/group/sex), as well as the mouse sperm abnormality test in Kunming male mice (5/group), following oral gavage of a single dose of up to 5,000 mg/kg body weight (Qin *et al.*, 2006). These studies were conducted in accordance with standard protocols set by the Ministry of Health of the People's Republic of China (2003). Yoon *et al.* (2005) examined the incidence of micronuclei formation and chromosomal aberrations in ICR male mice following exposure to chitosan oligosaccharide (0, 0.01, 0.1, and 1% w/v) through drinking water for up to 180 days. No differences in micronuclei frequency and chromosomal aberrations were observed between treated and control mice. The authors estimated the 1% (w/v) dose of chitosan oligosaccharide is equivalent to exposure of 10 mg/kg body weight/day. Furthermore, no differences in chromosomal aberrations were observed between treated and control mice in the F1, F2, and F3 generations also exposed to chitosan oligomers for up to 180 days under the same experimental paradigm. The authors did not mention following any guidelines or whether the studies were GLP-compliant.

Together, these *in vitro* and *in vivo* studies provide evidence that chitosan does not have mutagenic or genotoxic effects.

Table 3.7-1 Summary of Genotoxicity Studies for Chitosan

Model	Assay	Test article	Dose and route of administration	Findings	Reference
In vitro					
Salmonella typhimurium	• Ames test	Chitosan derived from fungal source	0, 10, 33,100, 333, 1000 µg/plate	• Ames test revealed no significant differences in revertant colonies	KitoZyme internal data (unpublished)-Kitozyme2008c
Salmonella typhimurium	• Ames test	Chitosan oligomer Source: shrimp DAC: 85% MW: 1.86 kDa	0, 0.5, 5, 50, 500, 5000 µg/plate	• Ames test revealed no significant differences in revertant colonies	Qin *et al.*, 2006
A. cepa Human lymphocyte cell cultures	• *A. cepa* assay for chromosome damage • Cytogenetic assay	Chitosan polymerized with poly(methacrylic acid) nanoparticles Source: NR DAC: 94% MW: 71.3 kDa	1.8, 19, 180 mg/L	• No differences in mean mitotic index values in *A. cepa* test • No numerical or structural changes in chromosomes	De Lima *et al.*, 2010
Euglena gracilis	• *E. gracilis* mutagenicity assay	N-carboxyethyl derivatives of chitosan Source: NR DAC: NR MW: 150 kDa	10, 50, 100, 200 µg/mL	• N-carboxyethyl chitosan did not cause formation of mutant colonies at any concentration tested • No change in cell viability observed • Co-treatment of carboxyethyl chitosan protected against acridine orange genotoxicity	Kogan *et al.*, 2004
In vivo					
Male ICR mice (20/group)	• Bone marrow micronuclei test • Chromosome aberration test (4 generations)	Chitosan oligomer Source: NR DAC: 90% MW: <10 kDa	0, 0.01%, 0.1%, 1% dietary chitosan oligosaccharide administered for 7, 60 and 180 days	• No differences in formation of micronuclei in bone marrow cells • No differences in chromosome aberrations in parents and F1-3	Yoon *et al.*, 2005

Table 3.7-1 Summary of Genotoxicity Studies for Chitosan

Model	Assay	Test article	Dose and route of administration	Findings	Reference
Kunming mice (5/sex/group)	• Micronucleus test	Chitosan oligomer (single dose) Source: shrimp DAC: 85% MW: 1.86 kDa	• 1.2, 2.5, 5 g/kg bw by oral gavage	• No differences in frequencies of micronucleus in mice	Qin *et al.*, 2006
Kunming male mice /group)	• Mouse sperm abnormality test	Chitosan oligomer (single dose) Source: shrimp DAC: 85% MW: 1.86 kDa	• 1.2, 2.5, 5 g/kg bw by oral gavage	• No differences in frequency of mouse sperm abnormalities	Qin *et al.*, 2006
Anti-genotoxic properties					
Chinese hamster lung cells (CHL)	• Sister chromatid exchange	Chitin and chitosan	20 mg/mL	• Chitin and chitosan was anti-genotoxic when co-treated with 4-nitroquinoline N-oxide, dinitropyrene, mitomycin C, or adriamycin	Ohe, 1996
Female CF1 mice (12 to 13 per group)	• Determination of aberrant crypts and proliferative indices in colon	Low molecular weight chitosan (LMWC) Source: NR DAC: 80% MW: 20 kDA High molecular weight chitosan (HMWC) Source: NR DAC: 80% MW: 20 kDA	Pretreatment with azoxymethane (known colon-specific carcinogen) for 2 weeks (i.p.) Diets supplemented with 2% LMWC or HMWC for 6 weeks	• 2% HMWC significantly decreased number of aberrant crypt foci, and decreased crypt height and circumference, in mice exposed to azoxymethane • 2% LMWC decreased (not significant) number of aberrant crypt foci in mice exposed to azoxymethane • 2% LMWC and HMWC significantly decreased number of mitotic figures per crypt in azoxymethane treated mice	Torzsas *et al.*, 1996

bw = body weight; DAC = Degree of deacetylation; HMWC = High molecular weight chitosan; i.p. = intraperitoneal; LMWC = Low molecular weight chitosan; MW = molecular weight; NR = not reported

3.8 Other Relevant Animal Studies

Studies on selective biological endpoints of orally administered chitosan or chitosan oligomers to animals are summarized Table IV.F-1. Although these studies were not designed as safety studies, the endpoints evaluated are relevant to the safety assessment of chitosan under the proposed food uses. Chitosan treatment did not alter body or organ weights, and no changes in urinalysis, blood biochemistry and hematological parameters were observed. Deuchi *et al.* (1995) reported significant reduction in serum levels of minerals (Ca, Fe, and Mg) and lipidsoluble vitamins (A, D, E, and K) in Sprague-Dawley rats fed diets supplemented with 5% chitosan. However, these findings have not been replicated in other animal studies (Gordon and Beach-Williford, 1984; Kimura *et al.*, 2004; Jung *et al.*, 2006). In several randomized, double-blind, placebo-controlled human clinical trials, supplementation of the diet with grams of chitosan did not affect the absorption of fat-soluble vitamins or minerals (Pittler *et al.*, 1999; Mhurchu *et al.*, 2004; Tapola *et al.*, 2008). Overall, no observations were identified in these studies which raise concerns over the safety of the proposed uses of chitosan in alcoholic beverage production.

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Rats						
Rats (Sprague-Dawley), male, 7/group	Dietary 28 days	Chitosan Source: shrimp shell DAC: 83% Size: 625 kDa	Group 1: 0 (control) Group 2: 5% (5,000[1])	• Body weight • Liver weight • Liver metabolizing enzymes	• Significantly lower final body weight in chitosan group • Significantly lower absolute and relative liver weight • Lower levels of CYP 3A, 1A1 in chitosan group, decrease in glutathione S-transferase	Yao *et al.*, 2010
Rats (Long Evans), female, 5/group	56 days	Chitosan, dietary Source: shrimp shells DAC: 85 to 98% Size: 350 kDa	Group 1: 0 Group 2: 2% (2,000[1])	Body weight and food consumption	NSD in weight and food consumption	Hossain *et al.*, 2007
				Plasma cholesterol	Plasma total cholesterol decreased by 16%	
				Liver lipids	NSD in liver lipids	
				Plasma fatty acid profile	NSD in plasma palmitic and steric acid levels, increases in oleic, linoleic, and docosapentaenoic acid; decreased arachidonic acid	
Rats (Sprague-Dawley) 8 males/group	Dietary 28 days	Chitosan Source: crab shell DAC: NR Size: NR	Group 1: 0 (control) Group 2: 2% (2,000[1]) Group 3: 5% (5,000[1])	• Food intake • Body weight gain • Plasma lipids • Microsomal CYP7A1 activity	• NSD body weight gain, food intake, food efficiency ratio • Chitosan-treated rats had significantly lower plasma total cholesterol and LDL-cholesterol concentration • Consumption of chitosan resulted in elevated activity of CYP7A1 by 123% in group 2, and 165% in group 3	Moon *et al.*, 2007

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Rats (Sprague-Dawley), Female, ovarectomized, 8/group	Dietary 42 days	Chito-oligosaccharides,	Group 1: 0 (control) Group 2: 2% (2,000[1])	Body weight, food consumption	NSD in weight gain, food intake, total calcium intake	Jung *et al.*, 2006
				Urinary and fecal calcium	Rate of calcium loss into feces significantly lower in ovariectomized rats in chitooligosaccharide group (retain calcium better)	
				Serum Calcium	NSD in treatment group	
				Bone mineral density	Chitooligosaccharide increased the bone marrow density in distal region of femur.	
Rats (Sprague-Dawley) 10/sex/group	Dietary 30 days	Chitosan oligomer Source: shrimp DAC: NR Size: 1.86 kDa	Group 1: 0% (control) Group 2: 0.75% (750[1]) Group 3: 1.5% (1,500[1]) Group 4: 3.0% (3,000[1])	• Daily food intake • Weekly body weight • Hematology test • Clinical chemistry tests • Organ weights • Histopathological examination	• NSD food intake, feces, hair, behavior, body weight • NSD in absolute or relative body weights • NSD in hematology and clinical chemistry parameters	Qin *et al.*, 2006
Rat (Sprague-Dawley) 9/sex/group	Oral (gavage) 28 days	Chitosan oligosaccharide Source: NR DAC: NR Size: <1 kDa	Group 1: 0 (control) Group 2: 500 Group 3: 1,000 Group 4: 2,000	• Clinical signs • Body weight • Hematological and biochemical parameters • Histopathological examinations	• NSD in behavior or external appearance • Normal body weight, food consumption • Normal urinalysis, hematology, blood chemistry, relative organ weights • Normal histopathological findings • NOAEL >2,000 mg/kg bw/day	Kim *et al.*, 2001

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Rats (Sprague-Dawley) 6/group	Dietary 28 days	Chitosan (high viscosity) Chitosan (low viscosity) Source: shrimp shell DAC: 90% Size: 480 kDa (high viscosity) 340 kDa (low viscosity)	Group 1: 0 (control) Group 2: 5% high viscosity chitosan (5,000[1]) Group 3: 5% low viscosity chitosan (5,000[1])	• Liver weight • Plasma lipid, transaminase, lactic acid, frutosamine, *beta*-hydroxybutyric acid, free fatty acid levels • Plasma and liver lipid peroxides • Liver and fecal lipids • Liver glucose-6-phosphate dehydrogenenase	• NSD in body weight • Decreased relative liver weight • Higher liver lipid peroxide in chitosan (high viscosity) group • NSD plasma lipid peroxide values • NSD found in other tissue weights • Chitosan decreased plasma total cholesterol, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol • Decreased liver total lipids, but no significant difference in liver triacylglycerol content	Chiang *et al.*, 2000
Rats (Sprague-Dawley), male, 8-9/group	Dietary 18 days	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (control) Group 2: Week 1: 10% (10,000[1]) Week 2+: 7.5% (7,500[1])	• Body weight, food intake • Liver lipids • Fecal fat • Cholesterol absorption	• Chitosan group had a slower rate of growth • Reduced food intake with 10% and 7.5% supplementation • Lower liver cholesterol contents in chitosan group • Higher fat excretion • No changes in intestinal contents supernantant viscosity	Gallaher *et al.*, 2000
Rats (Sprague-Dawley) 10/group	Dietary 14 days	Chitosan Source: NR DAC: 90% Size: NR	Group 1: 0 (cellulose control) Group 2: 5% (5,000[1])	• Body weight • Food efficiency • Apparent fat digestibility • Vitamin and mineral status	• Body weight gain reduced in chitosan group • Food efficiency ratio decreased in chitosan group • Apparent fat digestibility decreased in chitosan group • Chitosan group had lower Ca, Mg, Fe absorption, and lower bone mineral content • Liver retinol and retinyl palmitate lower in chitosan groups • Lower serum and liver vitamin E	Deuchi *et al.*, 1995

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
					observed in chitosan group • Lower serum triglyceride • higher plasma vitamin K concentration	
Rats (Wistar), Male	Dietary 21 days	Chitosan Source: NR DAC: 94% Size: 250 kDa	Group 1: 0 (control) Group 2: 2% (2,000[1]) Group 3: 5% (5,000[1])	• Body weight • Food intake • Liver weight • Fecal weight • Serum cholesterol • Fecal neutral sterol excretion • Fecal bile acid excretion	• NSD in growth, food intake, liver weight, dried fecal weight • NSD in fecal excretion of neutral sterols and bile acids • Composition of bile acids and neutral sterols in cecum was statistically different in 5% chitosan group; chitosan expanded the neutral sterol pool and cholesterol, and decreased coprostanol • Statistically significant decrease in serum cholesterol in 5% chitosan group	Fukada *et al.*, 1991
Rats (Sprague-Dawley) • 6-7/group • 6/group	Dietary • 22 days • 28 days	Chitosan Source: crab shell DAC:81-99% Size: NR	Group 1: 0 (control) Group 2: 2% (2,000[1]) Group 3: 5% (5,000[1])	• Food intake • Growth • Organ weights • Serum cholesterol levels • Serum and liver lipids	• NSD in body weight, food intake • Relative liver weight was lower in chitosan groups • Chitosan prevented the rise of serum cholesterol due to feeding cholesterol • Liver cholesterol concentrations decreased in chitosan groups	Sugano *et al.*, 1988
Rats (Sprague-Dawley), Male 10/group	Dietary, 58 days	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (control) Group 2: 1% (1,000[1])	Body weight	Weight gain reductions occurred in groups 5 and 6	Landes and Bough, 1976
				Food intake	Efficiency of food utilization was decreased in groups 5 and 6	

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Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan						
Species (Strain), Sex, and Number of Animals	**Route of Admin. and Study Duration**	**Test Article**	**Dose (mg/kg bw/d)**	**Parameters Measured Related to Safety**	**Reported Effects[a, b]**	**Reference**
			Group 3: 2.5% (2,500[1]) Group 4: 5% (5,000[1]) Group 5: 10% (10,000[1]) Group 6: 15% (15,000[1])	Hematology	Hemoglobin and packed cell volume decreased in groups 5 and 6; total serum protein decreased in group 6	
				Absolute and relative organ weights	Relative liver and kidney weights were reduced in group 6.	
Mice						
Mice (C57bl6/J), male 8/group	10 weeks	Fungal Chitosan from kitoZyme Source: *A. bisporus* DAC: NR Size: NR	Group 1: 0 (high-fat diet) Group 2: 5% (7,500; in high-fat diet)	Body weight gain, feed efficiency, fat mass development	Decreased body weight gain compared to non-supplemented high-fat diet; feed efficiency was significantly lower compared to control	Neyrinck *et al.*, 2009
				Liver weight, epididymal, visceral, and subcutaneous white adipose tissue weight	NSD in liver weight; white adipose tissue weight was systematically lower compared to controls	
				Oral glucose tolerance test	NSD in glucose tolerance	
				Plasma insulin, glucose, triglycerides, cholesterol, non-esterified fatty acids, and β-hydroxybuterate	NSD in insulin resistance index; decreased serum triglycerides, cholesterol; NSD in serum non-esterified fatty acids.	
				Lipid analysis in cecal	Fat staining of the tissue demonstrate	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan						
Species (Strain), Sex, and Number of Animals	**Route of Admin. and Study Duration**	**Test Article**	**Dose (mg/kg bw/d)**	**Parameters Measured Related to Safety**	**Reported Effects[a, b]**	**Reference**
				content, liver and muscle	that lipid accumulation was reduced in liver and muscle compared to controls	
Mice (Kunming), female 10/group	90 days	Chitosan, high-molecular weight (HCS) Source: NR DAC: 85.5% Size: 760 kDa Chitosan, middle molecular weight (MCS); Source: NR DAC: 85.2% Size: 32.7kDa Chito-oligomer (COS); Source: NR DAC: 85.7%	Group 1: 0 (control) Group 2: 1.05% HCS (1575[1]) Group 3: 1.05% MCS (1575[1]) Group 4: 1.05% COS (1575[1]) Group 5: 1.05% WSC (1575[1])	General condition	NSD in appearance and behavior	Zeng *et al.*, 2008b
				Body weight	NSD in chitosan groups compared to control	
				Food intake	NSD	
				Absolute and relative organ weights	In WSC group: statistically significant increase in relative thymus weight. Other groups: NSD in relative heart, liver, spleen, thymus, kidney, and lung weights.	
				Histopathology	NSD in chitosan groups compared to control	
				Trace iron	Iron levels in liver, heart, spleen, kidney not different in groups 2, 4, 5 when compared to control; iron level in liver and spleen elevated in MCS group.	
				Trace zinc	Zinc levels in liver, heart, spleen, kidney not different in groups 2, 4, 5 when compared to control; zinc level	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
		Size: 0.99 kDa Chitosan, water-soluble (WSC) Source: NR DAC: 52.6% Size: 39.1kDa			in liver, spleen, heart significant elevated in MCS group.	
				Trace copper	Copper levels in liver, heart, spleen, kidney not different in groups 2, 4, 5 when compared to control; copper level in liver, spleen significant elevated in MCS group	
Mice (C57Bl/6J), Males, 4/group	Oral (gavage) 140 days (20 weeks)	Water-soluble chitosan Source: NR DAC: NR Size: 46 kDa	Group 1: 0 (control) Group 2: 200 Group 3: 600	Body weight and food consumption	NSD in weight gain until week 17: group 3 had reduced body weight gain when fed high-fat diet.	Sumiyoshiand Kimura, 2006
				Plasma triglycerides, total cholesterol	NSD in plasma triglycerides; group 3 inhibited the increase of total cholesterol when fed a high-fat diet	
				Liver weight and lipids	Group 3 had significantly lower liver weight and hepatic triglyceride and total cholesterol	
				Liver and kidney damage markers	NSD in glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and blood nitrogen urea	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Mice (Swiss Webster), male and female, 29-30/group	Dietary, 70 days	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (control) Group 2: 10% (15,000[1])	Body weight	Chitosan group had reduction in weight gain at 10 weeks	Kimura *et al.*, 2004
				Small intestine length	Increased in chitosan group	
				Liver weight	Absolute and relative liver mass increased in chitosan group	
				Retinol concentration	NSD in whole-blood, tissue accumulation, and fecal and urinary excretion during 2-week retinol exposure period	
Mice (ICR) Female 13/group	Dietary, 63 days	Chitin-chitosan (80% chitosan) Source: NR DAC: NR Size: NR	Group 1: 0 (control) Group 2: 3% (4,500[1]) Group 3: 7% (10,500[1]) Group 4: 15% (22,500[1])	Body weight	Groups 2, 3, 4 significantly reduced the increase in body weight following high-fat diet	Han *et al.*, 1999
				Liver weight	Reduced in groups 3, 4 following a high-fat diet	
				Serum lipids, cholesterol	Serum triacylglycerol significantly reduced in groups 2, 3, 4	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Mice (BALB/c), male and female,	Dietary, 28 days	Chitosan Source: Crab shell DAC: 80% Size: 3.6 µm in diameter	Group 1: 0 (control) Group 2: 0.5% (750[1]) Group 3: 5.0% (7,500[1])	• Body weight • Food consumption • Fecal bacteria	• After 4 weeks of feeding, group 3 had a statistically significant reduction in body weight • Average food consumption in week 4 was statistically lower in group 3 than control group • Facultative anaerobes, and lactobacillus concentrations were statistically lower in group 3 than control. Anaerobe colonies were higher in group 3 than controls. NSD in bifidobacterium and enterobacteraceae. NSD between group 2 and controls	Tanaka *et al.*, 1997
Mice (CF_1), female, approx 12/group	Dietary, 42 days	Low-molecular weight chitosan (LMWC), and high-molecular weight chitosan (HMWC) Source: NR DAC: 80 % Size: MW of 20,000 (LMWC) and 50,000 (HMWC)	Group 1: 0 (control) Group 2: 2% LMWC (3,000[1]) Group 3: 2% HMWC (3,000[1])	Body weight	Chitosan groups had lowered body weight, but HMWC was not statistically significant	Torzsas *et al.*, 1996
				Frequency of aberrant crypt foci	NSD in mice; HMWC decreased the number of aberrant crypt foci in azoxymethane-treated mice	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
Mice (transgenic homozygous apo E-deficient), mixed gender 10 /control 13/experimental	Dietary, 182 days (26 weeks)	Chitosan Source: prawn shells DAC: 78% Size: NR	Group 1: 0 Group 2: 5% (7,500[1])	Body weight	Chitosan-fed mice had significantly higher body weight on day 126 and 154 of study (improved growth)	Ormrod *et al.*, 1998
				General condition	NSD	
				Select organ weights	NSD in liver, epididymal, uterine horn fat pad weights	
				Food consumption	Food intake of all chitosan mice was marginally more than that of controls	
Guinea Pigs						
Guinea pigs (Hartley) 6/group	Dietary, 35 days	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (control) Group 2: 5% (2,000[1])	Body weight, food intake, food efficiency ratio	NSD compared to controls	Jun *et al.*, 2010
				Relative organ weight and fat pad	NSD in relative organ weights NSD in fat pads except percentage of epididymal fat pad in chitosan group was significantly lower than control	
				Fecal excretion	Chitosan increased fecal weight, fecal fat excretion, fecal water excretion, fecal water content	
				Plasma cholesterol	Total cholesterol, LDL cholesterol, triacylglycerol decreased in chitosan	

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan						
Species (Strain), Sex, and Number of Animals	**Route of Admin. and Study Duration**	**Test Article**	**Dose (mg/kg bw/d)**	**Parameters Measured Related to Safety**	**Reported Effects[a, b]**	**Reference**
					group	
				Lipid peroxide and GSH levels	GSH level in liver of chitosan group was higher	
Broiler Chickens						
Broilers, male 64/treatment	Dietary, 42 days	Chito-oligosaccharides Source: NR DAC: NR Size: Between 10^3 and 10^4 Da	Group 1: 0 (control) Group 2: 5% Group 3: 10% Group 4: 15%	Daily checks of disease and mortality	NSD in chitooligosaccharide groups	Huang *et al.*, 2005
				Body weight, food consumption	NSD for group 1, 2, 3 compared to control; Group 4 exhibited lower feed consumption in first three weeks, but NSD from week 3-6	
				Ileal digestibilities	Ileal digestibility for calcium and phosphorus increased with increasing chitooligosaccharide concentration, but decreased phosphorus digestibility for group 4. Ileal digestibility profile of individual amino acids changed	
Broilers (Arbor Acre), male, 42/treatment	Dietary, 42 days	Chitosan	Group 1: 0 (control Group 2: 0.02%	Average Daily Gain	NSD during weeks 0 to 3; increased quadratically (non-linear) during weeks 3 to 6; most effect noted at 0.05 and 0.1%	Shi *et al.*, 2005

Table 3.8-1 Summary of Other Relevant Animal Studies for Chitosan

Species (Strain), Sex, and Number of Animals	Route of Admin. and Study Duration	Test Article	Dose (mg/kg bw/d)	Parameters Measured Related to Safety	Reported Effects[a, b]	Reference
		Source: NR DAC: NR Size: NR	Group 3: 0.05% Group 4: 0.1% Group 5: 0.3% Group 6: 0.5%	Average Daily Feed	NSD during weeks 0 to 6	
				Feed Conversion Efficiency	Tended to increase ($p = 0.057$) during weeks 0 to 3; increased quadratically (non-linear) during weeks 3 to 6; most effect noted at 0.05 and 0.1%	
Hens (White leghorn) Broilers Rabbits	Dietary, 239 days (34 weeks)	Chitosan Source: crab shells DAC: NR Size: NR	Hens and Broilers: Group 1: 0 Group 2: 1.4 Group 3: 3.6-4.2, Group 4: 14-18 Rabbits: Group 1: 0 Group 2: 0.8	Growth, appetite, appearance	All animals normal, except hens fed the large dose of chitosan due to incomplete digestion. Constriction was found in part of the jejunum, but no visible injury was found on the surface of mucous membranes and liver weight and color were normal Physiological trouble of hens were reversed within 1 week of chitosan-free feeding	Hirano *et al.*, 1990
				Hypolipidemic activity	Chitosan suppressed elevations in cholesterol and triacylglycerol due to high-cholesterol diet	

♂ = male animals; ♀ = female animals; DAC: Degree of deacetylation; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NR: not reported; NSD = no significant difference; RBC = red blood cell; VLDL = very low-density lipoprotein; WBC = white blood cell. [1]Doses of dietary exposure to chitosan were estimated when possible according to guidelines set by the Food and Drug Administration (U.S. FDA, 1993)

3.9 Human Clinical Studies

Studies evaluating the administration of chitosan to healthy human subjects have been widely reported in the literature. Although primary endpoints monitored in these studies relate to effects of chitosan on management of body weight and cholesterol metabolism, controlled placebo controlled dose-response investigations assessing the safety and tolerance in healthy subjects consuming up to 6.5 g of chitosan per day have been reported (Bokura and Kobayashi, 2003; Tapola *et al.*, 2008). These studies are summarized in Table IV.G-1. In general, chitosan consumption was well tolerated at levels typically ranging from 1 to 6 g per day, for durations as long as 24 weeks. The most commonly reported adverse effects related to chitosan consumption were transient and mild, and were primarily gastrointestinal in nature (*e.g.,* constipation, nausea, bloating, indigestion, and abdominal pain).

In a systematic review of 14 randomized controlled trials published before 2005, chitosan was found to significantly reduce body weight and total serum cholesterol. However, the effects were small and the clinical relevance is questionable (Mhurchu *et al.*, 2005). Only 2 studies measured fecal fat excretion; however, no conclusions could be drawn about the effects of chitosan consumption on this parameter as different measures were used in the study and both contained small sample sizes. When studies that reported adverse events were analyzed, no differences in the frequency of adverse events were found between the chitosan intervention groups compared to controls.

Jull *et al.* (2008) included 15 human clinical studies in a meta-analysis of effect of chitosan on obesity for interventions lasting longer than 4 weeks (Jull *et al.*, 2008). Thirteen of the 15 studies were included in the previous systematic review by Mhurchu *et al.* (2005). Chitosan treatment resulted in significantly greater weight loss (weighted mean difference -1.7 kg; 95% confidence interval (CI) -2.1 to -1.3 kg, P <0.00001), decrease in total cholesterol (-0.2 mmol/L [95% CI -0.3 to -0.1], P <0.00001), and a decrease in systolic (-6 mm Hg [95% CI -7 to -5], P <0.00001) and diastolic (-3 mm Hg [95% CI -4 to -2], P <0.00001) blood pressure compared with placebo. However, the authors noted that many of the included studies included were of poor quality, and that results obtained from high quality trials suggest the effect of chitosan on body weight is minimal and unlikely to be of clinical significance. Similar to the previous analysis, no clear conclusions could be drawn regarding the effect of chitosan on fecal fat excretion, and that the frequency of adverse events did not differ between chitosan treatment and placebo control groups.

Overall, the data provided by these studies indicate that chitosan consumption is safe and well tolerated at high dietary doses (up to 6.5 g/person/day). These doses exceed estimated exposure to chitosan from under the proposed uses by several orders of magnitude.

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
Healthy Subjects						
10 subjects, healthy volunteers, not taking antioxidants (such as vitamin E or C) during the 3 months before inclusion in the study	Oral preparation, 4 weeks, Open-label, placebo-controlled, cross-over study	Water-soluble chitosan Source: NR DAC: 95% Size: average MW of 20 kDa	Group 1: 0 Group 2: 0.54	• Blood pressure, BMI • HDL and LDL cholesterol, triglycerides • Atherogenic index • Calcium and phosphorous levels • Plasma antioxidant capacity	• NSD in blood pressure, BMI, levels of total cholesterol, phosphorous, or calcium • Decrease in levels of plasma glucose, and atherogenic index after 2 weeks and persisted until the end of study • Concentration of HDL cholesterol increased during treatment period; no significant difference in LDL-cholesterol • Lowered the ratio of oxidized to reduced albumin, and increased total plasma antioxidant activity	Anraku *et al.*, 2009
24 subjects, healthy males and females	Oral capsule, 12 days Double-blind, placebo-controlled, cross-over study	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 Group 2: 2.5	• Food intake • Weight • Fecal fat content	• NSD in weight or food intake • Very small increase in fecal fat content in men, but NSD in women • No adverse effects reported	Gades and Stern, 2005

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
8 subjects, healthy male volunteers	Oral biscuits, 14 days	Chitosan Source: sea crab shells DAC: NR Size: NR	Week 1: 0 Week 2: 3 Week 3: 6 Week 4: 0	• Mean energy and nutrient intake • Fecal microbiota, bacterial metabolites, fecal weight, moisture content, pH value	• Decrease in lecithinase-negative clostridia ("may lead to improvement in intestinal environment") • Decrease in fecal ammonia • Chitosan inhibits putrefactive activity of intestinal microbiota and may contribute to reduction of factors that lead to disease states	Terada *et al.*, 1995
8 subjects, healthy males	Biscuits, 14 days Random, placebo-controlled cross-over study	Chitosan Source: NR DAC: 90.5% Size: 500 kDa	Group 1: 0 Group 2: Week 1: 3 Week 2: 6	• Body weight • Nutrition survey • Serum lipid • Bile acid and neutral cholesterol in feces	• Intake of energy, protein, fat, and cholesterol did not change • Average total serum cholesterol level decreased, serum HDL-cholesterol increased, NSD in serum triglyceride and phospholipid • NSD in bile acid excretion, amount of secondary bile acid excreted as lithocholic acid significantly decreased • Excreted amount of metabolite of cholesterol,	Maezaki *et al.*, 1993

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
					coprostanol, was significantly lower	
Hypercholesterolemic Subjects						
56 subjects, mild hypercholesterolemia	Oral tablets 55 days Parallel, placebo-controlled, single-blind trial	Chitosan (ChitoClear fg 95 chitosan) Source: NR DAC: >95% Viscosity: <500 mPa·s	Group 1: 0 (placebo) Group 2: 4.5 Group 3: 6.75	• Hematology: blood count, plasma creatinine, urate, γ-glutamyl transferase, calcium, serum ferritin • Serum: *alpha*- and *beta*-carotene, vitamin A, vitamin E, 25-hydroxyvitamin D • Plasma total and HDL-cholesterol, total triglyceride concentrations • Body weight, blood pressure • RAND 36-item Health Survey • Incidence and severity of gastrointestinal, skin and other symptoms	• NSD in hematology, serum biochemistry, plasma lipids, body weight • Association in incidence of constipation, heartburn, nausea in first 4-week period in chitosan groups (not significant between groups after performing pair-wise comparisons) • Three subjects in chitosan group and 1 subject in placebo group reported skin symptoms	Tapola *et al.*, 2008
95 subjects, mild or moderate hypercholesterolemia	Oral tablet 12 weeks Multicenter, placebo-controlled, randomized study	HEP-40, low-molecular weight chitosan Source: NR DAC: 93% Size: 40 kDa	Group 1: 0 (placebo) Group 2: 1.2 Group 3: 1.6 Group 4: 2.4	• Blood cholesterol levels • Incidence of adverse events • Serum parameters	• NSD in non-serious adverse events • No serious adverse events reported • No clinically important changes in any laboratory safety parameters • NSD in serum 25(OH)D • HEP-40 reduced serum LDL-cholesterol and	Jaffer and Sampalis, 2007

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Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
					total cholesterol at weeks 4, 8. • At 12 weeks, NSD in lipid profile parameters	
90 women, Mild to moderate hypercholesterolemia	Oral capsules 8 weeks, Double-blind, placebo-controlled, randomized study	Chitosan Source: NR DAC: 89.5% Viscosity: 160 mPa·s	Group 1: 0 (placebo) Group 2: 1.2	• Serum chemistry profiles • Complete blood counts • Changes in physical findings and signs • Blood pressure	• NSD in body weight, BMI, blood pressure, food consumption • Chitosan therapy produced statistically significant reduction in total cholesterol at 8 weeks • NSD in HDL cholesterol, triglyceride levels	Bokura *et al.*, 2003
Overweight Subjects						
12 subjects, obese, without diabetes mellitus	Oral tablet 3 months Placebo-controlled, randomized, double-blind trial	Chitosan (Vitamin World, 750 mg chitosan) Source: NR DAC: NR Size: NR	Group 1: 0 (placebo) Group 2: 2.25	• Serum glucose, total cholesterol, HDL cholesterol, triglycerides	• NSD serum glucose levels, lipid profile • Significant decrease in triglycerides • No adverse events with interventions • Insulin sensitivity increased significantly	Hernandez-Gonzalez *et al.*, 2010
30 subjects, overweight, hyperlipemic, under physical training	Oral tablet 4 months Double-blind, placebo-controlled	Low molecular weight chitosan, polyglucosamine	Group 1: 0 (placebo) Group 2: 2	• Anthropometric measures • Blood pressure • LDL and HDL-cholesterol, blood glucose and triacylglycerol	• More significant reduction in body weight, waist circumference, LDL-cholesterol, triacylglycerol than placebo control • HDL increase was higher than placebo	Cornelli *et al.*, 2008

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
					control • Metabolic syndrome was reduced in 12 cases in the supplement group	
134 subjects, Overweight adults, 83% women	Oral capsules 60 days Double-blind, placebo-controlled study	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (placebo) Group 2: 3	• Body composition • Blood chemistries • Tracking forms (daily caloric intake, activity levels)	• Significant reduction in mean scale weight, fat mass • NSD in total cholesterol, HDL, LDL, or bone mineral density	Kaats *et al.*, 2006
250 subjects, Overweight adults, 82% women	Oral capsule, 24 weeks Randomized, double-blind, placebo-controlled trial	β-Chitosan Source: squid pens DAC: 75.5% Size: NR	Group 1: 0 (placebo) Group 2: 3	• Body weight • Blood pressure • Waist circumference • Serum lipids • Plasma glucose • Fat-soluble vitamins in serum • Fecal fat losses • Health-related quality of life questionnaire	• NSD in BMI, waist circumference, body fat, blood pressure, fat-soluble vitamins, fecal fat loss • Statistically significant decrease in total cholesterol levels, LDL-cholesterol, but not clinically significant • NSD in HDL-cholesterol • NSD in health-related quality of life questionnaire answers	Mhurchu *et al.*, 2004
68 subjects, Normoglycemic obese individuals	Oral tablet 12 weeks Randomized, double-blind, placebo controlled	Absorbitol, a salt of chitosan Source: shellfish DAC: NR Size: NR	Group 1: 0 (placebo) Group 2: 3	• Body weight • Waist/hip ratio • Blood pressure • Bioelectric impedance analysis • Serum total cholesterol, triglyceride, HDL cholesterol, glucose	• NSD in adverse effects reporting • NSD in weight, body composition, blood composition, blood pressure, lipid profile, fasting insulin levels	Ho *et al.*, 2001

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
59 subjects, overweight, mildly obese, females	Oral capsule, 8 weeks, Randomized, double-blind, placebo-controlled	Rapidly-soluble chitosan, LipoSan Ultra™ Source: NR DAC: > 78% Size: > 100 kDa	Group 1: 0 (placebo) Group 2: 3	• Body weight • Waist/hip ratio • Symptom Observational Survey questionnaire • Routine calorie and dietary fat intake; exercise diary • Fasting serum lipid levels • Fecal fat	• NSD in calorie and dietary fat intake • NSD in total Symptom Observational Survey results, though chitosan group reported more incidences of gastrointestinal discomfort, mild nausea, and heartburn; were alleviated by increasing water consumption • In placebo group, mean weight increased significantly by 1.5 kg while treatment group decreased mean weight by 1.0 kg • BMI was lower in chitosan group • Chitosan group exhibited an increasing trend in fecal fat excretion, but no statistical conclusion (sample size too small)	Schiller *et al.*, 2001
30 subjects, overweight volunteers	Oral capsules, 28 days Randomized, double-blind, placebo-controlled	Chitosan Source: NR DAC: NR Size: NR	Group 1: 0 (placebo) Group 2: 2	• Body mass index • Blood pressure • Quality of life • Serum cholesterol • Serum triglycerides • Vitamin A, D, E, *beta*-carotene	• NSD in body mass index, serum cholesterol, serum triglycerides, vitamin A, D, E, *beta*-carotene • Small increase in vitamin K after 4 weeks	Pittler *et al.*, 1999

Table 3.9-1 Summary of Human Studies on Chitosan

Number and Characteristics of Subjects	Route of Administration, Study Duration, and Study Design	Test Article and Properties	Dose (g/d)	Parameters Measured Related to Safety	Reported Effects	Reference
					in chitosan group compared with placebo • Minor adverse events reported in 9 subjects in chitosan group to be constipation	
Diabetic (Type 2) Subjects						
18 subjects, dyslipidemic type 2 diabetic subjects	Dietary supplementation, 12 weeks, Random, placebo-controlled	Chitosan Source: NR DAC: 90% Size: 1,000 kDa	Group 1: 0 Group 2: 1.8	• Body weight • Plasma cholesterol • HDL-cholesterol, LDL-cholesterol, triglyceride • Adverse events	• NSD in cholesterol, triglyceride concentration • Increase in HDL-cholesterol, concomitant reduction in LDL-cholesterol • Mild digestive discomfort	Ausar *et al.*, 2003

BMI: body mass index; DAC: degree of deacetylation; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NR: not reported; NSD: no significant difference

4 Other relevant safety studies

Other safety and toxicology data collected on chitosan of fungal source (*A. bisporus*) by means of *in vitro* and *in vivo studies* conducted on animals or humans to examine the toxicity of chitosan for different purposes (pharma, cosmetics,...) are summarized in Table 4-1 below. These data have been generated either directly on the product (as a powder), or on its aqueous polar extracts or on n cosmetic formulation (cream) containing chitosan.

4.1 Information Pertaining to the Safety of Residual beta-1,3-glucans in the processing aid

Fungal chitosan contain *beta*-1,3-glucans at maximum concentrations of 10 to 15% on a w/w% basis. Since shellfish derived chitosan preparations do not contain *beta* glucans, ancillary safety data on the toxicity of *beta*-1,3-glucans is provided. Jonker *et al.* (2010) reported on the repeated consumption of an insoluble *A. niger* derived chitin/*beta*-1,3-glucan preparation (KiOnutrime-CG) in Wistar rats in a Good Laboratory Practice (GLP)-compliant subchronic toxicity study. The study was conducted in accordance with the following guidelines: OECD Guideline for Testing of Chemicals No. 408 (OECD, 1998); U.S. Food and Drug Administration (FDA) IV.C.4.a (U.S. FDA, 2000); and EC Guideline No. B.26, EEC Directive 2001/59/EC, Official Journal of the European Communities, No. L225, 21.8.2001 (EC, 2001). Groups of male and female Wistar rats (20/sex/group) [Crl:WI(WU)] were administered chitin-glucan as a dietary admixture at concentrations of 0 (control), 1, 5, or 10% (equivalent to 0, 632, 3,217, and 6,589 mg/kg body weight/day, respectively, for males and 0, 684, 3,437, and 7,002 mg/kg body weight/day, respectively, for females) for a period of 13 weeks. Food intake in high-dose rats was significantly increased with no changes in body weight, in comparison to control rats. The author considered this finding to be toxicologically irrelevant due to the lower energy content of the high-dose diet compared to the control diet. A significant increase in the absolute weight of the full and empty cecum of mid- and high-dose males and high-dose females, and a significant increase in the full and empty cecum weights relative to body weight in the high-dose males and females were reported compared to controls. Cecal enlargement occurs in rodents administered large dietary quantities of non-digestible polysaccharides/polyols, and is an effect that is not considered relevant to humans (WHO, 1987). The authors concluded that under the conditions of the study, the NOAEL for KiOnutrime-CG was 10% in the diet, the highest concentration tested, which was equivalent to an overall estimated daily intake of 6,589 mg/kg body weight/day for males and 7,002 mg/kg body weight/day for females.

Similar findings have been reported in other toxicity studies evaluating the effect of orally administered insoluble fungal derived *beta*-glucan preparations in rodents (Feletti *et al.*, 1992; Babícek *et al.*, 2007). In a GLP and OECD No. 408 compliant subchronic toxicity study, a NOAEL of 100 mg/kg body weight (the maximum deliverable gavage dose) was derived for Fisher-344 rats administered a *Saccharomyces cerevisiae* derived *beta*-1,3-glucan preparation on a repeated basis over a period of 91 days (Babícek *et al.*, 2007). The chronic (52 weeks) toxicity of a *Candida albicans* derived *beta*-1,3-D-glucan insoluble isolate was evaluated by Feletti *et al.* (1992). Groups of Sprague-Dawley rats (20/sex/group) were randomized to treatment groups receiving gavage dose of *beta*-glucan at 0 (saline), 50, 100, or 200 mg/kg body weight/day. Similar to findings reported by Jonker *et al.* (2010), animals randomized to the high-dose male and female treatment groups (200 mg/kg body weight/day) experienced soft stools,

diarrhea, and fecal enlargement with variable hyperplasia of the colon mucosa. A NOAEL of 200 mg/kg body weight per day, the highest dose tested, can be determined from this study.

Table 4-1 Safety studies for Chitosan of fungal source			
In vitro studies	**Cell type;**	**Conditions, concentration**	**Conclusion**
Cytotoxicity according to the ISO 10993-5 standard : Biological Evaluation of Medical Devices: Tests for in vitro cytotoxicity	L-929 mouse fibroblasts	Aqueous extracts at 0.2g/ml	Under the conditions of this study, the chitosan extract showed no cytotoxicity (KitoZyme 2008d)
Mutagenicity- AMES test according to OECD 471 and test method B13/B14 of Directive 2000/32/EC	4 *Salmonella typhimurium* strains and one *Escherichia coli* WP2 strain	Aqueous extracts at 0.2g/ml chitosan	Based on the results obtained in this study, the chitosan extracts were found to be non- mutagenic and non-promutagenic under the test conditions (KitoZyme 2008c)
Irritant potential by HETCAM (Hen's Egg Chorio- Allantoic Membrane) test Luepke et al. Fd Chem Toxic 23, 287 (1985), JORF 26/12/1996)	Chorio-allantoic membrane of a hen's egg	Aqueous extracts at 0.2g/ml chitosan-0.3ml applied pur.	Under the conditions of this study, the chitosan after polar extraction, must be classified "practically non irritant" according to classification established in the JORF (26/12/1996) (Kitozyme 2008e)
In vivo studies	**Animal types**	**Conditions, concentration**	**Conclusion**

Acute oral toxicity according to OECD 423 (24/04/2002) and test method B1tris of directive 2004/73/EC	Rat	Chitosan powder at 2000 mg/kg body weight	The LD50 of chitosan is higher than 2000 mg/kg body weight by oral route in the rat ; no mortality; no clinical signs related to administration of tested product ; the body weight evolution of the animals remained normal ; no treatment-related changes (Kitozyme 2008a)
Acute toxicity after intravenous administration according to ISO 10993-11 concerning biological evaluation of medical devices	Mouse	Aqueous extracts at 0.2g/ml chitosan; injection of 50ml/kg body weight	The LD50 of the polar extract is higher than 50 ml/kg body weight by intravenous route in mouse; no mortality; no clinical signs related to administration of tested product (kitoZyme 2008b)
Human studies	**Nbre of volunterrs**	**Conditions, concentration**	**Conclusion**
Skin sensitizing potential according to Marzulli-Maibach method under dermatological control	50 adult healthy volunteers with normal skin	Cosmetic formulation at 5% chitosan	Under these study conditions, the product can be considered non irritant and non-sensitizing to the skin and can be labeled as hypoallergenic (kitoZyme 2008f)

Acute cutaneous tolerance according to single patch test method under dermatological control	10 adult healthy volunteers with normal skin	Cosmetic formulation containing chitosan	Under these study conditions, the product is considered as non-irritating to the skin (Kitozyme 2008g)
Environemental studies	**Species**	**Conditions, concentration**	**Conclusion**
Environmental acute toxicity – Daphnia mobility inhibition assay realized according to OECD 202.	*Daphnia magna*	Dispersion of chitosan in water	The inhibition level (IL_{50}) is > 10588 mg/l (kitozyme 2011)
Environmental acute toxicity – Growing inhibition of algae according to OECD 201.	*Pseudokirchner -iella subcapitata*	Dispersion of chitosan in water	The inhibition levels is > 10316 mg/l (Kitozyme 2011)

4. Information on the toxicity of the chemical processing aid and if necessary, its major metabolites:

Information on the toxicity is available on section 3 here above

5. Safety assessment reports by international agencies or other national government agencies:

There is no safety assessment report prepared by international agencies or other government agencies expect the one reported in section B1 and B2 above.

C. Information related to the safety of an enzyme processing aid

Not applicable. Fungal chitosan is not an enzyme processing aid

D. Additional information related to the safety of an enzyme processing aid derived from a microorganism

Not applicable. Fungal chitosan is not an enzyme processing aid.

F. Information related to the dietary exposure of the processing aid

1. A list of food groups likely to contain the processing aid or its metabolites

Fungal chitosan from *A. niger* is proposed for use as a processing-aid in the manufacture of wine, beer, cider and spirits, as well as grain and beet derived food grade ethanol.

Chitosan derived from *A. niger*, was shown to be chemically and structurally equivalent to shellfish derived chitosan. The principal difference between the two chitosan preparations is the presence of small quantities of *beta*-1,3-glucans in *A. niger* sources of chitosan that are not present in shellfish chitosan. Therefore, data establishing the safety of shellfish-derived chitosan are considered relevant to the safety evaluation of fungal chitosan for the proposed food uses described herein.

Shellfish derived chitosan is widely available in the food supply through use in dietary supplement products, industrial, pharmaceutical, agricultural, and cosmetic applications, and background exposures to chitosan are therefore expected to exceed those occurring from the proposed food uses of fungal chitosan. Thus, based on the absence/trivial exposure to chitosan under the proposed food uses, calculation of estimated intakes was not deemed necessary in the assessment of the safety of the material under the proposed food uses in wine/alcoholic beverage processing for the GRAS determination.

A number of animal, human, and *in vitro* studies relevant to the safety of shellfish chitosan, which has a long history of safe use in the food supply, have been published. Published studies examining the metabolism and kinetics; acute, subchronic, and chronic toxicity; reproductive toxicity in animals; and safety in human of shellfish-derived chitosan or chitosan oligosaccharides are presented in the dossier.

Shellfish derived chitosan has a long history of safe use in the food supply. It is currently approved for use as a natural food additive for general food use in Japan and Korea (Japan Food Chemical Research Foundation, 2011; KFDA, 2011), and has widespread use as a dietary supplement product in the United States, the European Union, and other regulatory jurisdictions throughout the world. Finally, fungal chitosan (derived from *Agaricus bisporus* and *Aspergillus niger* sources) has been granted Novel Food approval by the European Commission, for use in supplement products in the European Union based on its substantial equivalence to existing shellfish derived chitosan products that are currently in the market[8].

2. The levels of residues of the processing aids or its metabolites for each food or food group

Regardless of the technological purpose, the sediments that contain the chitosan are removed from the wine, must, or spirits at the end of the treatment by physical separation processes such as racking, centrifugation and/ or filtration. Since chitosan is insoluble at slightly acidic to neutral pH levels, as well as in aqueous and ethanol solutions, it is unlikely that any residual chitosan will remain in the treated products. High-performance liquid chromatography (HPLC) analyses for residual chitosan in wine processed with chitosan indicate that the final product is free from chitosan carry-over products up to the limit of detection of the analysis method (10 mg/L). Therefore, the estimated intake of chitosan from all proposed technological uses can be considered as negligible.

3. Information on likely level of consumption

No information.

4. Percentage of food group to use processing aid

There is no information on the expected use of this processing aid in Australian wine or imported product currently being sold in Australia.

5. Information on residues in foods in other countries

There is no information on residues in wines where it is approved as a processing aid in other countries.

6. Where consumption has changed, information on likely consumption

Not applicable

APPENDIX

Appendix 1 Report on Industrial test on Chitosan Fungal Auxiliary on must and wine.

Appendix 2 CELABOR HPLC Chitosan

Appendix 3 Resolution OIV-oeno 336A-2009

Appendix 4 Resolution OIV-oeno 337A-2009

Appendix 5 Resolution OIV-oeno 338A-2009

Appendix 6 Resolution OIV-oeno 339A-2009

Appendix 7 Resolution OIV-oeno 368-2009

Appendix 8 Commission Regulation CE 53/2011

Appendix 9 GRAS Notice n°397 Response 12192011

Appendix 10 Argentina approval resolution

Appendix 11 EFSA Scientific Opinion on the safety of 'Chitin-glucan' as a Novel Food ingredient

REFERENCES

Aam BB, Heggset EB, Norberg AL, Sørlie M, Vårum KM, Eijsink VG (2010). Production of chitooligosaccharides and their potential applications in medicine. Mar Drugs 8(5):1482-1517.

AFSCA (2011). *Législation de base de l'autocontrôle*. Brussels, Belgium: Agence fédérale pour la sécurité de la chaine alimentare (AFSCA). Available at: http://www.favv-afsca.be/autocontrole-fr/legislation/ [Updated to: 30.06.2011].

Anderson JW, Nicolosi RJ, Borzelleca JF (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food Chem Toxicol 43(2):187-201.

Anraku M, Fujii T, Furutani N, Kadowaki D, Maruyama T, Otagiri M et al. (2009). Antioxidant effects of a dietary supplement: reduction of indices of oxidative stress in normal subjects by water-soluble chitosan. Food Chem Toxicol 47(1):104-109.

Ausar SF, Morcillo M, León AE, Ribotta PD, Masih R, Vilaro Mainero M et al. (2003). Improvement of HDL- and LDL-cholesterol levels in diabetic subjects by feeding bread containing chitosan. J Med Food 6(4):397-399.

Babícek K, Cechová K, Simon RR, Harwood M, Cox DJ (2007). Toxicological assessment of a particulate yeast (1,3/1,6)-β-D-glucan in rats. Food Chem Toxicol 45(9):1719-1730.

Bokura H, Kobayashi S (2003). Chitosan decreases total cholesterol in women: a randomized, double-blind, placebo- controlled trial. Eur J Clin Nutr 57(5):721-725.

Chae SY, Jang M-K, Nah J-W (2005). Influence of molecular weight on oral absorption of water soluble chitosans. J Control Release 102(2):383-394.

Chiang MT, Yao HT, Chen HC (2000). Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. Biosci Biotechnol Biochem 64(5):965-971.

Choi HG, Kim JK, Kwak DH, Cho JR, Kim JY, Kim BJ et al. (2002). Effects of high molecular weight water-soluble chitosan on *in vitro* fertilization and ovulation in mice fed a high-fat diet. Arch Pharm Res 25(2):178-183.

Cornelli U, Belcaro G, Cesarone MR, Cornelli M (2008). Use of polyglucosamine and physical activity to reduce body weight and dyslipidemia in moderately overweight subjects. Minerva Cardioangiol 56(5, Suppl.): 71-78.

De Lima R, Feitosa L, do Espírito Santo Pereira A, de Moura MR, Ahmad Aouada F et al. (2010). Evaluation of the genotoxicity of chitosan nanoparticles for use in food packaging films. J Food Sci 75(6):N89-N96.

Deuchi K, Kanauchi O, Shizukuishi M, Kobayashi E (1995). Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. Biosci Biotechnol Biochem 59(7):1211-1216.

EC (2001). Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Text with EEA relevance). Off J Eur Communities 44(L225):1-333. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0059:EN:HTML.

EC (2002). Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Off J Eur Communities 45(L140):10-21. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:140:0010:0021:EN:PDF.

EC (2003a). Commission Directive 2003/57/EC of 17 June 2003 amending Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Off J Eur Union 46(L151):38-41. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:151:0038:0041:EN:PDF.

EC (2003b). Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Off J Eur Union 46(L285):33-37. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:285:0033:0037:EN:PDF.

EC (2007). *Notifications Pursuant to Article 5 of Regulation (EC) N° 258/97 of the European Parliament and of the Council (April 2007).* Brussels, Belgium: European Commission (EC). Available at: http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf.

EU (2011). Commission Regulation (EU) No 53/2011 of 21 January 2011 amending Regulation (EC) No 606/2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. Off J Eur Union 54(L19):1–6. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:019:0001:0006:EN:PDF.

Feletti F, De Bernardi di Valserra M, Contos S, Mattaboni P, Germogli R (1992). Chronic toxicity study on a new glucan extracted from Candida albicans in rats. Arzneimittelforschung 42(11):1363-1367.

Fukada Y, Kimura K, Ayaki Y (1991). Effect of chitosan feeding on intestinal bile acid metabolism in rats. Lipids 26(5):395-399.

Furda I (2000). Reduction of absorption of dietary lipids and cholesterol by chitosan and its derivatives and special formulations. In: Muzzaarelli RAA editor. *Chitosan Per Os: From Dietary Supplement to Drug Carrier.* Italy: Atec, Grottammare, pp. 41-63.

Gades MD, Stern JS (2005). Chitosan supplementation and fat absorption in men and women. J Am Diet Assoc 105(1):72-77.

Gallaher CM, Munion J, Hesslink R Jr, Wise J, Gallaher DD (2000). Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. J Nutr 130(11):2753-2759.

Gooday GW (1989). Control and inhibition of chitin synthesis in fungi and nematodes. In: Skjak-Braek G, Anthonsen T, Sandford P, editors. *Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications*. 4th International Conference on Chitin and Chitosan - Proceedings, Aug. 22-24, 1988, Trondheim, Norway. London, Engl. / New York (NY): Elsevier Applied Science, pp. 13-22.

Gordon DT, Beach-Williford C (1984). Action of amino polymers on iron status, gut morphology, and cholesterol levels in the rat. In: Zikakis JP, editor. *Chitin, Chitosan, and Related Enzymes*. Joint U.S. Japan Seminar on Advances in Chitin ..., Apr. 24-27, 1984, Newark, Delaware. Orlando (FL) / Toronto (ON): Academic Press, pp. 97-117.

Han LK, Kimura Y, Okuda H (1999). Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. Int J Obes Relat Metab Disord 23(2):174-179.

Hernández-González SO, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA (2010). Chitosan improves insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp technique in obese subjects. Nutr Res 0(6):392-395.

Hirano S, Itakura C, Seino H, Akiyama Y, Nonaka I, Kanbara N et al. (1990). Chitosan as an ingredient for domestic animal feeds. J Agric Food Chem 38(5):1214-1217.

Ho SC, Tai ES, Eng PH, Tan CE, Fok AC (2001). In the absence of dietary surveillance, chitosan does not reduce plasma lipids or obesity in hypercholesterolaemic obese Asian subjects. Singapore Med J 42(1):6-10.

Hossain S, Rahman A, Kabir Y, Shams AA, Afros F, Hashimoto M (2007). Effects of shrimp (Macrobracium rosenbergii)-derived chitosan on plasma lipid profile and liver lipid peroxide levels in normo- and hypercholesterolaemic rats. Clin Exp Pharmacol Physiol 34(3):170-176.

Huang RL, Yin YL, Wu GY, Zhang YG, Li TJ, Li LL et al. (2005). Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poult Sci 84(9):1383-1388.

Jaffer S, Sampalis JS (2007). Efficacy and safety of chitosan HEP-40 in the management of hypercholesterolemia: a randomized, multicenter, placebo-controlled trial. Altern Med Rev 12(3):265-273.

Japan Food Chemical Research Foundation (2011). *List of Existing Food Additives* [Complied and published by the Ministry of Health and Welfare on April 16, 1996]. Japan: Japan Food Chemical Research Foundation. Available at: http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/pages/list-exst.add [Effective from May 6, 2011].

Jonker D, Kuper CF, Maquet V, Nollevaux G, Gautier S (2010). Subchronic (13-week) oral toxicity study in rats with fungal chitin-glucan from *Aspergillus niger*. Food Chem Toxicol 48(10):2695-2701.

Jull AB, Ni Mhurchu C, Bennett DA, Dunshea-Mooij CA, Rodgers A (2008). Chitosan for overweight or obesity (review). In: *Cochrane Database of Systematic Reviews*, Iss. 3, Art. No. CD003892 [Version dated 16 July 2008, Iss. 3, Last assessed as up-to-date: 31 August 2007, DOI: 10.1002/14651858.CD003892.pub3].

Jun SC, Jung EY, Kang DH, Kim JM, Chang UJ, Suh HJ (2010). Vitamin C increases the fecal fat excretion by chitosan in guinea-pigs, thereby reducing body weight gain. Phytother Res 24(8):1234-1241.

Jung WK, Moon SH, Kim SK (2006). Effect of chitooligosaccharides on calcium bioavailability and bone strength in ovariectomized rats. Life Sci 78(9):970-976.

Kaats GR, Michalek JE, Preuss HG (2006). Evaluating efficacy of a chitosan product using a double-blinded, placebo-controlled protocol. J Am Coll Nutr 25(5):389-394.

Kean T, Thanou M (2010). Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev 62(1):3-11.

KFDA (2011). *Korea Food Additives Code, 2011*. Korea: Korea Food & Drug Administration (KFDA), p. 1556. Available at: http://www.kfda.go.kr/fa/ebook/egongjeon_intro.html.

Kim S-K, Park P-J, Yang H-P, Han S-S (2001). Subacute toxicity of chitosan oligosaccharide in Sprague-Dawley rats. Arzneimittelforschung 51(9):769-774.

Kimura Y, Nagata Y, Buddington RK (2004). Some dietary fibers increase elimination of orally administered polychlorinated biphenyls but not that of retinol in mice. J Nutr 134(1):135-142.

Kitozyme (2008a). *Final Report Acute Oral toxicity in the rat. Study n° TAO423-PH-08/0064:* CONFIDENTIAL. Prepared by S. Seguier –Phycher Bio développement (FR)

Kitozyme (2008b). *Final Report Acute toxicity in mice after intravenous injection. Study n° TAIVs-PH-08/0064:* CONFIDENTIAL. Prepared by S. Seguier –Phycher Bio développement (FR)

Kitozyme (2008c). *Final Report Ames Test B-00561: Chitosan (Cs)*: CONFIDENTIAL. Prepared by Madrid, Spain: vivotecnia Rearch S.L. for Cedex, France: Dermscan for submission to Herstal, Belgium: Kitozyme sa.

Kitozyme (2008d). *Test Report Cytotoxicity using the ISO 10993 standard- study n° 68117* CONFIDENTIAL. Prepared Biomatech , France for Kitozyme sa.

Kitozyme (2008e). Potentiel irritant par application sur la membrane chorio-allantoïdienne de l'œuf de poule. Etude n° HET-CAM-PH -08/0064 Prepared by S. Seguier –Phycher Bio développement (FR) for kitoZyme sa.

Kitozyme (2008f). Sensitized potential study of a cosmetic product HRIPT according to Marzulli-Maibach method. Study n° DN-953/08E3262. Report prepared by Dermscan for kitoZyme sa.

Kitozyme (2008g). Evaluation of the acute cutaneous tolerance of a cosmetic product on adult volunteers. Study n° DN-949/08E3262-2. Report prepared by Dermscan for kitoZyme sa.

Ko YT, Lin YL (2004). 1,3-β-glucan quantification by a fluorescence microassay and analysis of its distribution in foods. J Agric Food Chem 52(11):3313-3318.

Kogan G, Skorik YA, Zitnanová I, Krizková L, Duracková Z, Gomes CA et al. (2004). Antioxidant and antimutagenic activity of N-(2-carboxyethyl)chitosan. Toxicol Appl Pharmacol 201(3):303-310.

Kumirska J, Czerwicka M, Kaczyński Z, Bychowska A, Brzozowski K, Thöming J et al. (2010). Application of spectroscopic methods for structural analysis of chitin and chitosan. Mar Drugs 8(5):1567-636.

Landes DR, Bough WA (1976). Effects of chitosan - a coagulating agent for food processing wastes - In the diets of rats on growth and liver and blood composition. Bull Environ Contam Toxicol 15(5):555-563.

Lee KY, Shibutani M, Takagi H, Arimura T, Takigami S, Uneyama C et al. (2004). Subchronic toxicity study of dietary *N*-acetylglucosamine in F344 rats. Food Chem Toxicol 42(4):687-695.

Ma O, Lavertu M, Sun J, Nguyen S, Buschmann MD, Winnik F et al. (2008). Precise derivation of structurally distinct chitosans with rhodamine B isothiocyanate. Carbohydr Polym 72(4):616-624.

Maezaki Y, Tsuji K, Nakagawa Y, Kawai Y, Akimoto M, Tsugita T et al. (1993). Hypocholesterolemic effect of chitosan in adult males. Biosci Biotech Biochem 57(9):1439-1444.

Mhurchu CN, Poppitt SD, McGill A-T, Leahy FE, Bennett DA, Lin RB et al. (2004). The effect of the dietary supplement, Chitosan, on body weight: a randomised controlled trial in 250 overweight and obese adults. Int J Obes Relat Metab Disord 28(9):1149-1156.

Mhurchu CN, Dunshea-Mooij C, Bennett D, Rodgers A (2005). Effect of chitosan on weight loss in overweight and obese individuals: a systematic review of randomized controlled trials. Obes Res 6(1):35-42.

Moon MS, Lee MS, Kim CT, Kim Y (2007). Dietary chitosan enhances hepatic CYP7A1 activity and reduces plasma and liver cholesterol concentrations in diet-induced hypercholesterolemia in rats. Nutr Res Pract 1(3):175-179.

Muzzarelli RA (1997). Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell Mol Life Sci 53(2):131-140.

Naito Y, Tago K, Nagata T, Furuya M, Seki T, Kato H et al. (2007). A 90-day ad libitum administration toxicity study of oligoglucosamine in F344 rats. Food Chem Toxicol 45(9):1575-1587.

Naredi (2011). [Naredi Website]. Brussels, Belgium: Naredi (Belgian Federation for Food Supplements, Dietary and Organic Products). Available at: http://www.naredi.be/engels/home.htm.

Neyrinck AM, Bindels LB, De Backer F, Pachikian BD, Cani PD, Delzenne NM (2009). Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid-lowering action. Int Immunopharmacol 9(6):767-775.

NTP (2009). *Pathology Tables, Survival and Growth Curves from NTP Long-Term Studies – Rats: TOX-tbd – Chitosan*. Research Triangle Park (NC): National Toxicology Program (NTP). Available at: http://nik.niehs.nih.gov/index.cfm?objectid=B17A0477-F1F6-975E-7104B4E1BB87DB98 [Web page last updated on April 04, 2011].

OECD (1997). Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 471) [Updated & Adopted 31st July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en.

OECD (1998). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 408) [Adopted 21st September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.

Ohe T (1996). Antigenotoxic activities of chitin and chitosan as assayed by sister chromatid exchange. Sci Total Environ 181(1):1-5.

OIV (2011). *Resolutions – Oenology*. Paris, France: Organisation Internationale de la Vigne et du Vin/International Organization of Wine and Vine (OIV). Available at: http://news.reseau-concept.net/pls/news/p_entree?i_sid=&i_type_edition_id=20435&i_section_id=20440&i_lang=33.

Onishi H, Machida Y (1999). Biodegradation and distribution of water-soluble chitosan in mice. Biomaterials 20(2):175-182.

Ormrod DJ, Holmes CC, Miller TE (1998). Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis 138(2):329-334.

Ph. Eur (2004). Chitosan hydrochloride (01/2005:1774). In: *European Pharmacopoeia, 5th edition*. (European Treaty Series, no. 50, vols. 1 & 2). Strasbourg, France: Directorate for the Quality of Medicines of the Ph. Eur., pp. 1248-1249.

Pittler MH, Abbot NC, Harkness EF, Ernst E (1999). Randomized, double-blind trial of chitosan for body weight reduction. Eur J Clin Nutr 53(5):379-381.

Qin C, Gao J, Wang L, Zeng L, Liu Y (2006). Safety evaluation of short-term exposure to chitooligomers from enzymic preparation. Food Chem Toxicol 44(6):855-861.

Richardson SC, Kolbe HV, Duncan R (1999). Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution and ability to complex and protect DNA. Int J Pharm 178(2):231-243.

Sashiwa H, Saimoto H, Shigemasa Y, Ogawa R, Tokura S (1990). Lysozyme susceptibility of partially deacetylated chitin. Int J Biol Macromol 12(5):295-296.

Schiller RN, Barrager E, Schauss AG, Nichols EJ (2001). A randomized, double-blind, placebo-controlled study examining the effects of a rapidly soluble chitosan dietary supplement on weight loss and body composition in overweight and mildly obese individuals. J Am Nutraceut Assoc 4(1):42-49.

Schipper NG, Olsson S, Hoogstraate JA, deBoer AG, Vårum KM, Artursson P (1997). Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement. Pharm Res 14(7):923-929.

Shaikh SA, Despande MV (1993). Chitinolytic enzymes: their contribution to basic and applied research. World J Microbiol Biotechnol 9(4):468-475.

Shi BL, Li DF, Piao XS, Yan SM (2005). Effects of chitosan on growth performance and energy and protein utilization in broiler chickens. British Poultry Science. 46(4): 516-519

Simon RR, Marks V, Leeds AR, Anderson JW (2011). A comprehensive review of oral glucosamine use and effects on glucose metabolism in normal and diabetic individuals. Diabetes Metab Res Rev 27(1):14-27.

Simunek J, Kopecny J, Hodrová B, Bartonová H (2002). Identification and characterization of *Clostridium paraputrificum*, a chitinolytic bacterium of human digestive tract.. Folia Microbiol 47(5):559-564.

Sugano M, Watanabe S, Kishi A, Izume M, Ohtakara A (1988). Hypocholesterolemic action of chitosans with different viscosity in rats. Lipids 23(3):187-191.

Sumiyoshi M, Kimura Y (2006). Low molecular weight chitosan inhibits obesity induced by feeding a high-fat diet long-term in mice. J Pharm Pharmacol 58(2):201-207.

Takahashi M, Inoue K, Yoshida M, Morikawa T, Shibutani M, Nishikawa A (2009). Lack of chronic toxicity or carcinogenicity of dietary *N*-acetylglucosamine in F344 rats. Food Chem Toxicol 47(2):462-471.

Tanaka Y, Tanioka S, Tanaka M, Tanigawa T, Kitamura Y, Minami S et al. (1997). Effects of chitin and chitosan particles on BALB/c mice by oral and parenteral administration. Biomaterials 18(8):591-595.

Tapola NS, Lyyra ML, Kolehmainen RM, Sarkkinen ES, Schauss AG (2008). Safety aspects and cholesterol-lowering efficacy of chitosan tablets. J Am Coll Nutr 27(1):22-30.

Terada A, Hara H, Sato D, Higashi T, Nakayama S, Tsuju K et al. (1995). Effect of dietary chitosan on faecal microbiota and faecal metabolites of humans. Microb Ecol Health Dis 8(1):15-21.

Torzsas TL, Kendall CW, Sugano M, Iwamoto Y, Rao AV (1996). The influence of high and low molecular weight chitosan on colonic cell proliferation and aberrant crypt foci development in CF1 mice. Food Chem Toxicol 34(1):73-77.

Tozaki H, Komoike J, Tada C, Maruyama T, Terabe A, Suzuki T et al. (1997). Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. J Pharm Sci 86(9):1016-1021.

U.S. EPA (1997). *Aspergillus niger Final Risk Assessment*. Washington (DC): Environmental Protection Agency (U.S. EPA). Available at: http://www.epa.gov/biotech_rule/pubs/fra/fra006.htm.

U.S. EPA (2008). *Chitin and Chitosan [Includes Proposed Final Decision]*. Washington (DC): Environmental Protection Agency (U.S. EPA). Available at: http://www.epa.gov/oppsrrd1/registration_review/chitin/index.htm.

U.S. FDA (1993). Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: *Priority Based Assessment of Food Additives (PAFA) Database*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-D-Reference-F-FDA-vol205.pdf.

U.S. FDA (1997). Substances generally recognized as safe; Proposed rule (21 CFR Parts 170, 184, 186, and 570) [Docket No. 97N-0103]. Fed Regist (US) 62(74):18937-18964.

U.S. FDA (2000). IV.C.4.a Subchronic toxicity studies with rodents [Nov. 2003]. In: *Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000* [Updated to July, 2007]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm.

U.S. FDA (2011). *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR [See Table for CFR sections].

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
173—Secondary direct food additives permitted in food for human consumption	173.120	Carbohydrase and cellulase derived from Aspergillus niger
	173.280	Solvent extraction process for citric acid
184—Direct food substances affirmed as generally recognized as safe	184.1005	Acetic acid
	184.1033	Citric acid
	184.1763	Sodium hydroxide

Vårum KM, Myhr MM, Hjerde RJ, Smidsrød O (1997). In vitro degradation rates of partially N-acetylated chitosans in human serum. Carbohydr Res 299(1&2):99-101.

WHO (1987). Test procedures and evaluations. 5.5.3. Toxicological versus physiological responses. In: *Principles for the Safety Assessment of Food Additives and Contaminants in Food.* (Environmental health criteria, no 70). Geneva, Switz.: World Health Organization (WHO), International Programme on Chemical Safety (IPCS), p. 82. Available at: http://www.inchem.org/documents/ehc/ehc/ehc70.htm.

Wisker E, Feldheim W, Pomeranz Y, Meuser F (1985). Dietary fiber in cereals. In: Pomeranz Y, editor. *Advances in Cereal Science and Technology. Vol. VII.* St. Paul (MN): American Association of Cereal Chemists, pp. 169-238.

Xia W, Liu P, Liu J (2008). Advance in chitosan hydrolysis by non-specific cellulases. Bioresour Technol 99(15):6751-6762.

Yao HT, Lii CK, Chou RH, Lin JH, Yang HT, Chiang MT (2010). Effect of chitosan on hepatic drug-metabolizing enzymes and oxidative stress in rats fed low- and high-fat diets. J Agric Food Chem 58(8):5187-5193.

Yoon HJ, Park HS, Bom HS, Roh YB, Kim JS, Kim YH (2005). Chitosan oligosaccharide inhibits 203HgCl2-induced genotoxicity in mice: micronuclei occurrence and chromosomal aberration [published correction appears in Arch Pharm Res 28(10):1203]. Arch Pharm Res 28(9):1079-1085.

Zeng L, Qin C, Wang W, Chi W, Li W (2008a). Absorption and distribution of chitosan in mice after oral administration. Carbohydr Polym 71(3):435-440.

Zeng L, Qin C, He G, Wang W, Li W, Xu D (2008b). Effect of dietary chitosans on trace iron, copper and zinc in mice. Carbohydr Polym 74(2):279-282.

Zhang H, Neau SH (2002). In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. Biomaterials 23(13):2761-2766.

APPENDIX 1

Report on industrial test of chitosan from fungal source as a technological auxiliary on must and wine (2008/SA/0150)

Selon :

- Le règlement CE/606/2009 de la Commission Européenne article 4

Chitosan from fungal source

Assessment of 2 years experiments

(2008-2009, 2009-2010)

KitoZyme

Rue Haute Claire, 4
Parc Industriel des Hauts-Sarts, Zone 2
4040 Herstal
Belgique

Tel : +32/4-259-85-00
Fax : +32/4-259-85-09

http://www.kitozyme.com

1. Administrative data 3
1.1. Name and address of the manufacturer of the technological auxiliary 3
2. Description of technological auxiliary 3
2.1. Name and trade name of the technological auxiliary 3
2.2. Description of chitosan 3
2.3. Chemical formula 4
3. Effectiveness of chitosan from fungal source as a technological auxiliary - Assessment of 2 years experiments 4

1. Administrative data

1.1. Name and address of the manufacturer of the technological auxiliary

KitoZyme sa
Rue Haute Claire, 4
Parc Industriel des Hauts-Sarts, Zone 2
4040 Herstal
Belgique

Tel : +32 4 259 85 00
Fax : +32 4 259 85 09

http://www.kitozyme.com

2. Description of technological auxiliary

2.1. Name and trade name of the technological auxiliary

Name of the substance : **chitosan** from fungal source

Trade name : **KIOfine-Cs®**

IUPAC (International Union of Pure and Applied Chemistry)[1] name :

- (2R,3R,4R,5S,6R)-3-amino-6-(hydroxymethyl)oxane-2,4,5-triol

Chemical Abstract Service (CAS) registry number

- Chitosane: [9012-76-4]

2.2. Description of chitosan

Chitosan in the amino form, is a linear polysaccharide composed of two repeating units randomly distributed along the polymer chain and linked by α or β(1->4) bonds: D-glucosamine (GlcN) and et N-acetyl-D-glucosamine (GLcNAc) units.

Chitosan is obtained by deacetylation (hydrolysis of N-acetyl groups) of chitin whose main source is the mycelium of *Aspergillus niger*, the fungal microorganism that producing citric acid for the food and pharmaceutical markets.

[1] Source : http://www.chemindustry.com

2.3. Chemical formula

Empirical formula: $(C_6H_{11}O_4N)\cdot n$

The chemical structure of chitosan is illustrated in figure 1.

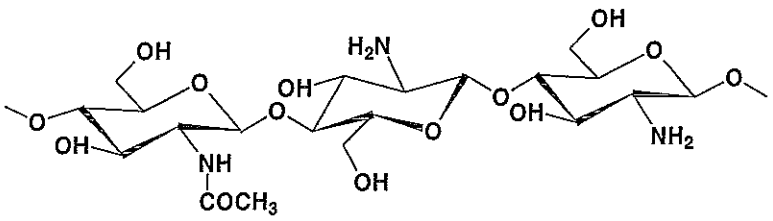

Figure 1 - Chemical structure of chitosan

3. Effectiveness of chitosan from fungal source as a technological auxiliary – Assessment of 2 years experiments

All the results obtained during two industrial test campaigns (2008-2009, 2009-2010) representing a cumulative volume of 4413.75hl of treated red wines have enabled the following interesting effects to be shown up:

- From an analytical point of view, chitosan of fungal origin has no bad influence on traditional analysis parameters, colour, colour intensity and optical density at 280nm (accurately measured).

- From an organoleptic point of view, wines treated with chitosan of fungal origin do not present any significant difference when tasting compared to the untreated control samples (triangle tests insignificant with 1% threshold) except in the following three cases: Nouveau Monde SCA Celliers, Bourdic Cooperative Cellars and the B barrel from Vignoble A. Maurel. For the three cases cited, the triangle test was completed by a preference test where the treated wine was preferred to the untreated wine each time.

- From a microbiological point of view, we can distinguish five types of situation:

1- Cases where the wines show an initial (T0) and final (T7) rate of low contamination

The cumulative volume for this category represents 931.75hl, being a total of 7 tanks out of 23 treated during the two industrial test campaigns (2008-2009 and 2009-2010).

In the above mentioned cases, the wines were particularly lacking in germs. The *Brettanomyces* count was carried out by direct plating of 100µl of wine on a specific gelose medium. The method used is not sufficiently sensitive to show the effectiveness of chitosan of fungal origin. The wine sample would have to be filtered on a 0.45µm porous membrane; then the membranes would have to be cultured on a specific gelose medium to concentrate the populations and lower the detection threshold in order to observe an effect.

2- Cases where the wines show an initial (T0) of low to moderate contamination

The cumulative volume for this category represents 1417hl, being a total of 8 tanks out of the 23 treated during the two industrial test campaigns (2008-2009 and 2009-2010).

Even though the initial *Brettanomyces* contamination rate was low to moderate, the *Brettanomyces* continued to develop after 7 days in the untreated samples (T7 control). Whereas, in the samples treated

with chitosan of fungal origin the *Brettanomyces* level dropped, after 7 days, under the detection threshold (<10UFC/ml). **These results clearly show the effectiveness of chitosan of fungal origin for eliminating *Brettanomyces* when the initial populations are low to moderate.**

3- Cases where the wines show an initial rate (T0) of high contamination

The cumulative volume for this category represents 580hl being a total of 2 tanks out of the 23 treated during the two industrial test campaigns (2008-2009 and 2009-2010).

These tests enabled noting that treatment with chitosan of fungal origin enables the *Brettanomyces* in the wine to be efficiently eliminated when the initial populations are high. In fact, seven days after processing it is noted that the treated samples have a very low *Brettanomyces* population compared to the reference sample which has maintained a high contamination level.

4-* Cases of wines with "natural decline" in *Brettanomyces

The cumulative volume for this category represents 630hl being a total of 2 tanks out of the 23 treated during the two industrial test campaigns (2008-2009 and 2009-2010).

For these tests we observed a moderate initial contamination rate (T0) for Bourdic (T0= 400UFC/ml) and very high for Bassan (T0= 10^6UFC/ml). However, it is noted that the contamination rate of the samples after 7 days is low both in the treated sample and in the control (T7 control and T7 treated <10UFC/ml). In view of these results, it can reasonably be supposed that when implementing the treatment, the declining phase of the growth cycle of the *Brettanomyces* was initiated. Therefore, these tests have not enabled the effectiveness of chitosan of fungal origin for *Brettanomyces* to be shown.

5- Specific cases

The cumulative volume for this category represents 855hl being a total of 4 tanks out of the 23 treated during the two industrial test campaigns (2008-2009 and 2009-2010).

For these tests, the initial contamination is either moderate (T0= 10UFC/ml and 320UFC/ml for the Alignan-du-Vent cooperative cellar and the Alba La Romaine wine producer's cooperative cellar respectively), or high (T0= 2020UFC/ml and >3000UFC/ml for the Puisserguier cooperative cellar and Château de Caladroy, respectively). However, it is noted that the contamination rate of the T7 samples (control and treated) has either slightly decreased (Alba La Romaine wine producer's cooperative cellar and Château Caladroy), or increased (Puisserguier cooperative cellar and Alignan-du-Vent cooperative cellar).

The lesser effectiveness of chitosan of fungal origin noted for these tests could be explained by an incorrect application of the product or by taking the treated T7 sample from the dregs instead of the liquid stage. This emphasizes the importance of correct application of the product for an optimum anti-fungal effect action against *Brettanomyces* in wine and the importance of sampling like decanting wine at the end of treatment with chitosan of fungal or after 7 days of action time.

In conclusion, the results obtained during the two industrial test campaigns (2008-2009 and 2009-2010) show to a great extent, the effectiveness of chitosan of fungal origin as a microbiological stabilisation agent by eliminating *Brettanomyces*, and this being irrespective of the initial rate of contamination.

In addition, chitosan of fungal origin has interesting properties which are directed towards respecting the analytical and organoleptic characteristics of wines.

APPENDIX 2

Centre de recherche et de contrôle agro-alimentaire, emballage, environnement et textile

KITOZYME sa
A l'attention de Mme Sylvia Legrain
Rue Haute Claire 4
Parc industriel des Hauts Sarts – zone 2
4040 Herstal

Votre demande du	Votre référence	Notre référence	Date
23/05/11	CMF 20110607	MD	31/05/11

RAPPORT D'ESSAI N° 10681

ESSAIS DEMANDES :

Détermination de la teneur en chitosane par HPLC avec détection ELSD.

ECHANTILLONS RECUS :

Référence Celabor	Informations données par le client	Date de réception
10681	SYLE0016-154	Le 23/05/11

ECHANTILLONNAGE EFFECTUE PAR : le client

Les résultats d'analyse valent pour les échantillons reçus. CELABOR n'est pas responsable de la représentativité des échantillons.

Ing. Caroline LONDON
Responsable Technique
(Tél. : 087/32.24.53)

Celabor s.c.r.l. : Zoning de Petit-Rechain - Avenue du Parc, 38 - B-4650 CHAINEUX (Belgique) - Tél. : 32 (0) 87.32.24.54 - Fax : 32 (0) 87.34.13.87
E-mail : info@celabor.be - www.celabor.be - RPM : Verviers - TVA : BE 0455.511.010 - Fortis Banque : 248-0603990-01
ING : 340-1380297-25 - ING IBAN : BE70 3401 3802 9725 - ING SWIFT : BBRUBEBB

celabor

RAPPORT N°	CLIENT	DATE
10681	KITOZYME	31/05/11

METHODE :

Norme :

- Chitosane : quantification par HPLC (Chromatographie Liquide Haute Performance) avec détecteur universel ELSD (Evaporative Light Scattering Detector) – MO268

Déviations à la norme : -

RESULTATS :

Date de fin de l'essai : le 30/05/11

Référence Celabor	Référence client	Chitosane (mg/l)
10681	SYLE0016-154	< 10

OIV

RESOLUTION OIV/OENO 336A/2009

MUSTS – FINING USING CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking note of the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practices and treatments:

PART II

Section 2: Musts

2.1.22. Fining using chitosan

Definition:

Addition of chitosan of fungal origin for the purpose of fining musts

Objectives:

a) To facilitate settling and clarification
b) To carry out a treatment to prevent protein haze

Prescriptions:

a) The doses to be used are determined after preliminary testing. The recommended dose used should be less than or equal to 100 g/hl.
b) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:

Accepted

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

APPENDIX 4

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OIV

RESOLUTION OIV/OENO 337A/2009

WINES – FINING USING CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological practice:

PART II

Section 3: Wines

3.2.12. Fining using chitosan

Definition:

Addition of chitosan of fungal origin for the purpose of fining wines

Objectives:

a) To reduce turbidity by precipitating particles in suspension.
b) To carry out a treatment to prevent protein haze by the partial precipitation of excess proteinaceous matter.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed 100 g/hl.
b) Sediments are eliminated by physical procedures.
c) Chitosan of fungal origin may be used alone or together with other admitted products.
d) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:
Accepted.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

OIV

RESOLUTION OIV/OENO 338A/2009

WINES – TREATMENT USING CHITOSAN

THE GENERAL ASSEMBLY,

in view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following oenological treatment:

PART II

Section 3: Wines

3.4.16. Treatment using chitosan

Definition:

Addition of chitosan of fungal origin to wines

Objectives:

a) To reduce heavy metal content, notably iron, lead, cadmium, copper,
b) To prevent iron haze, copper haze,
c) To reduce possible contaminants, especially ochratoxin A,
d) To reduce undesirable micro-organisms, notably *Brettanomyces*.

Prescriptions:

a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed :
   - 100 g/hl for the objectives a) and b)
   - 500 g/hl for the objective c)
   - 10 g/hl for the objective d)
b) Sediments are eliminated by physical procedures.
c) Chitosan of fungal origin may be used alone or together with other admitted products.
d) Chitosan must comply with the requirements of the International Oenological Codex.

OIV recommendations:
Accepted

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

OIV

RESOLUTION OIV/OENO 339A/2009

WINES - FINING: MODIFICATION OF THE EXISTING SHEET - CHITOSAN

THE GENERAL ASSEMBLY,

In view of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing International Office of Vine and Wine,

taking into account the favourable opinion of the "Food Security" group of experts,

taking into account the work carried out by the "Technology" group of experts,

and in view of draft resolution OENO/TECHNO/07/337A Wines – Fining using chitosan,

HAS HEREBY DECIDED, following a proposal made by Commission II "Oenology", to introduce into Part II of the "*International Code of Oenological Practices*" the following treatment:

PART II

Section 3: Wines

3.2.1. Fining

The addition to prescription b), chitosan

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Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

APPENDIX 7

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OIV

RESOLUTION OIV/OENO 368/2009

MONOGRAPH ON CHITOSAN

The GENERAL ASSEMBLY

In view of article 2, paragraph 2 IV of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

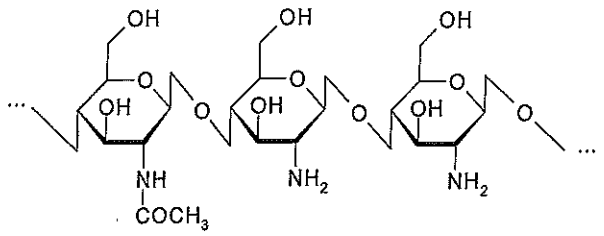
Considering the works of the group of experts "Specifications of Oenological Products",

DECIDE to add in the International Oenological Codex the following monograph:

CHITOSAN

$[C_6H_{11}NO_4]_n$

CAS number Chitosan: **[9012-76-4]**



Chitosan

1 PURPOSE, ORIGIN AND APPLICABILITY

Chitosan, a natural polysaccharide prepared of fungal origin, is initially extracted and purified from reliable and abundant food or biotechnological fungal sources such as *Agaricus bisporus* or *Aspergillus niger*.
Chitosan is obtained by hydrolysis of a chitin-rich extract. Chitin is a polysaccharide composed of several N-acetyl-D-glucosamine units interconnected by ß→ (1.4) type linkages.
Chitosan is composed of glucosamine sugar units (deacetylated units) and N-acetyl-D-glucosamine units (acetylated units) interconnected by ß→ (1.4) type linkages.

It is used as a fining agent in the treatment of musts for flotation clarification to reduce cloudiness and the content of unstable colloids.
It is also used for stabilising wines. This polymer actually helps eliminate undesirable micro-organisms such as *Brettanomyces.*

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2 SYNONYMS

Poly(N-acetyl-D-glucosamine)-poly(D-glucose).

3 LABELLING

The following information must be stated on the packaging label: exclusively fungal origin, product for oenological use, use and conservation conditions and use-by date.

4 CHARACTERS

4.1 Aspect and solubility

Chitosan comes in the form of a white, odourless and flavourless powder. Chitin-glucan is almost completely insoluble in aqueous or organic medium.

4.2 Purity and soluble residues

The purity of the product must be equal to or higher than 95 %.
Dissolve 5 g of chitin-glucan in 100 ml of bidistilled water and agitate for 2 minutes. Filter after cooling on a fine mesh filter or membrane.
Evaporate the filtrate and dry at 100-105 °C. The content of solubles should not be higher than 5 %.

5 TESTS

5.1 Determination of the acetylation degree and chitosan origin

5.1.1 Determination of the acetylation degree
The acetylation degree is determined by potentiometric titration, using the method described in Appendix I.

5.1.2 Determination of the source
Chitosan, as a natural polymer, is extracted and purified from fungal sources; it is obtained by hydrolysis of a chitin-rich extract. This chitosan is considered identical to chitosan from shellfish in terms of structures and properties.
An identification of the origin of chitosan is made based on 3 characteristics: content of residual glucans (refer to method in annex II), viscosity of chitosan in solution 1 % and settled density (following settlement).
Only fungal origin chitosan has both contents of residual glucan > at 2 %, a settled density ≥ at 0,7 g/cm^3 and viscosity in solution 1 % in acetic acid 1 % < at 15 cPs

5.2 Loss during desiccation

In a glass cup, previously dried for 1 hour in an oven at 100-105 °C and cooled in a desiccator, place 10 g of the analyte. Allow to desiccate in the drying oven at 100-105 °C to constant mass. Weigh the dry residue amount after cooling in the desiccator.

The weight loss must be lower than 10 %.

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The General Director of the OIV
Secretary of the General Assembly

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Note: all the limits stated below are reported in dry weight except for the microbiological analyses

5.3 Ashes

Incinerate without exceeding 600 °C the residue left from the determination of the loss during desiccation as described in 5.2. Allow to calcine for 6 hours. Allow the crucible to cool in a desiccator and weigh.

The total ash content should not be higher than 3 %.

5.4 Preparation of the test solution

Before determining the metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation is performed in a closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitosan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitosan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. This is submitted to microwave digestion with a maximum power of 1200 watts; Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

5.5 Lead

Lead is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The lead content must be lower than 1 mg/kg.

It is also possible to achieve lead determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.6 Mercury

Mercury is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The mercury content must be lower than 0.1 mg/kg.

It is also possible to achieve mercury determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.7 Arsenic

Arsenic is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The arsenic content must be lower than 1 mg/kg.

It is also possible to achieve arsenic determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

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The General Director of the OIV
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5.8 Cadmium

Cadmium is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The cadmium content must be lower than 1 mg/kg.

It is also possible to achieve cadmium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.9 Chromium

Chromium is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The chromium content must be lower than 10 mg/kg.

It is also possible to achieve chromium determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.10 Zinc

Zinc is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The zinc content must be lower than 50 mg/kg.

It is also possible to achieve zinc determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.11 Iron

Iron is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The iron content must be lower than 100 mg/kg.

It is also possible to achieve iron determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

5.12 Copper

Copper is determined by atomic absorption spectrophotometry, using the method described in appendix II.
The copper content must be lower than 30 mg/kg.

It is also possible to achieve copper determination by atomic absorption, using the method described in chapter II of the International Oenological Codex.

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5.13 MICROBIOLOGICAL CONTROL

5.13.1 Total bacteria count

The total bacteria count is performed according to the horizontal method by means of the colony count technique at 30 °C on the PCA medium described in appendix III.
Less than 1000 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.2 Enterobacteria

The enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C described in appendix IV.
Less than 10 CFU/g of preparation.

5.13.3 Salmonella

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Absence checked on a 25 g sample.

5.13.4 Coliform bacteria

Carry out the enumeration as described in chapter II of the International Oenological Codex.
Less than 100 CFU/g of preparation.

5.13.5 Yeasts

The enumeration of yeasts is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix VI.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

5.13.6 Moulds

The enumeration of moulds is carried out according to the horizontal method by means of the colony count technique at 25 °C on the YGC medium described in appendix VII.
Less than 100 CFU/g of preparation.

It is also possible to carry out the enumeration as described in chapter II of the International Oenological Codex.

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6 OCHRATOXIN A TESTING

Prepare an aqueous solution (distilled water) of chitosan at 1 % and agitate for 1 hour, then carry out determination using the method described in the Compendium of International Methods of Analysis of Wine and Musts.
Less than 5 µg/kg.

7 STORAGE

Keep container closed and store in a cool and dry place.

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Appendix I

DETERMINATION OF THE ACETYLATION DEGREE

1. PRINCIPLE

This method consists in determining the acetylation degree of chitosan by titration of the amino groups. The acetylation degree is the ratio of the number of N-acetyl-glucosamine units to the number of total monomers.
This method is based on the method described by Rinaudo et al., (1999).
The titration of a chitosan solution by means of NaOH at 0.1 M must be performed in order to identify two pH jumps from 0 to 14.
Chitosan is dissolved in 0.1M HCl, the amino groups (on the deacetylated glucosamine units (G)) are positively charged (HCl in excess)).
The chitosan solution (of known quantity) is titrated by NaOH of known concentration. In the first part of the reaction, the excess quantity of HCl is determined:
1.1. HCl (excess)+NaOH + $NH_3^+Cl^-$ --> NaCl + H_2O + $NH_3^+Cl^-$
After the first pH jump, the quantity of charged amino groups is determined:
1.2. HCl + H_2O + $NH_3^+Cl^-$+ NaOH --> NH_2 + $2H_2O$ + 2NaCl
The determination of the NaOH volume between the two jumps makes it possible to identify the quantity of charged amines.

2. REAGENTS ET MATERIALS

2.1. Commercial preparation of chitosan
2.2. Distilled or deionised water
2.3. Chlorhydric acid 0,3 M
2.4. Sodium Hydroxide 0,1M
2.5 Glass cylindrical flasks, pipettes, burettes...
2.6. Magnetic mixer and stir bar
2.7. pH-meter with temperature sensor.

3. SAMPLE PREPARATION

Before determination, the samples are prepared according to the protocol described hereafter:
100 mg of chitosan are placed into a cylindrical flask to which 3 ml of 0.3 M HCl and 40 ml of water are added. Agitate for 12 hours.

4. PROCEDURE

First introduce the pH electrode of the pH-meter as well as the temperature sensor into the cylindrical flask. Check that the pH value is lower than 3.
To bring to pH = 1, add a V1 volume (ml) of HCl 0.3 M and agitate.
Then to bring to pH = 7 with a V2 volume (ml) of 0.1 M NaOH
These operations can be carried out using an automatic titrator.

5. EXPRESSION OF RESULTS

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

The acetylation degree of chitosan is expressed in %. This formula is the ratio of the mass of acetylated glucosamine (aG) units in g actually present in the sample, to the mass in g that would be present if all the groups were acetylated, where:
Q = (V_{NaOH} X 0.1) / (1000 X M_{cs})
= specific concentration in amino groups

Mcs: dry weight of chitosan in g

$\mathbf{V_{NaOH}}$ = V2 – V1
= volume of 0.1 M NaOH between 2 pH jumps in ml
For a 1 g sample

With G = Glucosamine part; a = acetylated part

aG weight actually present (in g) =
1g – (Number of moles of G groups/g) x G molecular weight =
1g – Q x 162

aG weight if all the deacetylated groups were acetylated (in g) =
1g + (Number of moles of G groups/g) x molecular weight a =
1g + Q x 42

The acetylation degree will be equal to DA, where:

$$\mathbf{DA} = (1-162 \times Q) / (1+ 42 \times Q)$$

Rinaudo, M., G. Pavlov and J. Desbrieres. 1999. Influenced of acetic acid concentration on the solubilization of chitosan. *Polym.* 40, 7029-7032

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

Appendix II

DETERMINATION OF THE RESIDUAL GLUCAN CONTENT

1. PRINCIPLE

This method consists in determining the content of residual glucans in chitosan by means of spectrophotometry.
This method is based on a colorimetric reaction with a response depending on the degradation of the starch hydrolysates by hot concentrated sulphuric acid.
This degradation gives a brown yellow compound with a colour intensity proportional to the content of residual glucans.

2. REAGENTS ET MATERIALS

2.1 Glucan 97% (Société Mégazyme)
2.2 Commercial preparation of chitosan
2.3 Distilled or deionised water
2.4 Ethanol
2.5 Acetic acid 1%
2.6 Solution of phenol 5%
2.7 Glacial acetic acid 100%
2.8 Glass cylindrical flasks, pipettes, volumetric flasks,...
2.9 Magnetic mixer and stir bar
2.10 Chronometer

3. PREPARATION OF THE STANDARD RANGE

A stock solution of glucan (glucan with a purity of 97 % is provided by the company Megazyme) is prepared according to the precise protocol described hereafter:
500 mg of glucan are introduced into a volumetric flask of 100 ml into which 6 ml of ethanol and 80 ml of distilled water are added.
Agitate and boil out to allow glucan dissolution
Allow to cool, adjust to the filling mark with water
Agitate for 30 minutes.
Pour 1 ml of this solution into a 50 ml volumetric flask and adjust to the filling mark with 1 % acetic acid.
The solution is ready to use to produce the standard range according to the protocol hereafter.

Stock solution V (ml)	Water V (ml)	Glucan M (µg)
0	1	0
0.1	0.9	10
0.3	0.7	30
0.5	0.5	50
0.7	0.3	70

4. SAMPLE PREPARATION

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Before determination, the samples are prepared according to the protocol described hereafter:
100 mg of chitosan are placed into a 50 ml volumetric flask to which 25 ml of 1 % acetic acid are added.
Agitate for 12 hours then adjust to the filling mark.

5. PROCEDURE

In a test tube, add 1 ml of the analyte solution, 1 ml of phenol at 5 % and 5 ml of concentrated sulphuric acid.
Agitate this mixture using a vortex for 10 s, then allow to cool for 1 hour.
The absorbance A is measured at 490 nm.

6. EXPRESSION OF THE RESULTS

Determine the glucan content in µg/g from the calibration curve (0-70 µg). This content is expressed in µg/g of chitosan.

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The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

Appendix III
METAL DETERMINATION BY ATOMIC EMISSION SPECTROSCOPY

1. PRINCIPLE

This method consists in measuring atomic emission by an optical spectroscopy technique.

2. SAMPLE PREPARATION

Before the determination of metals, the sample is dissolved by acid digestion (HNO_3, H_2O_2 and HCl). Mineralisation takes place in closed microwave system. The sample undergoes neither crushing nor drying before mineralisation.
The reagents used for the mineralisation of chitosan are as follows: HNO_3 (65 %) (Suprapur), HCl (37 %) (Suprapur), H_2O_2 (35 %). The 0.5 to 2 g sample of chitosan is placed in a flask to which are added 25 ml of HNO_3, 2 ml of HCl and 3 ml of H_2O_2. The whole is then submitted to microwave digestion (Power of 60 % for 1 min, 30 % for 10 min, 15 % for 3 min, and 40 % for 15 min). The solution is then diluted in a volumetric flask with bidistilled water to a final volume of 25.0 ml.
The metal contents can then be determined.

3. PROCEDURE

The dissolved samples are nebulised and the resulting aerosol is transported in a plasma torch induced by a high frequency electric field. The emission spectra are dispersed by a grating spectrometer and the line intensity is evaluated by a detector (photomultiplier). The detector signals are processed and controlled by a computer system. A background noise correction is applied to compensate for the background noise variations.

4. EXPRESSION OF THE RESULTS

The metal concentrations in chitosan are expressed in mg/kg.

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The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

Appendix IV
Total bacteria count by counting the colonies obtained at 30 °C

PCA medium

Composition:

Peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar-agar	15 g
Adjusted to	pH 7.0
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 48 to 72 hours. Count the CFU number.

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The General Director of the OIV
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Appendix V

Enumeration of *Enterobacteria* is carried out according to the horizontal method by means of the colony count technique at 30 °C

VRBG medium

Composition:

Peptone	7 g
Yeast extract	3 g
Glucose	10 g
Sodium Chloride	5 g
Crystal Violet	0.002 g
Neutral Red	0.03 g
Agar-agar	13 g
Bile salts	1.5 g
Adjusted to	pH 7.4
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method. After inoculation, they are incubated at 30 °C in aerobiosis for 18 to 24 hours. Count the CFU number.

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Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

Appendix VI
Enumeration of yeasts by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of yeasts.

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The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

Appendix VII
Enumeration of the moulds by counting

YGC medium

Composition:

Yeast extract	5.0 g
D-glucose	20 g
Agar-agar	14.9 g
Choramphenicol	0.1 g
Adjusted to	pH 6.6
Water	complete to 1000 ml

The medium is sterilised before use in an autoclave at 120 °C for 20 min.

The Petri dishes are inoculated by pour plate method and spiral plating method.
After inoculation, they are incubated at 25 °C in aerobiosis for 3 to 5 days without being turned over.
Count the number of moulds.

Certified in conformity
Zagreb, 3rd July 2009
The General Director of the OIV
Secretary of the General Assembly

Federico CASTELLUCCI

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(Non-legislative acts)

REGULATIONS

COMMISSION REGULATION (EU) No 53/2011

of 21 January 2011

amending Regulation (EC) No 606/2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) [1], in particular the third and fourth paragraphs of Article 121 thereof,

Whereas:

(1) According to Article 3 of Commission Regulation (EC) No 606/2009 [2], the authorised oenological practices are laid down in Annex I to that Regulation. The International Organisation of Vine and Wine (OIV) has adopted new oenological practices. In order to meet the international standards in this field and to provide EU producers with the new possibilities available to third country producers, these new oenological practices should be authorised in the EU under the conditions of use defined by the OIV.

(2) Regulation (EC) No 606/2009 authorises clarification by means of pectolytic enzymes and enzymatic preparations of beta-glucanase. These enzymes and other enzymatic preparations are also used for maceration, clarification, stabilisation, filtration and for revealing the aromatic precursors of grapes present in the must and the wine. These oenological practices have been adopted by the OIV and they should be authorised under the conditions of use defined by the OIV.

(3) Wines entitled to the protected designations of origin 'Malta' and 'Gozo' have a sugar content greater than 45 g/l and are produced in small quantities. Likewise, certain French white wines with a protected geographical indication may have a total alcoholic strength by volume greater than 15 % vol. and a sugar content greater than 45 g/l. In order to ensure the preservation of these wines, the Member States concerned, i.e., Malta and France, respectively, requested a derogation to the maximum sulphur dioxide contents given in Annex I B to Regulation (EC) No 606/2009. These wines should be mentioned in the list of wines having a maximum sulphur dioxide content of 300 milligrams per litre.

(4) Wines entitled to the traditional expression 'Késői szüretelésű bor' have a very high sugar content and are produced in small quantities. In order to ensure the preservation of these wines, Hungary requested a derogation to the maximum sulphur dioxide content. A maximum sulphur dioxide content of 350 milligrams per litre should be authorised for these wines.

(5) Wines entitled to the protected designation of origin 'Douro' followed by the statement 'colheita tardia' derogate from the maximum sulphur dioxide content. Wines entitled to the protected designation of origin 'Duriense' have the same characteristics as these wines. On the basis of this, Portugal requested a derogation from the maximum sulphur dioxide content. A maximum sulphur dioxide content of 400 milligrams per litre should be authorised for these wines.

(6) In order to render the names of vine varieties clearer, the names of the varieties should be given in the different languages of the countries where these varieties are used.

(7) Certain provisions concerning certain liqueur wines differ from the requirements laid down in the specifications for these wines. These provisions should be amended in accordance with the requirements in question.

[1] OJ L 299, 16.11.2007, p. 1.
[2] OJ L 193, 24.7.2009, p. 1.

(8) Regulation (EC) No 606/2009 should be amended accordingly.

(9) The making of wine from grapes harvested during the 2010 wine-growing year has already begun. In order not to distort competition between wine producers, the new oenological practices should be authorised for all these producers starting at the beginning of the 2010 wine-growing year. This regulation should apply retroactively from 1 August 2010, which marks the start of the 2010 wine-growing year.

(10) The measures provided for in this Regulation are in accordance with the opinion of the Regulatory Committee established by Article 195(3) of Regulation (EC) No 1234/2007,

HAS ADOPTED THIS REGULATION:

Article 1

Regulation (EC) No 606/2009 shall be amended as follows:

(a) Annex I A is amended in accordance with Annex I to this Regulation;

(b) Annex I B is amended in accordance with Annex II to this Regulation;

(c) Annex II is amended in accordance with Annex III to this Regulation;

(d) Annex III is amended in accordance with Annex IV to this Regulation.

Article 2

This Regulation shall enter into force on the day following its publication in the *Official Journal of the European Union*.

It shall apply from 1 August 2010.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 21 January 2011.

For the Commission
The President
José Manuel BARROSO

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ANNEX I

Annex I A to Regulation (EC) No 606/2009 shall be amended as follows:

(1) The table shall be amended as follows:

(a) line 10 shall be replaced by the following:

'10	clarification by means of one or more of the following substances for oenological use: — edible gelatine, — plant proteins from wheat or peas, — isinglass, — casein and potassium caseinates, — egg albumin, — bentonite, — silicon dioxide as a gel or colloidal solution, — kaolin, — tannin, — chitosan of fungoid origin, — chitin-glucan of fungoid origin		The use of chitosan in the treatment of wines is limited to 100 g/hl. The use of chitin-glucan in the treatment of wines is limited to 100 g/hl'

(b) the following entries shall be added:

'44	Treatment using chitosan of fungoid origin	Under the conditions set out in Appendix 13	
45	Treatment using chitin-glucan of fungoid origin	Under the conditions set out in Appendix 13	
46	Acidification by means of electro-membranary treatment	Conditions and limits laid down in points C and D of Annex XVa to Regulation (EC) No 1234/2007 and Articles 11 and 13 of this Regulation Under the conditions set out in Appendix 14	
47	Use of enzymatic preparations for oneological purposes in maceration, clarification, stabilisation, filtration and to reveal the aromatic precursors of grapes present in the must and the wine	Without prejudice to the provisions of Article 9(2) of this Regulation, enzymatic preparations and the enzyme activities of these preparations (i.e., pectolyase, pectin methylesterase, polygalacturonase, hemicellulase, cellulase, betaglucanase and glycosidase) must comply with the corresponding purity and identification specifications of the International Oenological Codex published by the OIV'	

(2) Appendix 1 shall be deleted.

(3) The following Appendices 13 and 14 shall be added:

'Appendix 13

Requirements for the treatment of wines with chitosan of fungoid origin and for the treatment of wines with chitin-glucan of fungoid origin

Areas of application:

(a) reduction in the heavy metal content, particularly iron, lead, cadmium and copper;

(b) prevention of ferric casse and copper casse;

(c) reduction of possible contaminants, especially ochratoxin A;

(d) reduction in the populations of undesirable micro-organisms, in particular *Brettanomyces*, solely by means of treatment with chitosan.

Requirements:

— The dose levels to be used are determined after a qualification test. The maximum dose level used may not exceed:

  — 100 g/hl for applications (a) and (b),

  — 500 g/hl for application (c),

  — 10 g/hl for application (d),

— sediments are removed using physical processes.

Appendix 14

Requirements for acidification by means of electro-membranary treatment

— The cationic membranes must be constituted in such a way as to enable only the extraction of cations, in particular cation K^+.

— The bipolar membranes are impermeable to the anions and cations of must and wine.

— The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

— The membranes used must comply with the requirements of Regulation (EC) No 1935/2004 and of Directive 2002/72/EC and with the national provisions adopted for the implementation of the Directive. The membranes must also comply with the requirements of the monograph "Electrodialysis Membranes" of the International Oenological Codex published by the OIV.'

ANNEX II

Part A, point 2, of Annex I B to Regulation (EC) No 606/2009 shall be amended as follows:

(1) point (c) shall be amended as follows:

(a) in the 13th indent, the following sub-indents shall be added:

'— Vin de pays de l'Agenais,

— Vin de pays des terroirs landais,

— Vin de pays des Landes,

— Vin de pays d'Allobrogie,

— Vin de pays du Var;'

(b) the following indent shall be added:

'— wines originating in Malta with a total alcoholic strength by volume greater than or equal to 13,5 % vol. and a sugar content greater than or equal to 45 g/l and entitled to the protected designation of origin "Malta" and "Gozo";'

(2) in point (d), the following indent shall be added:

'— wines entitled to the traditional expression "Késői szüretelésű bor".'

(3) in point (e), the ninth indent shall be replaced by the following:

'— white wines entitled to the protected designation of origin "Douro" or to the protected geographical indication "Duriense" followed by the statement "colheita tardia";'

ANNEX III

In Appendix 1 to Annex II to Regulation (EC) No 606/2009, the names of the following vine varieties shall be inserted in the list in the appropriate alphabetical order:

'"Albariño", "Macabeo B", "Toutes les Malvasías" and "Tous les Moscateles".'

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ANNEX IV

Annex III to Regulation (EC) No 606/2009 shall be amended as follows:

(a) the second indent of Part A, point 4(a) shall be replaced by the following:

'— concentrated grape must, rectified concentrated grape must or must from raisined grapes to which neutral alcohol of vine origin has been added to prevent fermentation, for Spanish wine described by the traditional expression "vino generoso de licor" and provided that the increase in the total alcoholic strength by volume of the wine in question is not greater than 8 % vol.,';

(b) Part B shall be amended as follows:

(i) in point 3, the second paragraph shall be replaced by the following:

'However, as concerns liqueur wines with the protected designation of origin "Málaga" and "Jerez-Xérès-Sherry", the must of raisined grapes to which neutral alcohol of vine origin has been added to prevent fermentation, obtained from the Pedro Ximénez vine variety, may come from the "Montilla-Moriles" region.';

(ii) in point 10, the first indent shall be replaced by the following:

'— obtained from "vino generoso", as referred to in point 8, or from wine under flor capable of producing such a "vino generoso", to which has been added either must of raisined grapes to which neutral alcohol of vine origin has been added to prevent fermentation, or rectified concentrated grape must or "vino dulce natural",';

(c) Appendix 1 shall be amended as follows:

(i) in point A in the list for Spain, the following rows shall be inserted in the appropriate alphabetical order:

'Condado de Huelva	Pedro Ximénez Moscatel Mistela
Empordà	Mistela Moscatel'

(ii) in point B.5 in the list for Spain, the following row shall be inserted in the appropriate alphabetical order:

'Empordà	Garnacha/Garnatxa'

(d) Appendix 2 shall be amended as follows:

(i) in point A 2, liqueur wine with the protected designation of origin 'Trentino' shall be removed from the list for Italy;

(ii) in point A 3, the following list shall be added:

ITALY

Trentino';

(e) in Appendix 3, the names of the following vine varieties shall be added:

'Moscateles — Garnacha'

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COMMISSION REGULATION (EU) No 54/2011

of 21 January 2011

amending Regulation (EU) No 447/2010 opening the sale of skimmed milk powder by a tendering procedure, as regards the date of entry into storage of intervention skimmed milk powder

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) [1], and in particular Article 43(f) and (j), in conjunction of Article 4 thereof,

Whereas:

(1) Article 1 of Commission Regulation (EU) No 447/2010 of 21 May 2010 [2] lays down that intervention skimmed milk powder placed on sale should have entered into storage before 1 May 2009.

(2) Given the current situation on the skimmed milk powder market in terms of demand and prices and the level of intervention stocks, it is appropriate that skimmed milk powder entered into storage before 1 November 2009 is made available for sale.

(3) Regulation (EU) No 447/2010 should therefore be amended accordingly.

(4) In order to make the skimmed milk powder available for sale without delay, this regulation should enter into force immediately after its publication in the *Official Journal of the European Union*.

(5) The measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Common Organisation of Agricultural Markets,

HAS ADOPTED THIS REGULATION:

Article 1

Article 1 of Regulation (EU) No 447/2010 is replaced by the following:

'*Article 1*

Scope

Sales by a tendering procedure of skimmed milk powder entered into storage before 1 November 2009 are open, under the conditions provided for in Title III of Regulation (EU) No 1272/2009.'

Article 2

This Regulation shall enter into force on the day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 21 January 2011.

For the Commission,
On behalf of the President,
Štefan FÜLE
Member of the Commission

[1] OJ L 299, 16.11.2007, p. 1.
[2] OJ L 126, 22.5.2010, p. 19.

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COMMISSION REGULATION (EU) No 55/2011

of 21 January 2011

establishing the standard import values for determining the entry price of certain fruit and vegetables

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) [1],

Having regard to Commission Regulation (EC) No 1580/2007 of 21 December 2007 laying down implementing rules for Council Regulations (EC) No 2200/96, (EC) No 2201/96 and (EC) No 1182/2007 in the fruit and vegetable sector [2], and in particular Article 138(1) thereof,

Whereas:

Regulation (EC) No 1580/2007 lays down, pursuant to the outcome of the Uruguay Round multilateral trade negotiations, the criteria whereby the Commission fixes the standard values for imports from third countries, in respect of the products and periods stipulated in Annex XV, Part A thereto,

HAS ADOPTED THIS REGULATION:

Article 1

The standard import values referred to in Article 138 of Regulation (EC) No 1580/2007 are fixed in the Annex hereto.

Article 2

This Regulation shall enter into force on 22 January 2011.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 21 January 2011.

For the Commission,
On behalf of the President,
José Manuel SILVA RODRÍGUEZ
Director-General for Agriculture and Rural Development

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[1] OJ L 299, 16.11.2007, p. 1.
[2] OJ L 350, 31.12.2007, p. 1.

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ANNEX

Standard import values for determining the entry price of certain fruit and vegetables

(EUR/100 kg)

CN code	Third country code (1)	Standard import value
0702 00 00	MA	61,3
	TN	120,5
	TR	96,2
	ZZ	92,7
0707 00 05	EG	158,2
	JO	87,5
	TR	96,8
	ZZ	114,2
0709 90 70	MA	37,4
	TR	122,4
	ZZ	79,9
0709 90 80	EG	66,7
	ZZ	66,7
0805 10 20	AR	41,5
	BR	41,5
	EG	57,7
	MA	54,7
	TR	68,3
	ZA	41,5
	ZZ	50,9
0805 20 10	MA	74,8
	TR	79,6
	ZZ	77,2
0805 20 30, 0805 20 50, 0805 20 70, 0805 20 90	CN	69,6
	IL	67,2
	JM	101,1
	MA	109,6
	PK	69,0
	TR	73,7
	ZZ	81,7
0805 50 10	AR	45,3
	TR	52,6
	UY	45,3
	ZZ	47,7
0808 10 80	AR	78,5
	CA	96,7
	CL	82,0
	CN	97,4
	MK	54,3
	US	140,6
	ZZ	91,6
0808 20 50	CN	58,3
	NZ	97,8
	US	127,9
	ZA	101,0
	ZZ	96,3

(1) Nomenclature of countries laid down by Commission Regulation (EC) No 1833/2006 (OJ L 354, 14.12.2006, p. 19). Code 'ZZ' stands for 'of other origin'.

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COMMISSION REGULATION (EU) No 56/2011

of 21 January 2011

fixing the allocation coefficient to be applied to applications for import licences for olive oil lodged from 17 to 18 January 2011 under the Tunisian tariff quota and suspending the issue of import licences for the month of January 2011

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Regulation (EC) No 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) [1],

Having regard to Commission Regulation (EC) No 1301/2006 of 31 August 2006 laying down common rules for the administration of import tariff quotas for agricultural products managed by a system of import licences [2], and in particular Article 7(2) thereof,

Whereas:

(1) Article 3(1) and (2) of Protocol No 1 [3] to the Euro-Mediterranean Agreement establishing an association between the European Communities and their Member States, of the one part, and the Republic of Tunisia, of the other part [4], opens a tariff quota at a zero rate of duty for imports of untreated olive oil falling within CN codes 1509 10 10 and 1509 10 90, wholly obtained in Tunisia and transported direct from that country to the European Union, up to the limit laid down for each year.

(2) Article 2(2) of Commission Regulation (EC) No 1918/2006 of 20 December 2006 opening and providing for the administration of tariff quota for olive oil originating in Tunisia [5] lays down monthly quantitative limits for the issue of import licences.

(3) Import licence applications have been submitted to the competent authorities under Article 3(1) of Regulation (EC) No 1918/2006 in respect of a total quantity exceeding the limit laid down for the month of January in Article 2(2) of that Regulation.

(4) In these circumstances, the Commission must set an allocation coefficient allowing import licences to be issued in proportion to the quantity available.

(5) Since the limit for the month of January has been reached, no more import licences can be issued for that month,

HAS ADOPTED THIS REGULATION:

Article 1

The quantities for which import licence applications were lodged for 17 and 18 January 2011 under Article 3(1) of Regulation (EC) No 1918/2006 shall be multiplied by an allocation coefficient of 21,673003 %.

The issue of import licences in respect of amounts applied for as from 24 January 2011 shall be suspended for January 2011.

Article 2

This Regulation shall enter into force on 22 January 2011.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 21 January 2011.

For the Commission,
On behalf of the President,
José Manuel SILVA RODRÍGUEZ
Director-General for Agriculture and Rural Development

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[1] OJ L 299, 16.11.2007, p. 1.
[2] OJ L 238, 1.9.2006, p. 13.
[3] OJ L 97, 30.3.1998, p. 57.
[4] OJ L 97, 30.3.1998, p. 2.
[5] OJ L 365, 21.12.2006, p. 84.

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DECISIONS

COUNCIL IMPLEMENTING DECISION

of 18 January 2011

amending Decision 2007/884/EC authorising the United Kingdom to continue to apply a measure derogating from Articles 26(1)(a), 168 and 169 of Directive 2006/112/EC on the common system of value added tax

(2011/37/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Directive 2006/112/EC of 28 November 2006 on the common system of value added tax [1], and in particular Article 395(1) thereof,

Having regard to the proposal from the European Commission,

Whereas:

(1) In a letter registered by the Commission's Secretariat-General on 22 July 2010, the United Kingdom requested authorisation to extend a derogating measure in order to continue to restrict the right of deduction of VAT by the hirer or lessee on charges for the hire or lease of a passenger car where the car is not used entirely for business purposes.

(2) The Commission informed the other Member States of the request made by the United Kingdom by letter dated 12 October 2010. By letter dated 15 October 2010, the Commission notified the United Kingdom that it had all the information necessary to consider the request.

(3) Council Decision 2007/884/EC of 20 December 2007 authorising the United Kingdom to continue to apply a measure derogating from Articles 26(1)(a), 168 and 169 of Directive 2006/112/EC on the common system of value added tax [2], authorised the United Kingdom to restrict to 50 % the right of the hirer or lessee to deduct input VAT on charges for the hire or lease of a passenger car where the car is not used entirely for business purposes. The United Kingdom was also allowed not to treat as supplies of services for consideration the private use of a car hired or leased by a taxable person for his business purposes. This simplification measure removed the need for the hirer or the lessee to keep records of private mileage travelled in business cars and to account for tax on the actual private mileage of each car.

(4) According to the information provided by the United Kingdom, the restriction to 50 % still corresponds to the actual circumstances as regards the business and the non-business use by the hirer or lessee of the vehicles concerned. It is therefore appropriate that the United Kingdom be authorised to apply the measure for a further limited period, until 31 December 2013.

(5) Where the United Kingdom considers a further extension beyond 2013 is necessary, a report which includes a review of the percentage applied should be submitted to the Commission together with the extension request no later than 1 April 2013.

(6) On 29 October 2004, the Commission adopted a proposal for a Council Directive amending Directive 77/388/EEC, now Directive 2006/112/EC, that includes the harmonisation of the categories of expenses for which exclusions of the right of deduction may apply. Under this proposal, exclusions on the right to deduct may be applied to motorised road vehicles. The derogating measures provided for in this Decision should expire on the date of the entry into force of such amending Directive, if that date is earlier than the date of expiry provided for in this Decision.

(7) The derogation has no impact on the Union's own resources accruing from value added tax.

(8) Decision 2007/884/EC should therefore be amended accordingly,

[1] OJ L 347, 11.12.2006, p. 1.
[2] OJ L 346, 29.12.2007, p. 21.

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HAS ADOPTED THIS DECISION:

Article 1

Article 3 of Decision 2007/884/EC is replaced by the following:

'*Article 3*

This Decision shall expire on the date of entry into force of Union rules determining the expenditure relating to motorised road vehicles that is not eligible for full deduction of VAT, or on 31 December 2013, whichever is the earlier.

Any request for the extension of the measures provided for in this Decision shall be submitted to the Commission by 1 April 2013.

Any request for extension of those measures shall be accompanied by a report which includes a review of the percentage restriction applied on the right to deduct VAT on the hire or lease of cars not entirely used for business purposes.'.

Article 2

This Decision shall take effect on the day of its notification.

This Decision shall apply as from 1 January 2011.

Article 3

This Decision is addressed to the United Kingdom of Great Britain and Northern Ireland.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

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COUNCIL IMPLEMENTING DECISION

of 18 January 2011

authorising France to apply differentiated levels of taxation to motor fuels in accordance with Article 19 of Directive 2003/96/EC

(2011/38/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Directive 2003/96/EC of 27 October 2003 restructuring the Community framework for the taxation of energy products and electricity [1], and in particular Article 19 thereof,

Having regard to the proposal from the European Commission,

Whereas:

(1) Council Decision 2005/767/EC [2] authorises France to apply, for a period of 3 years, differentiated levels of taxation to gas oil and unleaded petrol. France had requested the authorisation in the context of an administrative reform involving the decentralisation of certain specific powers previously exercised by central government. Decision 2005/767/EC expired on 31 December 2009.

(2) By letter dated 12 August 2009, France requested authorisation to continue to apply differentiated rates of taxation under the same conditions for a further 6 years after 31 December 2009.

(3) Decision 2005/767/EC was adopted on the basis that the measure requested by France met the requirements set out in Article 19 of Directive 2003/96/EC. In particular, it was considered that that measure would not hinder the proper functioning of the internal market. It was also considered that it was in conformity with the relevant Community policies.

(4) The national measure is part of a policy designed to increase administrative effectiveness by improving the quality and reducing the cost of public services, as well as a policy of subsidiarity. It offers regions an additional incentive to improve the quality of their administration in a transparent fashion. In this respect, Decision 2005/767/EC requires that the reductions be linked to the socioeconomic circumstances of the regions in which they are applied. Overall, the national measure is based on specific policy considerations.

(5) The tight limits set for the differentiation of rates on a regional basis as well as the exclusion of gas oil used for commercial purposes from the measure imply that the risk of competitive distortions in the internal market is very low. Moreover, the application of the measure so far has shown a strong tendency on behalf of regions to levy the maximum rate allowable, which has further decreased any potential for competitive distortions.

(6) No obstacles to the proper functioning of the internal market have been reported as regards, more particularly, the circulation of the products in question in their capacity as products subject to excise duty.

(7) When originally requested, the national measure had been preceded by a tax increase equal to the margin for regional reductions. Against this background and in light of the conditions of the authorisation as well as experience gathered, the national measure does not, at this stage, appear to be in conflict with Union energy and climate policies.

(8) It follows from Article 19(2) of Directive 2003/96/EC that each authorisation granted under that Article must be strictly limited in time. Due to the possible future developments of the Union framework on energy taxation, this authorisation should be limited to a period of 3 years. It is furthermore appropriate to avoid any time gap with respect to the application of the authorisation,

HAS ADOPTED THIS DECISION:

Article 1

1. France is hereby authorised to apply reduced rates of taxation to unleaded petrol and gas oil used as fuel. Gas oil for commercial use within the meaning of Article 7(2) of Directive 2003/96/EC shall not be eligible for any such reductions.

2. Administrative regions may be permitted to apply differentiated reductions provided the following conditions are fulfilled:

(a) the reductions are no greater than EUR 35,4 per 1 000 litres of unleaded petrol or EUR 23,0 per 1 000 litres of gas oil;

[1] OJ L 283, 31.10.2003, p. 51.
[2] OJ L 290, 4.11.2005, p. 25.

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(b) the reductions are no greater than the difference between the levels of taxation of gas oil for non-commercial use and gas oil for commercial use;

(c) the reductions are linked to the objective socio-economic conditions of the regions in which they are applied;

(d) the application of regional reductions does not have the effect of granting a region a competitive advantage in intra-Union trade.

3. The reduced rates must comply with the requirements of Directive 2003/96/EC, and in particular the minimum rates laid down in Article 7.

Article 2

This Decision shall take effect on the day of its notification.

It shall apply from 1 January 2010.

It shall expire on 31 December 2012.

Article 3

This Decision is addressed to the French Republic.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

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COUNCIL DECISION

of 18 January 2011

appointing one Austrian member and two Austrian alternate members of the Committee of the Regions

(2011/39/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 305 thereof,

Having regard to the proposal from the Austrian Government,

Whereas:

(1) On 22 December 2009 and 18 January 2010, the Council adopted Decisions 2009/1014/EU and 2010/29/EU appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2010 to 25 January 2015 [1].

(2) A member's seat on the Committee of the Regions has become vacant following the end of the term of office of Mr Franz VOVES.

(3) Two alternate members' seats have become vacant following the end of the term of office of Mr Hermann SCHÜTZENHÖFER and Mr Walter PRIOR,

HAS ADOPTED THIS DECISION:

Article 1

The following are hereby appointed to the Committee of the Regions for the remainder of the current term of office, which runs until 25 January 2015:

(a) as a member:

— Herr Landesrat Dr Christian BUCHMANN, *Landesrat in der Steiermärkischen Landesregierung;*

and

(b) as alternate members:

— Frau Landesrätin Mag. Elisabeth GROSSMANN, *Landesrätin in der Steiermärkischen Landesregierung,*

— Herr Klubobmann Christian ILLEDITS, *Abgeordneter zum Burgenländischen Landtag; Klubobmann der SPÖ-Fraktion.*

Article 2

This Decision shall take effect on the day of its adoption.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

[1] OJ L 348, 29.12.2009, p. 22, and OJ L 12, 19.1.2010, p. 11.

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COUNCIL DECISION

of 18 January 2011

appointing a Slovak alternate member of the Committee of the Regions

(2011/40/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 305 thereof,

Having regard to the proposal of the Slovak Government,

Whereas:

(1) On 22 December 2009 and on 18 January 2010, the Council adopted Decisions 2009/1014/EU and 2010/29/EU appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2010 to 25 January 2015 [1].

(2) An alternate member's seat on the Committee of the Regions has become vacant following the end of the term of office of Mr Pavol FREŠO,

HAS ADOPTED THIS DECISION:

Article 1

The following is hereby appointed to the Committee of the Regions as an alternate member for the remainder of the current term of office, which runs until 25 January 2015:

— Mr Juraj BLANÁR

predseda Žilinského samosprávneho kraja.

Article 2

This Decision shall take effect on the day of its adoption.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

[1] OJ L 348, 29.12.2009, p. 22 and OJ L 12, 19.1.2010, p. 11.

123

COUNCIL DECISION

of 18 January 2011

appointing three Dutch members and six Dutch alternate members of the Committee of the Regions

(2011/41/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 305 thereof,

Having regard to the proposal from the Dutch Government,

Whereas:

(1) On 22 December 2009 and 18 January 2010, the Council adopted Decisions 2009/1014/EU and 2010/29/EU appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2010 to 25 January 2015 [1].

(2) Three members' seats on the Committee of the Regions have become vacant following the end of the term of office of Ms Annemarie JORRITSMA-LEBBINK, Ms Luzette WAGENAAR-KROON and Mr Rob BATS.

(3) Four alternate members' seats have become vacant following the end of the term of office of Ms Ellie FRANSSEN, Mr Job COHEN, Ms Rinda DEN BESTEN and Mr Hendrikus DE LANGE.

(4) Two alternate members' seats will become vacant following the appointment of Mr Hans KOK and Mr Henk KOOL as members of the Committee of the Regions,

HAS ADOPTED THIS DECISION:

Article 1

The following are hereby appointed to the Committee of the Regions for the remainder of the current term of office, which runs until 25 January 2015:

(a) as members:

— Mr H.A.J. (Hans) KOK, Burgemeester (Mayor of 't Hof van Twente),

— Mr H.P.M (Henk) KOOL, Wethouder (alderman of The Hague),

— Mr S.B. (Sipke) SWIERSTRA, Gedeputeerde (member of the Executive Council) of the Province of Drenthe;

and

(b) as alternate members:

— Mr H.A.J. (Henk) AALDERINK, Burgemeester (Mayor of Bronckhorst),

— Mr J.P. (Jean Paul) GEBBEN, Burgemeester (Mayor of Renkum),

— Mr J.P.W. (Jan Willem) GROOT, Wethouder (alderman of Amstelveen),

— Ms L.W.C.M. (Loes) van der MEIJS, Wethouder (alderman of Doetinchem),

— Mr N.A. (André) van de NADORT, Burgemeester (Mayor of Ten Boer),

— Mr F. (Frank) de VRIES, Wethouder (alderman of Groningen).

Article 2

This Decision shall take effect on the day of its adoption.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

[1] OJ L 348, 29.12.2009, p. 22, and OJ L 12, 19.1.2010, p. 11.

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COUNCIL DECISION

of 18 January 2011

appointing one Polish member and one Polish alternate member of the Committee of the Regions

(2011/42/EU)

THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 305 thereof,

Having regard to the proposal from the Polish Government,

Whereas:

(1) On 22 December 2009 and 18 January 2010, the Council adopted Decisions 2009/1014/EU and 2010/29/EU appointing the members and alternate members of the Committee of the Regions for the period from 26 January 2010 to 25 January 2015 [1].

(2) A member's seat on the Committee of the Regions has become vacant following the end of the term of office of Mr Jerzy KROPIWNICKI.

(3) An alternate member's seat will become vacant following the appointment of Mr Tadeusz TRUSKOLASKI as a member of the Committee of the Regions,

HAS ADOPTED THIS DECISION:

Article 1

The following are hereby appointed to the Committee of the Regions for the remainder of the current term of office, which runs until 25 January 2015:

(a) as a member:

— Tadeusz TRUSKOLASKI, *Prezydent Miasta Białegostoku;*

and

(b) as alternate member:

— Pawel ADAMOWICZ, *Prezydent Miasta Gdańska.*

Article 2

This Decision shall take effect on the day of its adoption.

Done at Brussels, 18 January 2011.

For the Council
The President
Gy. MATOLCSY

[1] OJ L 348, 29.12.2009, p. 22, and OJ L 12, 19.1.2010, p. 11.

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COMMISSION DECISION

of 21 January 2011

amending Decision 2010/468/EU providing for the temporary marketing of varieties of *Avena strigosa* Schreb. not included in the common catalogue of varieties of agricultural plant species or in the national catalogues of varieties of the Member States

(notified under document C(2011) 156)

(Text with EEA relevance)

(2011/43/EU)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Directive 66/402/EEC of 14 June 1966 on the marketing of cereal seed [1], and in particular Article 17(1) thereof,

Whereas:

(1) Commission Decision 2010/468/EU [2] authorises, until 31 December 2010, the marketing in the Union of seed of varieties of *Avena strigosa* Schreb. (hereinafter 'A. *strigosa*') not included in the common catalogues of varieties of agricultural plant species or in the national catalogues of varieties of the Member States.

(2) The temporary difficulties in the general supply of A. *strigosa* which were the reason for the adoption of Decision 2010/468/EU, continue. It is therefore necessary to extend the period of application of the authorisation provided for in that Decision.

(3) It appears from the information provided to the Commission by the Member States that, for 2011, an additional total quantity of 5 130 tonnes is necessary to resolve these supply difficulties, as Belgium has indicated to the Commission that it needs for that period a quantity of 300 tonnes, France a quantity of 3 700 tonnes, Germany a quantity of 300 tonnes, Italy a quantity of 280 tonnes, Spain a quantity of 300 tonnes and Portugal a quantity of 250 tonnes.

(4) Decision 2010/468/EU should therefore be amended accordingly.

(5) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Seeds and Propagating Material for Agriculture, Horticulture and Forestry,

HAS ADOPTED THIS DECISION:

Article 1

Decision 2010/468/EU is amended as follows:

1. Article 1 is amended as follows:

   (a) in paragraph 1, the words '31 December 2010' are replaced by '31 December 2011';

   (b) paragraph 2 is replaced by the following:

   '2. The total quantity of seed authorised for marketing in the Union pursuant to this Decision shall not exceed 4 970 tonnes in 2010. The total quantity of seed authorised for marketing in the Union pursuant to this Decision shall not exceed 5 130 tonnes in 2011.';

2. in the second paragraph of Article 3 the words '31 December 2010' are replaced by '31 December 2011'.

Article 2

This Decision is addressed to the Member States.

Done at Brussels, 21 January 2011.

For the Commission

John DALLI

Member of the Commission

[1] OJ 125, 11.7.1966, p. 2309/66.
[2] OJ L 226, 28.8.2010, p. 46.

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COMMISSION DECISION

of 19 January 2011

concerning certain protection measures against foot-and-mouth disease in Bulgaria

(notified under document C(2011) 179)

(Text with EEA relevance)

(2011/44/EU)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Council Directive 89/662/EEC of 11 December 1989 concerning veterinary checks in intra-Community trade with a view to the completion of the internal market [1], and in particular Article 9(4) thereof,

Having regard to Council Directive 90/425/EEC of 26 June 1990 concerning veterinary and zootechnical checks applicable in intra-Community trade in certain live animals and products with a view to the completion of the internal market [2], and in particular Article 10(4) thereof,

Whereas:

(1) On 5 January 2011 Bulgaria reported a case of foot-and-mouth disease in a wild boar shot in Burgas region in the South-East of Bulgaria within a zone of reinforced surveillance along the border with Turkey. The Commission therefore adopted Decision 2011/8/EU of 6 January 2011 concerning certain interim protection measures against foot-and-mouth disease in Bulgaria [3].

(2) On 9 January 2011 Bulgaria reported outbreaks of foot-and-mouth disease in livestock in the same area. The new epidemiological situation requires to review the measures previously adopted, also in the light of the information provided by Bulgaria and the discussions with Member States at the meeting of the Standing Committee on the Food Chain and Animal Health of 12 January 2011.

(3) The foot-and-mouth disease situation in Bulgaria is liable to endanger the herds of other Member States in view of trade in live biungulate animals and the placing on the market of certain of their products.

(4) Bulgaria has taken measures in the framework of Council Directive 2003/85/EC of 29 September 2003 on Community measures for the control of foot-and-mouth disease [4], in particular the measures provided for in Section 3 of Chapter II and in Article 85(4) thereof.

(5) The whole territory of Bulgaria is subject to the restrictions of Articles 2, 4, 5, 6, 8b and 11 of Commission Decision 2008/855/EC of 3 November 2008 concerning animal health control measures relating to classical swine fever in certain Member States [5]. However, being listed in Part II of the Annex to that Decision allows Bulgaria to dispatch under certain health conditions fresh pig meat and meat preparations and products produced from such meat.

(6) The disease situation in Bulgaria makes it necessary to reinforce the control measures for foot-and-mouth disease taken by the competent authorities in Bulgaria.

(7) It is appropriate to define as a permanent measure the high and low risk areas in the affected Member State and to provide for a prohibition on the dispatch of susceptible animals from the high and low risk areas and on the dispatch of products derived from susceptible animals from the high risk area. The Decision should also provide for the rules applicable to the dispatch from those areas of safe products that either had been produced before the restrictions, from raw material sourced from outside the restricted areas or that had undergone a treatment proven effective in inactivating possible foot-and-mouth disease virus.

(8) The size of the defined risk areas is a direct function of the outcome of tracing of possible contacts to the infected holding and takes into account the possibility to implement sufficient controls on the movement of animals and products. At this point of time and based on information provided by Bulgaria, the whole of Burgas region should currently remain a high risk area.

(9) The prohibition of dispatch should only cover products derived from animals of susceptible species coming from or obtained from animals originating in the high risk areas listed in Annex I and should not affect transit through these areas of such products coming from or obtained from animals originating in other areas.

(10) Council Directive 64/432/EEC [6] concerns animal health problems affecting intra-Community trade in bovine animals and swine.

(11) Council Directive 91/68/EEC [7] concerns animal health conditions governing intra-Community trade in ovine and caprine animals.

[1] OJ L 395, 30.12.1989, p. 13.
[2] OJ L 224, 18.8.1990, p. 29.
[3] OJ L 6, 11.1.2011, p. 15.
[4] OJ L 306, 22.11.2003, p. 1.
[5] OJ L 302, 13.11.2008, p. 19.
[6] OJ 121, 29.7.1964, p. 1977/64.
[7] OJ L 46, 19.2.1991, p. 19.

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(12) Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC [1] concerns, amongst others, trade in other biungulates and in semen, ova and embryos of sheep and goats, and in embryos of porcine animals.

(13) Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin [2] concerns, amongst others, the health conditions for the production and marketing of fresh meat, minced meat, mechanically separated meat, meat preparations, farmed game meat, meat products, including treated stomachs, bladders and intestines, and dairy products.

(14) Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption [3] concerns, amongst others, the health marking of food of animal origin.

(15) Council Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption [4] provides for specific treatment of meat products that ensure inactivation of the foot-and-mouth disease virus in products of animal origin.

(16) Commission Decision 2001/304/EC of 11 April 2001 on marking and use of certain animal products in relation to Decision 2001/172/EC concerning certain protection measures with regard to foot-and-mouth disease in the United Kingdom [5] concerns a specific health mark to be applied to certain products of animal origin that shall be restricted to the national market. It is appropriate to lay down in a separate Annex a similar marking in the case of foot-and-mouth disease in Bulgaria.

(17) Council Directive 92/118/EEC [6] lays down animal health and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A(I) to Directive 89/662/EEC and, as regards pathogens, to Directive 90/425/EEC.

(18) Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption [7] provides for a range of treatments of animal by-products suitable to inactivate the foot-and-mouth disease virus.

(19) Council Directive 88/407/EEC [8] lays down the animal health requirements applicable to intra-Community trade in and imports of deep-frozen semen of domestic animals of the bovine species.

(20) Council Directive 89/556/EEC [9] concerns the animal health conditions governing intra-Community trade in and imports from third countries of embryos of domestic animals of the bovine species.

(21) Council Directive 90/429/EEC [10] lays down the animal health requirements applicable to intra-Community trade in and imports of semen of domestic animals of the porcine species.

(22) The model health certificates for trade within the Union in semen, ova and embryos of animals of the ovine and caprine species and in ova and embryos of animals of the porcine species are laid down in Commission Decision 2010/470/EU of 26 August 2010 laying down model health certificates for trade within the Union in semen, ova and embryos of animals of the equine, ovine and caprine species and in ova and embryos of animals of the porcine species [11].

(23) In so far as medicinal products defined in Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products [12], Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [13], and Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use [14] no longer fall under the scope of Regulation (EC) No 1774/2002 they should be excluded from animal health related restrictions set up by this Decision.

[1] OJ L 268, 14.9.1992, p. 54.
[2] OJ L 139, 30.4.2004, p. 55.
[3] OJ L 139, 30.4.2004, p. 206.
[4] OJ L 18, 23.1.2003, p. 11.
[5] OJ L 104, 13.4.2001, p. 6.
[6] OJ L 62, 15.3.1993, p. 49.
[7] OJ L 273, 10.10.2002, p. 1.
[8] OJ L 194, 22.7.1988, p. 10.
[9] OJ L 302, 19.10.1989, p. 1.
[10] OJ L 224, 18.8.1990, p. 62.
[11] OJ L 228, 31.8.2010, p. 15.
[12] OJ L 311, 28.11.2001, p. 1.
[13] OJ L 311, 28.11.2001, p. 67.
[14] OJ L 121, 1.5.2001, p. 34.

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(24) Article 6 of Commission Decision 2007/275/EC of 17 April 2007 concerning lists of animals and products to be subject to controls at border inspection posts under Council Directives 91/496/EEC and 97/78/EC [1] provides for a derogation from the veterinary checks for certain products containing animal products. It is appropriate to allow dispatch from the high risk areas of such products under a simplified certification regime.

(25) The possible risk of spread of foot-and-mouth disease within the European Union through the movements of consignments of products of animal origin of a non-commercial character should be considered in view of the foot-and-mouth disease situation in Bulgaria. Therefore such movements should be prevented in order to avoid further spread of the disease. Bulgaria should ensure that compliance with the restrictions imposed by this Decision on certain products derived from animals of species susceptible to foot-and-mouth disease is also ensured as regards the non-commercial movement of these products. Member States should cooperate in monitoring personal luggage of passengers travelling in particular from the high risk areas and in information campaigns carried out to prevent introduction of products of animal origin into the territory of Member States other than Bulgaria.

(26) Member States other than Bulgaria should support the disease control measures carried out in the affected areas by ensuring that live susceptible animals are not dispatched to those areas.

(27) Council Decision 2009/470/EC of 25 May 2009 on expenditure in the veterinary field [2] provides for a mechanism to compensate affected holdings for losses incurred as a result of disease control measures.

(28) The foot-and-mouth disease situation has been reviewed at the meeting of the Standing Committee on the Food Chain and Animal Health of 12 January 2011 and the measures provided for in Decision 2011/8/EU were adapted in the light of the information received from Bulgaria on the development of the epidemiological situation. Decision 2011/8/EU should therefore be repealed and replaced by this Decision.

(29) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS DECISION:

Article 1

Live animals

1. Bulgaria shall ensure that the conditions set out in paragraphs 2 to 7 of this Article are met, without prejudice to the measures taken by that Member State within the framework of:

(a) Directive 2003/85/EC; and

(b) Decision 2008/855/EC.

2. No live animals of the bovine, ovine, caprine and porcine species and other biungulates shall move between the areas listed in Annex I and Annex II.

3. No live animals of the bovine, ovine, caprine and porcine species and other biungulates shall be dispatched from or moved through the areas listed in Annex I and Annex II.

4. By way of derogation from paragraph 3, the competent authorities of Bulgaria may authorise the direct and uninterrupted transit of biungulate animals through the areas listed in Annex I and Annex II on main roads and railway lines.

5. The health certificates, as provided for in Directive 64/432/EEC for live bovine animals and, without prejudice to Article 8b and 9 of Decision 2008/855/EC, for porcine animals and in Directive 91/68/EEC for live ovine and caprine animals, accompanying animals consigned from parts of the territory of Bulgaria not listed in Annex I and Annex II to other Member States shall bear the following words:

'Animals conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

6. The health certificates accompanying biungulates other than those covered by the certificates referred to in paragraph 5, consigned from parts of the territory of Bulgaria not listed in Annex I and Annex II to other Member States shall bear the following words:

'Live biungulates conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

7. Animals accompanied by an animal health certificate as referred to in paragraphs 5 and 6 may be moved to other Member States only if the local veterinary authority in Bulgaria has, 3 days before the move, notified the central and local veterinary authorities in the Member State of destination.

8. By way of derogation from paragraph 2 the competent authorities of Bulgaria may authorise the transport of animals of species susceptible to foot-and-mouth disease from holding situated in areas listed in Annex II to a slaughterhouse situated in the areas listed in Annex I.

9. By way of derogation from paragraph 2, the competent authority of Bulgaria may authorise the transport of pigs from holdings outside the surveillance zone established in accordance with Article 21 of Directive 2003/85/EC for immediate slaughter at designated slaughterhouses situated in the areas listed in Annex II under the following conditions:

[1] OJ L 116, 4.5.2007, p. 9.
[2] OJ L 155, 18.6.2009, p. 30.

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(a) the pigs originate from holdings in the area listed in Annex I from which consignments of fresh pigmeat and meat preparations and meat products consisting of, or containing meat of those pigs may be dispatched in accordance with Article 6 of Decision 2008/855/EC.

The central veterinary authority of Bulgaria shall communicate to the other Member States and to the Commission the list of holdings which they have approved for the purpose of application of this paragraph;

(b) during the 21 days prior to the date of transport to the slaughterhouse, the animals have remained under the supervision of the competent veterinary authority on a single holding which is situated in the centre of a circle around the holding of at least 10 km radius, where there has been no outbreak of foot-and-mouth disease during at least 30 days prior to the date of loading;

(c) no animals of species susceptible to foot-and-mouth disease have been introduced into the holding referred to in the introductory sentence of this paragraph during the 21 days prior to the date of loading, except in the case of pigs coming from a supplying holding which complies with the conditions laid down in point (b), in which case the period of 21 days may be reduced to 7 days;

(d) the transport of pigs is only authorised after the satisfactory completion of the measures provided for in Article 22(2) of Directive 2003/85/EC.

Article 2

Meats

1. For the purposes of this Article, 'meats' means 'fresh meat', 'minced meat', 'mechanically separated meat' and 'meat preparations' as defined in points 1.10, 1.13, 1.14 and 1.15 of Annex I to Regulation (EC) No 853/2004.

2. Bulgaria shall not dispatch meats of the bovine, ovine, caprine and porcine species and other biungulates coming from or obtained from animals originating in the areas listed in Annex I.

3. Meats not eligible for dispatch from Bulgaria in accordance with this Decision shall be marked in accordance with the second subparagraph of Article 4(1) of Directive 2002/99/EC or in accordance with Annex IV.

4. Without prejudice to Articles 6 and 8b of Decision 2008/855/EC, the prohibition set out in paragraph 2 shall not apply to meats bearing the health mark in accordance with Chapter III of Section I of Annex I to Regulation (EC) No 854/2004, provided that:

(a) the meat is clearly identified, and has been transported and stored since the date of production separately from meat which is not eligible, in accordance with this Decision, for dispatch outside the areas listed in Annex I;

(b) the meat complies with one of the following conditions:

(i) it was obtained before 9 December 2010; or

(ii) it is derived from animals that have been reared for at least 90 days, or since birth if less than 90 days of age, prior to the date of slaughter and which have been slaughtered, or in the case of meat obtained from wild game of species susceptible to foot-and-mouth disease killed, outside the areas listed in Annexes I and II; or

(iii) it complies with the conditions set out in points (c), (d) and (e);

(c) the meat was obtained from domestic ungulates or from farmed game of species susceptible to foot-and-mouth disease, as specified for the respective category of meat in one of the appropriate columns 4 to 7 in Annex III, and complies with the following conditions:

(i) the animals have been reared for at least 90 days prior to the date of slaughter, or since birth if less than 90 days of age, on holdings situated within the areas specified in columns 1, 2 and 3 of Annex III, where there has been no outbreak of foot-and-mouth disease during at least 90 days prior to the date of slaughter;

(ii) during the 21 days prior to the date of transport to the slaughterhouse, or in the case of farmed game of species susceptible to foot-and-mouth disease prior to the date of on-farm slaughtering, the animals have remained under the supervision of the competent veterinary authorities on a single holding which is situated in the centre of a circle around the holding of at least 10 km radius, where there has been no outbreak of foot-and-mouth disease during at least 30 days prior to the date of loading;

(iii) no animals of species susceptible to foot-and-mouth disease have been introduced into the holding referred to in point (ii) during the 21 days prior to the date of loading, or in the case of farmed game of species susceptible to foot-and-mouth disease prior to the date of on-farm slaughtering, except in the case of pigs coming from a supplying holding which complies with the conditions laid down in point (ii), in which case the period of 21 days may be reduced to 7 days.

However, the competent authority may authorise the introduction into the holding referred to in point (ii) of animals of species susceptible to foot-and-mouth disease which comply with the conditions set out in points (i) and (ii) and which:

— come from a holding where no animals of species susceptible to foot-and-mouth disease have been introduced during the 21 days prior to the date of transport to the holding referred to in point (ii), except in the case of pigs coming from a supplying holding in which case the period of 21 days may be reduced to 7 days; or

— were subjected with negative results to a test for antibodies against the foot-and-mouth disease virus carried out on a blood sample taken within 10 days prior to the date of transport to the holding referred to in point (ii); or

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— come from a holding that was subjected with negative results to a serological survey pursuant to a sampling protocol suitable to detect 5 % prevalence of foot-and-mouth disease with at least a 95 % level of confidence;

(iv) the animals or, in the case of farmed game of species susceptible to foot-and-mouth disease slaughtered on the farm, the carcasses have been transported under official control in means of transport that have been cleansed and disinfected before loading from the holding referred to in point (ii) to the designated slaughterhouse;

(v) the animals have been slaughtered less than 24 hours following the time of arrival at the slaughterhouse and separately from animals the meat of which is not eligible for dispatch from the area listed in Annex I;

(d) the meat, if positively marked in column 8 of Annex III, was obtained from wild game of species susceptible to foot-and-mouth disease, that was killed in areas where there has been no outbreak of foot-and-mouth disease for at least a period of 90 days before the date of killing and at a distance of at least 20 km from areas not specified in columns 1, 2 and 3 of Annex III;

(e) meat referred to in points (c) and (d) must in addition comply with the following conditions:

(i) the dispatch of such meat is only to be authorised by the competent veterinary authority of Bulgaria, if

— the animals referred to in point (c)(iv) have been transported to the establishment without contact to holdings situated in areas not specified in columns 1, 2 and 3 of Annex III, and

— the establishment is not situated in a protection zone;

(ii) the meat is at all times clearly identified, handled, stored and transported separately from meat which is not eligible for dispatch from the area listed in Annex I;

(iii) during post-mortem inspection by the official veterinarian in the establishment of dispatch, or in the case of on-farm slaughtering of farmed game of species susceptible to foot-and-mouth disease on the holding referred to in point (c)(ii), or in the case of wild game of species susceptible to foot-and-mouth disease at the game-handling establishment, no clinical signs or post-mortem evidence of foot-and-mouth disease were established;

(iv) the meat has remained in the establishments or holdings referred to in point (e)(iii) for at least 24 hours following the post-mortem inspection of the animals referred to in points (c) and (d);

(v) any further preparation of meat for dispatch outside the area listed in Annex I shall be suspended:

— in the case where foot-and-mouth disease has been diagnosed in the establishments or holdings referred to in point (e)(iii), until the slaughter of all animals present and the removal of all meat and dead animals has been completed, and at least 24 hours have elapsed since the completion of the total cleansing and disinfection of those establishments and holdings under the control of an official veterinarian, and

— in the case of slaughter in the same establishment of animals susceptible to foot-and-mouth disease coming from holdings situated in areas listed in Annex I that do not comply with the conditions set out in point 4(c) or (d), until the slaughter of all such animals and the cleansing and disinfection of those establishments have been completed under the control of an official veterinarian;

(vi) the central veterinary authorities shall communicate to the other Member States and the Commission a list of those establishments and holdings which they have approved for the purposes of application of points (c), (d) and (e).

5. Compliance with the conditions set out in paragraphs 3 and 4 shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

6. Without prejudice to Articles 6 and 8b of Decision 2008/855/EC, the prohibition set out in paragraph 2 of this Article shall not apply to fresh meat obtained from animals reared outside the areas listed in Annex I and Annex II and transported, by way of derogation from Article 1(2) and (3), directly and under official control without contact to holdings situated in areas listed in Annex I to a slaughterhouse situated in the areas listed in Annex I outside the protection zone for immediate slaughter, provided that such fresh meat is only placed on the market in the areas listed in Annex I and Annex II and complies with the following conditions:

(a) all such fresh meat is marked in accordance with the second subparagraph of Article 4(1) of Directive 2002/99/EC or in accordance with Annex IV to this Decision;

(b) the slaughterhouse:

(i) is operated under strict veterinary control;

(ii) suspends any further preparation of meat for dispatch outside the areas listed in Annex I in the case of slaughter in the same slaughterhouse of animals susceptible to foot-and-mouth disease coming from holdings situated in areas listed in Annex I until the slaughter of all such animals and the cleansing and disinfection of the slaughterhouse have been completed under the control of an official veterinarian;

(c) the fresh meat is clearly identified, and transported and stored separately from meat which is eligible for dispatch outside Bulgaria.

Compliance with the conditions set out in the first subparagraph shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

The central veterinary authority of Bulgaria shall communicate to the Commission and to the other Member States the list of the establishments which they have approved for the purposes of application of this paragraph.

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7. Without prejudice to Article 6 of Decision 2008/855/EC, the prohibition set out in paragraph 2 shall not apply to fresh meat obtained from cutting plants situated in the areas listed in Annex I under the following conditions:

(a) only fresh meat as described in paragraph 4(b) is processed in that cutting plant, on the same day.

Cleansing and disinfection shall be carried out after processing of any meat not meeting this requirement;

(b) all meat bears the health mark in accordance with Chapter III of Section I of Annex I to Regulation (EC) No 854/2004;

(c) the cutting plant is operated under strict veterinary control;

(d) the fresh meat is clearly identified, and transported and stored separately from meat which is not eligible for dispatch outside the areas listed in Annex I.

Compliance with the conditions set out in the first subparagraph shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

The central veterinary authority of Bulgaria shall communicate to the other Member States and the Commission the list of the establishments which they have approved for the purpose of application of this paragraph.

8. Meat dispatched from Bulgaria to other Member States shall be accompanied by an official certificate, which shall bear the following words:

'Meat conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

9. Without prejudice to Articles 6 and 8b of Decision 2008/855/EC, the prohibition set out in paragraph 2 of this Article shall not apply to fresh meat obtained from pigs reared in the areas listed in Annex I and transported in accordance with Article 1(9) to a slaughterhouse situated in the areas listed in Annex II for immediate slaughter, provided that such fresh meat complies with the following conditions:

(a) the fresh meat is marked in accordance with the second subparagraph of Article 4(1) of Directive 2002/99/EC or in accordance with Annex IV to this Decision and is placed on the market only in the areas listed in Annex I and Annex II;

(b) the slaughterhouse:

(i) is operated under veterinary control;

(ii) suspends any further preparation of meat for dispatch outside the areas listed in Annex I in the case of slaughter in the same slaughterhouse of animals susceptible to foot-and-mouth disease coming from other holdings situated in areas listed in Annex I until the slaughter of all such animals and the cleansing and disinfection of the slaughterhouse have been completed under the control of an official veterinarian;

(c) the fresh meat is clearly identified and is transported and stored separately from meat which is eligible for dispatch outside Bulgaria.

Compliance with the conditions set out in paragraph 1 shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

The central veterinary authority of Bulgaria shall communicate to the other Member States and to the Commission the list of the establishments which they have approved for the purpose of application of this paragraph.

Article 3

Meat products

1. Bulgaria shall not dispatch meat products, including treated stomachs, bladders and intestines, of animals of the bovine, ovine, caprine and porcine species and other biungulates ('meat products') coming from the areas listed in Annex I or prepared using meat obtained from animals originating in those areas.

2. Without prejudice to Articles 6 and 8b of Decision 2008/855/EC, the prohibition set out in paragraph 1 shall not apply to meat products, including treated stomachs, bladders and intestines, bearing the health mark in accordance with Chapter III of Section I of Annex I to Regulation (EC) No 854/2004, provided that the meat products:

(a) are clearly identified and have been transported and stored since the date of production separately from meat products not eligible, in accordance with this Decision, for dispatch outside the areas listed in Annex I;

(b) comply with one of the following conditions:

(i) they are made from meats described in Article 2(4)(b); or

(ii) they have undergone at least one of the relevant treatments laid down for foot-and-mouth disease in Part I of Annex III to Directive 2002/99/EC.

Compliance with the conditions set out in the first subparagraph shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

The central veterinary authorities shall communicate to the other Member States and the Commission a list of the establishments which they have approved for the purpose of application of this paragraph.

3. Meat products dispatched from Bulgaria to other Member States shall be accompanied by an official certificate, which shall bear the following words:

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'Meat products, including treated stomachs, bladders and intestines, conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

4. By way of derogation from paragraph 3 it shall be sufficient, in the case of meat products which comply with the requirements of paragraph 2 and have been processed in an establishment operating Hazard Analysis and Critical Control Points (HACCP) and an auditable standard operating procedure which ensures that standards for treatment are met and recorded, that compliance with the conditions required for the treatment laid down in point (b)(ii) of the first subparagraph of paragraph 2 is stated in the commercial document accompanying the consignment, endorsed in accordance with Article 9(1).

5. By way of derogation from paragraph 3 it shall be sufficient, in the case of meat products heat treated in accordance with point (b)(ii) of the first subparagraph of paragraph 2 in hermetically sealed containers so as to ensure that they are shelf stable, to be accompanied by a commercial document stating the heat treatment applied.

Article 4

Colostrums and milk

1. Bulgaria shall not dispatch colostrums and milk from animals of species susceptible to foot-and-mouth disease intended or not intended for human consumption from the areas listed in Annex I.

2. The prohibition set out in paragraph 1 shall not apply to milk produced from bovine, ovine and caprine animals kept in areas listed in Annex I which has been subjected to a treatment in accordance with:

(a) Part A of Annex IX to Directive 2003/85/EC, if the milk is intended for human consumption; or

(b) Part B of Annex IX to Directive 2003/85/EC, if the milk is not intended for human consumption or is intended for feeding to animals of species susceptible to foot-and-mouth disease.

3. The prohibition set out in paragraph 1 shall not apply to milk from bovine, ovine and caprine animals prepared in establishments situated in the areas listed in Annex I under the following conditions:

(a) all milk used in the establishment must either conform to the conditions set out in paragraph 2 or be obtained from animals reared and milked outside the areas listed in Annex I;

(b) the establishment is operated under strict veterinary control;

(c) the milk must be clearly identified, and transported and stored separately from milk and dairy products which are not eligible for dispatch outside the areas listed in Annex I;

(d) transport of raw milk from holdings situated outside the areas listed in Annex I to the establishments situated in the areas listed in Annex I is carried out in vehicles which were cleansed and disinfected prior to operation and had no subsequent contact with holdings in the areas listed in Annex I keeping animals of species susceptible to foot-and-mouth disease.

Compliance with the conditions set out in the first subparagraph shall be checked by the competent veterinary authority under the supervision of the central veterinary authorities.

The central veterinary authorities shall communicate to the other Member States and the Commission a list of the establishments which they have approved for the purpose of application of this paragraph.

4. Milk dispatched from Bulgaria to other Member States shall be accompanied by an official certificate, which shall bear the following words:

'Milk conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

5. By way of derogation from paragraph 4 it shall be sufficient, in the case of milk which complies with the requirements of paragraph 2 and has been processed in an establishment operating HACCP and an auditable standard operating procedure which ensures that standards for treatment are met and recorded, that compliance with those requirements is stated in the commercial document accompanying the consignment, endorsed in accordance with Article 9(1).

6. By way of derogation from paragraph 4 it shall be sufficient, in the case of milk which complies with the requirements in paragraph 2(a) or (b) and which has been heat treated in hermetically sealed containers so as to ensure that it is shelf stable, to be accompanied by a commercial document stating the heat treatment applied.

Article 5

Dairy products

1. Bulgaria shall not dispatch dairy products produced from colostrums and milk from animals of species susceptible to foot-and-mouth disease intended or not intended for human consumption from the areas listed in Annex I.

2. The prohibition set out in paragraph 1 shall not apply to dairy products:

(a) produced before 9 December 2010; or

(b) prepared from milk complying with the provisions in Article 4(2) or (3); or

(c) for export to a third country where import conditions permit such products to be subject to treatment other than those laid down in Article 4(2) which ensures the inactivation of the foot-and-mouth disease virus.

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3. Without prejudice to Chapter II of Section IX of Annex III to Regulation (EC) No 853/2004, the prohibition set out in paragraph 1 of this Article shall not apply to the following dairy products intended for human consumption:

(a) dairy products produced from milk of a controlled pH less than 7 and subject to a heat treatment at a temperature of at least 72 °C for at least 15 seconds, on the understanding that such treatment was not necessary for finished products, the ingredients of which comply with the respective animal health conditions laid down in Articles 2, 3 and 4 of this Decision;

(b) dairy products produced from raw milk of bovine, ovine or caprine animals which have been resident for at least 30 days on a holding situated, within an area listed in Annex I, in the centre of a circle of at least 10 km radius in which no outbreak of foot-and-mouth disease has occurred during 30 days prior to the date of production of the raw milk, and subject to a maturation or ripening process of at least 90 days during which the pH is lowered below 6.0 throughout the substance, and the rind of which has been treated with 0,2 % citric acid immediately prior to wrapping or packaging.

4. The prohibition set out in paragraph 1 shall not apply to dairy products prepared in establishments situated in the areas listed in Annex I under the following conditions:

(a) all milk used in the establishment either complies with the conditions laid down in Article 4(2) or is obtained from animals outside the areas listed in Annex I;

(b) all dairy products used in the final products either comply with the conditions set out in paragraph 2(a) and (b) or paragraph 3 or are made from milk obtained from animals outside the areas listed in Annex I;

(c) the establishment is operated under strict veterinary control;

(d) the dairy products are clearly identified and transported and stored separately from milk and dairy products which are not eligible for dispatch outside the areas listed in Annex I.

Compliance with the conditions set out in the first subparagraph shall be checked by the competent authority under the responsibility of the central veterinary authorities.

The central veterinary authorities shall communicate to the other Member States and the Commission a list of the establishments which they have approved for the purposes of application of this paragraph.

5. The prohibition set out in paragraph 1 shall not apply to dairy products prepared in establishment situated outside the areas listed in Annex I using milk obtained before 9 December 2010, provided that the dairy products are clearly identified and transported and stored separately from dairy products which are not eligible for dispatch outside those areas.

6. Dairy products dispatched from Bulgaria to other Member States shall be accompanied by an official certificate, which shall bear the following words:

'Dairy products conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

7. By way of derogation from paragraph 6 it shall be sufficient, in the case of dairy products which comply with the requirements of paragraph 2(a) and (b) and paragraphs 3 and 4 and have been processed in an establishment operating HACCP and an auditable standard operating procedure which ensures that standards for treatment are met and recorded, that compliance with those requirements is stated in the commercial document accompanying the consignment, endorsed in accordance with Article 9(1).

8. By way of derogation from paragraph 6 it shall be sufficient, in the case of dairy products which comply with the requirements of paragraph 2(a) and (b) and paragraphs 3 and 4 and which have been heat treated in hermetically sealed containers so as to ensure that they are shelf stable, to be accompanied by a commercial document stating the heat treatment applied.

Article 6

Semen, ova and embryos

1. Bulgaria shall not dispatch semen, ova and embryos of the bovine, ovine, caprine and porcine species and other biungulates ('semen, ova and embryos') from the areas listed in Annex I and Annex II.

2. Without prejudice to Article 5 of Decision 2008/855/EC, the prohibitions set out in paragraph 1 shall not apply to:

(a) semen, ova and embryos produced before 9 December 2010;

(b) frozen bovine semen and in-vivo derived embryos, frozen porcine semen, and frozen ovine and caprine semen and embryos imported into Bulgaria in accordance with the conditions laid down in Directives 88/407/EEC, 89/556/EEC, 90/429/EEC or 92/65/EEC respectively, and which since their introduction into Bulgaria have been stored and transported separately from semen, ova and embryos not eligible for dispatch in accordance with paragraph 1;

(c) frozen semen and embryos obtained from bovine, porcine, ovine and caprine animals kept for at least 90 days prior to the date of and during collection outside the areas listed in Annex I and Annex II and which:

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(i) have been be stored in approved conditions for a minimum period of 30 days prior to the date of dispatch; and

(ii) have been collected from donor animals standing in centres or on holdings which have been free from foot-and-mouth disease for at least 3 months prior to the date of collection of the semen or embryos and 30 days after the date of collection and which are situated in the centre of an area of 10 kilometres radius in which there has been no case of foot-and-mouth disease for at least 30 days prior to the date of collection.

(d) Before the dispatch of the semen or embryos referred to in points (a), (b) and (c) the central veterinary authorities shall communicate to the other Member States and the Commission a list of centres and teams approved for the purpose of application of this paragraph.

3. The health certificate provided for in Directive 88/407/EEC and accompanying frozen bovine semen dispatched from Bulgaria to other Member States shall bear the following words:

'Frozen bovine semen conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

4. Without prejudice to Article 9(b) of Decision 2008/855/EC, the health certificate provided for in Directive 90/429/EEC and accompanying frozen porcine semen dispatched from Bulgaria to other Member States shall bear the following words:

'Frozen porcine semen conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

5. The health certificate provided for in Directive 89/556/EEC and accompanying bovine in-vivo derived embryos dispatched from Bulgaria to other Member States shall bear the following words:

'Bovine in-vivo derived embryos conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

6. The health certificate provided for in Directive 92/65/EEC and accompanying frozen ovine or caprine semen dispatched from Bulgaria to other Member States shall bear the following words:

'Frozen ovine/caprine semen conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

7. The health certificate provided for in Directive 92/65/EEC and accompanying frozen ovine or caprine embryos dispatched from Bulgaria to other Member States shall bear the following words:

'Frozen ovine/caprine embryos conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

8. Without prejudice to Article 9(c) of Decision 2008/855/EC, the health certificate provided for in Directive 92/65/EEC and accompanying frozen porcine embryos dispatched from Bulgaria to other Member States shall bear the following words:

'Frozen porcine embryos conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

Article 7

Hides and skins

1. Bulgaria shall not dispatch hides and skins of animals of the bovine, ovine, caprine and porcine species and of other biungulates ('hides and skins') from the areas listed in Annex I.

2. The prohibition set out in paragraph 1 shall not apply to hides and skins which:

(a) were produced in Bulgaria before 9 December 2010; or

(b) comply with the requirements provided for in point 2(c) or (d) of Part A of Chapter VI of Annex VIII to Regulation (EC) No 1774/2002; or

(c) were produced outside the areas listed in Annex I in accordance with the conditions laid down in Regulation (EC) No 1774/2002, and have since introduction into Bulgaria been stored and transported separately from hides and skins not eligible for dispatch in accordance with paragraph 1.

Treated hides and skins shall be separated from untreated hides and skins of animals of species susceptible to foot-and-mouth disease.

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3. Bulgaria shall ensure that hides and skins to be dispatched to other Member States shall be accompanied by an official certificate which bears the following words:

'Hides and skins conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

4. By way of derogation from paragraph 3 it shall be sufficient, in the case of hides and skins which comply with the requirements of points (1)(b) to (e) of Part A of Chapter VI of Annex VIII to Regulation (EC) No 1774/2002, to be accompanied by a commercial document stating compliance with those requirements.

5. By way of derogation from paragraph 3 it shall be sufficient, in the case of hides and skins which comply with the requirements of point 2(c) or (d) of Part A of Chapter VI of Annex VIII to Regulation (EC) No 1774/2002, that compliance with those requirements is stated in the commercial document accompanying the consignment, endorsed in accordance with Article 9(1).

Article 8

Other animal products

1. Bulgaria shall not dispatch products of animals of the bovine, ovine, caprine and porcine species and other biungulates not mentioned in Articles 2 to 7 produced after 9 December 2010 and coming from the areas listed in Annex I, or obtained from animals originating in the areas listed in Annex I.

Bulgaria shall not dispatch dung and manure of the bovine, ovine, caprine and porcine species and other biungulates from the areas listed in Annex I.

2. The prohibition set out in the first subparagraph of paragraph 1 shall not apply to:

(a) animal products which:

(i) have been subjected to a heat treatment:

— in a hermetically sealed container with a Fo value of 3,00 or more, or

— in which the centre temperature is raised to at least 70 °C; or

(ii) were produced outside the areas listed in Annex I in accordance with the conditions laid down in Regulation (EC) No 1774/2002, and which since introduction into Bulgaria have been stored and transported separately from animal products not eligible for dispatch in accordance with paragraph 1;

(b) blood and blood products as defined in points 4 and 5 of Annex I to Regulation (EC) No 1774/2002 which have been subjected to at least one of the treatments provided for in point 4(a) of Part A of Chapter IV of Annex VIII to Regulation (EC) No 1774/2002, followed by an effectiveness check, or have been imported in accordance with Part A of Chapter IV of Annex VIII to Regulation (EC) No 1774/2002;

(c) lard and rendered fats which have been subject to the heat treatment prescribed in point 2(d)(iv) of Part B of Chapter IV of Annex VII to Regulation (EC) No 1774/2002;

(d) animal casings complying with the conditions in Part A of Chapter 2 of Annex I to Directive 92/118/EEC and which have been cleaned, scraped and then either salted, bleached or dried, followed by steps to prevent the recontamination of the casings;

(e) sheep wool, ruminant hair and pigs bristles which have undergone factory washing or have been obtained from tanning and unprocessed sheep wool, ruminant hair and pigs bristles which are securely enclosed in packaging and dry;

(f) petfood conforming to the requirements of points 2, 3 and 4 of Part B of Chapter II of Annex VIII to Regulation (EC) No 1774/2002;

(g) composite products which are not subject to further treatment containing products of animal origin, on the understanding that the treatment was not necessary for finished products, the ingredients of which comply with the respective animal health conditions laid down in this Decision;

(h) game trophies in accordance with points 1, 3 or 4 of Part A of Chapter VII of Annex VIII to Regulation (EC) No 1774/2002;

(i) packed animal products intended for use as in-vitro diagnostic, laboratory reagents;

(j) medicinal products as defined in Directive 2001/83/EC, medical devices manufactured utilising animal tissue which is rendered non-viable as referred to in Article 1(5)(g) of Directive 93/42/EEC, veterinary medicinal products as defined in Directive 2001/82/EC, and investigational medicinal products as defined in Directive 2001/20/EC.

3. Bulgaria shall ensure that the animal products referred to in paragraph 2 to be dispatched to other Member States shall be accompanied by an official certificate which bears the following words:

'Animal products conforming to Commission Decision 2011/44/EU of 19 January 2011 concerning certain protection measures against foot-and-mouth disease in Bulgaria (*).

(*) OJ L 19, 22.1.2011, p. 20.'

4. By way of derogation from paragraph 3, it shall be sufficient, in the case of the products referred to in paragraph 2(a) to (d) and (f) of this Article that compliance with the conditions for the treatment stated in the commercial document required in accordance with the respective Union legislation is endorsed in accordance with Article 9(1).

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5. By way of derogation from paragraph 3 it shall be sufficient, in the case of products referred to in paragraph 2(e) to be accompanied by a commercial document stating either the factory washing or origin from tanning or compliance with the conditions laid down in points 1 and 4 of Part A of Chapter VIII of Annex VIII to Regulation (EC) No 1774/2002.

6. By way of derogation from paragraph 3 it shall be sufficient, in the case of products referred to in paragraph 2(g) which have been produced in an establishment operating HACCP and an auditable standard operating procedure which ensures that pre-processed ingredients comply with the respective animal health conditions laid down in this Decision, that this is stated on the commercial document accompanying the consignment, endorsed in accordance with Article 9(1).

7. By way of derogation from paragraph 3, it shall be sufficient, in the case of products referred to in paragraph 2(i) and (j), to be accompanied by a commercial document stating that the products are for use as in-vitro diagnostic, laboratory reagents, medical products or medical devices, provided that the products are clearly labelled 'for in-vitro diagnostic use only' or 'for laboratory use only', as 'medicinal products' or as 'medical devices'.

8. Derogating from the provisions in paragraph 3, it shall be sufficient, in the case of composite products that fulfil the conditions set out in Article 6(1) of Decision 2007/275/EC that they are accompanied by a commercial document, which bears the following words:

'These composite products are shelf stable at ambient temperature or have clearly undergone in their manufacture a complete cooking or heat treatment process throughout their substance, so that any raw material is de-natured'.

Article 9

Certification

1. Where reference is made to this paragraph, the competent authorities of Bulgaria shall ensure that the commercial document required by Union legislation for trade between Member States is endorsed by the attachment of a copy of an official certificate stating that:

(a) the products concerned have been produced:

(i) in a production process that has been audited and found in compliance with the appropriate requirements in Union animal health legislation and suitable to destroy the foot-and-mouth disease virus; or

(ii) from pre-processed materials which had been certified accordingly; and

(b) provisions are in place to avoid possible re-contamination with the foot-and-mouth disease virus after treatment.

Such certification of the production process shall bear a reference to this Decision, shall be valid for 30 days, shall state the expiry date and shall be renewable after inspection of the establishment.

2. In case of products for retail sale to the final consumer, the competent authorities of Bulgaria may authorise consolidated consignments of animal products other than fresh meat, minced meat, mechanically separated meat and meat preparations, each of which is eligible for dispatch in accordance with this Decision, to be accompanied by a commercial document endorsed by the attachment of a copy of an official veterinary certificate confirming that:

(a) the premises of dispatch have in place a system to ensure that goods can only be dispatched if they are traceable to documentary evidence of compliance with this Decision; and

(b) the system referred to in (a) has been audited and found satisfactory.

Such certification of the traceability system shall bear a reference to this Decision, shall be valid for 30 days, shall state the expiry date and shall be renewable only after the establishment had been audited with satisfactory results.

The competent authorities of Bulgaria shall communicate to the other Member States and the Commission the list of establishments which they have approved for the purpose of application of this paragraph.

Article 10

Cleansing and disinfection

1. Without prejudice to Article 11 of Decision 2008/855/EC, Bulgaria shall ensure that vehicles which have been used for the transport of live animals in the areas listed in Annex I and Annex II are cleansed and disinfected after each operation, and that such cleansing and disinfection is recorded in accordance with Article 12(2)(d) of Directive 64/432/EEC.

2. Bulgaria shall ensure that vehicles which have been used within the areas listed in Annex I and Annex II for the transport of animals and parts of animals of species susceptible to foot-and-mouth disease referred to in Article 5(1)(e) of Regulation (EC) No 1774/2002 and of other animal by-products and processed animal by-products derived from animals of species susceptible to foot-and-mouth disease are cleansed and disinfected after each operation, and that such cleansing and disinfection and all contacts with holdings keeping animals of species susceptible to foot-and-mouth disease are recorded in the journey log of the vehicle concerned.

Article 11

Certain exempted products

The restrictions laid down in Articles 3, 4, 5 and 8 shall not apply to the dispatch from the areas listed in Annex I of the animal products referred to in those Articles if such products were:

(a) not produced in Bulgaria and remained in their original packaging indicating the country of origin of the products; or

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(b) produced in an approved establishment situated in the areas listed in Annex I from pre-processed products not originating from those areas, which:

(i) have, since introduction into the territory of Bulgaria, been transported, stored and processed separately from products which are not eligible for dispatch outside the areas listed in Annex I;

(ii) are accompanied by a commercial document or official certificate as required by this Decision.

Article 12

Cooperation between Member States

Member States shall cooperate in monitoring personal luggage of passengers travelling from the areas listed in Annex I and in information campaigns carried out to prevent introduction of products of animal origin into the territory of Member States other than Bulgaria.

Article 13

Measures to be taken by Member States other than Bulgaria

1. Without prejudice to Article 1(4), Member States other than Bulgaria shall ensure that live animals of species susceptible to foot-and-mouth disease are not dispatched to the areas listed in Annex I.

2. Member States other than Bulgaria shall take appropriate precautionary measures in relation to susceptible animals dispatched from Bulgaria between 9 December 2010 and 6 January 2011. Those measures may include any of the following:

(a) isolation and clinical inspection;

(b) where necessary, laboratory testing to detect or rule out infection with the foot-and-mouth disease virus.

Article 14

Implementation

Member States shall amend the measures which they apply to trade so as to bring them into compliance with this Decision. They shall immediately inform the Commission thereof.

Article 15

Repeal

Decision 2011/8/EU is repealed.

References to the repealed Decision shall be construed as references to this Decision.

Article 16

This Decision shall apply until 31 March 2011.

Article 17

Addressees

This Decision is addressed to the Member States.

Done at Brussels, 19 January 2011.

For the Commission

John DALLI

Member of the Commission

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ANNEX I

The following areas in Bulgaria:

Region of Burgas.

ANNEX II

The following areas in Bulgaria:

Regions of Kardjali, Haskovo, Yambol, Sliven, Shumen and Varna.

ANNEX III

The following areas in Bulgaria:

1	2	3	4	5	6	7	8
GROUP	ADNS	Administrative Unit	B	S/G	P	FG	WG
Bulgaria	00002	Region of Burgas	—	—	—	—	—
	—	—	—	—	—	—	—
	—	—	—	—	—	—	—
	—	—	—	—	—	—	—
	—	—	—	—	—	—	—

ADNS = Animal Disease Notification System Code (Decision 2005/176/EC)
B = bovine meat
S/G = sheep and goat meat
P = pig meat
FG = farmed game of species susceptible to foot-and-mouth disease
WG = wild game of species susceptible to foot-and-mouth disease

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ANNEX IV

The health mark referred to in Article 2(3):

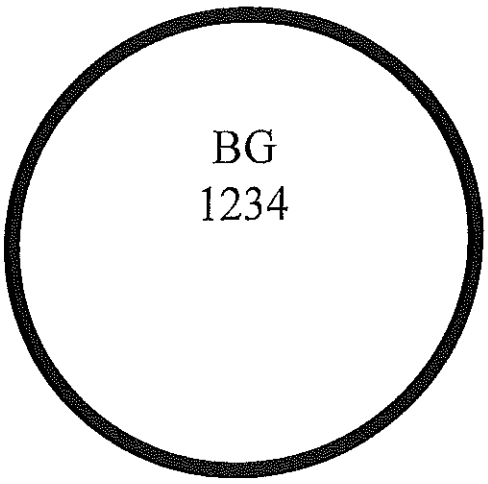
Dimensions:

BG = 7 mm

Establishment No = 10 mm

Circle outer diameter = 50 mm

Line thickness of Circle = 3 mm



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ACTS ADOPTED BY BODIES CREATED BY INTERNATIONAL AGREEMENTS

DECISION No 1/2010 OF THE COMMUNITY/SWITZERLAND INLAND TRANSPORT COMMITTEE

of 22 December 2010

amending Annex 1 to the Agreement between the European Community and the Swiss Confederation on the carriage of goods and passengers by rail and road

(2011/45/EU)

THE COMMITTEE,

Having regard to the Agreement between the European Community and the Swiss Confederation on the carriage of goods and passengers by rail and road, and in particular Article 52(4) thereof,

Whereas:

(1) The first indent of Article 52(4) of the Agreement provides for the Joint Committee to adopt decisions revising Annex 1.

(2) Annex 1 has been amended last by Decision 1/2009 of the Joint Committee of 17 June 2009.

(3) New legal acts of the European Union have been adopted in the areas covered by the Agreement. Annex 1 must therefore be reworded to bring it into line with the changes in the relevant legislation of the European Union,

HAS DECIDED AS FOLLOWS:

Article 1

Annex 1 to the Agreement is hereby repealed and replaced by the text annexed to this Decision.

Article 2

This Decision shall enter into force on 1 January 2011.

Done at Berne, 22 December 2010.

The Chairman	*The Head of the Delegation of the European Union*
Peter FÜGLISTALER	Enrico GRILLO PASQUARELLI

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ANNEX

'ANNEX I

APPLICABLE PROVISIONS

In accordance with Article 52(6) of this Agreement, Switzerland shall apply legal provisions equivalent to the following:

Relevant provisions of Community law

SECTION 1 — ADMISSION TO THE OCCUPATION

— Council Directive 96/26/EC of 29 April 1996 on admission to the occupation of road haulage operator and road passenger transport operator and mutual recognition of diplomas, certificates and other evidence of formal qualifications intended to facilitate for these operators the right to freedom of establishment in national and international transport operations (OJ L 124, 23.5.1996, p. 1), as last amended by Council Directive 98/76/EC of 1 October 1998 (OJ L 277, 14.10.1998, p. 17).

SECTION 2 — SOCIAL STANDARDS

— Council Regulation (EEC) No 3821/85 of 20 December 1985 on recording equipment in road transport (OJ L 370, 31.12.1985, p. 8), as last amended by Commission Regulation (EC) No 68/2009 of 23 January 2009 (OJ L 21, 24.1.2009, p. 3).

— Regulation (EC) No 484/2002 of the European Parliament and of the Council of 1 March 2002 amending Council Regulations (EEC) No 881/92 and (EEC) No 3118/93 for the purposes of establishing a driver attestation (OJ L 76, 19.3.2002, p. 1).

For the purposes of this Agreement,

(a) only Article 1 of Regulation (EC) No 484/2002 shall apply;

(b) the European Community and the Swiss Confederation shall exempt from the obligation to hold a driver attestation all citizens of the Swiss Confederation, of a European Community Member State and of a Member State of the European Economic Area;

(c) the Swiss Confederation may not exempt citizens of States other than those mentioned in point b) from the obligation to hold a driver attestation without prior consultation with and approval by the European Community.

— Directive 2003/59/EC of the European Parliament and of the Council of 15 July 2003 on the initial qualification and periodic training of drivers of certain road vehicles for the carriage of goods or passengers, amending Council Regulation (EEC) No 3820/85 and Council Directive 91/439/EEC and repealing Council Directive 76/914/EEC (OJ L 226, 10.9.2003, p. 4).

— Directive 2006/22/EC of the European Parliament and of the Council of 15 March 2006 on minimum conditions for the implementation of Council Regulations (EEC) No 3820/85 and (EEC) No 3821/85 concerning social legislation relating to road transport activities and repealing Council Directive 88/599/EEC (OJ L 102, 11.4.2006, p. 35).

— Regulation (EC) No 561/2006 of the European Parliament and of the Council of 15 March 2006 on the harmonisation of certain social legislation relating to road transport and amending Council Regulations (EEC) No 3821/85 and (EC) No 2135/98 and repealing Council Regulation (EEC) No 3820/85 (OJ L 102, 11.4.2006, p. 1).

— Commission Regulation (EC) No 68/2009 of 23 January 2009 adapting for the ninth time to technical progress Council Regulation (EEC) No 3821/85 on recording equipment in road transport (OJ L 21, 24.1.2009, p. 3).

SECTION 3 — TECHNICAL STANDARDS

Motor vehicles

— Council Regulation (EC) No 2411/98 of 3 November 1998 on the recognition in intra-Community traffic of the distinguishing sign of the Member State in which motor vehicles and their trailers are registered (OJ L 299, 10.11.1998, p. 1).

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— Council Directive 91/542/EEC of 1 October 1991 amending Directive 88/77/EEC on the approximation of the laws of the Member States relating to the measures to be taken against the emission of gaseous pollutants from diesel engines for use in vehicles (OJ L 295, 25.10.1991, p. 1).

— Council Directive 92/6/EEC of 10 February 1992 on the installation and use of speed limitation devices for certain categories of motor vehicles in the Community (OJ L 57, 2.3.1992, p. 27), as last amended by Directive 2002/85/EC of the European Parliament and of the Council of 5 November 2002 (OJ L 327, 4.12.2002, p. 8).

— Council Directive 92/24/EEC of 31 March 1992 relating to speed limitation devices or similar speed limitation on-board systems of certain categories of motor vehicles (OJ L 129, 14.5.1992, p. 154).

— Council Directive 92/97/EEC of 10 November 1992 amending Directive 70/157/EEC on the approximation of the laws of the Member States relating to the permissible sound level and the exhaust system of motor vehicles (OJ L 371, 19.12.1992, p. 1).

— Council Directive 96/53/EC of 25 July 1996 laying down for certain road vehicles circulating within the Community the maximum authorised dimensions in national and international traffic and the maximum authorised weights in international traffic (OJ L 235, 17.9.1996, p. 59), as last amended by Directive 2002/7/EC of the European Parliament and of the Council of 18 February 2002 (OJ L 67, 9.3.2002, p. 47).

— Directive 2000/30/EC of the European Parliament and of the Council of 6 June 2000 on the technical roadside inspection of the roadworthiness of commercial vehicles circulating in the Community (OJ L 203, 10.8.2000, p. 1).

— Directive 2003/20/EC of the European Parliament and of the Council of 8 April 2003 amending Council Directive 91/671/EEC on the approximation of the laws of the Member States relating to compulsory use of safety belts in vehicles of less than 3,5 tonnes (OJ L 115, 9.5.2003, p. 63)

— Commission Directive 2003/26/EC of 3 April 2003 adapting to technical progress Directive 2000/30/EC of the European Parliament and of the Council as regards speed limiters and exhaust emissions of commercial vehicles (OJ L 90, 8.4.2003, p. 37).

— Directive 2009/40/EC of the European Parliament and of the Council of 6 May 2009 on roadworthiness tests for motor vehicles and their trailers (Recast) (OJ L 141, 6.6.2009, p. 12).

Transport of dangerous goods

— Council Directive 95/50/EC of 6 October 1995 on uniform procedures for checks on the transport of dangerous goods by road (OJ L 249, 17.10.1995, p. 35), as last amended by Council Directive 2008/54/EC of the European Parliament and of the Council of 17 June 2008 (OJ L 162, 21.6.2008, p. 11).

— Directive 2008/68/EC of the European Parliament and of the Council of 24 September 2008 on the inland transport of dangerous goods (OJ L 260, 30.9.2008, p. 13).

For the purposes of this Agreement the following derogations to Directive 2008/68/EC apply in Switzerland:

1. Road transport

Derogations for Switzerland under Article 6(2)(a) of Directive 2008/68/EC of 24 September 2008 on the inland transport of dangerous goods

RO-a-CH-1

Subject: Transport of diesel fuel and heating oil with UN number 1202 in tank containers.

Reference to Annex I, I.1, to this Directive: 1.1.3.6 and 6.8

Content of the Annex to the Directive: Exemptions related to the quantities transported per transport unit, regulations concerning the construction of tanks.

Content of the national legislation: Tank containers which are not constructed according to 6.8 but according to national legislation, which have a capacity of less than or equal to 1210 l and which are used to transport heating oil or diesel fuel with UN number 1202 may benefit from the exemptions in 1.1.3.6 ADR.

Initial reference to the national legislation: Appendix 1, paragraphs 1.1.3.6.3(b) and 6.14, of the Ordinance on the carriage of dangerous goods by road (SDR; RS 741.621).

Expiry date: 1 January 2017.

RO-a-CH-2

Subject: Exemption from the requirement to carry a transport document for certain quantities of dangerous goods as defined in 1.1.3.6.

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Reference to Annex I, I.1, to this Directive: 1.1.3.6 and 5.4.1.

Content of the Annex to the Directive: Requirement to have a transport document.

Content of the national legislation: The transport of uncleaned empty containers belonging to Transport Category 4 and filled or empty gas cylinders for breathing apparatuses for use by emergency services or as diving equipment, in quantities not exceeding the limits set in 1.1.3.6, is not subject to the obligation to carry a transport document provided for in 5.4.1.

Initial reference to the national legislation: Appendix 1, paragraph 1.1.3.6.3(c) of the Ordinance on the carriage of dangerous goods by road (SDR; RS 741.621).

Expiry date: 1 January 2017.

RO-a-CH-3

Subject: Transport of uncleaned empty tanks by companies servicing storage facilities for liquids hazardous to water.

Reference to Annex I, I.1, to this Directive: 6.5, 6.8 and 8.2 and 9.

Content of the Annex to the Directive: Construction, equipping and inspection of tanks and vehicles; driver training.

Content of the national legislation: Vehicles and uncleaned empty tanks/containers used by companies servicing storage facilities for liquids hazardous to water to contain liquids while stationary tanks are being serviced are not subject to the construction, equipping and inspection regulations or to the labelling and orange-plate identification regulations stipulated by the ADR. They are subject to particular labelling and identification regulations, and the driver of the vehicle is not obliged to have undertaken the training described in 8.2.

Initial reference to the national legislation: Appendix 1, paragraph 1.1.3.6.3.10, of the Ordinance on the carriage of dangerous goods by road (SDR; RS 741.621).

Expiry date: 1 January 2017.

Derogations for Switzerland under Article 6(2)(b)(i) of Directive 2008/68/EC of 24 September 2008 on the inland transport of dangerous goods

RO-bi-CH-1

Subject: Transport of domestic waste containing dangerous goods to waste disposal installations.

Reference to Annex I, I.1, to this Directive: 2, 4.1.10, 5.2 and 5.4.

Content of the Annex to the Directive: Classification, combined packaging, marking and labelling, documentation.

Content of the national legislation: The rules include provisions relating to the simplified classification of domestic waste containing (domestic) dangerous goods by an expert recognised by the competent authority, to the use of appropriate receptacles and to driver training. Domestic waste which cannot be classified by the expert may be transported to a treatment centre in small quantities identified by package and by transport unit.

Initial reference to the national legislation: Appendix 1, paragraph 1.1.3.7, of the Ordinance on the carriage of dangerous goods by road (SDR; RS 741.621).

Comments: These rules may only be applied to the transport of domestic waste containing dangerous goods between public treatment sites and waste disposal installations.

Expiry date: 1 January 2017.

RO-bi-CH-2

Subject: Return transport of fireworks.

Reference to Annex I, I.1, to this Directive: 2.1.2, 5.4.

Content of the Annex to the Directive: Classification and Documentation.

Content of the national legislation: With the aim of facilitating the return transport of fireworks with UN numbers 0335, 0336 and 0337 from retailers to suppliers, exemptions regarding the indication of the net mass and product classification in the transport document are envisaged.

Initial reference to the national legislation: Appendix 1, paragraph 1.1.3.8, of the Ordinance on the carriage of dangerous goods by road (SDR; RS 741.621).

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Comments: Detailed checking of the exact contents of each item of unsold product in each package is practically impossible for products intended for retail trade.

Expiry date: 1 January 2017.

RO-bi-CH-3

Subject: ADR training certificate for journeys undertaken with the purpose of transporting vehicles which have broken down, of carrying out repairs, of gaining tank vehicle/tank expertise, and journeys made in tank vehicles by experts responsible for examination of the vehicle in question.

Reference to Annex I, I.1, to this Directive: 8.2.1.

Content of the Annex to the Directive: Drivers of vehicles must attend training courses.

Content of the national legislation: ADR training and certificates are not required for journeys undertaken with the purpose of transporting vehicles that have broken down or test drives related to repairs, journeys made in tank vehicles with a view to gaining tank vehicle/tank expertise, and journeys made by experts responsible for tank vehicle examination.

Initial reference to the national legislation: Instructions of 30 September 2008 of the Federal Department of Environment, Transport, Energy and Communication (DETEC) on the carriage of dangerous goods by road.

Comments: In some cases, vehicles which have broken down or are undergoing repairs and tank vehicles being prepared for technical inspection or being checked at the time of the inspection still contain dangerous goods.

The requirements in 1.3 and 8.2.3 are still applicable.

Expiry date: 1 January 2017.

2. Rail transport

Derogations for Switzerland under Article 6(2)(a) of Directive 2008/68/EC of 24 September 2008 on the inland transport of dangerous goods

RA-a-CH-1

Subject: Transport of diesel fuel and heating oil with UN number 1202 in tank containers.

Reference to Annex II, Section II.1, to this Directive: 6.8.

Content of the Annex to the Directive: Regulations concerning the construction of tanks.

Content of the national legislation: Tank containers which are not constructed according to 6.8 but according to national legislation, which have a capacity of less than or equal to 1210 l and which are used to transport heating oil or diesel fuel with UN number 1202 are authorised.

Initial reference to the national legislation: Annex to the DETEC Ordinance of 3 December 1996 relating to the transport of dangerous goods by rail and cableway installation (RSD, RS 742.401.6) and Appendix 1, Chapter 6.14, of the Ordinance relating to the carriage of dangerous goods by road (SDR, RS 741.621).

Expiry date: 1 January 2017.

RA-a-CH-2

Subject: Transport document.

Reference to Annex II, Section II.1, to this Directive: 5.4.1.1.1.

Content of the Annex to the Directive: General information required in the transport document.

Content of the national legislation: Use of a collective term in the transport document and an annexed list containing the information prescribed as stipulated above.

Initial reference to the national legislation: Annex to the DETEC Ordinance of 3 December 1996 relating to the transport of dangerous goods by rail and cableway installation (RSD, RS 742.401.6).

Expiry date: 1 January 2017.

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SECTION 4 — ACCESS AND TRANSIT RIGHTS WITH REGARD TO RAILWAYS

— Council Directive 95/18/EC of 19 June 1995 on the licensing of railway undertakings (OJ L 143, 27.6.1995, p. 70).

— Council Directive 95/19/EC of 19 June 1995 on the allocation of railway infrastructure capacity and the charging of infrastructure fees (OJ L 143, 27.6.1995, p. 75).

— Council Directive 91/440/EEC of 29 July 1991 on the development of the Community's railways (OJ L 237, 24.8.1991, p. 25).

SECTION 5 — OTHER FIELDS

— Council Directive 92/82/EEC of 19 October 1992 on the approximation of the rates of excise duties on mineral oils (OJ L 316, 31.10.1992, p. 19).

— Directive 2004/54/EC of the European Parliament and of the Council of 29 April 2004 on minimum safety requirements for tunnels in the Trans-European Road Network (OJ L 167, 30.4.2004, p. 39).'

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DECISION No 1/2011 OF THE EU-SWITZERLAND JOINT COMMITTEE

of 14 January 2011

amending Tables III and IV(b) of Protocol 2 to the Agreement between the European Economic Community and the Swiss Confederation concerning certain processed agricultural products

(2011/46/EU)

THE JOINT COMMITTEE,

Having regard to the Agreement between the European Economic Community and the Swiss Confederation signed in Brussels on 22 July 1972 [1] hereinafter referred to as 'the Agreement', as amended by the Agreement between the European Community and the Swiss Confederation amending the Agreement as regards the provisions applicable to processed agricultural products [2] signed in Luxembourg on 26 October 2004, and its Protocol 2, and in particular Article 7 of that Protocol,

Whereas:

(1) For the implementation of Protocol 2 to the Agreement, domestic reference prices have been fixed for the Contracting Parties.

(2) Actual prices have changed on the domestic markets of the Contracting Parties as regards raw materials for which price compensation measures are applied.

(3) It is therefore necessary to update the reference prices and amounts listed in Tables III and IV(b) of Protocol 2 accordingly,

HAS ADOPTED THIS DECISION:

Article 1

Protocol 2 to the Agreement is amended as follows:

(a) Table III is replaced by the text set out in Annex I to this Decision;

(b) in Table IV, point (b) is replaced by the text set out in Annex II to this Decision.

Article 2

This Decision enters into force on the day of its publication in the *Official Journal of the European Union*.

It shall apply from 1 February 2011.

Done at Brussels, 14 January 2011.

For the Joint Committee
The Chairman
M. O'SULLIVAN

---

[1] OJ L 300, 31.12.1972, p. 189.
[2] OJ L 23, 26.1.2005, p. 19.

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ANNEX I

TABLE III

EU and Swiss domestic reference prices

Agricultural raw material	Swiss domestic reference price	EU domestic reference price	Article 4(1) Applied on Swiss side Difference Swiss/EU reference price	Article 3(3) Applied on EU side Difference Swiss/EU reference price
	CHF per 100 kg net	CHF per 100 kg net	CHF per 100 kg net	EUR per 100 kg net
Common wheat	48,05	28,20	19,85	0,00
Durum wheat	—	—	1,20	0,00
Rye	41,45	27,40	14,05	0,00
Barley	—	—	—	—
Maize	—	—	—	—
Common wheat flour	97,00	54,50	42,50	0,00
Whole-milk powder	611,55	362,40	249,15	0,00
Skimmed-milk powder	428,95	297,60	131,35	0,00
Butter	1 055,15	480,10	575,05	0,00
White sugar	—	—	—	—
Eggs	—	—	38,00	0,00
Fresh potatoes	43,20	28,60	14,60	0,00
Vegetable fat	—	—	170,00	0,00'

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ANNEX II

TABLE IV

(b) The basic amounts for agricultural raw materials taken into account for the calculation of the agricultural components:

Agricultural raw material	Applied on the Swiss side Article 3(2) Applied basic amount	Applied on the EU side Article 4(2) Applied basic amount
	CHF per 100 kg net	EUR per 100 kg net
Common wheat	17,00	0,00
Durum wheat	1,00	0,00
Rye	12,00	0,00
Barley	—	—
Maize	—	—
Common wheat flour	36,00	0,00
Whole-milk powder	212,00	0,00
Skimmed-milk powder	112,00	0,00
Butter	489,00	0,00
White sugar	—	—
Eggs	32,00	0,00
Fresh potatoes	12,00	0,00
Vegetable fat	145,00	0,00'

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CORRIGENDA

Corrigendum to Commission Regulation (EU) No 47/2011 of 20 January 2011 fixing representative prices in the poultrymeat and egg sectors and for egg albumin, and amending Regulation (EC) No 1484/95

(Official Journal of the European Union L 18 of 21 January 2011)

On page 18, recital 4:

for: '(4) The Management Committee for the Common Organisation of Agricultural Markets has not delivered an opinion within the time limit set by its Chair,',

read: '(4) The measures provided for in this Regulation are in accordance with the opinion of the Management Committee for the Common Organisation of Agricultural Markets,'.

2011/46/EU:

★ **Decision No 1/2011 of the EU-Switzerland Joint Committee of 14 January 2011 amending Tables III and IV(b) of Protocol 2 to the Agreement between the European Economic Community and the Swiss Confederation concerning certain processed agricultural products** 40

Corrigenda

Corrigendum to Commission Regulation (EU) No 47/2011 of 20 January 2011 fixing representative prices in the poultrymeat and egg sectors and for egg albumin, and amending Regulation (EC) No 1484/95 (OJ L 18, 21.1.2011) 43

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EN

APPENDIX 9

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

[redacted]

DEC 1 9 2011

KitoZyme S.A.
Rue Haute Claire, 4
Parc Industriel des Hauts-Sarts, Zone 2
BE-4040 Herstal BELGIUM

Re: GRAS Notice No. GRN 000397

Dear Dr. Maquet:

The Food and Drug Administration (FDA) is responding to the notice, dated July 28, 2011, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on August 3, 2011, filed it on August 8, 2011, and designated it as GRAS Notice No. GRN 000397.

The subject of the notice is chitosan from *Aspergillus niger* (chitosan). The notice informs FDA of the view of KitoZyme S.A. (KitoZyme) that chitosan from *A. niger* is GRAS, through scientific procedures, for use as a secondary direct food ingredient[1] in alcoholic beverage production at levels between 10 and 500 grams per hectoliter (100 liters).

As part of its notice, KitoZyme includes the report of a panel of individuals (KitoZyme's GRAS Panel) who evaluated the data and information that are the basis for KitoZyme's GRAS determination. KitoZyme considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. KitoZyme's GRAS panel discusses identity, specifications, method of manufacture, dietary exposure, and safety of chitosan, including the history and current regulatory status of *A. niger* and chitosan in food, and concludes that chitosan is GRAS under the intended conditions of use.

Chitosan is identified by the CAS registry number 9012-76-4. KitoZyme describes chitosan as an insoluble, non-digestible fiber derived from the post-fermentation biomass of non-viable *A. niger* used to manufacture food-grade citric acid. KitoZyme states that strains of *A. niger* used in the production of citric acid are nonpathogenic and nontoxigenic, and have a long history of safe use worldwide. Hydrolysis of the raw *A. niger* material produces chitosan, which is then washed, precipitated, concentrated, and dried. KitoZyme states the degree of acetylation for chitosan to be 0 to 30 mole percent (%). All materials and processing aids used in the manufacture of chitosan are food-grade. The notifier provides product specifications for chitosan, including

[1] 21 CFR 173: A secondary direct food additive has a technical effect in food during processing but not in the finished food (e.g., processing aid). The technical use of chitosan in GRN 000397 is for microbiological stabilization, removal of contaminants, and/or clarification of the alcoholic beverage.

microbiological limits, heavy metals, and chemical characterization. KitoZyme states that chitosan from *A. niger* is chemically equivalent to chitosan from shellfish, based on Fourier transform infrared spectroscopy and nuclear magnetic resonance analyses. KitoZyme noted the presence of beta-1,3-D-glucans (present in ~10 to 15% concentration on a weight by weight basis) that is absent from shellfish sources of chitosan.

KitoZyme reported on a published 13-week subchronic toxicity study in Wistar rats. Twenty rats per sex were fed *A. niger*-sourced chitin and beta-glucan in a 30:70 ratio. KitoZyme reported the No Observable Adverse Effect Level to be the highest dose tested, 6,589 and 7,002 milligrams/ kilogram body weight/day for the male and female rats, respectively.

When chitosan is used in the production of alcoholic beverages, it is removed from the wine, must, beer, cider, or spirits at the end of the treatment using physical separation processes, such as racking, centrifugation, or filtering.

KitoZyme notes that chitosan preparations are insoluble in both water and ethanol, and because the material is removed from solution, intake modeling is not considered necessary. In addition, high performance liquid chromatography of wine processed with chitosan indicated that the final product was free from chitosan carry-over products at the limit of detection. In addition, KitoZyme notes that neither chitosan nor beta-1,3-D-glucans are digested by the human gastrointestinal tract, therefore, absorption and systemic exposure to chitosan would not occur.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

The Food and Drug Administration Amendments Act of 2007, which was signed into law on September 27, 2007, amends the FD&C Act to, among other things, add section 301(ll). Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In its review of KitoZyme's notice that chitosan is GRAS for the intended uses, FDA did not consider whether section 301(ll) or any of its exemptions apply to foods containing chitosan. Accordingly, this response should not be construed to be a statement that foods that contain chitosan if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusion

Based on the information provided by KitoZyme, as well as other information available to FDA, the agency has no questions at this time regarding KitoZyme's conclusion that chitosan from *A. niger* is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of chitosan. As always, it is the continuing responsibility of KitoZyme to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000397, as well as a copy of the information in this notice that conforms to the information in the GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying at http://www.fda.gov/grasnoticeinventory.

Sincerely,

[redacted]

Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Attachment 10

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"2011-Year of Decent Work, and the Health and Safety of Workers"
C.P.

[Emblem]
Ministry of Agriculture, Livestock and Fisheries

MENDOZA, 22 November 2011

HAVING REVIEWED File No. S93:0003081/2011, and

CONSIDERING:

That by means of the action quoted above, a request was made for authorisation to use Chitosan as an oenological practice in order to reduce the presence of yeasts of the Brettanomyces genus.

That Chitosan is a natural polysaccharide of fungal origin, extracted and purified by means of safe and abundant food or biotechnological fungal sources such as Agaricus bisporus or Aspergillus niger, used as a clarification agent and a biological stabiliser of wine.

That this compound has proved to be a useful tool, easy to apply and effective against the Brettanomyces yeasts present in wines, as well as for reducing the concentration of heavy metals and contaminants, and avoiding ferric and cupric casse, without modifying their organoleptic profile and analytical characteristics.

That the Health and Safety Commission of the INTERNATIONAL ORGANISATION OF VINE AND WINE (OIV) gave its approval in relation to the use of this product, by means of OENO Resolution No. 338A dated 3 July 2009.

That the OIV, in its INTERNATIONAL CODE OF ENOLOGICAL PRACTICES, accepts as an oenological practice the addition of Chitosan of fungal origin to wine.

That likewise, the EUROPEAN UNION, in its Regulation No. 53 dated 21 January 2011, appendix 13, also authorises the treatment of wines with the said product as a licit oenological practice.

That the NATIONAL VITICULTURE INSTITUTE (INV), according to Article 21 of the General Law on Wines No. 14.878, may eliminate, modify or extend permitted oenological corrections or practices and establish legal limits for the components of wine.

That the Sub-management for Legal Affairs of the INV has taken the action incumbent upon it.

Therefore, and in exercising the powers conferred by Laws Nos. 14.878 and 24.566 and Decree no. 1.306/08,

THE PRESIDENT OF THE
NATIONAL INSTITUTE OF VITICULTURE
DECIDES AS FOLLOWS:

1. The addition of Chitosan to wine as a licit oenological practice is authorised, in order to comply with the following objectives:

   a) To reduce the concentration of heavy metals, such as iron, lead, cadmium and copper.
   b) To avoid ferric and cupric casse.
   c) To reduce the quantities of possible contaminants, especially of Ochratoxin A.
   d) To reduce the presence of undesirable microorganisms such as Brettanomyces.

2. The maximum dose of Chitosan to be used should be:

   a) ONE HUNDRED GRAMS PER HECTOLITRE (100 g/hl) for objectives a) and b).
   b) FIVE HUNDRED GRAMS PER HECTOLITRE (500 g/hl) for objective c).
   c) TEN GRAMS PER HECTOLITRE (10g/hl) for objective d).

3. The Chitosan must comply with the specifications of the INTERNATIONAL ENOLOGICAL CODEX.

4. To be registered, communicated, published, given to the National Division of the Official Register for publication and once complied with, to be filed.

RESOLUTION No. C.51

[signature]
C.P.N. GUILLERMO DANIEL GARCIA
PRESIDENT
NATIONAL INSTITUTE OF VITICULTURE

Attachment 11

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European Food Safety Authority

EFSA Journal 2010; 8(7):1687

SCIENTIFIC OPINION

Scientific Opinion on the safety of 'Chitin-glucan' as a Novel Food ingredient[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2], [3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to carry out the additional assessment for 'Chitin-glucan' as a food ingredient in the context of Regulation (EC) No. 258/97. The Novel Food ingredient called "KiOnutrime-CG™" has a content of more than 90 % chitin-glucan, which is the main component in the cell walls of the mycelium of *Aspergillus niger* derived from a fermentation process. The compositional data and the manufacturing process do not give rise to concerns. The ingredient is intended to be marketed as a food supplement to increase the daily intake of fibre. The intended intake of chitin-glucan is 2 to 5 g/day. At the highest dose administered in a 13-week rat study, i.e. about 6.6 g/kg body weight (bw), no adverse effects were observed. This dose is approximately 80-fold higher than the maximum intended level of intake for humans on a g/kg bw basis. The Panel concludes that Novel Food KiOnutrime-CG™ is safe as a food ingredient at the proposed conditions of use and the proposed intake levels.

KEY WORDS

Chitin-glucan, novel food ingredient, Kitozyme

[1] On request from the European Commission, Question EFSA-Q-2009-00762. Adopted on 9 July 2010.

[2] Panel members: Carlo Virginio Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank for the preparatory work on this scientific opinion: The members of the Working Group on Novel Foods: Karl-Heinz Engel, Ines Golly, Marina Heinonen, Pagona Lagiou, Rosangela Marchelli, Bevan Moseley, Monika Neuhäuser-Berthold, Annette Pöting, Seppo Salminen, Hendrik Van Loveren and Hans Verhagen, and EFSA's staff member Wolfgang Gelbmann for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the safety of "Chitin-Glucan" as a Novel Food ingredient. EFSA Journal 2010; 8(7):1687. [17 pp.]. doi:10.2903/j.efsa.2010.1687. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of 'Chitin-glucan' as a novel food ingredient in the context of Regulation (EC) No. 258/97 taking account of the comments/objections of a scientific nature raised by the Member States.

The Novel Food ingredient "KiOnutrime-CG™" has a content of more than 90 % chitin-glucan, which is the main component in the cell walls of the mycelium of *Aspergillus niger* derived from a fermentation process. The *A. niger* strain is non-toxic and non-pathogenic and has a history of safe use in production of food ingredients. The compositional data and the manufacturing process do not give rise to concerns.

The ingredient is intended to be marketed as a food supplement in the form of a powder in different formats such as gelatine capsules or tablets. According to the applicant it is designed to increase the daily intake of fibres. The intended intake of chitin-glucan is 2 to 5 g/day.

At the highest dose administered in the 13-week rat study, i.e. about 6.6 g/kg bodyweight (bw), no adverse effects were observed. This dose is approximately 80-fold higher than the maximum intended level of intake for humans on a g/kg bw basis. On basis of the data provided and taking into account the nature of the novel food ingredient, the Panel considers that there are no safety concerns under the proposed conditions of use.

The Panel concludes that Novel Food KiOnutrime-CG™ is safe as a food ingredient at the proposed conditions of use and the proposed intake levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 15 January 2008, KitoZyme submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market 'Chitin-glucan' as a novel food ingredient.

On 23 January 2009, the competent authorities of Belgium forwarded to the Commission their initial assessment report, which came to the conclusion that an additional assessment was required.

On 12 March 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted additional comments.

In consequence, a Community Decision was required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- No data given on the composition of the product's lipid and protein fraction.
- Unclear data on specification and composition, thus it remains unclear whether the composition complies with the specification.
- Only a single production batch was examined in the manufacturer's test facility.
- No indication of GMP conditions or a HACCP plan.
- Insufficient information on mycotoxin and total heavy metal and lead testing (not in accordance with European Pharmacopoeia, no information whether laboratory was accredited, insufficient information on the applied methods).
- Insufficient data on the product's stability. No results from analyses showing the stability of the product under realistic storage conditions.
- Information on the 'levels of exposure' or 'anticipated intakes' of other age groups such as children, the elderly, pregnant and lactating women was also not evident.
- The applicant should consider the effect of chitin-glucan on metabolism of lipids, fat-soluble vitamins and minerals.
- The provided human study with 20 male adults is insufficient to demonstrate safety. No information on whether chitin-glucan might influence the bioavailability and serum levels of other nutrients such as minerals and fat-soluble vitamins.
- Uncertainty of the intestinal fate of chitin-glucan, vague information to what extent chitin-glucan is fermented in the large bowel.
- Lack of characterisation of the up to 6 % protein fraction, concerns regarding potential allergenicity and cross-reactivity in relation to IgE sensitisation to *Aspergillus fumigatus,* a major respiratory and skin allergen.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Chitin-glucan' as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC chitin-glucan from *Aspergillus niger* is allocated to Class 2.1 'a complex (non-GM derived) novel food ingredient, the source of the novel food having a history of food use in the community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of Belgium, the concerns and objections of the other Member States and the responses of the applicant to these questions and those of Belgium. The data are required to comply with the information required for novel foods of Class 2.1 i.e. structured schemes I, II, III, IX, X, XI, XII and XIII. It is noted that the novel ingredient is intended by the applicant to be marketed for consumption as a food supplement in the form of a powder in different prescription formats (gelatine capsules, tablets and possibly other). This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of chitin-glucan with regard to any claimed benefits.

1. Specification of the Novel Food (NF)

Chitin-glucan is a purified ingredient, presented in the form of a powder, which is composed largely of two polysaccharides:

- chitin, composed of repeating units of N-acetyl-D-glucosamine (CAS number 1398-61- 4);
- beta(1,3)-glucan, composed of repeating units of D-glucose (CAS number 9041-22-9).

Chitin-glucan is the main component in the cell walls of the mycelium of a fungus from the Ascomycetes family: *Aspergillus niger* (*A. niger*). The two polymers are linked covalently and form a three-dimensional network.

Chitin-glucan is obtained from the mycelium of non-genetically-modified strains of *A. niger*, a microorganism employed in the food and pharmaceutical industries for the production of citric acid. The applicant uses two sources for its novel food ingredient.

The Novel Food ingredient (KiOnutrime-CG™, KiOnutrime-CG®, KiOnutrime-CG) is a white odourless powder with a yellowish tinge which has a dry matter content ≥ 90 %. It is insoluble in aqueous and organic media. It is intended to be marketed in different food supplement formats.

The applicant proposed the following specifications for the novel food ingredient (see **Table 1**).

Table 1: Specification for KiOnutrime-CG™

Parameter	Specification	Methods
Loss on drying (%)	≤ 10	Gravimetric method
Chitin-glucan content (%)	≥ 90	Internal Method: total weight minus ash, minus protein
Ratio of chitin-glucan	30:70 to 60:40	Internal Method based on 13C NMR
Ash (%)	≤ 3	Gravimetric method
Lipids (%)	≤ 1	Gravimetric method
Proteins (%)	≤ 6	Colorimetric method
Total heavy metals (ppm)	≤ 20	ICP-MS
Mercury (ppm)	≤ 0.2	ICP-MS
Lead (ppm)	≤ 1	ICP-MS
Arsenic (ppm)	≤ 1	ICP-MS
Cadmium (ppm)	≤ 0.5	ICP-MS

Aerobic count (cfu/g)	< 1000	ISO 4833
E. coli (cfu/g)	< 10	ISO 16649
Yeast and mould count (cfu/g)	< 1000	ISO 7954

In response to Member State comments, the applicant provided certificates from its *A. niger*-mycelium sources and information on the laboratory methods and accreditation of laboratories that conducted the analyses.

As concerns batch variation, five non-consecutive batches were analysed to demonstrate the ability of KitoZyme to produce within these specifications. Analytical results are presented in **Table 2** and indicate that the specifications were met.

The analytical methods used for the analyses followed ISO (International Standard Organization) norms. Analyses were run mostly in external laboratories that were accredited under ISO 17025 (amino acid profile, fatty acid profile, carbohydrate profile, mycotoxins, sterols, DNA, heavy metals, microbiology, water activity); the few internal methods were done according to existing in-house protocols. For parameters for which no standard tests are available, KitoZyme in-house QC laboratory has developed internal validated methods.

Table 2 : Batch testing

Parameter	Batch N° L09093CG	Batch N° L09068CG	Batch N° L09070CG	Batch N° L09071CG	Batch N° L09072CG	Mean	Max.	Min.
Loss on drying (%)	5.0	8.0	7.0	7.0	10.0	7.4	10.0	5.0
Chitin-glucan content (%)	94.0	94.0	95.0	94.0	94.0	94.0	95.0	94.0
Ratio of chitin-glucan	30:70	35:65	30:70	30:70	32:68	NA	NA	NA
Ash (%)	2.5	3.0	2.0	3.0	3.0	2.7	3.0	2.0
Lipids (%)	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.7
Proteins (%)	3.0	3.5	3.0	3.4	3.5	3.3	3.5	3.0
Total heavy metals (ppm)	2.3	1.9	2.1	2.0	2.0	2.0	2.3	1.9
Mercury (ppm)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead (ppm)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Arsenic (ppm)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Cadmium (ppm)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
Aerobic count (cfu/g)	< 10	30	<10	10	<10	20	30	< 10
Yeast and mould count (cfu/g)	< 10	<10	<10	<10	<10	< 10	< 10	< 10

Protein fraction

The protein content is ≤ 6%. The method for protein determination was based on colorimetry after reaction with ninhydrin and UV-absorption at 564 nm. Conventional methods for determination of nitrogen/protein content such as the Bradford protein assay, the Lowry method, the Kjeldahl method, and the BCA (bicinchoninic acid) method could not be used because of the presence of the amid group of chitin which interferes with these assays as well as the use of sodium hydroxide for several

hours for the extraction of chitin-glucan from the mycelium which is expected to denature or partly hydrolyse any protein component from the source.

As concerns amino acid composition, ten batches of chitin-glucan extracted from two different sources of *A. niger* have been analyzed, i.e. three batches of chitin-glucan from a first source and 7 batches of chitin-glucan from a second source. The eight most abundant amino acids are Leucine, Phenylalanine, Glutamic acid, Aspartic acid, Isoleucine, Valine, Alanine, and Tyrosine; the standard deviations between assays were small. The total amino acid content represents 2.9 % of chitin-glucan (2.9 g per 100 g of chitin-glucan; average values from 10 batches). This value is very close to the average value of 3.3 % reported by the applicant and which is the average protein content as determined by the KitoZyme method on the same batches.

Lipid fraction

The lipid content in chitin-glucan of KiOnutrime-CG™ in the specification is ≤ 1% as assessed by gravimetry after solvent extraction.

A literature review on lipids of *A. niger* mycelium from different strains shows that all strains contain phospholipids, glycolipids, and neutral lipids (triglyceride, diglyceride, monoglyceride, sterols and pigments) (Chattopadhyay et al., 1985; 1987). According to the applicant, the main difficulty in the determination of these fractions is related to the overall low lipid content in chitin-glucan, therefore requiring multiple solvent extractions to obtain a lipid fraction compatible with quantitative analysis. In addition, determination of these compounds was not possible using normalized methods (except for sterols). The applicant provided a review article showing that *A. niger* contains C_{16} to C_{18} saturated and unsaturated fatty acids. Small amounts of long chain (C_{20} to C_{24}) and short chain (C_{10} to C_{14}) saturated and unsaturated acids are also present. Linoleic acid, oleic acid, and palmitic acid are the major acids, while stearic acid and linolenic acid are the minor ones (Chattopadhyay et al., 1987).

For the determination of the lipid fraction, the following analyses have been performed:

- Total hydrolysis followed by fatty acid extraction according to the method described in Regulation EC N° 152/20092.

- Determination of the lipid content extracted by the Folch method (Folch, 1957); preparation and analysis by gaseous phase chromatography of the methyl esters of fatty acids according to NF EN ISO 5509 and NF ISO 5508; determination of sterol content (individual and total sterols) according to NF EN ISO 12228. The results on the fatty acid profile indicate that the 5 most abundant fatty acids are oleic acid (55.1 %), linoleic acid (19.9 %), palmitic acid (12.7%), stearic acid (5.1 %) and lignoceric acid (3.3%), albeit that some batches had too low a lipid content to allow analyses; other fatty acids are present at < 1% each. Three major fatty acids represent ~ 90 % of the total fatty acids: oleic acid, linoleic acid and palmitic acid. The ratio of unsaturated fatty acids/saturated fatty acids is 77/23. The sequence of the first 5 fatty acids is the same as the one reported in literature for *A. niger*. The results on the determination of the phytosterol content show that the phytosterols represent a low fraction of the extracted lipid fraction (73.9 mg/100 g of chitin-glucan), ergosterol being the main constituent of the phytosterols (74 %).

In conclusion and according to the applicant, the lipid fraction of chitin-glucan is similar to the lipid profile of *A. niger* reported in the literature (Chattopadhyay, 1985; 1987).

Carbohydrate

The applicant determined the total carbohydrate value by adding the content of glucose, saccharose, lactose, galactose, maltose and fructose measured by ionic chromatography. The determination of the carbohydrate content in chitin-glucan according to this method has been performed on ten batches of

chitin-glucan extracted from the two different sources of *A. niger*. The total carbohydrate value is less than 0.2 % for all batches.

The Panel considers that the protein, lipid and carbohydrate fractions do not indicate a safety concern as the ingredients found are regular constituents of the diet.

Heavy metals

The applicant reported that specifications for heavy metals and microbial contamination are in compliance with regulation EC N°629/2008 (Table 2).

Total heavy metal content as well as each specific metal listed in the certificate of analysis are determined by ICP-MS. The limits of detection were for Hg (0.1 ppm) and for Pb, As and Cd (0.25 ppm).

Mycotoxins

The mycotoxin analyses have been selected based on publication pointing out that certain strains of *A. niger* produce both ochratoxin and fumonisins (Frisvad, 2007). The results are presented in Table 3 for *A. niger* and chitin-glucan samples. The results for mycotoxins such as aflatoxin (B1, B2, G1, G2), fumonisin B1 and B2 and ochratoxin are below detection limit. The number of samples tested was not provided; the detection limit is assumed to be the value indicated in the Table after "<".

Table 3 : Mycotoxin analysis in samples of A. niger and chitin-glucan

Sample analysed (µg/kg)	A. niger	Chitin-glucan
Aflatoxin B1	< 0.1	< 0.1
Aflatoxin B2	< 0.1	<0.1
Aflatoxin G1	< 0.1	<0.1
Aflatoxin G2	< 1	<1
Ochratoxin	< 1	<1
Fumonisin B1	< 100	<100
Fumonisin B2	< 100	<100

The Panel considers that the contaminants measured (heavy metals, mycotoxins, micro-organisms) do not indicate a safety concern.

2. Effect of the production process applied to the NF

The source of the novel food ingredient [mycelium of *A. niger*] is obtained by means of fermentation, in accordance with different processes depending on the producers concerned, followed by stages involving extraction from citric acid and washing. At the end of this production process, the source is dried and packaged.

Chitin-glucan is obtained by a process of digestion by hydrolysis of the source (the mycelium of *A.niger)*, purification in an aqueous medium and drying. The process is described by patent No WO/2003/068824 filed by KitoZyme, albeit that this patent is very broad with respect to process conditions such as temperature, time of treatment and percentage of alkali. Upon request more specific production process data have been obtained from the applicant; these were marked by the applicant as "confidential" and caused the Panel no concern.

After drying, the resulting powder has a dry matter content of more than 90 %. The chitin-glucan powder is then packed in double-layer polyethylene bags, which are sealed and stored at ambient temperature. A sample is taken to check compliance with the specifications.

The applicant follows the auto-control guide established by the Belgian Federation for Food Supplements, Dietary and Organic Products, NAREDI, for the traceability, mandatory notification and risk analysis based on the HACCP method.

As concerns stability of chitin-glucan, the applicant claims that a shelf life of two years has been established for chitin-glucan by the applicant based on 3 stability studies and on the very low water activity of chitin-glucan:

Stability Study N°1 started July 2007: two batches were stored at room temperature. Follow up for microbiological content every 6 months. Both batches are within the specification during two years.

Stability Study N°2: one batch of chitin-glucan stored at two temperature conditions, i.e. (1) at room temperature and (2) at 40°C in closed polyethylene container. The duration of this study was extending over 13 months (RT) and over 6 months (40°C). Parameters followed during the stability study were loss on drying which allows characterisation of the water tightness of the packaging and the hygroscopic characteristics of the chitin-glucan; total microbiological content and yeasts/moulds. Results for these three parameters are within the specifications showing that chitin-glucan is stable for 13 months at room temperature and 6 months at 40°C.

Stability Study N°3: Three batches (a sample size of 20 g) according to ICH Q1A (stability testing of new drug substances and products) in recommended storage conditions, i.e. in the final closure container at 25 +/- 2°C. The testing program, start and end dates are reported in Table 4. A first long-term study at 25 +/- 2°C is set up for a duration of 36 months. A second study at 40 +/- 2°C (and 75 +/- 5% Relative Humidity) is set up under accelerated conditions for a duration of 6 months. The samples are stored in qualified ICH climatic chambers. At pre-determined time intervals, samples are collected and analysed for loss on drying, water activity and microbiological parameters (aerobic microbial count, total yeasts and moulds count, *Escherichia coli*, *Listeria monocytogenes*, Enterobacteriaceae, *Salmonella*) according to specified ISO methods.

Table 4 : Testing program for the stability study of KiOnutrime-CG®

Storage conditions	Temperature	Relative Humidity	Test intervals (T in month)	Start date	End date
Accelerated conditions	40 ± 2°C	75 ± 5 %	T0, T3, and T6	June 09	December 2009
Recommended storage conditions	25 ± 2°C	60 ± 5 %	T0, T3, T6, T9, T12, T18, T24, T30 and T36	June 09	June 2012

3. History of the organism used as the source of the NF

The *A. niger* strain used as the raw material for manufacture of KiOnutrime-CG™ is non-genetically modified, non-pathogenic and non-toxic for humans and animals. It does not produce ochratoxin A. This species has been commonly used in food production since the 1920s. The strain used in this process is a "privately developed strain of a proprietary nature" that was specifically selected for citric acid production. The citric acid has been sold in the US, EU and other countries since 1993.

Also, the mycelium of *A. niger* is currently used as a feed alternative to supplement the diet of ruminants with protein and nutritional fibre.

A. niger has a long history of use in the food industry as a source of enzymes. Several enzymes derived from the organism are authorized for use in the manufacture of food ingredients in Europe and the United States. FAO/WHO has repeatedly reviewed and accepted enzyme preparations from *A. niger*. The FDA in the United States has accepted numerous enzymes for food use, recognizing that α-amylase, cellulase, amyloglucosidase, catalase, glucose oxidase, lipase and pectinase from *A. niger* can be 'generally regarded as safe' (GRAS) under the condition that non-pathogenic and non-toxigenic strains and current good manufacturing practices be used in production (Schuster et al., 2002).

4. Anticipated intake/extent of the use of the NF

According to the applicant, chitin-glucan is intended for consumption as a food supplement in the form of a powder in different prescription formats (gelatine capsules, tablets and possible other) and is designed to increase the daily intake of fibre. It is supposed to be consumed either in a short-term manner (intestinal comfort) or in a prolonged manner as a fibre nutritional supplement. The intended intake of chitin-glucan is 2 to 5 g/day, split into two or three doses, taken preferably with food and with some liquid to help it swell.

The applicant indicates that the recommended dietary intake for fibre in European countries is between 25 - 35 g/day (for adults), In addition, the applicant provides data that the dietary fibre intakes vary from 10 - 20 g/day in young children (< 10 - 12 years), from 15 - 30 g/day in adolescents, and from 16 - 29 g/day in adults (EFSA, 2010).

Although not specifically indicated by the applicant the Panel assumes that the target group is the general population.

5. Information from previous exposure to the NF or its source

Chitin-glucan is obtained from a defined source: the mycelium of *Aspergillus niger,* the microorganism used to produce citric acid which is produced by several industrial companies. A number of producers of citric acid are commercializing the mycelium of *A. niger* as a feed ingredient.

6. Nutritional information on the NF

Food supplements containing KiOnutrime-CG™ are intended by the applicant to increase the dietary fibre intake. The caloric value is low given that chitin-glucan is an insoluble fibre which contains indigestible carbohydrates. Dietary fibre has a low caloric value (EFSA, 2010). The intended intake of chitin-glucan is 2 to 5 g/day.

Chitin-glucan being insoluble, it is not expected to be digested by human enzymes to any significant extent. Digestion of insoluble fibres does not occur in the small intestine; therefore, the majority of the material is expected to travel intact through the gastrointestinal tract to the colon to be subject to fermentation by the resident microbiota.

The fermentation of beta-glucan is well described in literature, and the metabolic products of fermentation are expected to be innocuous compounds (H_2, CO_2, CH_4, and volatile fatty acids). Chitin is fairly resistant to microbial fermentation and it is therefore expected to be excreted as such in faeces.

Proof that fermentation of chitin-glucan takes place in the colon is provided by results from the subacute and subchronic *in vivo* studies on Wistar rats (see below) showing the increase of caecal content. Such effects have also been confirmed by a 4 weeks *in vivo* study in the high-fat mouse model. Chitin-glucan is a fibre according the definition given into Directive EC N°2008/1004, therefore the energy content (or metabolisable energy) of chitin-glucan can be estimated to be around 2 kcal/g of product coming solely from fermentation in the colon.

On the basis of this information, it is concluded that the novel food product is not nutritionally disadvantageous.

7. Microbiological information on the NF

The applicant provided methods (*Annex 4 of the original application*) and results on an unknown number of batches as presented in **Table 5.**

Table 5 : Microbiological methods and testing results from one batch

Microbiology	Methods	Result
Total mesophilic bacteria (cfu/g)	NF-V-05-051	≤ 1000
Yeasts and moulds (cfu/g)	NF-V-05-059	≤ 1000
Pathogens		
Enterobacteriaceae	NF-V08-054	≤ 10
Total coliforms at 30°C (cfu/g)		≤ 1000
E.coli (cfu/g)	ISO 16649-2 NF-V-08-053	≤ 10
Listeria monocytogenes	Derived from ISO-13720	None / 25g
Salmonella	NMKL 71	None / 25g

The Panel notes that the value ≤ 10 cfu/g for E. coli does not comply with the European Pharmacopeia.

8. Toxicological information on the NF

8.1. Genotoxicity

The applicant provided a study report on an AMES test of a product called "chitin-glucan KiOfine" (a test substance which was reportedly similar to KiOnutrime-CG) carried out by the company Vivotechnia (ES) under GLP and according to OECD guideline 471 and method B13/B14 of Directive EC N° 2000/327. Up to 2.5 mg in DMSO per plate was tested in both the absence and presence of a metabolic activation system. Chitin-glucan was not mutagenic under these conditions.

In addition the applicant provided a literature review commissioned by the applicant on test substances related to chitin-glucan, albeit not similar. These were non-genotoxic: beta-glucan from barley in a mouse bone marrow micronucleus test (Delaney et al., 2004), 6-O-carboxymethylchitin-glucan in a mouse bone marrow micronucleus test (Chorvatovicova et al., 1998), and chitooligomers in an Ames test, a mouse micronucleus test, and in a mouse sperm abnormality test (Qin et al., 2006).

It is concluded that these results do suggest non-genotoxicity of the novel food ingredient.

8.2. Animal studies

8.2.1. Acute toxicity studies

Acute oral toxicity in rats and acute intravenous toxicity in mice were studied with a test product called "Chitin-Glucan KiOfine-26" in 2005 by the company Phycher BioDéveloppement (FR).

Table 6 : Acute toxicity testing

Protocol	Administration - species	Dose	Result
OECD 425	Oral - rats (n=6)	990-5000 mg/kg-bw of chitin-glucan	LD_{50} > 5000 mg/kg-bw
ISO10993-11	Intravenous – mice (n= 10)	50 ml/kg-bw of an aqueous extract of chitin-glucan[4]	LD_{50} > 50 ml/kg-bw

In addition, the applicant conducted a HET-CAM test with chitin-glucan on eye irritation in which it came out as non-irritant to the eyes.

8.2.2. Sub-acute toxicity (OECD 407)

A 28-day oral toxicity study in rats (in accordance with OECD protocol 407 and under GLP) was conducted with chitin-glucan. In this trial, the rats were given repeated doses of 0% (control group), 1 %, 5 % and 10 % of chitin-glucan in the feed. These percentages correspond to doses of 0.8, 4 and 8 g/kg body weight per day (TNO, 2009a).

No significant difference was found in terms of body weight, daily food intake, the weight of the organs and the biochemical parameters of blood and plasma. Statistically significant caecum enlargements were noted in the highest dose group (males) and two highest dose groups (females); caecal enlargement is not uncommon with large doses of fibres/poorly digestible carbohydrates, viz is considered a physiological rather than toxic response. There also was no histological anomaly observed in the organs. It was concluded from the test that chitin-glucan was not toxic, even at the highest dose (8 g/kg body weight/day).

The applicant also reported a 4-week study in which rats on a high fructose diet were administered KiOnutrime-CG at 10 % in the diet. No adverse effects were reported for KiOnutrime-CG.

8.2.3. Sub-chronic toxicity (OECD 408)

A 13-week oral toxicity study with chitin-glucan (KiOnutrime-CG™) in rats (in accordance with OECD protocol 408 and under GLP) was provided (TNO, 2009b).

Chitin-glucan was fed at constant dietary levels of 0 % (control), 1 % w/w (low-dose), 5 % w/w (mid-dose) and 10 % w/w (high-dose) to groups of 20 male and 20 female Wistar rats. These dietary levels were equal to overall mean intake levels of 0.63, 3.2 and 6.6 g chitin-glucan/kg body weight per day

[4] The extract of chitin-glucan is obtained by dispersing the chitin-glucan powder (3 g/15 mL) in a physiological buffer for 72 hours at 73°C, then harvesting the supernatant after centrifugation, in accordance with Annex A of Standard NF EN ISO-10993 part 10, page 23.

in males, and 0.68, 3.4 and 7.0 g chitin-glucan/kg body weight per day in females of the low-, mid- and high-dose group, respectively.

None of the rats died during the study and there were no treatment-related clinical signs. Body weight was not affected by the test substance. There were no treatment-related toxicological effects. As with the 28-day study, the full and empty weight of the caecum were increased in mid- and high-dose males, dose dependently, and in high-dose females, which caecal enlargement was considered a physiological response to the feeding of a high amount of poorly digestible carbohydrate. It is concluded that at an intake of 10 % of chitin-glucan in the diet, the highest dose tested, no adverse effects were observed. This dietary level was equivalent to an overall intake of 6.6 and 7.0 g chitin-glucan/kg body weight/day in males and females, respectively.

It is concluded from the animal studies (acute toxicity, subacute and subchronic studies) that these do not indicate a safety concern.

8.2.4. Allergenicity

A. niger is not currently known to be used as a direct food ingredient, but it is commonly detectible in fruits, certain vegetables, green coffee beans, onions, mango, corn and other cereals, peanuts, dried fruit products, and other food stuffs (Frisvad et al., 2007). The consumption of the organism is therefore expected to occur in the diet of most individuals.

There are some known allergens of *A. niger*: Asp n 14, Beta-Xylosidase, which enzyme is an additive used in the food industry and which presents an allergenic activity when it is inhaled (Horner et al., 1995; Kurup et al., 2000) whereas no allergenicity during the oral ingestion was listed in the scientific literature for this enzyme. Other allergens are Asp n 18, vacuolar serin protease, Pectinase; Glucoamylase, MG 66 000; Xylanase; Phytase; Cellulase; Flaviastase renamed (Doekes et al., 1999). The 3-phytase B is authorized as a feed additive and produced by *A. niger*.

An 8-year follow-up study on clinical reactions to *A. niger* was conducted in a biotechnology plant producing citric acid by fermentation of molasses with *A. niger* and belonging to Tate & Lyle. The authors concluded that *A. niger* was a weak antigen and that simple hygiene measures protect the workforce (Seaton and Wales, 1994). *A. niger* and *A. fumagitus* share some antigens and therefore cross-reactivity is possible.

For glucosamine hydrochloride, a novel food ingredient derived from a fermentation process with *A. niger*, the Panel noted that despite the fact that there was no evidence for the presence of protein in the assessed novel food ingredient, the possibility of allergenicity could not be fully excluded (EFSA, 2009). For the concerned Novel Foods ingredient "Chitin-Glucan" the specification indicate ≤ 6 % protein.

The Panel concludes that an allergenic risk cannot be ruled out, but is expected not to be higher than the consumption of other *A. niger* derived products.

8.3. Human studies

Under the supervision of the Diabetology, Nutrition and Metabolic Diseases Department, the Clinical Pharmacology Unit at Liège University Teaching Hospital has conducted a 4-week trial in 30 healthy male volunteers. Twenty volunteers received 4.5 g chitin-glucan (KiOnutrime-CG) per day and 10 were controls. There were no data on randomization or blinding of subjects. The study investigated markers for cardiovascular risk and metabolic syndrome. The study report indicated "After analysis of the study results, no toxicity of the compound was observed. All hepatic (γGT, TGO, TGP) and renal (creatine, urea) parameters were normal. Some side effects were however mentioned by some volunteers: among the 20 participants receiving KiOnutrime-CG™, seven subjects felt side effects

associated with ingestion of the compound. These gastrointestinal side effects mentioned by the volunteers were mild, transient and are common after ingestion of a certain quantity of fibers. It is noted that no side effects were reported in the control group. However, the study report did not specify what product or substance was used for the placebo. This human study was poorly reported as a small 3-page report with little detail; therefore this study is considered of very limited value to demonstrate the safety in humans.

According to the applicant, chitin-glucan has been classified as 'non-irritant to the skin' on the basis of a '24-hour occlusive single patch test' carried out on 10 volunteers, which corresponds to the lowest level of irritation, and chitin-glucan has been classified as 'non-irritant' to the skin and as 'non-sensitising' to the skin in accordance with the Marzulli-Maibach method in a 6 weeks trial on 50 volunteers (Study Report, Kitozyme, 2004).

8.4. Effect on the bioavailability of micronutrients

Some Member States had questions related to the bioavailability of micronutrients (lipids, fat-soluble vitamins and minerals).

In order to provide more information on the potential reduction of the bioavailability of nutrients, a literature review was performed by the applicant. Since no specific literature exists on chitin-glucan, the review was focused on beta-glucans of similar structure like those from plants, yeasts and mushrooms, as well as on carboxymethyl-chitin-glucan and chitin. Studies suggested that various pectins, gums, lactulose, oligofructose and indigestible sugars improve mineral bioavailability (Greger et al, 1999). However it is noted that this review addressed soluble fibre, and that chitin-glucan is an insoluble fibre.

The consumption of dietary fibre (like beta-glucans) has been reported to reduce the bioavailability of various minerals, an effect which is predominantly a function of the phytate content of the food containing beta-glucans. Therefore, the applicant has performed a titration of phytic acid in chitin-glucan in order to ensure the absence of phytate. KitoZyme shows that the mycelium of *A. niger* used for the production of chitin-glucan does not contain phytate, the concentration is below 0.05 % (m/m) which corresponds to the detection limit. *A. niger* is also reported to be a source of phytase (Pandey et al., 2001), an enzyme active against phytic acid.

Based on the scientific literature and on data provided by the applicant, particularly compositional data and the proposed intake levels, the Panel considers that the Novel Ingredient "chitin-glucan" under the proposed conditions of use has no relevant impact on bioavailability of nutrients.

8.5. Other information

In addition to the data provided on the novel food ingredient, the applicant provided literature data on compounds related to the novel food ingredient (beta-glucan, glucosamine, N-acetyl-glucosamine, chitin). Chitin-glucan is a copolymer composed of metabolites of D-glucosamine, N-acetyl-D-glucosamine and glucose. The polysaccharides related to chitin-glucan and on which toxicological data are available are chitin (of crustacean origin), chitosan (derived from chitin, of crustacean origin), beta-glucans (of vegetable and fungal origin) and oligomers of chitosan. No safety concerns arise from these data.

DISCUSSION

The applicant intends to market the Novel Food "KiOnutrime-CG™" with a content of more than 90 % chitin-glucan as a food supplement in different formats. According to the applicant it is designed to increase the daily intake of fibre. The intended intake of chitin-glucan is 2 to 5 g/day. The compositional data and the manufacturing process do not give rise to concerns.

At the highest dose administered in the 13-week study in rats, i.e. about 6.6 g/kg bw, no adverse effects were observed. This dose is approximately 80-fold higher than the max intended level of intake for humans on a g/kg bw basis. On basis of the data provided and taking into account the nature of the novel food ingredient, the Panel considers that there are no safety concerns under the proposed conditions of use.

There are no specific safety data for children but there are also no suggestions that they may be particularly susceptible to adverse effects.

CONCLUSIONS

The Panel concludes that Novel Food KiOnutrime-CG™ is safe as a food ingredient at the proposed conditions of use and the proposed intake levels.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on 'Chitin-glucan' (KiOnutrime-CG™) received on 10th September 2009. Submitted by KitoZyme. Additional data were provided on 22 of December 2009 and on 16 June 2010.
2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of 'Chitin-glucan'. SANCO E4/AK/mm (2009) D/540563, dated 27 August 2009.
3. Initial assessment report carried out by Belgium: Advisory Report of the Superior Health Council on a marketing authorization application for chitin-glucan as a novel food ingredient under Regulation EC No 258/97
4. Member States' comments and objections
5. Response by the applicant to the initial assessment report and the Member States' comments and objections

REFERENCES

Chattopadhyay P, Banerjee SK, Sen K, Chakrabarti P (1985). Lipid profiles of Aspergillus niger and its unsaturated fatty acid auxotroph, UFA2. Canadian Journal of Microbiology 31:352-355.

Chattopadhyay P, Banerjee SK, Sen K, Chakrabarti P (1987). Lipid profiles of conidia of Aspergillus niger and a fatty acid auxotroph. Canadian Journal of Microbiology 33:1116-1120.

Chorvatovicova D, Machova E, Sandula J (1998) Ultrasonication: the way to achieve antimutagenic effect of carboxymethyl-chitin-glucan by oral administration. Mutation Research 412:83-89

Delaney B, de Vogel N, Krul CA (2004) Evaluation of the in vivo genetic toxicity of concentrated barley β-glucan. Food and Chemical Toxicology 42(1):155-156.

Doekes G., Kamminga N. Helwegen L. and Heederik D. (1999) Occupational IgE sensitisation to phytase, a phosphatase derived from Aspergillus niger; Occupational and Environmental Medicine 56:454-459.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of glucosamine hydrochloride from Aspergillus niger as food ingredient. The EFSA Journal (2009) 1099, 1-19. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2010. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on Dietary Reference Values for carbohydrates and dietary fibre. The EFSA Journal 2010; 8(3):1462 [77 pp.]. doi:10.2903/j.efsa.2010.1462. Available online: www.efsa.europa.eu

Folch PI J, Lees M, Sloane-Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Frisvad JC, Smedsgraard J, Samson RA, Larsen TO, Thrane U (2007). Fumonisin B2 production by Aspergillus niger. Journal of Agricultural and Food Chemistry 55:9727-9732.

Greger JL (1999) Nondigestible carbohydrates and mineral biovailability. Journal of Nutrition 129(7):1434S-1435S.

Horner WE, Helbling A, Salvaggio JE, Lehrer SB (1995). Fungal allergens. Clinical Microbiology Reviews 8:161-179.

Kurup VP, Shen HD, Banerjee B (2000). Respiratory fungal allergy. Microbes and Infection 2:1101-1110.

Pandey A, Szakacs G, Soccol CR, Rodriguez-Leon JA, Soccol VT (2001). Production, purification and properties of microbial phytases. Bioresource Technology 77(3):203-214.

Qin C, Gao J, Wang L, Zeng L, Liu Y (2006) Safety evaluation of short-term exposure to chitooligomers from enzymic preparation. Food and Chemical Toxicology 44: 855–861.

Schuster E., Dunn-Coleman N., Frisvard J.C. and van Dijck P.W.M. (2002) On the safety of Aspergillus niger. Review. Applied Microbiology and Biotechnology 59:426-435.

Seaton A. and Wales D. (1994) Clinical reactions to Aspergillus niger in a biotechnology plant: an eight year follow up. Occupational and Environmental Medicine 51:54-56.

Study Report, Kitozyme (2004). Clinical Evaluation of the sensitizing potential of the cosmetic product "Chitin-Glucan Kiofine CB9-4" according to Marzulli-Maibach method (Study #1689).

TNO (Netherlands Organisation for Applied Scientific Research), 2009a. A 28-day oral toxicity study in rats. Study Report provided by Kitozyme.

TNO (Netherlands Organisation for Applied Scientific Research), 2009b. Repeated-dose (13-week) oral toxicity study in rats with chitin-glucan. Study Report provided by Kitozyme.