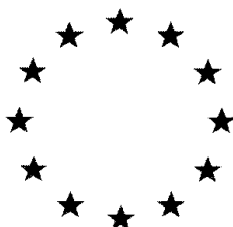


European Commission



VOLUME 3 – Annex B (AS)

Laminarin

B.6 Toxicology and metabolism

Rapporteur Member State: The Netherlands

April 2016

**Draft Re-Assessment Report and Proposed decision of the Netherlands
prepared in the context of the possible renewal of laminarin under Regulation
(EC) 1107/2009**

Version history page

Date	Version history
April 2016	Initial RAR

TABLE OF CONTENTS – VOLUME 3 B.6

B.6	Toxicology and metabolism data	4
B.6.1	Absorption, distribution, metabolism and excretion in mammals	4
B.6.2	Acute toxicity.....	12
B.6.3	Short-term toxicity.....	21
B.6.4	Genotoxicity	29
B.6.5	Long-term toxicity and carcinogenicity	32
B.6.6	Reproductive toxicity	33
B.6.7	Neurotoxicity	39
B.6.8	Other toxicological studies.....	39
B.6.9	Medical data and information	42
B.6.10	References relied on	45

B.6 Toxicology and metabolism data

Nearly all studies were already evaluated in the original DAR (2002). For this RAR, the text in the DAR was considered too concise in places, and therefore the original DAR was not copied to the letter.

B.6.1 Absorption, distribution, metabolism and excretion in mammals

In the original DAR it was indicated that no specific ADME study has been conducted in mammals. The information below is copied mainly from the DAR.

Laminarin, a linear β D-1, 3-linked glucan was extracted and purified from the brown alga *Laminaria digitata*.

Algae such as *Laminaria*, belonging to the brown seaweeds (*Phaeophyceae*), are commonly found on the beaches. Some of them are edible and used unprocessed in food preparations, others are used mainly in livestock feed and occasionally in human food.

Marine algae polymers are mainly non-starch polysaccharides. The majority of them escapes digestion by pancreatic and small-bowel enzymes in the human gut and therefore arrive in the large bowel where their digestive fate is dependent on the depolymerizing and fermentative abilities of the large intestinal microbiota.

Soluble polysaccharides in brown seaweed consist of laminarans, some storage polysaccharides, and fucans, which are cell wall components. Brown algae, unlike the red and green algae, do not synthesise starch-type polysaccharides; instead they store carbohydrate as laminaran.

Chemically, laminarans are β -1,3 linked glucans containing different proportions of β -1,6 linkages and mannitol residues on the reducing ends. Laminarin is an essentially linear glucan composed of ca. 33 β -1,3-linked Glc residues. These polymers are neutral and soluble in hot water.

For certain animals such as rodents, plant material constitute a major portion of their diets. Microbial digestion of plant material is significant and occurs mainly in the caecum (rats) and large intestine (humans) after prior host digestion in stomach and small intestine. A similar digestion pattern takes place in humans, but only small amounts of plant fibres are degraded. In contrast, with ruminant animals, intake of plant material is extensive, and digestion occurs prior to gastric digestion. The ruminant is dependent on micro-organisms in the forestomach or rumen for the digestion of plant polysaccharides that are consumed. Further digestion of plant materials can occur in the caecum and large intestine, but is considerably less in magnitude.

Overall, plant polysaccharides are degraded to varying extents to soluble forms, mostly oligosaccharides, by the hydrolytic microbial species. Laminarinases that hydrolyse laminarin occur in bacteria, fungi, algae, higher plants, and molluscs (Black and Dewar, 1954).

The small intestine of humans (and animals) does not possess specific β -D-glucans-hydrolases required for hydrolysis of oligosaccharides to their constituent simple sugars. As a result, virtually

100% of ingested polysaccharides reaches the colon unchanged. In the colon, bacteria, in a process called fermentation, mainly degrade them to mono- and oligo-saccharides, and then, these degraded saccharides of lower molecular size are utilised by a variety of intestinal bacteria to give a mixture of short chain fatty acids (acetate, propionate and butyrate), L-lactate, carbon dioxide and hydrogen (Kuda et al.,1992). Unfermented carbohydrates increase faecal bulk.

In France, the Superior Council of Public Hygiene gave a favourable advice for the use of *Laminaria digitata* in human food:

For an adult, supply should be ≤ 30 mg (dry product) or 210 mg/day for a fresh product. Children < 4 year, supply should be below 15 mg dry compound corresponding to 105 mg fresh compound.

In addition, EFSA concluded in 2012 that no reference values are required for sea algae extracts, used as PPPs, including an extract from *Laminaria digitata*.

http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2492.pdf

B.6.1.1 Absorption, distribution, metabolism and excretion by oral route

Absorption

As no study has been conducted on mammals, the absorption of the chemical cannot be evaluated. However, due to the size of the molecule ($\approx 5\,000\text{ g}\cdot\text{mol}^{-1}$), absorption by the skin or through inhalation can be discarded, and only the oral route has to be considered, with a first possibility of absorption at the stomach level.

The only paper found with an oral administration was on salmon with an aminated β -(1 \rightarrow 3)-polyglucose, which could be called "aminated laminaran", where only limited product appeared in the blood and the organs, indicating poor absorption, but substantial amounts were found in the cell walls of the descending intestine; this location in a place rich in immunocompetent cells is of interest for the immunomodulator potential of this chemical.

In the DAR it was stated that in the open literature a review article deals specifically with the digestive fate of soluble polysaccharides such as laminarin from marine macro-algae, and on the involvement of the colonic microflora and physiological consequences for the host (Michel and Macfarlane,1996):

Non-starch polysaccharides such as laminarin are resistant to hydrolytic enzymes produced by monogastric animals, and their digestive fate is dependent on the depolymerizing and fermentative abilities of the large intestinal microbiota. The monosaccharides are the only units that normally cross the intestinal membrane.

Distribution.

Short Chain Fatty Acids (SCFAs) including acetate, propionate and butyrate are produced in the caecum and colon of nonruminant animals and humans and account for approximately 80% of the colonic anion concentration. They are produced in nearly constant molar ratio 60:25:15. Among their various properties, SCFAs are readily absorbed by intestinal mucosa, are relatively high in caloric content, are metabolised by oxidation in colonocytes and hepatocytes, stimulate sodium and water absorption in the colon and are trophic to the intestinal mucosa (D'Argesio and Mazzacca 1999).

Once absorbed, SCFAs are used preferentially as fuel for colonic epithelial cells and have a trophic effect on the epithelium. Butyrate oxidation in colonocytes produced ketone bodies and CO₂ (Cook and Sellin, 1998). Beside their action on gut morphology and function, SCFAs influence gastrointestinal motility producing a laxative effect. The increase in faecal output is likely to be due to an increase in biomass (Cummings et al, 2001).

There are several studies on the distribution of laminarin in salmon after oral or iv administration. Laminarin accumulated in organs with large amounts of lymphoid tissue, such as pronephros, spleen, gills and the wall of the posterior intestine after oral administration. From 2 to 12 days after iv injection, radioactivity was higher in spleen, anterior kidney, posterior kidney, liver and the posterior intestinal mucosa than in blood. Only traces of radioactivity were observed in the bile. They also showed by fluorescence of FITC-laminaran that the compound in the kidney was trapped in macrophages and in endothelial cells; these cells are known to possess β -glucan receptors, including in mammals. Laminaran also accumulated in the same type of cells in the spleen.

Metabolism

Bacteroides species from the human colon ferment a variety of polysaccharides, which are not digested in the stomach or small intestine. Dietary fibre components are an important source of carbohydrate for colon bacteria.

Laminarin is fermented by some species of anaerobic bacteria containing laminarinase activity from the human colon. Laminarinase is an enzyme complex that can involve as many as three types of activity: β -glucosidase, which hydrolyse low molecular weight glucose containing substrates (di- or trisaccharides), an exoglucanase that cleaves single glucose units from the non-reducing end of laminarin and an endoglucanase that release laminaribiose, laminaritriose or higher oligomers of glucose from laminarin. Polysaccharide degrading enzymes from several *bacteroides* species have been studied and in most cases the enzyme activities were cell bound rather than extracellular. Laminaran-degrading species mainly belong to the genus *Bacteroides* and *Bifidobacteria* (*B. thetaiotaomicron*, *B. distasonis*, *Bacteroides* 0061-1, *Bacteroides* T4-1). These organisms are major components of the gut microbiota. The concerted activities of these polymer degrading species, together with other saccharolytic and hydrogenotrophic organisms in the large bowel, gives rise to a range of fermentation products, Short Chain Fatty Acids (SCFAs) (Table B.6.1.1-1). SCFAs are the predominant products of bacterial metabolism from human as well as rat colon. SCFAs are important

anions in the colonic lumen, affecting both colonocyte morphology and function. The three main acids (acetate, propionate, and butyrate) stimulate colonic sodium and fluid absorption and exert proliferative effects on the colonocyte (Sheppach, 1994). After uptake by colonic mucosa, they undergo a variety of metabolic fates in host tissues (see table B.6.1.1-1) (Djouzi et al.1995 and Michel and Macfarlane, 1996).

Table B.6.1.1-1: Metabolic fate of the major carbohydrate fermentation products formed by bacteria in the human large intestine (From Michel and Macfarlane, 1996)

Fermentation product	Metabolic significance
Short Chain Fatty Acids	<ul style="list-style-type: none"> - About 85% of total colonic production are absorbed from the large bowel. Stimulate salt and water absorption in the colon - Intestinal motility is increased - Crypt cell formation and mucosal weight increased - Accelerated mucosal healing - Antibacterial effects
Acetate	<ul style="list-style-type: none"> - Quantitatively most important fermentation product - oxidised in brain, heart, kidney, liver, muscle and peripheral tissues
Propionate	<ul style="list-style-type: none"> - Mainly cleared by liver, but not a major gluconeogenic substrate - Suppresses cholesterol synthesis
Butyrate	<ul style="list-style-type: none"> - substrate for membrane lipid synthesis - affects gene expression and induces differentiation in a wide range of human and other mammalian cells - induces apoptosis - Principal fuel for colonic epithelial cells, particularly in distal bowel
Hydrogen	<ul style="list-style-type: none"> - Electron donor in other biochemical reactions in colonic bacteria - Excreted in breath and flatus - Excretion: Converted to H₂S, acetate, or methane by colonic SRB, acetogenic and methanogenic bacteria respectively
Carbon dioxide	<ul style="list-style-type: none"> - Required in some bacterial fermentation reactions, e.g. methanogenesis, acetogenesis, bacteroides metabolism. Involved in SCFA and sodium absorption in the colon - Excreted in breath and flatus

The involvement of colonic bacteria in laminarin breakdown is illustrated in rats by increases in faecal bacterial excretion following inclusion of laminarin (10%) in the diet (Kuda et al, 1992).

Conclusion:

In human, non-digestible polysaccharides such as laminarin escape enzymatic digestion in the upper gastrointestinal tract; colonic fermentation produces short-chain fatty acids, which are then absorbed. Absorption reaches approximately 90%.

Metabolism is important, involving gut microbiota (colonic fermentation) which degrade the polymers giving rise to SCFAs.

Distribution is large: carbohydrate fermentation products are oxidised in brain, heart, kidney, liver, muscle, peripheral tissues.

Excretion occurs via breath and flatus after conversion of SCFAs into H_2S , CO_2 , CH_4 , and acetate. Unfermented carbohydrates increase faecal bulk likely as a result of increased biomass.

In contrast, digestible carbohydrates are hydrolysed by alpha-amylases secreted by pancreas and salivary glands and oligosaccharidases responsible for hydrolysing the residual sugars. Active glucose absorption occurs in the small intestine (Szepesi, 1996).

Metabolism in plants

Numerous enzymes able to degrade laminarans have been isolated from bacteria, fungi, algae, molluscs and higher plants. The enzymes that hydrolyse laminarin are termed laminarinases including β -D-glucanases and β -D-glucosidases. Laminarase occurs in bacteria, fungi, algae, higher plants and molluscs (Black and Dewar, 1954). These enzymes are involved in the intracellular mobilisation of food reserves (not only in algae but also in higher plants and fungi, all of which synthesise β -D-glucans) and are also encountered in the extracellular breakdown of plant debris and in the digestive metabolism of invertebrates. Enzyme preparations have been reported from cereals, potato tubers etc. The enzyme removes D-glucose units by endwise attack from the non-reducing ends of the chains (Black and Dewar, 1954).

B-Glucan endohydrolases from plants are involved in cell wall degradation. They release oligosaccharides from their substrate and are probably of central importance for the initial solubilization of the (1 \rightarrow 3, 1 \rightarrow 4) β -glucans. The soluble products of the initial hydrolysis are then acted on by glucanglucohydrolases, which preferentially attacks the longer gluco-oligomers (cellotriase) releasing glucose. β -Glucan exohydrolases and β -glucosidases may be important additional enzymes for the conversion of released oligosaccharides to glucose.

B-Glucanases which are widely distributed in plant hydrolyse also polysaccharides that are abundant in fungal cell walls (Hrmova et al., 1997).

In conclusion, in plants, laminarin may undergo degradation by polysaccharide and oligosaccharide hydrolases leading to production of glucose.

Metabolism in ruminants

In ruminants, feedstuffs are all initially exposed to the fermentative activity in the rumen prior to gastric and intestinal digestion. Dietary polysaccharides are generally degraded by the ruminal micro-organisms into characteristic endproducts. Fermentative production of SCFAs is the principal mechanism of intestinal digestion in ruminants (D'Argenio and Mazzacca, 1999).

Consequently, additional uptake of polysaccharides by animals by way of feeding is of no concern and livestock metabolism studies are not necessary.

Metabolism in fish

Several reports on (1→3)-β-D-glucans deal with their immunostimulatory effects in fish and indirect evidence that (1→3)-β-D-glucan is absorbed from the posterior intestine was showed in the Atlantic salmon. In fish, it was suggested that laminaran and aminated (1→3)-β-D-glucans, after i.v. administration, are distributed to tissues rich in immunocompetent cells like the spleen and the anterior kidney. The cellular accumulation of laminaran may be related to the endocytic function displayed by sinusoidal endothelial cells and macrophages (Dalmo et al, 1995).

Overall conclusion

Laminarins are cell wall components that are degraded by the colonic microflora in monogastric animals. The large bowel fermentation involves bacteria producing laminarinases, and B-glucosidases, which fully degrade the substrate into Short Chain Fatty Acids (SCFAs). SCFAs are absorbed and further metabolized before excretion into breath and flatus.

In plants, laminarin may undergo degradation by polysaccharide and oligosaccharide hydrolases leading to production of glucose.

Fermentative production of SCFAs is the principal mechanism of intestinal digestion in ruminants.

Additional information from the literature search

No new information was found in publications concerning absorption, distribution, metabolism and excretion for laminarin in mammals. However, as presented in the Section 9 - Literature data, a suckling model in young rat was used in 2007 to assess the absorption and tissues distribution of enterally administered ¹²⁵I-Phycarine. Despite the fact that these studies didn't follow toxicokinetic Guidelines (such as OECD No.417), results suggested that only very limited amount of glucan was absorbed by the gut and transferred into systemic blood. The majority of radioactivity was detected in the gastrointestinal tract and the liver. It gives some information on (1-3)-beta-D-glucan when orally administered to young rats.

<i>Previous evaluation:</i>	<i>Submitted for the purpose of renewal, as a result of the literature search</i>
RMS remarks	Acceptable as supplementary study

reference	:	Vetvicka et al, 2007	exposure	:	Oral (gavage)
Report number	:	N/a	doses	:	12,000 cpm of ¹²⁵ I-Phycarin
test substance	:	(1-3)-beta-D-glucan	GLP statement	:	yes
species	:	Rat, Sprague–Dawley (15-days old)	guideline	:	Not in accordance with OECD 417
group size	:	40 pups (male and female)	acceptability	:	acceptable

Author(s)	[REDACTED]
Year	2007
Journal	Int J Biol Macromol. 2007 Mar 10;40(4):291-8. Epub 2006 Aug 23.
Relevance check	Yes

Reliability check	2
Reasons for restricted or no reliability/limitations	Toxicokinetic Guidelines such as OECD No.417 are not followed in this publication where intestinal absorption and tissues distribution of (1-3)-beta-D-glucan are studied in young rats. For example, Sprague-Dawley rats were younger (2 weeks instead of 6-12 weeks at the time of dosing) and the selected time points were reduced (until 120 minutes after administration).
Summary	Using a suckling rat model for evaluation of the absorption and tissues distribution of enterally administered ¹²⁵ I-Phycarine, the majority of Phycarine was detected in the stomach and duodenum 5 min after administration. This amount sharply decreased during first 30 min. A significant amount of Phycarine entered proximal intestine in a shortly after the gavage. Its transit through proximal intestine was decreasing with time and simultaneously increasing in the ileum. Systemic blood levels were very low (less than 0.5%).
Test protocol GLP, GEP, Guidelines (US EPA, OECD, ...)	No GLP. No specific Guidelines
Test substance Identification of test substance, source, purity, stability	<p>Phycarine was extracted and purified from the marine brown alga <i>Laminaria digitata</i>. Briefly <i>L. digitata</i> sporophytes, harvested in late summer, were extracted with hot water for 2 h. The water extracts were fractionated by two ultrafiltrations, first with a cutoff of 300 kDa and second with a cutoff of 1 kDa. Resulting retentate was desalted and freeze-dried. Molecular weight of laminarin was 5300 Da, as measured by molecular size chromatography coupled with a refractometric detector. Purity, size and structure were further analyzed by ¹³C NMR spectroscopy and HPAEC-PAD. Using the <i>Limulus</i> lysate test, the LPS contamination was determined to be below 0.005 U/mL.</p> <p>Phycarine iodination was performed using 1 mg of IODO-GEN (1, 3, 4, 6 – tetrachloro - 3α, 6α - diphenylglycouril) dissolved in 5mL of chloroform. 50 μL of this solution was transferred into glass tubes. Chloroform was evaporated and a thin film of IODO-GEN was deposited on the walls of the glass tubes. Tubes were stored at 0°C until use. 100 μg of Phycarine dissolved in PBS were incubated with 11.1 MBq of NaI¹²⁵ at 25°C for 1 h. Following the incubation, the mixture was purified by gel filtration on a PD 10 column using 0.1 M phosphate buffer (pH 7.2), as a mobile phase.</p>
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	<p>Sprague–Dawley rats (Charles River Labs, Pontage, MI) (15-day-old)</p> <p>Forty pups, both male and female originating from four different litters, were used in these studies. Pups were fasted for 20 h before the beginning of experiment. Fasted pups were kept in plastic cages, which were placed with their bottom half on an electric heating pad to help the pups regulate their normal body temperature.</p> <p>At the beginning of experiment, pups were fed with 200 μL of 0.9% saline containing approximately 12,000 cpm of ¹²⁵I-Phycarine.</p> <p>At selected time points (5, 10, 30, 60, and 120 min after Phycarine administration), the pups were anesthetized and decapitated. A 100 μL of systemic blood was collected. Then, the stomach, duodenum, the small intestine, liver, and kidney were quickly removed. The small intestine was divided into two halves (proximal and distal) and all collected samples were counted on a Packard Cobra II gamma counter.</p> <p>Results were expressed as a percentile of the original dosage. To evaluate the fate of administered Phycarine, total recovery of</p>

	labeled Phycarine was calculated as a percentile of administered dose.
Controls Positive control, negative control	No control animals were used in these studies.
Dosing system Exposure (dose, duration, frequency)	The animals were fed by gavage with 200 µL of 0.9% saline containing approximately 12,000 cpm of ¹²⁵ I-Phycarine.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Student's <i>t</i> -test was used to statistically analyze the data.
Results Determined effect concentration, dose response observed	The majority of Phycarine was detected in the stomach and duodenum 5 min after the administration. This amount was sharply decreasing during first 30 min (from 76% to 24%), then slower decreasing trend continued up to 120 min. A significant amount of iodine-labeled Phycarine (approx. 18%) entered proximal intestine in a very short time after the gavage. Phycarine transit through proximal intestine was decreasing with time and simultaneously increasing in the ileum. This suggested that a significant portion of Phycarine passed throughout the proximal intestine into ileum. At 30, 60 and 120 min, about 25–29% of administered Phycarine was detected in the ileum. The amount of Phycarine detected in the liver was approximately 0.5–1.5% and similar results were detected in kidney (0.2–1%). Levels of Phycarine in systemic blood levels were very low (less than 0.5%). In the initial time point of this study (5 min), the majority of labelled Phycarine (95%) is localized in the stomach and proximal small intestine. The total recovery of administered Phycarine was gradually decreasing with time, reaching approx. 60% after 120 min.
Overall conclusion	Although this study didn't follow toxicokinetic Guidelines (such as OECD No.417), the test was performed in the young rats during the suckling period when the intestinal barrier function and transport function are not fully established. Results suggested that only very limited amount of glycan was absorbed by the gut and transferred into systemic blood. The majority of radioactivity was detected in the gastrointestinal tract and the liver. It gives some information on toxicokinetic properties of (1-3)-beta-D-glucan when orally administered to young rats.

B.6.1.2 Absorption, distribution, metabolism and excretion by other routes

No study available, not necessary.

B.6.2 Acute toxicity**B.6.2.1 Oral**

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████ 1998a	exposure	:	Single oral (gavage)
Report number	:	970352 ST	doses	:	0 or 2000 mg/kg bw
test substance	:	Phycarine, batch no.96S51	GLP statement	:	yes
species	:	Rat, Sprague-Dawley	guideline	:	in accordance with OECD 401
group size	:	5/sex/dose	acceptability	:	acceptable

Report : ██████████ (1998a) ; Phycarine[®] 96S51 - Acute toxicity study - Safety test in the rat by the oral route

██████████
Unpublished report N°.970352 ST, 12/03/1998

Dates of experimental work : 07/01/1998 to 21/01/1998

Guidelines : OECD N° 401 (1987)

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®] ; Batch N°96S51; Purity on dry matter : 91 %

methods : 2000 mg/kg bw of laminarin was administered orally in a volume of 10 mL/kg bw as a suspension in water for injection. A group of control animals under the same conditions as the test animals was treated with 10 mL/kg bw of water for injection and served as a reference. Animals were monitored daily for 14 days after administration of the compound.

Findings : No mortalities were observed. No clinical signs were observed throughout the observation period. No effects on body weight development were noted. At gross necropsy no visible lesions were observed.

Table 6.2.1-1: Acute oral toxicity of laminarin

Males			Females		
Dose	Mortality	Time of death	Dose	Mortality	Time of death
2000 mg/kg b.w.	0/5	-	2000 mg/kg b.w.	0/5	-

Conclusion

The oral LD50 of laminarin in rats was determined to be greater than 2000 mg/kg bw.. The classification according to Regulation (EC) No 1272/2008 is :

Symbol : none

Indication of danger : none

Risk phrase : none

No new information was found in publications concerning acute oral toxicity in rat for laminarin. However, as presented in the Section 9 - Literature data, (1,3/1,6)-beta-D-glucan (structure close to

1,3 beta-D-glucan) was administered to Fisher-344 rats (5/sex) with a single dose of 2000 mg/kg bw followed by a 14-day period (OECD Guideline No.420). Since no adverse or toxic effects were observed after acute oral administration, the oral LD₅₀ of (1,3/1,6)-beta-D-glucan in rats was determined to be greater than 2000 mg/kg bw.

The paper describes two studies: a single oral toxicity study, which is evaluated below, and a 91-day repeated dose toxicity study, which is evaluated in B.6.3.2.

Previous evaluation:	Submitted for the purpose of renewal, as a result of the literature search
Evaluation RMS:	Acceptable

reference	:		exposure	:	Oral (gavage)
Report number	:	n/a	doses	:	0, or 2000 mg/kg bw
test substance	:	(1,3/1,6)-beta-D-glucan	GLP statement	:	yes
species	:	Rats, Brl-Han:WIST@Jcl	guideline	:	in accordance with OECD 420
group size	:	5/sex/dose	acceptability	:	acceptable

Author(s)	
Year	2007
Journal	Food Chem Toxicol. 2007 Sep;45(9):1719-30. Epub 2007 Mar 23.
Relevance check	Yes
Reliability check	2
Reasons for restricted or no reliability/limitations	The substance tested corresponds to a (1,3/1,6)-beta-D-glucan structure (close to 1,3 beta-D-glucan).
Summary	For the acute study, Wistar rats were administered (1,3/1,6)-beta-D-glucan via gavage at a dose of 2000 mg/kg bw, and any evidence of toxicity was monitored over a 14-day period. (1,3/1,6)-beta-D-glucan was well tolerated, indicating that the LD50 value is greater than 2000 mg/kg bw.
Test protocol GLP, GEP, Guidelines (US EPA, OECD, ...)	GLP: yes OECD Guideline No.420 (OECD, 2001) OECD Guideline No.408 for the Testing of Chemicals, "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD, 2001)
Test substance Identification of test substance, source, purity, stability	WGP® 3–6 (Lot #20030731) from Biopolymer Engineering, Eagan MN WGP® 3–6 powder is a highly purified extract of <i>Saccharomyces cerevisiae</i>
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	<u>Acute toxicity study</u> Five male and five female rats Brl-Han:WIST@Jcl were allocated to control and treatment Groups. Clinical observations were made frequently on the day of administration and once a day during the 14-day observation period. The rats were weighed on days 7 and 14 following oral gavage, and the mean body weight values of the experimental and control groups were statistically analyzed using the t-test (p = 0.05).
Controls Positive control, negative control	Control animals (negative): yes
Dosing system Exposure (dose, duration, frequency)	<u>Acute toxicity study</u> 0 (control) and 2000 mg/kg bw
Statistical analyses Sample size/replicates, statistical analysis of data	Means and standard deviations of body weights were calculated. Standard analysis of variance (ANOVA) was used for statistical evaluation of the data. When a significant dose-effect was found,

(significance level, variability)	further statistical analysis using Tukey's method and Dunnett's multiple comparisons were used for a detailed assessment of data obtained and for an assessment of their mutual relationship.
Results Determined effect concentration, dose response observed	<u>Acute toxicity study</u> No deaths or clinical abnormalities for either male or female animals were observed during the experimental period. No significant differences in body weight gain occurred as a result of test item administration. In addition, the findings of gross necropsy performed at study termination did not reveal any signs of toxicity. Under the conditions of this acute oral test, the LD50 value is greater than 2000 mg/kg bw.
Overall conclusion	<u>Acute toxicity study</u> Under the conditions of this acute oral test, the LD50 value is greater than 2000 mg/kg bw.

B.6.2.2 Dermal

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 2001d	exposure	:	Single dermal (semi-occlusive)
Report number	:	20000698 ST	doses	:	0 or 5000 mg/kg bw
test substance	:	Laminarin, batch no.99S24	GLP statement	:	yes
species	:	Rat, Sprague-Dawley	guideline	:	in accordance with OECD 402
group size	:	5/sex/dose	acceptability	:	Acceptable

Report : ██████████. (2001d). H11 (Batch 99S24) - Acute dermal toxicity study in the rat

Unpublished report N°20000698 ST, 26/07/2001

Dates of experimental work : 04/01/2001 to 18/01/2001

Guidelines : OECD N°402 (1987)

Directive 92/69/EEC, method B3

Deviations : the maximum dose tested was 5000 mg/kg bw instead of 2000 mg/kg bw.

GLP : Yes (certified laboratory)

Material and Test material : Laminarin, Batch N°99S24 ; Purity on dry matter : 94.9 %

methods : 5000 mg/kg bw. of laminarin was administered cutaneously in a volume of 8 mL/kg bw. as a viscous solution in sterile water on a piece of absorbant gauze to a group of 5 males and 5 females. A group of control animals under the same conditions as the test animals was treated with 8 mL/kg bw. of water and served as a reference. Animals were monitored daily for 14 days after administration of the compound.

Findings : No mortality occurred during the study. No clinical signs and no dermal reactions were observed during the course of the study Mean weight gain in treated animals was normal. At necropsy no macroscopic organ or tissue abnormality was seen.

Table 6.2.2-1: Acute dermal toxicity of laminarin

Males			Females		
Dose	Mortality	Time of death	Dose	Mortality	Time of death
5000 mg/kg b.w.	0/5	-	5000 mg/kg b.w.	0/5	-

Conclusion

The dermal LD₅₀ of the laminarin in rats was determined to be greater than 5000 mg/kg bw.. The classification according to Regulation (EC) No 1272/2008 is :

Symbol : none

Indication of danger : none

Risk phrase : none

Additional study

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 1998b	exposure	:	Single subcutaneous injection
Report number	:	970353 ST	doses	:	0 or 1000 mg/kg bw
test substance	:	Phycarine, batch no.96S51	GLP statement	:	yes
species	:	Rat, Sprague-Dawley	guideline	:	Directive 87/18/ EEC Directive 87/18/ EEC
group size	:	5/sex/dose	acceptability	:	Acceptable as additional information

Report : ██████████ (1998b) ; Phycarine[®] 96S51 - Acute toxicity study - Safety test in the rat by the sub-cutaneous route

██████████

Unpublished report N°.970353 ST, 12/03/1998

Dates of experimental work : 07/01/1998 to 21/01/1998

Guidelines : Directive 87/18/ EEC;
Recommendations CC81/30 Appendix 2 of OECD
Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®]; Batch N°96S51 ; Purity on dry matter : 91 %

methods : 1000 mg/kg bw. of laminarin was administered subcutaneously in a volume of 5 mL/kg bw as a suspension in water for injection. A group of control animals under the same conditions as the test animals was treated with 5 mL/kg bw. of water for injection and served as a reference. Animals were monitored daily for 14 days after administration of the compound.

Findings : No mortalities were observed. No clinical signs were observed throughout the observation period. No effects on body weight development were noted. At gross necropsy no visible lesions were observed.

Table 6.2.2.1-1: Acute subcutaneous toxicity of Laminarin

Males	Females
-------	---------

Dose	Mortality	Time of death	Dose	Mortality	Time of death
1000 mg/kg bw.	0/5	-	1000 mg/kg bw.	0/5	-

Conclusion

The subcutaneous LD₅₀ of the laminarin in rats was determined to be greater than 1000 mg/kg bw.

B.6.2.3 Inhalation

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 1999	exposure	:	Inhalation (4h, head-nose only)
Report number	:	980001 EX	doses	:	1.02 mg/L
test substance	:	Phycarine [®] ; Batch N°96S51	GLP statement	:	yes
species	:	Rat, Wistar	guideline	:	in accordance with OECD 403
group size	:	5/sex/dose	acceptability	:	acceptable

Report : ██████████ (1999) ; Evaluation of acute inhalation toxicity of Phycarine[®] in rats ;

██████████

Unpublished report N°980001 EX, 20/03/1999

Dates of experimental work : 28/10/1998 to 16/11/1998

Guidelines : OECD N° 403 (1981)

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®]; Batch N°96S51 ; Purity on dry matter : 91 %

methods : Groups of 5 male and 5 female Charles River Wistar rats were exposed to the test material. The test article was dissolved in 10 % concentration in distilled water and was administered as an aerosol of this aqueous solution for 4 hours with the maximum attainable concentration of 1.02 mg/L in the air; 58% of the particles had a diameter < 22µm. Animals were examined for mortality, clinical signs, body weight gain and pathological alterations of organs at the end of a 14-day observation period.

Findings : No mortalities were observed (Table 6.2.3-1). No clinical signs were observed throughout the observation period. No effects on body weight development were noted. At gross necropsy no visible lesions were observed.

Table 6.2.3-1: Acute inhalation toxicity of laminarin

Males			Females		
Dose	Mortality	Time of death	Dose	Mortality	Time of death
1.02 mg/L/4h	0/5	-	1.02 mg/L/4h	0/5	-

Conclusion

The acute inhalation LC₅₀ of laminarin in rats was determined to be greater than 1.02 mg/L/4h.

The limit concentration of 5 mg/L/4h could not be reached and 1.02 mg/L/4h was the maximum attainable concentration. Therefore, in accordance with Regulation (EC) No 1272/2008, laminarin is

considered neither a toxic nor a harmful substance based on this acute inhalation toxicity study and the classification is :

Symbol : none

Indication of danger : none

Risk phrase : none

B.6.2.4 Skin irritation

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 1998a	exposure	:	Dermal (4h, semi-occlusive)
Report number	:	970349 ST	doses	:	0.5 g
test substance	:	Phycarine [®] , Batch N°96S51	GLP statement	:	yes
species	:	Rabbit, New Zealand A bino	guideline	:	in accordance with OECD 404
group size	:	3 animals	acceptability	:	acceptable

Report : ██████████ (1998a) ; Phycarine[®] 96S51 - Cutaneous primary irritation in the rabbit ;

██████████

Unpublished report N°970349 ST, 24/02/1998

Dates of experimental work : 23/12/1997 to 30/12/1997

Guidelines : OECD N° 404 (1992)

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®] ; Batch N°96S51 ; Purity on dry matter : 91 %

methods : 0.5 g of the compound laminarin was applied to the right flank of each of three rabbits. Adjacent surfaces of untreated skin of each animal served as a control for the trial. The compound was moistened with 0.5 mL of water for injection in order to allow good contact between the test substance and the skin. Semi-occlusive dressings held the compound in place for 4 hours on the flanks of each animal.

Findings : Results obtained appear in Table 6.2.4-1 below:

Table 6.2.4-1: Cutaneous tolerance to laminarin

Mean indices per parameter (24 - 48 - 72 h)

Rabbit number	Mean index (Mi)	
	Erythema	Oedema
970367	0.00	0.00
970388	0.00	0.00
970389	1.00	0.00

Conclusion

Under the experimental conditions adopted, the classification of the compound laminarin is : non-irritant for the skin of the rabbit.

The classification according to Regulation (EC) No 1272/2008 is :

Symbol : none

Indication of danger : none

Risk phrase : none

B.6.2.5 Eye irritation

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	: [REDACTED] 1998b	exposure	: Ocular
Report number	: 970350 ST	doses	: 100 mg
test substance	: Phycarine [®] , Batch N°96S51	GLP statement	: yes
species	: Rabbit, New Zealand SPF	guideline	: in accordance with OECD 405
group size	: 3 animals	acceptability	: acceptable

Report : [REDACTED] (1998b) ; Phycarine[®] 96S51-Ocular primary irritation in the rabbit ; [REDACTED]
[REDACTED]

Unpublished report N°970350 ST, 03/03/1998

Dates of experimental work : 29/12/1997 to 01/01/1998

Guidelines : OECD N° 405 (1987)

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®]; Batch N°96S51; Purity on dry matter : 91 %

methods : 0.1 g of laminarin was instilled into the conjunctival sac of the left eye of each of three rabbits used. The untreated right eye served as a control. 24 h after instillation in the conjunctival sac of the eye, the substance was washed out with tap water.

Findings : Results obtained appear in Table 6.2.5-1 below:

Table 6.2.5-1: Ocular tolerance of laminarin

Mean indices per parameter (24 – 48 - 72 h)

Rabbit number	Mean index (Mi)			
	Chemosis	Redness	Iris	Cornea
970376	0.00	0.33	0.00	0.00
970377	0.00	0.33	0.00	0.00
970368	0.00	0.00	0.00	0.00

Conclusion

Under the experimental conditions adopted, the classification of laminarin is non-irritant for the eye of the rabbit.

The classification according to Directive 99/45/EC is :

Symbol : none

Indication of danger : none

Risk phrase : none

B.6.2.6 Skin sensitisation

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

reference	:	██████, 1998c	exposure	:	intradermal and topical induction, topical challenge (occlusive, 48h)
Report number	:	970351 ST	doses	:	See study design
test substance	:	Phycarine [®] , Batch N°96S51	GLP statement	:	yes
species	:	guinea pig White, Hartley	guideline	:	in accordance with OECD 406
group size	:	10/sex	acceptability	:	acceptable

Report : ██████ (1998c) ; Phycarine[®] 96S51 - Study of cutaneous sensitisation using the Magnusson and Kligman maximisation test in the guinea pig.

Unpublished report N°970351 ST, 23/04/1998

Dates of experimental work : 06/01/1998 to 30/01/1998

Guidelines : OECD N° 406 (1992)

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : Phycarine[®]; Batch N°96S51 ; Purity on dry matter : 91 %

methods : The maximum slight to moderately irritant concentration (based on the cutaneous reaction 24 hours after intradermal administration) and used during the primary induction phase was laminarin at 25 % (p/v) in water for injection.

The maximum slight to moderately irritant concentration (based on reading of cutaneous reactions 1 hour after removing the dressing of a 48-hour epicutaneous exposure and used during the second induction phase and the sensitization phase performed by the topical route on D9) was laminarin at 25 % (p/v) in water for injection.

The Maximum Non-Irritant Concentration (M.N.I.C.) determined by epicutaneous application (based on reading of cutaneous reactions 24 and 48 hours after removing the dressing of a 24-hour exposure) and used during the challenge phase was laminarin at

25 % (p/v) in water for injection.

Determination of the degree of allergenicity at 24 and 48 hours was based upon the percentage of animals in the group showing a reaction, rather than on its severity.

The sensitivity and the reliability of the experimental method is verified, at least every 6 months, using a positive control group in which animals are treated with dinitrochlorobenzene (D.N.C.B., 1%).

Findings Findings are summarised in the following Table 6.2.6-1.

Table 6.2.6-1: Cutaneous reactions and degree of allergenicity at 24 and 48 hours with laminarin

Group	Time	Number of animals/grade				% of sensitised animals	CLASS (degree of allergenicity)
		0	1	2	3		

Positive Control (1)	24 h	0	8	2	0	100	V
	48 h	2	6	2	0	80	-
Treated (2)	24 h	20	0	0	0	0	I
	48 h	20	0	0	0	0	-

(1) 1% dinitrochlorobenzene (D.N.C.B.) in alcoholic solution (tested on 02/12/1997 - D1)

(2) Laminarin

Conclusion

Under the experimental conditions adopted, laminarin showed only minimal allergenicity of Class I at 24 hours. Therefore it is considered that the test substance is free of any sensitising capacity in the Guinea Pig.

The classification according to Regulation (EC) No 1272/2008 is :

Symbol : none

Indication of danger : none

Risk phrase : none

B.6.2.7 Phototoxicity

According to Regulation (EU) No.283/2013, “the in vitro study shall be required where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than $10 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, no toxicity testing is required.”

Since laminarin doesn't encounter these physico-chemical characteristics, no phototoxicity study is required for the present submission. This is accepted by the RMS.

B.6.3 Short-term toxicity**B.6.3.1 Oral 28-day study****Rat**

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████ 2000	exposure	:	4 weeks (oral gavage)
Report number	:	RTC 7286/T/240/99	doses	:	0, 1000 mg/kg bw/d
test substance	:	Laminarin, Batch N°99S21	GLP statement	:	Yes
species	:	Rat, Sprague-Dawley	guideline	:	in accordance with OECD 407 (1995)
group size	:	5/sex/dose	acceptability	:	acceptable

Report : ██████████ (2000) ; H11 – 4-week oral toxicity study in rats

██████████

Unpublished report N° RTC 7286/T/240/99, 19/01/2000

Dates of experimental work : 09/08/1999 to 06/09/1999

Guidelines : OECD N° 407 (1995). It also reflects the updated 2008 version, as the main difference is the inclusion in 407 (2008) of optional endocrine mediated effects determination (eg oestrus cycle, hormone levels). As this is optional in the 2008 version and the current dossier does not indicate such effects, the study is considered acceptable.

Deviations : none

GLP : Yes (certified laboratory)

Material and Test material : H11 ; Batch N°99S21 ; Purity on dry matter: 97.6 %

methods : The toxicity of laminarin, when given by daily oral administration to rats, has been investigated over a period of 4 consecutive weeks.

A single group of 5 male and 5 female Sprague Dawley rats received the test substance daily by oral gavage at a dose level 1000 mg/kg bw/day for a minimum of 28 consecutive days. A second similarly constituted group received 0.5% carboxymethylcellulose in distilled water and acted as a control. Neurotoxicity tests were performed including: removal, handling reactivity, lachrymation, palpebral closure, salivation, piloerection, rearing, clonic movements, tonic movements, gait, mobility impairment, rousal, vocalisation, stereotypes, unusual respiration, bizarre behavior, urination, defecation.

Findings :

Mortality: no deaths occurred during the study.

Clinical signs: daily post-dose observations revealed no clinical signs.

Neurotoxicity tests and motor activity measurements performed at the end of treatment did not show changes attributable to treatment.

Body weight: no statistically significant differences were observed between the control and the treated group.

Food consumption: was not affected by treatment.

Haematology: no changes of toxicological significance were seen. The statistically significant increase in RBCcount in females (see Table B.6.3.1-1) was <3% and therefore considered incidental. It was within the range of historical controls according to the study report, although no further details on historical controls were provided.

Clinical chemistry: no treatment related changes were seen. The statistically significant increase in potassium observed in males was <6% and therefore considered to be incidental. It was within the range of historical controls according to the study report, although no further details on historical controls were provided (table B.6.3.1-1).

Organ weights: no biological significant effects observed.

Histopathology: no treatment related effect.

Table B.6.3.1-1: 4-week toxicity study in rats.

Findings	0 mg/kg bw/d		1000 mg/kg bw/d	
	M	F	M	F
Body weight (g):	349 ± 7.3	231.3 ± 3.6	362.9 ± 22	228.7 ± 12.33
Haematology: Red blood cell count	8.75 ± 0.25	8.06 ± 0.15	8.69 ± 0.22	8.28* ± 0.13
Clinical chemistry: K ⁺	3.998 ± 0.10	3.624 ± 0.176	4.226* ± 0.134	3.836 ± 0.107
Organ weight – absolute (g): Heart	1.315 ± 0.02	1.290 ± 0.121	0.925 ± 0.039	0.921 ± 0.036

*P<0.05

Conclusion

No toxicologically significant findings were observed in any of the parameters investigated following treatment with laminarin at a level of 1000 mg/kg bw/day. The results of this study determine 1000 mg/kg bw/day as the No Observed Adverse Effect Level (NOAEL).

B.6.3.2 Oral 90-day study

Rat

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 2001a	exposure	:	90 days (oral gavage)
Report number	:	20000389T	doses	:	0, 1000 mg/kg bw/d
test substance	:	Laminarin, Batch N°99S24	GLP statement	:	Yes
species	:	Rat, Sprague-Dawley	guideline	:	in accordance with OECD 408 (1998)
group size	:	10/sex/dose	acceptability	:	acceptable

Report : ██████████ (2001a) ; H11- 90-day repeated dose oral toxicity study in the rat ██████████

Unpublished report N°20000389T, 16/03/2001

Dates of experimental work : 28/07/2000 to 30/10/2000

Guidelines : OECD N° 408 (1998)

Deviations :

- Weighing of animals: animals were weighed on D8 instead of D7 for planning reason.
- Formulation of the test substance: on three days (21 to 23 August 2000), the formulation of the test substance was 6% more concentrate than expected (weighing error).
- Haematology: because problems (clotting of numerous blood samples) on the first predose sampling time, a new blood sampling for haematology and coagulation parameters was scheduled the day after the first one on non-fasted animals.

GLP : Yes (certified laboratory)

Material and methods : 10 male and 10 female Sprague-Dawley rats received Laminarin (batch n°. 99S24; 94.9 % purity) by gavage at 1000 mg/kg bw/d for 90 days. Control group received the vehicle (water) only.

Functional and neurobehavioral tests were performed during the 13th week: behaviour profile including awareness, mood, motor activity, motor incoordination; neurological profile including central excitation, muscle tone, body posture and reflexes; autonomic profile.

Findings:

Mortality: No death was recorded during the study.

Clinical signs: No treatment-related clinical signs were recorded during the study.

Body weights: Body weights of treated rats were similar to those of the controls.

Food consumption Food consumption was slightly reduced in male and female rats from week 2 up to week 13.

Water consumption was statistically significantly reduced for females from week 2 up to the end of the study. In male rats, water consumption was reduced since week 3 up to the end of the study without reaching statistical significance. In rodents, water consumption is closely associated with food consumption. This was confirmed by the fact that reduced water intake was not associated with a decreased urinary volume, or specific gravity alteration.

Haematology: No biological or statistically significant effects were observed.

Blood chemistry: At the end of treatment period, mean calcium level in females was increased. This difference was mainly due to a high calcium level in one female.

Urinalysis did not reveal any treatment-related effect.

Organ weight: Mean absolute and relative heart weight of treated females were statistically significantly greater than that of control females. This effect was not associated to *histopathological* or biochemical findings.

Table B.6.3.2-1: 90-day rat study by gavage.

Endpoints/dose	control		1000 mg/kg bw/d	
	M	F	M	F
Body weight (g)	483 ± 0.12	282.7 ± 29.5	498 ± 45	284.5 ± 5.6
Food consumption (g)	128.8	95	122.7	89
Water consumption (ml)	155.2	129.4	143	107.7* (↓ 16.7%)
Food/water consumption ratio	0.829/1.2	0.734/1.3	0.858/1.2	0.831/1.2
Haematology			No compound related effect	
Blood chemistry				
calcium	2.60 ± 0.14	2.56 ± 0.13	2.57 ± 0.10	2.72 ± 0.12*
Urinalysis			No compound related effect	
Organ weight				
Heart (absolute)(g)	1.813 ± 0.193	1.078 ± 0.08	1.868 ± 0.169	1.188 ± 0.13*
Heart (relative % bw)	0.400 ± 0.027	0.414 ± 0.04	0.399 ± 0.030	0.452 ± 0.04 *
Histopathology			No treatment-related effects	

**P* < 0.05

Comments: Overall, for males as well as for females, a slight body weight increase, not reaching statistically significance, is observed, although food consumption is decreased (not statistically significant). These effects suggest that gavage with laminarin contributes to the energy content of the diet, the animals have to eat less to meet their caloric requirements.

According to the open literature, addition of soluble forms of fibre to diets often has been found to improve absorption of minerals. Ingestion of resistant sugars resulted in caecal hypertrophy, reduced pH of caecal contents and increased permeability of intracellular junctions to passive absorption of minerals such as calcium (Greger, 1999). A slight increased calcium absorption is not considered as adverse.

Cardiac hypertrophy is defined as an increase in heart muscle. This increase is predominantly due to an increase in the contractile elements and mitochondria. Hypertrophy usually develops as a means of compensation: a thicker muscular wall can generate more power. Abnormal heart weight is usually the result of congenital abnormalities, abnormal heart valve function or systemic hypertension, though it may also arise from long-term chemical influences (thyroid hormone, growth hormone, and catecholamines).

In this case, there is no consistency between sexes, no correlation with clinical observations or with other clinical pathology findings and 2/10 female rats seems to be outside the group. Moreover, this effect was not observed in the 28-day rat study. So, we conclude that the difference between control and treated female rats is not biologically significant.

Conclusion

No toxicologically significant findings were observed in any of the parameters investigated following treatment with laminarin at a level of 1000 mg/kg bw/d. The results of this study determine 1000 mg/kg bw/d as the No Observed Adverse Effect Level (NOAEL).

No new information was found in publications concerning short-term toxicity in rat for laminarin. However, as presented in the Section 9 - Literature data, (1,3/1,6)-beta-D-glucan (structure close to 1,3 beta-D-glucan) was administered to Fisher-344 rats (10/sex/group) at doses of 0, 2, 33.3, or 100 mg/kg body weight for 91 consecutive days. Since no adverse or toxic effects were observed after subchronic oral administration, a no observed adverse effect level (NOAEL) of 100 mg/kg bw/day was determined. It confirms the absence of toxicity in rats treated with beta-glucan structures.

<i>Previous evaluation:</i>	<i>Submitted for the purpose of renewal, as a result of the literature search</i>
Evaluation RMS:	Acceptable

reference	: Babicek et al, 2007	exposure	: Oral (gavage)
Report number	: n/a	doses	: 0, 2, 33.3, or 10 mg/kg bw/d
test substance	: (1,3/1,6)-beta-D-glucan	GLP statement	: yes
species	: Fisher-344 rats	guideline	: in accordance with OECD 408
group size	: 10/sex/dose	acceptability	: Acceptable

Author(s)	
Year	2007
Journal	Food Chem Toxicol. 2007 Sep;45(9):1719-30. Epub 2007 Mar 23.
Relevance check	Yes
Reliability check	2
Reasons for restricted or no reliability/limitations	The substance tested corresponds to a (1,3/1,6)-beta-D-glucan structure (close to 1,3 beta-D-glucan).
Summary	<p>For the sub-chronic toxicity, Fisher-344 rats (10/sex/group) were randomly allocated to receive daily gavage treatment with (1,3/1,6)-beta-D-glucan at doses of 0, 2, 33.3, or 100 mg/kg bw/d for 91 consecutive days. Control and high-dose satellite recovery groups of each sex also were included. Full toxicological monitoring and endpoint investigations were performed throughout and upon completion of the study.</p> <p>No negative effects on animal weights or food consumption attributable to the test substance were evident at any dose. In addition, no mortality, clinical pathology, functional/behavioral, microscopic, or gross observations indicating toxicity were observed.</p> <p>Sporadic changes in some biochemical and hematological parameters were observed; however, since the effects were within the physiological ranges in historical controls, were not dose-responsive, or were not observed in both sexes, they were determined to be of no toxicological significance.</p> <p>In conclusion, no adverse or toxic effects were observed after subchronic oral administration of 2, 33.3, or 100 mg/kg bw/d of (1,3/1,6)-beta-D-glucan in Fisher-344 rats, and therefore, a no observed adverse effect level (NOAEL) of 100 mg/kg bw/d, the highest dose tested, was determined.</p>
Test protocol GLP, GEP, Guidelines (US EPA, OECD, ...)	GLP: yes OECD Guideline No.408 for the Testing of Chemicals, "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD, 2001)
Test substance Identification of test substance, source, purity, stability	WGP® 3–6 (Lot #1033-003) from Biopolymer Engineering, Eagan MN WGP® 3–6 powder is a highly purified extract of <i>Saccharomyces cerevisiae</i>
Test system characterization and study design	<u>Subchronic toxicity study</u> - Test system One-hundred and sixty (160; 80 males and 80 females) SPF

<p>Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol</p>	<p>Fisher CDF (F-344) / CrIBR rats were obtained from Charles River Deutschland. The animals were acclimatized for 13-16 days, and were 5-6 weeks of age and weighed between 80 and 100 g at the initiation of treatment. Sixty males and sixty females were randomly selected according to weight criteria and allocated to the control and treatment groups.</p> <p><u>- Information on conditions and maintenance</u> Rats were housed as groups of five animals per cage in standard plastic cages. The temperature inside the experimental room was kept at 22°C (±2°C) and the relative humidity was maintained at 30-70% (with an aim of 50-60%). Additional environmental controls involved the use of artificial lighting (12 h light/12 h dark), controlled air changes (>15 per hour), and microbiological environmental monitoring. Animals received sterilized standard diets and sterilized drinking water ad libitum.</p> <p><u>- Study protocol</u> Groups of 10 male and 10 female Fisher-344 rats were randomized to 1 of 4 groups receiving 0 (control), 2, 33.3, or 100 mg/kg bw/d of WGP® 3–6 powder for 91 consecutive days by oral gavage. Separate control and 100 mg/kg body weight/day recovery groups for each sex also were included, and were maintained for an additional 14 days without treatment following the 91-day dosing period.</p> <p>Clinical observations, body weights, and food consumption were monitored throughout the treatment period, and at the selected termination dates (92 or 106 days) the animals were euthanized and subjected to a full post-mortem examination.</p>
<p>Controls Positive control, negative control</p>	<p>Control animals (negative): yes</p>
<p>Dosing system Exposure (dose, duration, frequency)</p>	<p><u>Subchronic toxicity study</u> 0 (control), 2, 33.3, or 100 mg/kg bw/d of WGP® 3–6 powder for 91 consecutive days by oral gavage.</p>
<p>Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)</p>	<p>Means and standard deviations of means for groups, mean values of body weights, food consumption, absolute organ weights, relative organ weights, and hematological and biochemical parameters were calculated.</p> <p>Standard analysis of variance (ANOVA) was used for statistical evaluation of the data. When a significant dose-effect was found, further statistical analysis using Tukey's method and Dunnett's multiple comparisons were used for a detailed assessment of data obtained and for an assessment of their mutual relationship.</p>
<p>Results Determined effect concentration, dose response observed</p>	<p><u>Subchronic toxicity study</u> Mean body weights of males and females were measured weekly without significant differences. No significant differences in mean weekly food consumption were observed.</p> <p>WGP® 3–6 was well tolerated throughout the treatment period, with no mortality or signs of morbidity observed.</p> <p>A number of functional and behavioral tests were performed towards the end of the treatment period. No evidence of neuromuscular impairment was observed between control and treated animals.</p> <p>Some haematological and clinical chemistry changes occurred, but were within historical control values and were not dose-responsive, and did not occur in both sexes. The effects were considered to be of no toxicological significance.</p> <p>No pathological findings were present in any animals subject to necropsy after termination of the recovery period.</p> <p>If some absolute and relative organ weights were increased, no</p>

	alterations in any of the organs indicating a relationship with effects of WGP [®] 3–6 were revealed by gross necropsy. No increases in the incidence of histopathological findings were observed in the group treated with the highest dose of WGP [®] 3–6 compared to the controls.
Overall conclusion	<u>Subchronic toxicity study</u> Since no adverse or toxic effects were observed after subchronic oral administration of 2, 33.3, or 100 mg/kg bw/d of (1,3/1,6)-beta-D-glucan in Fisher-344 rats, a no observed adverse effect level (NOAEL) of 100 mg/kg bw/d was determined.

Dog

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
<i>Evaluation RMS:</i>	No remarks on original assessment.

reference	:	██████████, 2001b	exposure	:	90 days (oral gavage)
Report number	:	20000390T	doses	:	0, 1000 mg/kg bw/d
test substance	:	Laminarin, Batch N°99S24	GLP statement	:	Yes
species	:	Dog, Beagle	guideline	:	in accordance with OECD 409 (1998)
group size	:	10/sex/dose	acceptability	:	acceptable

Report : ██████████ (2001b); H11- 90-day repeated dose oral toxicity study in the dog ██████████;

Unpublished report N°20000390T, 16/03/2001.

Dates of experimental work : 02/08/2000 to 03/11/2000

Guidelines : OECD N°409

GLP : Yes (certified laboratory)

Material and Test material : H11 ; Batch N°99S24 ; Purity on dry matter : 94.9 %

methods : Laminarin was administered daily by the oral route for 90 consecutive days to one group of animals treated at the maximum limit dose of 1 000 mg/kg bw/day. A group of control animals was given the vehicle (water) under the same conditions. Each group included 4 male and 4 female dogs.

Examinations include : mortality, observations of animals, body weight changes, food and water consumption, clinical pathology, ophtalmology, macroscopic findings, weight of organs and histopathology.

Findings:

Mortality: 1 animal in the control group was sacrificed for humane reasons: the female presented convulsions and decreases in spontaneous locomotion activity the day before the sacrifice.

Clinical signs: the main findings reported in control and treated animals were diarrhoea and/or soft stools. These findings are common in young laboratory dogs. However a slight increase in incidence was recorded in treated animals when compared with controls.

Comment: Degradation of polysaccharides in the large bowel gives rise to a range of fermentation products. Fermentation increases bacterial mass in the bowel;

undegraded polysaccharide physically dilute gut contents; increased mass in the gut as well as SCFA production stimulates peristalsis and increases gut transit rates. Polysaccharides fermentation is generally a desirable process in the colon and is intimately linked to large bowel physiology (Michel and Macfarlane, 1996)

Haematology: some slight variations, within historical control values were observed, not considered to be treatment –related.

Blood chemistry: no statistically significant differences were found compared with the control group.

Urinalysis, ophthalmology did not reveal any treatment-related effect.

Organ weight: Some small organ weights variations were observed. Since no findings were reported at histopathology examination, these differences were considered to be incidental.

Table B.6.3.2-2: 90-day dog study by gavage.

Endpoints/ dose	control		1000 mg/kg bw/d	
	M	F	M	F
Mortality		1 day 31		
Clinical signs				
Diarrhoea	2	1	2	0
Soft stool	3	1	1	4
Mucous stool	0	0	1	1
Vomiting	1	0	2	0
Body weight (kg)	14.11 ± 0.52	11.78 ± 2.07	14.09 ± 1.17	12.47 ± 1.61
Food consumption (g), week 13	1922 ± 158	1773 ± 205	1904 ± 193	1871 ± 261
Water consumption (ml), week 13	3617 ± 779	2557 ± 1071	3642 ± 643	3640 ± 854
Haematology			No compound related effect	
Blood chemistry			No compound related effect	
Urinalysis			No compound related effect	
Organ weight				
spleen (absolute) (g)	45.32 ± 6.67	29.000 ± 1.92	30.34 ± 3.79*	31.507 ± 5.85
spleen (relative, % bw)	0.330 ± 0.014	0.270 ± 0.052	0.220 ± 0.015*	0.260 ± 0.0268
Adrenal (rel,% bw)	0.0086 ± 0.00042	0.0098 ± 0.0014	0.0097 ± 0.00050*	0.0103 ± 0.00156
Histopathology			No compound related effect	

*P<0.05

Conclusion

Laminarin administered for 90 consecutive days to male and female Beagle dogs at the level of 1 000 mg/kg bw/d was well tolerated. The NOAEL was 1 000 mg/kg bw/d.

B.6.3.3 Other routes

No route other than the oral route has been used for short-term toxicity studies.

B.6.4 Genotoxicity**B.6.4.1 In vitro studies**

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

Report : MARZIN D. (2000) ; Mutagenicity test on bacteria (*Salmonella typhimurium* his and *Escherichia coli* trp) using B.N. Ames's technique with H11

Institut Pasteur de Lille, France

Unpublished report N°IPL-R 991011/H11, 03/05/2000

Dates of experimental work : 30/08/1999 to 07/10/1999

Guidelines : Experimental protocol in compliance with test method B.14, dir. 92/69/EEC

Deviations: none

GLP : Yes (laboratory in the process for certification)

Material and methods : *S.typhimurium* strains TA 1535, TA 100, TA 1537, TA 98 and *E.coli* strain WP2 and WP2 uvrA were used in the standard plate test and preincubation test with and without S9 mix from Arochlor 1254 induced rat liver.

Laminarin (batch n°. 99521, purity > 90%) powder was solubilized in water and tested at 0, 50, 150, 500, 1500, 5000 µg/plate. No bacteriotoxicity was observed. 2 experiments were performed. The second assay with S9 mix was performed using a pre-incubation method. Positive controls were sodium azide, 9 amino-acridine, 2 nitro-fluorene, mitomycin C, K chromate, 2 anthramine and benzoapyrene. Evaluation criteria well defined.

Findings

Both without and with metabolic activation in two independent assays, no biologically significant increase in the number of revertants was noted in the four *Salmonella typhimurium* and the two *Escherichia coli* strains tested, in the presence of the test compound laminarin.

It is noted that with metabolic activation a slight but statistically significant increase in the number of revertants was observed at the maximum dose tested of 5000 µg/plate in the first assay in strains WP2 (pKM101) and WP2uvrA (pKM101) and at the three tested doses of 150, 1500 and 5000 µg/plate in the second assay in the last strain. However this effect was not biologically significant, not dose related and, as biological relevance should be considered first according to the OECD's recommendations, it was not attributed to a significant mutagenic activity.

Conclusion

The compound laminarin did not induce mutagenic activity in the four *Salmonella typhimurium* and the two *Escherichia coli* strains tested.

***In vitro* chromosomal aberration assay in CHO cells**

In the dossier that was submitted to US EPA, an *in vitro* gene mutation test in L5178Y TK mouse lymphoma cells (Haddouk, 2002) was present. According to the EPA evaluation, the study revealed that laminarin did not damage chromosomes or the mitotic apparatus of bone marrow cells.

The applicant should submit this study.

In a paper looking for anti-tumor activity of natural products, Larripa *et al.* tested laminarin in an anaphase-telophase test in CHO cells. No chromosomal damage was observed at any dose up to 100 µg/mL.

Previous evaluation:	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

Report : Larripa et al (1987) ; Biological activity in *Macrocystis pyrifera* from Argentina: sodium alginate, fucoidan and laminaran. II. Genotoxicity

Guidelines : unknown

GLP : unknown

Material and methods : CHO cells were treated with 1, 50 or 100 µg/ml crude laminaran from *Laminara digitata* (product # L 9634) was purchased from Sigma Chemical Co., St Louis, MO, USA. The cultures were incubated at 37°C for 24 hours. The cells were then fixed in ethanol (96%) during 1 hour at room temperature. 100 anaphase cells per culture were analysed. Statistical analysis was carried out using the Chi-square test.

Findings

Table B.6.4.1-1: *in vitro* CA assay with laminaran.

Compound	Dose	Abnormal anaphases (nb and % abnormal anaphase-telophases observed)				
		Normal	Lagging	Bridges	Tripolar s	%
Physiological saline	-	90	3	6	1	10
Laminaran :	1	92	5	3	0	8
	50	90	3	5	2	10
	100	92	4	4	0	8

Conclusion:

Laminaran did not induce chromosome aberrations *in vitro* in CHO cells.

B.6.4.2 *In vivo* studies in somatic cells

Previous evaluation:	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

Report : [REDACTED] (2001); LAMINARIN: Bone marrow micronucleus test by oral route in mice; [REDACTED]
Unpublished report N°21149 MAS; 15/03/2001
Dates of experimental work : 27/12/2000 to 22/01/2001

Guidelines : EEC B12 (1992)

GLP : Yes (certified laboratory)

Material and methods : Test material : Laminarin ; Batch N°99S10 ; Purity on dry matter: 99 %
After a preliminary test allowing to define the dose levels, three groups of five male and five female Swiss Ico: OF1 (IOPS Caw) mice received two oral treatments of laminarin at 500, 1000 and 2000 mg/kg bw/d, at a 24-hour interval. A control group received the vehicle (water) under the same experimental conditions, and a positive control group received cyclophosphamide at 50 mg/kg only once.
The animals were killed 24 hours after the last (or unique) treatment. Bone marrow smears were then prepared.
For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE+NE).

Findings

The mean values of MPE as well as the PE/NE ratio for the vehicle and positive controls were consistent with the historical data.

Cyclophosphamide induced a highly significant increase ($p < 0.001$) in the frequency of MPE, indicating the sensitivity of the test system under these experimental conditions. The study was therefore considered valid.

For the active substance, all the dose-levels were expressed taking into account the purity of 94%. The top dose-level for the cytogenetic test was selected according to the criteria specified in the international guidelines; since no observable toxic effects were noted, the top dose-level was 2000 mg/kg bw/d. The two other dose-levels were 1000 and 500 mg/kg bw/d.

For both males and females, the mean values of MPE as well as the PE/NE ratio in the groups treated with the test substance, were equivalent to those of the vehicle group.

Conclusion

Laminarin does not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two oral administrations, with a 24-hour interval, at 500, 1000 or 2000 mg/kg bw/d.

B.6.4.3 *In vivo* studies in germ cells

Due to the absence of any effect of laminarin both in the *in vitro* studies and in the *in vivo* studies in somatic cells (what is not very surprising when looking at the very natural structure of the active substance which can only give rise to oligosaccharides and ultimately to glucose upon metabolism), no specific genotoxicity testing has been conducted in germ cells.

B.6.5 Long-term toxicity and carcinogenicity

The nature of laminarin, an algae cell wall polysaccharide, rapidly and extensively fermented by intestinal bacteria, gives rise to fermentation products such as, butyrate, propionate, acetate, CO₂, H₂S etc. Such kinds of products are also formed during digestion of vegetables, fruits and legumes. Long term toxicity resulting from a chronic polysaccharide overload as a result of the use of laminarin in plants can be ruled out. The amount of SCFAs, which could be additionally produced in result of the use of laminarin on plants, is not relevant if compared with the amount of polysaccharides in vegetables and legumes, which are consumed daily.

Due to the structure of laminarin, which can be considered as a storage carbohydrate similar to starch or glycogen, to the very high NOAELs observed in the 90-day studies on rats and dogs (absence of any treatment-related effect in two species), and to the recognition of *Laminaria digitata* as a human food, no long term toxicity study and no carcinogenicity study have been conducted. Moreover no mutagen effects were observed in both *in vitro* and *in vivo* studies.

Therefore, based on the favourable profile of laminarin, also known for its antioxidant properties, and in order to avoid useless tests in vertebrates, no long term toxicity study and no carcinogenicity study are deemed necessary.

B.6.6 Reproductive toxicity**B.6.6.1 Generational studies**

Due to the structure of laminarin, which can be considered as a storage carbohydrate similar to starch or glycogen, to the very high NOAELs observed in the 90-day studies on rats and dogs (absence of any treatment-related effect in two species), to the favourable conclusions of both rat and rabbit developmental toxicity studies (see B.6.6.2) (absence of any treatment-related maternal or foetal effects in two species) and to the recognition of *Laminaria digitata* as a human food, no generational studies have been conducted. Moreover no mutagen effects were observed in both *in vitro* and *in vivo* studies.

Therefore, based on the favourable profile of laminarin, also known for its antioxidant properties, and in order to avoid useless tests in vertebrates, no generational study is deemed necessary.

B.6.6.2 Developmental toxicity studies**Rat**

Previous evaluation:	In DAR (2002)
Evaluation RMS:	No remarks on original assessment.

reference	: Audeval-Gerard, 2001c	exposure	: Gd 6-17 (oral gavage)
Report number	: 20000387 T	doses	: 0, 1000 mg/kg bw/d
test substance	: Laminarin, Batch N°99S24	GLP statement	: Yes
species	: Rat, Sprague-Dawley	guideline	: in accordance with OECD 414 (1981)
group size	: 21-23 pregnant females/dose	acceptability	: acceptable

Report : [REDACTED] (2001c) ; H11-Study for the effects on embryo-foetal development in the rat by the oral route.

[REDACTED]

Unpublished report N°20000387 T ; 16/03/2001

Dates of experimental work: 01/08/2000 to 23/08/2000

Guidelines : OECD N° 414 (1981)

Deviations :

- Test substance administration: the test substance was administered to females daily from Day 6 to Day 17 of pregnancy instead of Day 6 to Day 15. Reason: mistake in the protocol.

- Analysis of results: water consumption results were analysed by two-way analysis of variance.

Reason: the daily measurement of water consumption enabled to perform this.

- Examination of the foetuses: Sexing of all foetuses was performed at external examination in order to verify that external characteristics are in accordance with internal characteristics of the gender.

GLP : Yes (certified laboratory)

Material and methods Test material : H11 ; Batch N°99S24 ; Purity on dry matter : 94.9 %

The study involved 2 groups including a control group. Treatment allocation was decided at random as follows :

Group	Number of animals and sex	Dose in mg/kg body weight / day	Volume administered
1	23 pregnant females	0	5 mL/kg bw.
2	21 pregnant females	1000	5 mL/kg bw.

The test substance was administered by oral gavage as a solution in sterile water on day 6 through day 17 p.c.. Control animals were given sterile water under the same conditions. Solution to be administered was prepared daily.

Findings

Laminarin did not induce maternal toxicity. Reproduction parameters were not altered. No embryotoxicity was observed. No developmental toxic effects were reported (Table B.6.6.2-1)

Table B.6.6.2-1: developmental toxicity of laminarin in rats.

Endpoints/dose	control	1000 mg/kg bw/d
Pregnant rats (nb)	23	21
Bw at day 20 (g)	375.3±34.1	373.6±35.6
Food consumption (g)	51.7±12.4	50.5±8.3
Water consumption	50.1±9.4	45.6±9.1
Uterus weight	75.4±14	73±16
Corpora lutea	17.4±5.6	16.9±3.6
Litter data		
Implantation sites	13.5±2.6	13.1±2.9
% pre-implantation loss	19±14.9	21.4±17.7
% post implantation loss	6.9±6.2	7.6±8.4
No. live fetuses	12.6±2.6	12.1±2.8
No. resorptions		
Early	0.9±0.9	1±1
Late	0.0±0.2	0.0±0.2
Foetal parameters:		
foetuses / litter examined	140/23	123/21
Male/females	65/75	65/58
Caudal-cranial measurement	36.5±1.4	36.8±1.5
Foetus weight	3.93±0.26	3.94±0.27
Placental weight	0.597±0.07	0.596±0.079
External examination		

haematoma	1 fetus/4 dams 2 foetuses /2dams	1fetus/1dam 2foetuses/1dam
Visceral and skeletal examination foetuses / litter examined	150/23 No effects	131/21 No effects

Conclusion:

Laminarin is not toxic in the rat developmental study. No maternal and no foetal abnormalities were reported.

NOAEL maternal tox >1000 mg/kg bw/d.

NOAEL develop >1000 mg/kg bw/d.

Rabbit

<i>Previous evaluation:</i>	<i>In DAR (2002)</i>
Evaluation RMS:	No remarks on original assessment.

reference	:	██████████, 2001e	exposure	:	Gd 6-19 (oral gavage)
Report number	:	20000388 T	doses	:	0, 1000 mg/kg bw/d
test substance	:	Laminarin, Batch N°99S24	GLP statement	:	Yes
species	:	Rabbit, New Zealand White	guideline	:	in accordance with OECD 414 (1981)
group size	:	13-16 pregnant females/dose	acceptability	:	acceptable

Report : ██████████ (2001e) ; H11-Study for the effects on embryo-foetal development in the rabbit by the oral route.

██████████
Unpublished report N°20000388 T ; 04/10/2001

Dates of experimental work: 13/11/2000 to 15/12/2000

Guidelines : OECD N° 414 (1981)

Deviations :

- Tap water was not filtered. Reason: mistake in the protocol.
- Analysis of results: water consumption results were analysed by two-way analysis of variance.
- Reason: the daily measurement of water consumption enabled to perform this.
- Examination of the foetuses: Sexing of all foetuses was performed at external examination in order to verify that external and internal characteristics are in accordance with the gender.

GLP : Yes (certified laboratory)

Material and methods : Test material: H11 ; Batch N°99S24 ; Purity on dry matter: 94.9 %

The objective of this study was to detect in the rabbit treated with laminarin any adverse effects on the pregnant female and on the development of the embryo and foetus consequent to exposure of the female to the test substance during the period of organogenesis, that is from implantation to closure of the hard palate, *i.e.* from Day 6 to Day 19 of pregnancy.

The study involved 2 groups including a control group. Treatment allocation was decided at random as follows :

Group	Number of animals and sex	Dose in mg/kg bw/d	Volume administered
1	16 pregnant females	0	5 mL/kg bw
2	13 pregnant females	1000	5 mL/kg bw

The test substance was administered by oral gavage as a solution in sterile water. Control animals were given sterile water under the same conditions. Solution to be administered was prepared daily.

Findings

One female had a food intake almost nil from D1 to D14. This was correlated with a body weight loss. After that, food intake of this female was quite similar to other females and bodyweight gain was greater than other females (recovery). At terminal necropsy, this female was pregnant but showed a total litter loss. As anorexia and bodyweight loss started before initiating the dosing, involvement of treatment is considered unlikely.

No *mortality* was recorded. During the treatment period, food consumption of treated females was significantly lower than that of control females at the threshold of 5%. Before and after treatment, animals given laminarin at the dose of 1000 mg/kg bw/d had similar food consumption to control animals. *Water consumption* was unaltered.

No significant difference was noted between treated and control animals. Number of implantation sites and of live foetuses of treated females was significantly greater than that of control females. Total litter loss in female n° 20001576 was probably not treatment related as anorexia and correlated bodyweight loss was observed before treatment initiation.

Caudo-cranial measurement, weight of foetuses and weight of placenta were not different from control.

External examination of foetuses: forelimb flexure was reported in one foetus of 3 treated females. This minor variation is commonly reported in rabbit foetuses and involvement of treatment is unlikely. Some slight inter-group differences in ossification parameters were recorded, but there were no consistent associations or trends indicative of any advancement or retardation of foetal ossification related to maternal treatment.

Incompletely ossified thoracic vertebral centra: the applicant considers that the control values for this parameter in the study were in fact quite low. Normally, they expect to see approximately 10% of control fetuses from approximately 50% of litters with incomplete/anomalous ossification of at least one thoracic vertebral centrum. The value recorded for the limit dose group in the study is much more in line with this background control data.

In the limit dose group there appeared to be slight increase in the proportion of fetuses with supernumerary ribs at the thoraco-lumbar border (rib count 13/13), compared with the concurrent control group. Consequently, there was a decrease in the proportion of fetuses with 12/12 ribs. In association with this finding, a number of fetuses with supernumerary ribs had additional (8th) lumbar vertebra. Comparison of the litter incidences of these findings revealed no increase in the number of affected litters in the limit group, indicating that the increase in affected fetuses occurred with litters already containing fetuses with supernumerary ribs/lumbar vertebrae, rather than there being an increase in the number of affected litters.

When the values recorded for the limit dose group were compared with the control values from a study performed in the same lab, same strain of rabbits in September 2000, the distribution of rib numbers were essentially similar in both groups; whilst the proportion of fetuses with an additional lumbar vertebra was lower in the limit dose group. It was considered, therefore, that the apparent shift in rib and lumbar vertebral numbers seen in the limit dose group was of no toxicological significance. (Table B.6.6.2-2)

Table B.6.6.2-2: developmental toxicity of laminarin in rabbits.

Endpoints/dose	control	1000 mg/kg bw/d
Nb pregnant females	16	13
Bw at day 29 (g)	3.762±0.210	3.743±0.432
Food consumption (g)		
D1-D5	556±161	532±206
D6-D19	1883±318	1601±167*
D20-D28	875±106	1017±256
Water consumption D28	252±90	295±117
Uterus weight	464.1±111	507.8±190.4
Nb corpora lutea	11.37±2.73	12.08±2.75
Litter data		
Implantation sites	9.5±2.6	11.5±2.4*
% pre-implantation loss	16.3±15.7	9.1±12.5
% post implantation loss	7.4±10.7	13.9±26.5
No. live fetuses	8.69±2.36	10±3.85
No. resorptions		
Early	0.37±0.72	0.46±0.52
Late	0.44±1.03	0.23±0.44
Foetal parameters:		
Caudal-cranial measurement	88.6±3.3	87.8±3.8
Foetus weight	6.75±4.39	6.15±5.25
Placental weight	5.34±0.58	5.19±0.77
External examination		

Forelimb flexure		1 fetus/3 females	
Visceral and skeletal examination			
Incompletely ossified thoracic vertebral centrum: no. litters affected (%litters incidence)	2/16(12.5%)	5/12(41.7%)	
Incompletely ossified thoracic vertebral centrum :actual number foetuses affected	2/139(1.4%)	12/130(9.2%)	
	Control from this study	Laminarin	Control from study 20000153T
Fetuses with supernumerary ribs (rib count 13/13)	42.4%	61.5%	61.1%
Fetuses with 12/12 ribs	40.3%	24.6%	25.8%
Additional lumbar vertebra 8	7.2%	15.4%	22.1%
Unusual morphological changes: no.fetuses affected			
exencephaly	1		
abnormal thoracic vertebrae and ribs	1	2	
abnormal cervical vertebrae		1	
abnormal thoracic vertebrae		2	

Conclusion

Laminarin was well tolerated. The overall picture does not indicate a consistent trend, indicative of either treatment-related retardation or advancement of ossification. No maternal toxicity and no treatment-related foetal abnormalities were reported.

NOAEL maternal tox >1000 mg/kg bw/d.

NOAEL develop >1000 mg/kg bw/d.

B.6.7 Neurotoxicity

No such study has been conducted as the structure of laminarin is not similar or related to structures capable of inducing delayed neurotoxicity. Moreover, neurotoxicity tests and motor activity measurements were performed at the end of treatment during 28 and 90 day in the rat study and at the end of the 90-day dog study. These studies did not show changes attributable to treatment.

B.6.8 Other toxicological studies

At this stage, no other toxicological study has been deemed necessary on the active substance or on metabolites, which would be smaller-sized oligo-saccharides or glucose itself.

B.6.8.1 Toxicity studies of metabolites and relevant impurities

No study is deemed necessary.

B.6.8.2 Supplementary studies on the active substance

No supplementary study is deemed necessary on the active substance as no ARfD has to be derived, like no ADI was allocated by the RMS in the past.

As presented in the Section 9 - Literature data, 1,3/1,6)-beta-D-glucan (structure close to 1,3 beta-D-glucan) was administered to BALB-c mice (8/group) at doses of 0 mg/kg bw/d (control), of 50 mg/kg bw/d β -D-glucan (oral gavage for 10 days), of a single dose of 900 mg/kg bw ip acetaminophen (liver toxicant), or of 50 mg/kg bw/d -D-glucan (oral gavage for 10 days) followed by a single acetaminophen injection. No difference was observed between mice from control group and mice treated just with 50 mg/kg bw/d -D-glucan (oral gavage for 10 days); the findings of the current study illustrate that β -D-glucan has no particular toxicity after sub-acute exposure, as observed in rat and dog (see B.6.3). Moreover it acts as an antioxidant agent by protecting hepatic tissues against acetaminophen-induced oxidative injury. Therefore it merits consideration as a potential agent in limiting the drug-induced oxidative damage of the liver.

<i>Previous evaluation:</i>	<i>Submitted for the purpose of renewal, as a result of the literature search</i>
RMS remarks	Acceptable as supplementary study

Author(s)	[REDACTED]
Year	2006
Journal	Eur J Pharmacol. 2006 Aug 14;543(1-3):133-40. Epub 2006 Jun 2.
Relevance check	Yes
Reliability check	2
Reasons for restricted or no reliability/limitations	The substance tested corresponds to a (1,3/1,6)-beta-D-glucan structure (close to 1,3 beta-D-glucan). The study doesn't correspond to a toxicity study

	performed according to Guidelines such US EPA or OECD.
Summary	<p>The protective effect of β-glucan against oxidative injury caused by acetaminophen was studied in mice liver. BALB-c mice (25–30 g) were treated either with vehicle (control group) or with β-D-glucan (50 mg/kg bw/d, p.o.) for 10 days or with a single dose of 900 mg/kg bw, i.p. acetaminophen or with β-D-glucan (50 mg/kg bw/d, p.o.) for 10 days and on the 11th day they received an overdose of acetaminophen (900 mg/kg bw, i.p.). Four hours after the acetaminophen injection, mice were decapitated and their blood was taken to determine serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and tumor necrosis factor-alpha (TNF-α) levels. Tissue samples of the liver were taken for histological examination or for the determination of levels of malondialdehyde, an end product of lipid peroxidation; glutathione (GSH), a key antioxidant; and myeloperoxidase activity, an index of tissue neutrophil infiltration. The formation of reactive oxygen species in hepatic tissue samples was monitored by using the chemiluminescence technique with luminol and lucigenin probes.</p> <p>Acetaminophen caused a significant decrease in the GSH level of the tissue, which was accompanied with significant increases in the hepatic luminol and lucigenin chemiluminescence values, malondialdehyde level, MPO activity and collagen content. Similarly, serum ALT, AST levels, as well as LDH and TNF-α, were elevated in the acetaminophen-treated group when compared with the control group. On the other hand, β-D-glucan treatment reversed all these biochemical indices, as well as histopathological alterations that were induced by acetaminophen. Moreover no difference was observed between mice from control group and mice treated just with 50 mg/kg b.w., p.o. β-D-glucan for 10 days</p> <p>In conclusion, these results suggest that β-D-glucan exerts cytoprotective effects against oxidative injury through its antioxidant properties and may be of therapeutic use in preventing acetaminophen toxicity.</p>
Test protocol GLP, GEP, Guidelines (US EPA, OECD, ...)	GLP: no No Guidelines (US EPA, OECD, ...)
Test substance Identification of test substance, source, purity, stability	1,3-1,6 β -D-glucan in microparticulate form, prepared from <i>Saccharomyces cerevisiae</i> yeast and suspended in saline.
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	<p>BALB-c mice of either sex (25–30 g) were divided into 4 groups, each consisting of eight animals:</p> <ol style="list-style-type: none"> 1) control (vehicle) group; 2) 50 mg/kg bw/d β-D-glucan administered by intragastric gavage for 10 days (β-D-glucan

	<p>group);</p> <p>3) a single dose of 900 mg/kg bw ip Acetaminophen (acetaminophen group);</p> <p>4) β-glucan, in a dose of 50 mg/kg bw/d administered by intragastric gavage for 10 days prior and 30 min after acetaminophen injection (acetaminophen +β-D-glucan group).</p> <p>Mice were decapitated at 4 h after acetaminophen injection; their trunk blood was taken; the serum was separated and stored at -70°C. The tissue samples of the liver were obtained for biochemical and histological analyses.</p>
Controls Positive control, negative control	Negative control: saline solution Positive control: acetaminophen
Dosing system Exposure (dose, duration, frequency)	A dose of β -glucan 50 mg/kg bw/d administered by intragastric gavage for 10 days
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Statistical analysis carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego; CA; USA). All data expressed as means \pm S.E.M. Groups of data compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < 0.05$ regarded as significant.
Results Determined effect concentration, dose response observed	When treated with acetaminophen, Increased levels of serum ALT and AST levels are observed in mice, indicating a deterioration of the hepatic functions due to the toxic effects of the drug. The increases in tissue luminol and lucigenin chemiluminescence, lipid peroxidation, myeloperoxidase activity and collagen contents accompanied by a significant reduction in glutathione levels, implicate oxidative liver injury while the histopathological data of the tissues confirm the acetaminophen-induced organ damage. Furthermore, serum TNF- α and LDH activity, as a marker of cytokine release and generalized tissue damage, showed a significant increase in acetaminophen-treated animals. When previously treated with β -D-glucan during 10 days, acetaminophen-induced liver damage and dysfunction are reduced due to a limitation of glutathione depletion and lipid peroxidation.
Overall conclusion	In conclusion, the findings of the current study illustrate that β -D-glucan has no particular toxicity after subacute exposure; moreover it acts as an antioxidant agent by protecting hepatic tissues against acetaminophen-induced oxidative injury. Therefore it merits consideration as a potential agent in limiting the drug-induced oxidative damage of the liver.

B.6.8.3 Studies on endocrine disruption

The conclusions of both rat and rabbit developmental toxicity studies showed that no effects were observed *in utero* and according to laminarin structure no effects on the post-natal development are expected either. Laminarin is thus not expected to have endocrine disrupting properties.

B.6.9 Medical data and information**B.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies**

Due to the very low acute and sub-chronic toxicity of laminarin, no special surveillance on manufacturing plant personnel has been nor will be undertaken.

B.6.9.2 Data collected on humans

No specific information or data are collected on humans. However, as presented in the Section 9 - Literature data, the ability of (1→3)- β -D-glucan to produce pollen-like response was assessed after that sensitive persons received a nasal deposition. The percentage of eosinophils and amount of eotaxin were measured in nasal lavage; no effect could be demonstrated. The absence of an inflammatory response after (1→3)- β -D-glucan application confirms earlier findings in inhalation studies.

Previous evaluation:	Submitted for the purpose of renewal, as a result of the literature search
RMS remarks	Acceptable as supplementary study

Author(s)	
Year	2004
Journal	Mediators Inflamm. Feb 24, 2005; 2005(1): 50–52.
Relevance check	Yes
Reliability check	2
Reasons for restricted or no reliability/limitations	Not a regular study, but assessing hyperreactivity of (1→3)- β -D-glucan on specific subpopulation groups
Summary	To assess if (1→3)- β -D-glucan, a microbial cell wall agent normally present in pollen, has the ability to produce pollenlike response, sensitive persons received a nasal deposition of two doses of (1→3)- β -D-glucan. The percentage of eosinophils and amount of eotaxin were measured in nasal lavage 30 minutes and 24 hours after challenge. No effect could be demonstrated. The absence of an inflammatory response after (1→3)- β -D-glucan application confirms earlier findings in inhalation studies.
Test protocol GLP, GEP, Guidelines (US EPA, OECD, ...)	GLP: no No Guidelines (US EPA, OECD, ...)
Test substance Identification of test substance, source, purity, stability	A purified preparation of (1→3)- β -D-glucan from <i>Candida albicans</i> was used. A 5-mg aliquot was suspended in 5mL of 0.3N NaOH, ultrasonicated for 30 minutes, and diluted in phosphate buffered

	<p>saline (PBS) till a concentration of 5000 and 500 ng/mL. Each of these doses was further diluted in PBS and 200 μL was inserted in a Biodose nasal applicator that produced an aerosol of 100 μL for each of the two actuations.</p> <p>The resulting doses of 50 and 5 ng were chosen based upon the data on the amount of (1\rightarrow3)-β-D-glucan in pollen where a typical spring exposure of 5 000 pollen/m³ was calculated to correspond to 5 ng (1\rightarrow3)-β-D-glucan/m³. Control applications contained PBS only.</p>
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	<p>Test persons ($n = 11$) were recruited by advertising among university students. Inclusion criteria were known reactivity to pollen, age of 18–35 years, never smoking and without any current disease or regular medication. The study was approved by the Ethical Committee.</p> <p>The subjects were exposed in both nostrils on three occasions, at least one week apart, randomly to the high and low doses and control fluid.</p> <p>The subjects underwent nasal lavage 30 minutes prior to exposure (baseline) and 30 minutes and 24 hours afterwards. After nasal lavage, the collected fluid was stored in plastic tubes in ice until centrifugation of 200 g for 10 minutes. The supernatant was removed and stored frozen at -70°C. The cell pellet was resuspended in PBS and a cell smear was prepared.</p> <p>A cytosine cell smear preparation of the nasal lavage fluid was stained with May-Grunewald-Giemsa and 200 cells were counted in an optical microscope at 1000\times magnification, determining the proportion of eosinophils.</p> <p>The amount of eotaxin in the NAL was also analysed using an ELISA commercial preparation</p>
Controls Positive control, negative control	Control: yes (negative)
Dosing system Exposure (dose, duration, frequency)	Exposure in both nostrils on three occasions, at least one week apart.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	-
Results Determined effect concentration, dose response observed	<p>A very large proportion of eosinophils was found among two subjects in the control tests (80% and 25.5%) as compared to the average of the group which was 1.6. None of these persons showed an increase in the proportion of eosinophils after application of (1\rightarrow3)-β-D-glucan. No differences were seen between (1\rightarrow3)-β-D-glucan exposures and control exposures.</p>
Overall conclusion	<p>The present study is of an exploratory nature and the number of subjects small. The dose in the nose was calculated basing the exposure on a typical spring exposure of 5000 pollen/m³ which means that the doses of (1\rightarrow3)-β-D-glucan used were well in the range of the normal environmental dose.</p> <p>In summary, the results do not support the</p>

	hypothesis that (1→3)- β -D-glucan induces the inflammatory response seen after exposure to pollen in sensitised subjects.
--	--

B.6.9.3 Direct observations

In the frame of the initial submission, no incident occurred during the production and handling of the pilot batches, and during the experimental field applications. Since laminarin is produced industrially, no clinical case has been observed and no incident occurred.

B.6.9.4 Epidemiological studies

Due to the very low acute and sub-chronic toxicity of laminarin, no epidemiological studies were performed and submitted in the present dossier.

B.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

In the acute or short-term toxicity studies, no sign of any toxicity has ever been seen and a target organ could not be defined. Therefore no signs of poisoning can be described.

In case where laminarin would still be suspected, analytical methods are available for detecting the active substance and its degradates (oligosaccharides, glucose) are available; for this purpose, a method using total hydrolysis would be recommended.

B.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

In case of accidental ingestion, there is no risk if small quantity is ingested. Wash out mouth with water.

If inhalation, take the patient to get fresh air.

In case of skin contact, take of soiled clothes and wash the skin with water.

If eye splashing occurs, wash thoroughly with water. Seek medical advice if irritation develops.

As no sign of poisoning has ever been seen, no antidote can be proposed.

B.6.9.7 Expected effects of poisoning

Laminarin being of very low acute and sub-chronic toxicity, no effect of poisoning is likely to occur. The only effect which could be expected would be a hyperglycemia which could be detrimental for individuals suffering from diabetes.

B.6.10 References relied on**B.6.10.1 Literature search**

Report :	Laboratoires Goëmar SAS (2015); Section 9 Literature data
Guidelines :	- EFSA (2011). Guidance of EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092. - AGES (2013). External scientific report, Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010, EFSA supporting publication 2013:EN-511.
GLP :	not relevant

Previous evaluation:	<i>submitted for the purpose of renewal, essential</i>
RMS remark	Literature search is acceptable

Search strategy

The search terms were only looked for in the titles and/or abstracts.

General search terms

Common name:	Laminarin
	OR
ISO name:	Laminarin
	OR
CAS Number:	9008-22-4
	OR
Chemical name (IUPAC):	(1→3)-β-D-glucan (according to IUPAC-IUB Joint Commission on Biochemical Nomenclature)
	OR
Others:	EC No (EINECS or ELINCS): 232-712-4 Chemical name (CA): laminaran Development code: H11

Trade names: IODUS 2 OR VACCIPLANT OR VAXIPLANT

Search process for all sections

Data requirement(s) captured in the search	PubMed, ScienceDirect.
Active substance	Justification for choosing the source: <i>please refer to table 7.6-03</i>
	Date of the search: 11/03/2014
	Date of the latest database update included in the search: 11/03/2014
	Total number of summary records retrieved: 3925

Search terms for all sections

Database: PubMed Search restrictions: all fields	Search terms	Number of summary records retrieved
Active substance common and ISO name	1. Laminarin	308
Active substance chemical name (CA)	2. Laminaran	24

Active substance other names or codes	3. H11	308 – not relevant denomination – will not be used further.
CAS No.	4. 9008-22-4	0
Chemical Name (IUPAC)	5. (1→3)-β-D-glucan	514
EC No	6. 232-712-4	0
CIPAC No.:	7. 671	not relevant denomination – will not be used further.
Trade names	8. IODUS 2 9. VACCIPLANT 10. VAXIPLANT	0 1 0 In view of the very low number of matches, the trade names will not be used further.

Section specific search terms

General words for toxicology were used, such as:

- tox* OR hazard OR adverse OR health
- NOAEL OR NOEL OR LOAEL OR LOEL OR BMD*
- "in vivo" OR "in vitro"

as well as key words related to specific data requirements, such as:

- acute OR subacute OR subchronic OR chronic
- oral OR dermal OR gavage OR diet* OR inhal*
- skin* OR eye* OR irrit* OR sensi* OR allerg*
- rat OR rats OR dog* OR rabbit* OR guinea pig* OR mouse OR mice
- metabolism OR metabolite* OR metabolic OR distribution OR adsorption OR excretion OR elimination OR kinetic OR PBPK
- CYP OR cytochrome OR enzym*
- gen* OR muta* OR chromos* OR clastogen* OR DNA
- carcino* OR cancer*
- mechanis*
- immun*
- neur* OR behav*
- endocrin* OR hormon*
- reproduct* OR development* OR malformation* OR anomal* OR fertil* OR foet* OR fet* OR matern* OR pregnan* OR embryo*
- epidem* OR medical* OR poison*
- exposure OR operator* OR bystander* OR resident* OR worker* OR occupat*
- mixture*
- photo toxicity
- mechanistic tests for endocrine disruption

Search process for toxicology

Data requirements captured by the search	Search: PubMed
All data requirements together	Justification for choosing the source: PubMed is a free search engine which has over 23 million records going back to 1966 and a database of citations and abstracts for biomedical literature from MEDLINE and additional life science journals.
	Date of the search: 11/03/2014
	Date span of the search: 11/03/2004 - 11/03/2014
	Date of the latest database update included in the search: 11/03/2014
	Total number of summary records retrieved: 1763

	Total number of <u>not relevant</u> summary records: 1758
	Total number of <u>not clearly relevant</u> summary records: 1
	Total number of relevant summary records: 4

Search terms for toxicology

Database: PubMed Search restrictions: all fields	Search terms Laminarin OR Laminaran OR (1→3)-β-D-glucan		Number of summary records retrieved in Pubmed database
Active substance common and ISO name	1.	Laminarin	308
Active substance chemical name (CA)	2.	Laminaran	24
Chemical Name (IUPAC)	3.	(1→3)-β-D-glucan	514
Without trade name	4.	tox* OR hazard OR adverse OR health	65
	5.	NOAEL OR NOEL OR LOAEL OR LOEL OR BMD*	3
	6.	"in vivo" OR "in vitro"	70
	7.	acute OR subacute OR subchronic OR chronic	26
	8..	oral OR dermal OR gavage OR diet* OR inhal*	43
	9.	skin* OR eye* OR irrit* OR sensi* OR allerg*	143
	10.	rat OR rats OR dog* OR rabbit* OR guinea pig* OR mouse OR mice	68
	11.	metabolism OR metabolite* OR metabolic OR distribution OR adsorption OR excretion OR elimination OR kinetic OR PBPK	36
	12.	CYP OR cytochrome OR enzym*	79
	13.	gen* OR muta* OR chromos* OR clastogen* OR DNA	109
	14.	carcino* OR cancer*	40
	15.	mechanis*	49
	16.	immun*	38
	17.	neur* OR behav*	9
	18.	endocrin* OR hormon*	1
	19.	reproduct* OR development* OR malformation* OR anomal* OR fertil* OR foet* OR fet* OR matern* OR pregnan* OR embryo*	44
	20.	epidem* OR medical* OR poison*	32
	21.	exposure OR operator* OR bystander* OR resident* OR worker* OR occupat*	54
	22.	mixture*	8
	23.	photo toxicity	0

Database: PubMed Search restrictions: all fields	Search terms Laminarin OR Laminaran OR (1→3)-β-D-glucan		Number of summary records retrieved in Pubmed database
	24	mechanistic tests for endocrine disruption	0

Relevance criteria for the section toxicology

Data requirement	Criteria for relevance
ADME (OECD IIA 5.1)	1. Defined test material 2. In vivo tests in relevant test species for toxicological testing (i.e rat, mouse, rabbit, dog) 3. In vitro tests 4. PBPK modelling 5. Specific endpoint can be clearly related to this data requirement
Acute toxicity, irritation, sensitisation (a.s.) (OECD IIA 5.2)	1. Defined test material 2. Relevant test species for toxicological testing (i.e rat, mouse, rabbit, dog, guinea pig) 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Short-term toxicity (OECD IIA 5.3)	1. Defined test material 2. Relevant test species for toxicological testing 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Genotoxicity (OECD IIA 5.4)	1. Defined test material 2. In vitro tests 3. In vivo tests in relevant test species for toxicological testing 4. Specific endpoint can be clearly related to this data requirement
Long-term toxicity and carcinogenicity (OECD IIA 5.5)	1. Defined test material 2. Relevant test species for toxicological testing (i.e. rat, mouse) 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Reproductive toxicity (OECD IIA 5.6)	1. Defined test material 2. Relevant test species for toxicological testing 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Neurotoxicity (OECD IIA 5.7)	1. Defined test material 2. In vivo tests in relevant test species for toxicological testing 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Studies on metabolites and impurities (OECD IIA 5.8)	1. Defined test material 2. In vitro tests 3. In vivo tests in relevant test species for toxicological testing 4. Relevant (physiologic) route of exposure 5. Specific endpoint can be clearly related to this data requirement

Data requirement	Criteria for relevance
Supplementary studies on active substance (e.g. immunotoxicity studies on mixture effects) (OECD IIA 5.10)	1. Defined test material 2. Relevant test species for toxicological testing 3. Specific endpoint can be clearly related to this data requirement
Endocrine disrupting properties (OECD IIA 5.10)	1. Defined test material 2. In vitro tests 3. In vivo tests in relevant test species for toxicological testing (i.e. rat, mouse, rabbit, dog) 4. Relevant (physiologic) route of exposure 5. Specific endpoint can be clearly related to this data requirement
Medical data (OECD IIA 5.9)	1. Defined test material 2. Epidemiological studies 3. Poisonings, clinical cases 4. Relevant (physiologic) route of exposure
Acute toxicity, irritation, sensitisation (PPP) (OECD IIIA 7.1)	1. Defined test material 2. Relevant test species for toxicological testing (i.e. rat, mouse, rabbit, dog, guinea pig) 3. Relevant (physiologic) route of exposure 4. Specific endpoint can be clearly related to this data requirement
Exposure assessment (PPP) (OECD IIIA 7.3-7.5)	1. Defined test material 2. Field studies 3. Calculations 4. Specific endpoint can be clearly related to this data requirement
Dermal absorption (PPP) (OECD IIIA 7.6)	1. Defined test material 2. In vitro tests 3. In vivo tests in relevant test species for toxicological testing (i.e. rat) 4. Specific endpoint can be clearly related to this data requirement

Lists of relevant and non-relevant studies

List of studies considered as relevant or of unclear relevance, classified by author(s) - toxicology

Authors	Data requirement (indicated by the corresponding OECD data point number)	Year	Title	Source
Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	OECD IIA 5.2 OECD IIA 5.3	2007	Toxicological assessment of a particulate yeast (1,3/1,6)- beta-D-glucan in rats	Food Chem Toxicol. 2007 Sep;45(9):1719- 30. Epub 2007 Mar 23.
Beijer L. and Rylander R.	OECD IIA 5.9	2004	(1→3)-β-D-Glucan does not induce acute inflammation after nasal deposition	Mediators Inflamm. Feb 24, 2005; 2005(1): 50–52.

Authors	Data requirement (indicated by the corresponding OECD data point number)	Year	Title	Source
Douwes J.	OECD IIA 5.9	2005	1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence	Indoor Air. 2005 Jun;15(3):160-9.
Toklu HZ, Sehirli AO, Velioğlu-Oğünç A, Cetinel S, Sener G.	OECD IIA 5.8	2006	Acetaminophen-induced toxicity is prevented by beta-D-glucan treatment in mice	Eur J Pharmacol. 2006 Aug 14;543(1-3):133-40. Epub 2006 Jun 2.
Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P, Yvin JC.	IIA 5.1	2007	Orally administered marine (1-3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity.	Int J Biol Macromol. 2007 Mar 10;40(4):291-8. Epub 2006 Aug 23.

List of the studies excluded from the risk assessment after detailed assessment of the full-text documents, classified by author(s) - toxicology

Authors	Year	Title	Source	Reason(s) for not including this study in the dossier
Douwes J	2005	(1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence	Indoor Air. 2005 Jun;15(3):160-9.	It concerned the potential health effects of indoor (1-->3)-beta-D-glucan exposure associated with indoor fungal exposure. It is not particularly relevant according to the PPP intended uses. Moreover based on this exposure, the available epidemiological data did not permit conclusions to be drawn regarding the presence (or absence) of an association between environmental (1-->3)-beta-D-glucan exposure and specific adverse health effects.

Criteria for study reliability

For estimation of reliability (inherent quality of data) of open literature, 'ToxRTool' was used. ToxRTool is the software-based tool being the outcome of a research project initiated by ECVAM and funded by the European Commission. In general, ToxRTool is based upon the Klimisch criteria for assessing reliability:

1. Reliable without restrictions
2. Reliable with restrictions
3. Not reliable

According to Klimisch, a study is reliable without restrictions if it is generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP). It was noticed by several authorities, including EFSA and working groups of the European Commission,

that non-guideline data (e.g. from academic laboratories) following good scientific principles in design, conduct and reporting and employing appropriate statistics, should be judged on their scientific merit and not automatically considered of lower quality to a Test Guideline studies conducted by a GLP accredited facility.

In the ToxRTool, even if using the Klimisch criteria for assessing reliability, the performance of a study according to GLP is not quoted. This means that in the ToxRTool a well performed non-guidance study can reach the same Klimisch score as a study performed under GLP.

Due to the transparent, relatively simple and efficient utilisation of the ToxRTool it was used for all case studies.

The quality of human epidemiology data was assessed according to the Bradford Hill²⁰ considerations of causal inference (i.e. strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy).

Estimation of study reliability

Estimation of reliability of open literature using ToxRTool software

Reference	Score ToRToll evaluator	Score expert judgment evaluator
Int J Biol Macromol. 2007 Mar 10;40(4):291-8. Epub 2006 Aug 23	2/3	2 (reliable with restriction)
Food Chem Toxicol. 2007 Sep;45(9):1719-30. Epub 2007 Mar 23.	1	2 (reliable with restriction)
Mediators Inflamm. Feb 24, 2005; 2005(1): 50–52.	1	2 (reliable with restriction)
Indoor Air . 2005 Jun;15(3):160-9.	Not relevant	3
Eur J Pharmacol. 2006 Aug 14;543(1-3):133-40. Epub 2006 Jun 2.	1	2 (reliable with restriction)

List of reliable and reliable with limitations studies

List of all studies considered relevant and reliable or relevant and reliable with limitations, classified by author(s) - toxicology

Authors	Data requirement (indicated by the corresponding OECD data point number)	Year	Title	Source
Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	OECD IIA 5.2 OECD IIA 5.3	2007	Toxicological assessment of a particulate yeast (1,3/1,6)-beta-D-glucan in rats	Food Chem Toxicol. 2007 Sep;45(9):1719-30. Epub 2007 Mar 23.
Beijer L. and Rylander R.	OECD IIA 5.9	2004	(1→3)-β-D-Glucan does not induce acute inflammation after nasal deposition	Mediators Inflamm. Feb 24, 2005; 2005(1): 50–52.
Toklu HZ, Sehirli AO, Velioğlu-Oğünç A, Cetinel S, Sener G.	OECD IIA 5.8	2006	Acetaminophen-induced toxicity is prevented by beta-D-glucan treatment in mice	Eur J Pharmacol. 2006 Aug 14;543(1-3):133-40. Epub 2006 Jun 2.

Authors	Data requirement (indicated by the corresponding OECD data point number)	Year	Title	Source
Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P, Yvin JC.	OECD IIA 5.1	2007	Orally administered marine (1-3)-beta- D-glucan Phycarine stimulates both humoral and cellular immunity.	Int J Biol Macromol. 2007 Mar 10;40(4):291- 8. Epub 2006 Aug 23.

**List of studies considered as relevant or of unclear relevance, classified by data requirement(s)
- toxicology**

Data requirement (indicated by the corresponding OECD data point number)	Authors	Year	Title	Source
OECD IIA 5.1	Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P, Yvin JC.	2007	Orally administered marine (1-3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity.	Int J Biol Macromol. 2007 Mar 10;40(4):291- 8. Epub 2006 Aug 23.
OECD IIA 5.2	Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	2007	Toxicological assessment of a particulate yeast (1,3/1,6)- beta-D-glucan in rats	Food Chem Toxicol. 2007 Sep;45(9):1719- 30. Epub 2007 Mar 23.
OECD IIA 5.3	Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	2007	Toxicological assessment of a particulate yeast (1,3/1,6)- beta-D-glucan in rats	Food Chem Toxicol. 2007 Sep;45(9):1719- 30. Epub 2007 Mar 23.
OECD IIA 5.8	Toklu HZ, Sehirli AO, Velioğlu- Oğünç A, Cetinel S, Sener G.	2006	Acetaminophen-induced toxicity is prevented by beta- D-glucan treatment in mice	Eur J Pharmacol. 2006 Aug 14;543(1-3):133- 40. Epub 2006 Jun 2.
OECD IIA 5.9	Beijer L. and Rylander R.	2004	(1→3)-β-D-Glucan does not induce acute inflammation after nasal deposition	Mediators Inflamm. Feb 24, 2005; 2005(1): 50–52.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
No data are submitted							

B.6.10.2 Existing and New data submitted for the purpose of renewal

Point	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner
B.6.1.1	Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P, Yvin JC.	2007	Orally administered marine (1->3)-beta-D-glucan Phycarine stimulates both humoral and cellular immunity. Non-GLP Published	Y	N		/
B.6.2.1	██████████	1998	Acute toxicity study - Safety test in the rat by the oral route report N°.970352 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.2.1	Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	2007	Toxicological assessment of a particulate yeast (1,3/1,6)-beta-D-glucan in rats. Non-GLP Published	Y	N		/
B.6.2.2.	██████████ █	2001	H11 (Batch 99S24) - Acute dermal toxicity study in the rat ██████████ Report n°2000698 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.2.2.	██████████	1998	Phycarine® 96S51 - Acute toxicity study - Safety test in the rat by the sub-cutaneous route ██████████ Report n°970353 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.2.3.	██████████	1999	Evaluation of acute inhalation toxicity of Phycarine® in rats ██████████ Report n°980001 EX GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.

Point	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner
B.6.2.4.	████████	1998a	Phycarine®96S51 - Cutaneous primary irritation in the rabbit ████████ Report n°970349 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.2.5.	████████	1998b	Phycarine® 96S51-Ocular primary irritation in the rabbit ████████ Report n°970350 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.2.6.	████████	1998c	Phycarine® 96S51 - Study of cutaneous sensitisation using the Magnusson and Kligman maximisation test in the guinea pig ████████ Report n°970351 ST GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.3.1.	████████	2000	H11 – 4-week oral toxicity study in rats ████████ Report n° RTC 7286/T/240/99 GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.3.2.	████████ █	2001a	H11- 90-day repeated dose oral toxicity study in the rat ████████ Report n° 20000389T GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.3.2	Babíček K, Cechová I, Simon RR, Harwood M, Cox DJ	2007	Toxicological assessment of a particulate yeast (1,3/1,6)-beta-D-glucan in rats. No GLP Published	Y	N		/
B.6.3.2	████████ █	2001b	H11- 90-day repeated dose oral toxicity study in the dog ████████ Report n° 20000390T GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.4.	Marzin D.	2000	Mutagenicity test on bacteria (Salmonella typhimurium his and Escherichia coli trp) using B.N. Ames's technique with H11 Institut Pasteur de Lille Report n° IPL-R 991011/H11 GLP Unpublished	N	N		Laboratoires Goëmar S.A.S.

Point	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner
B.6.4.	Larripa et al.	1987	Biological activity in Macrocystis pyrifera from Argentina: sodium alginate, fucoidan and laminaran. II. Genotoxicity GLP Unpublished	N	N		Laboratoires Goëmar S.A.S.
B.6.4.2	Haddouk H.	2001	LAMINARIN: Bone marrow micronucleus test by oral route in mice CIT, Evreux, France, Report n°21149 GLP Unpublished	N	N		Laboratoires Goëmar S.A.S.
B.6.6.2	██████████	2001c	H11-Study for the effects on embryo-foetal development in the rat by the oral route. ██████████ Report n°20000387 T GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.6.2	██████████	2001e	H11-Study for the effects on embryo-foetal development in the rabbit by the oral route. ██████████ Report n°20000388T GLP Unpublished	Y	N		Laboratoires Goëmar S.A.S.
B.6.8.2	Toklu HZ, Sehirli AO, Velioğlu- Oğünç A, Cetinel S, Sener G.	2006	Acetaminophen-induced toxicity is prevented by beta- D-glucan treatment in mice No GLP Published	Y	N		/
B.6.9.2	Beijer L. and Rylander R.	2004	(1→3)-β-D-Glucan Does Not Induce Acute Inflammation After Nasal Deposition No GLP Published	Y	N		/
B.6.9.2	Douwes J.	2005	1-->3)-Beta-D-glucans and respiratory health: a review of the scientific evidence No GLP Published	N	N		/