# Monograph

May 2003

## Laminarin

## Volume 1

Report and
Proposed Decision

**Rapporteur Member State: Belgium**

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# LEVEL 1

# Laminarin

# Statement of subject matter and purpose for which the monograph was prepared

**1.1 Purpose of the monograph (Document A)**

This monograph is submitted to support the application for the first inclusion of the new active substance Laminarin in Annex I of Directive 91/414/EEC

**1.2 Summary and assessment of information relating to the collective provision of dossiers (Document B)**

Not applicable as Laminarin is a new active substance with only one applicant.

**1.3 Identity of the active substance (Annex IIA 1)**

**1.3.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1)**

Applicant : Laboratoires GOËMAR S.A.
Z.A.C. La Madeleine
Avenue du Général Patton
35400 Saint-Malo
FRANCE

Contact person : Dr. Jean-Claude YVIN
Phone : +33 (0) 2 99 21 53 70
Fax : +33 (0) 2 99 82 56 17

**1.3.2 ISO common name and synonyms (Annex IIA 1.3)**

Proposed name : LAMINARIN (< original dossier)
Until now, this proposal has not yet been submitted to ISO. In fact, notifier now intends to propose another name (probably SOLAMIN) and to submit this new proposal to ISO

Synonym : LAMINARAN (Ref : Merck Index)

**1.3.3 Chemical name (Annex IIA 1.4)**

IUPAC nomenclature : (1→3)-β-D-glucan
(according to IUPAC-IUB Joint Commission on Biochemical Nomenclature)

CA nomenclature : -
(notifier has submitted a description of the structure to CAS, in order to confirm that the CAS-number [9008-22-4] is acceptable for the structure of the Laminaran extracted from *Laminaria digitata* and to obtain a CA name)

**1.3.4 Manufacturer's development code number (Annex IIA 1.5)**

Active substance : LAMINARIN
Code number : H11

Formulation : PHYLIQ
no code number specified

**1.3.5 CAS, ECC and CIPAC numbers (Annex IIA 1.6)**

CAS number : 9008-22-4
EINECS number : 232-712-4
CIPAC number : 671

**1.3.6 Molecular formula, molecular mass and structural formula (Annex IIA 1.7)**

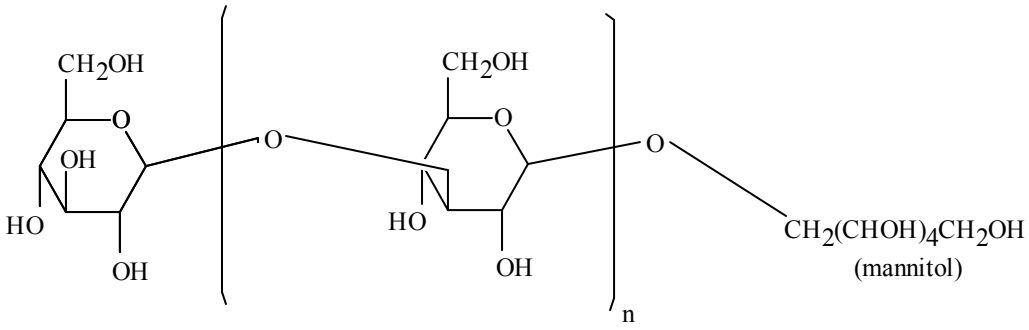
Laminarans are a class of low-molecular weight β-glucans which appear "to be the food reserve of all brown algae" (Painter, 1983). Among the different species, they differ by the number of units (20 to 30 in general), the proportion of branching points (determining the level of water solubility), the position of linkage (1, 3, 6), and the presence and proportion of terminal mannitol residues. The different types of laminarans have been extensively reviewed in the literature (see for instance Percival and Mc Dowell, 1985).

In the case of Laminarin (= laminaran from *Laminaria digitata*), the size heterogeneity and branching structure have been particularly studied by ElectroSpray-Ionisation-Mass-Spectrometry, ESIMS (Read et al., 1996).
It appears to be a β-(1→3)-linked D-glucan with occasional β-(1→6)-linked branches, composed of a major M-series containing 20-30 glucosyl residues linked to a mannitol terminal residue, and a minor G-series containing 22-28 glucosyl residues. Both series have a mean degree of polymerisation of 25 glucosyl residues and an approximately 3:1 molar ratio of M-series to G-series molecules is maintained across the range of molecular sizes. M-series molecules contain 0 to 4 branches, with an average of 1.3 branches per molecule ; branched G-series molecules are also detected. This study also showed that 75 % of branches are single glucosyl residues.

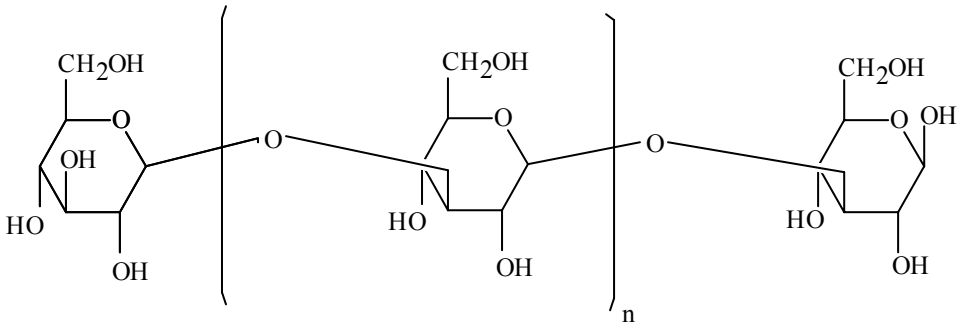
Molecular formula : $(C_6H_{12}O_6)_n$ n = 25 to 30

Molecular mass : 4500 – 5000 g/mol

Structural formula :




**M-series molecules**




**G-series molecules**

**1.3.7 Manufacturer of the active substance (Annex IIA 1.2)**

The active substance is a natural compound extracted from a sea brown alga, named *Laminaria digitata*, by :

Laboratoires GOËMAR S.A.
Z.A.C. La Madeleine
Avenue du Général Patton
35400 Saint-Malo
FRANCE

Location of plant : idem

Contact person : Dr. Jean-Claude YVIN
Phone : +33 (0) 2 99 21 53 70
Fax : +33 (0) 2 99 82 56 17

**1.3.8 Method or methods of manufacture (Annex IIA 1.8)**

Confidential information, see Annex C

**1.3.9 Specification of the purity of the active substance (Annex IIA 1.9)**

Minimum purity : 860 g/kg on dry matter

Nominal purity : 930 g/kg on dry matter

**1.3.10 Identity of isomers, impurities and additives (Annex IIA 1.10)**

Confidential information, see Annex C

**1.3.11 Analytical profile of batches (Annex IIA 1.11)**

Confidential information, see Annex C

**1.4 Identity of the plant protection product PHYLIQ (Annex IIA 3.1; Annex IIIA 1)**

**1.4.1 Current, former and proposed trade names and development code numbers (Annex IIIA 1.3)**

Trade name : not yet determined

Development code : PHYLIQ

**1.4.2 Manufacturer or manufacturers of the plant protection product (Annex IIIA 1.2)**

Manufacturer of the preparation : Laboratoires GOËMAR S.A.
Z.A.C. La Madeleine
Avenue du Général Patton
35400 Saint-Malo
FRANCE

Location of plant :idem

Manufacturer of the active substance : idem

Contact person : Dr. Jean-Claude YVIN
Phone : +33 (0) 2 99 21 53 70
Fax : +33 (0) 2 99 82 56 17

**1.4.3 Type of the preparation and code (Annex IIIA 1.5)**

Soluble concentrate (SL)

**1.4.4 Function (Annex IIIA 1.6)**

Elicitor of the crop's self-defence mechanism

**1.4.5 Composition of the preparation (Annex IIIA 1.4)**

Table 1.4.5-1 : Composition of PHYLIQ

| **Component** | **Content (g/L)** | **Function** |
|---|---|---|
| Laminarin<br>- pure a.s.<br>- technical a.s. (nominal purity : 93% on dry matter) | <br>37<br>39.8 | Active substance |
| Other components | Confidential information, see Annex C | |

**1.5 Uses of the plant protection product PHYLIQ**

**1.5.1 Fields of use (Annex IIA 3.3; Annex IIIA 3.1)**

Agriculture

**1.5.2 Effects on harmful organisms (Annex IIA 3.2; Annex IIIA 3.2)**

Laminarin is an elicitor of the crop's self-defence mechanisms and as such has no direct effect on harmful organisms. It stimulates the natural defences of the plant against pathogens, i.e. it enhances defence reactions which inhibit the development of the pathogens.
Laminarin being a natural oligosaccharide with a molecular weight of # 5 000 $g.mol^{-1}$, it is probably not transported in the plants as such. However, due to the systemic properties of the action, it is believed that smaller-sized oligosaccharides resulting from the hydrolysis of Laminarin in the plant would be responsible for

this phenomenon.

**1.5.3 Summary of intended uses (Annex IIA 3.4; Annex IIIA 3.3 to 3.7, 3.9)**

See summary of proposed Good Agricultural Practices

**SUMMARY OF PROPOSED GOOD AGRICULTURAL PRACTICES FOR PESTICIDES USES**

**(Application on agricultural crops)**

| Crop and/or situation (a) | Member State or Country | F, G or I (b) | Pests or Group of pest controlled ( c) | Formulation | | Application | | | Application rate per treatment | | | PHI (days) (k) | Remarks (l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Type (d-f) | Conc. Of a.s. (i) | Method kind (f-g) | Growth stage (j) | Number min max | kg a.s./hl min max | water l/ha min max | kg a.s./ha min max | | |
| Wheat | FR, GB , BE, DE, NL | F | Foliar fungi | SL | 37 | Foliar spraying | BBCH 29-30 | 1 | 0.0074-0.074 | 50-500 | 0.037 | - | |
| Barley | FR, GB , BE, DE, NL | F | Foliar fungi | SL | 37 | Foliar spraying | BBCH 29-30 | 1 | 0.0074-0.074 | 50-500 | 0.037 | - | |

### 1.5.4 Information on authorizations in EU Member States (Annex IIIA 12.1)

Laminarin being a new active substance, no authorizations are currently available

# LEVEL 2

# Laminarin

# Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

### 2.1.1 Identity

Laminarin is a natural compound, i.e. a low-molecular weight β-glucan extracted from a sea brown alga named *Laminaria digitata*.
The "active substance as manufactured" has a minimum purity of 860 g/kg dry matter. This purity value, as well as the proposed impurity profile, is supported by an acceptable analytical profile of batches.
The representative formulation PHYLIQ is a soluble concentrate (SL) containing 37 g/L Laminarin.

### 2.1.2 Physical and chemical properties

Active substance
Laminarin, an oligosaccharide with a molecular mass of ca. 5000 g/mol, is an odourless, white to beige powder. Its melting point and boiling point cannot be determined due to decomposition starting at ca. 216°C. Its low vapour pressure and Henry's law constant indicate that Laminarin is very slightly volatile. Laminarin is readily soluble in water and slightly to moderately soluble in organic solvents. Its very low octanol/water partition coefficient indicates no significant potential for bioaccumulation. Laminarin is hydrolytically stable, whatever the pH (as far as hydrolysis to glucose is concerned) and the light should have no influence on this stability. Laminarin shows no dissociation in water and is not considered as surface active. The a.s. is not highly flammable, not self heating and exhibits no explosive or oxidizing properties.

Formulation
PHYLIQ is a soluble concentrate (SL) to be used alone or in combination with fungicides for the protection of cereals against pathogens. It is a clear brown liquid with a slight odour and it is not to be classified as a dangerous preparation.
The technical properties of the formulation indicate that no particular problems are to be expected when it is used as recommended : it is not very acidic, its dilution is stable, and it produces no foam. The only peculiarity is that the product has to be considered as surface active.
Its physical stability observed after 14 days at 54°C suggests that storage under practical conditions should pose no problems; chemical stability on the other hand appears to be problematic after accelerated storage (a.s. content decreases by 13.8% after 14 days at 54°C); shelf life study at ambient temperature in commercial packaging is still ongoing. Justification is required for the fact that the difference between the determined a.s. content and that declared exceeds the FAO tolerance.
When used in tank mixes, no problem is expected although in some cases a particular procedure for mixing might be required (i.e. respecting a particular order of adding the tank mix partners).

### 2.1.3 Details of uses and further information

***Details of uses***

Laminarin is intended to be used as an elicitor of the crop's self-defence mechanisms against pathogens on cereals.
Details of intended uses supported by available data are summarized in point 1.5.3 in Level 1.

***Further information***

Acceptable information has been provided for the "active substance as manufactured" (recommendations concerning handling,, storage, transport or fire, procedures for destruction or decontamination and emergency measures in case of an accident) and for the plant protection product (packaging, cleaning procedures, recommendations concerning handling, storage, transport or fire, emergency measures in case of an accident and procedures for destruction or decontamination).

### 2.1.4 Classification and Labelling

Table 2.1.4-1 : Classification and labelling of laminarin made by the Rapporteur

| | | |
|---|---|---|
| *Classification :* | | |
| *Labelling :* | | |
| Hazard symbols : | None | |
| Indication of danger : | None | |
| Risk phrases : | None | |
| Safety phrases : | S2 | Keep out of the reach of children |
| | S13 | Keep away from food, drink and animal feedingstuffs |
| | S20/21 | When using, do not eat, drink or smoke |
| | S46 | If swallowed, seek medical advice immediately and show the container or label |

Table 2.1.4-2 : Classification and labelling of Phyliq SL made by the Rapporteur

| | |
|---|---|
| *Classification :* | |
| *Labelling :* | |
| Hazard symbols : | None |
| Indication of danger : | None |
| Risk phrases : | None |
| Safety phrases : | None |

**2.2 Methods of analysis**

**2.2.1 Analytical methods for analysis of the active substance as manufactured**

Validated methods are available for the determination of the purity and the significant impurities of the technical a.s.
No CIPAC methods exist for Laminarin.

**2.2.2 Analytical methods for formulation analysis**

A validated method is available for the determination of the Laminarin content in the formulation (SL).
No CIPAC methods exist for Laminarin formulations.

**2.2.3 Analytical methods for residue analysis**

Relevant residues of Laminarin in food of plant and animal origin and in the environmental compartments are not expected to occur. The setting of MRL's is not necessary and no residue is defined, neither with relevance to MRL nor with relevance to the environment (cfr. point 2.4.1; point 2.5.1). Therefore, residue analytical methods for the determination of the a.s. in *food of plant and animal origin* for enforcement purposes, as well as in *soil, water and air* for monitoring purposes are not required.

Methods for the determination of residues in *body fluids and tissues* are not required since the a.s. is not classified as toxic or highly toxic.

**2.3 Impact on human and animal health**

**2.3.1 effects having relevance to human and animal health arising from exposure to the active substance or to their transformation products.**

Laminarin or phycarin, a linear β D-1, 3-linked glucan was extracted and purified from the brown alga *laminaria digitata*.
Laminarin acts early against cereal diseases inducing natural defence reactions. By inducing systemic resistance on cereals, this allows protection during growth.
Laminarins are cell wall components, which are degraded by the colonic microflora in monogastric animals.

*Absorption, distribution, metabolism and excretion:*

*In human,* non-digestible polysaccharides such as laminarin escape enzymatic digestion in the upper gastrointestinal tract. The large bowel fermentation involves bacteria producing laminarinases, and β-glucosidases, which fully degrade the substrate into short Chain Fatty Acids (SCFAs), which are then absorbed by the colonocytes before further metabolism. Absorption may reach 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.

Numerous enzymes able to degrade laminarans have been isolated from bacteria, fungi, algae, molluscs and *higher plants*. β-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→ 3, 1→4) β-glucans. The soluble products of the initial hydrolysis are then acted on by glucanglucohydrolases, which preferentially attacks the longer gluco-oligomers (cellotriose) releasing glucose. β-Glucan exohydrolases and β-glucosidases may be important additional enzymes for the conversion of released oligosaccharides to glucose. Active glucose absorption occurs in the small intestine of mammals.

*Ruminants:* the ruminal microorganisms degrade dietary polysaccharides prior to gastric and intestinal digestion. Fermentation of foodstuffs in the rumen yields short-chain fatty acids (primarily acetic, propionic and butyric acids), carbon dioxide, methane, ammonia, and occasionally lactic acid.

*Dermal absorption*

Due to the high molecular weight of the active substance (5000 $g.mol^{-1}$), its hydrophily and the absence of solvents in the preparation of Phyliq LS, the dermal absorption of laminarin is probably quite low. Hydrophilic compounds like polysaccharides do not easily pass through the lipid bilayers of cell membranes and must be transported across cell membranes on carrier proteins.

*Acute toxicity of laminarin*

Laminarin is characterised by a rather low acute toxicity.
According to EU classification, laminarin should not be classified.

Table B.2.3.1-1 Summary of acute toxicity of Laminarin

| Type of test | Batch n°, purity | Results LD50 | Classification | References |
|---|---|---|---|---|
| Acute oral rat | batch n°96S51; purity: 91% | >2000 mg/kg bw | - | Delille, 1998a |
| Acute dermal, rat | batch n°99S24; purity: 94.9% | >5000 mg/kg bw | - | Audeval, 2001d |
| Acute subcutaneous rat* | batch n°96S51; purity: 91% | >1000 mg/kg bw | - | Delille, 1998b |
| Acute inhalation rat , aerosol, head-nose | batch n°96S51; purity: 91% | >1.02 mg/l/4h | - | Müller, 1999 |
| Skin irritation, rabbit | batch n°96S51; purity: 91% | Non irritant | - | Baudet , 1998a |
| Eye irritation, rabbit | batch n°96S51; purity: 91% | Non irritant | - | Baudet , 1998b |
| Skin sensitisation M & K test | batch n°96S51; purity: 91% | Not sensitizer | - | Baudet , 1998c |

*The study is accepted as additional information.

*Short-term toxicity of laminarin*

Laminarin was given by gavage to rats at 1000 mg/kg bw/d for 28 days and 90 days. A slight body weight increase was observed associated to a slight food consumption and water intake reduction. Laminarin as a polysaccharide might contribute to increase the energy content of the diet, reducing food intake. In rodents, water consumption is closely associated to food consumption. Therefore, these effects are not considered to be adverse.
In dogs, laminarin was given by gavage at 1000 mg/kg bw/d for 90 days. Slight increased incidence of soft faeces was reported which can be explained by the increased gastrointestinal motility produced by short chain fatty acids (resulting from cecal fermentation), as well as an increase in biomass which increases the faecal output. Therefore, the observed effects are not considered to be adverse.

Table 2.3.1-2: Summary of short-term toxicity of laminarin:

| Type of test | Batch n°. purity | Findings | NOAEL (mg/kg bw/d) | LOAEL (mg/kg bw/d) | References |
|---|---|---|---|---|---|
| 28 day feeding rat study | Batch n°: 99S21 97.6% | No effects | >1000 mg/kg bw/d | - | Longobardi, 2000 |
| 90 day feeding rat study | Batch n°: 99S24 94.9% | No effects | >1000 mg/kg bw/d | - | Audeval, 2001a |
| Dog, 90day dog feeding study | Batch n°: 99S24 94.9% | No effects | >1000 mg/kg bw/d | - | Audeval, 2001b |

*Genotoxicity of laminarin*

Laminarin was tested for its mutagenic potential *in vitro* and negative results were observed in the bacterial point mutation assay (Ames test). *In vivo*, Laminarin did not induce micronuclei in mice bone marrow after oral administration.
Negative results were also observed in a chromosomal aberration test *in vitro* in CHO cells, as reported in the public literature.

Table 2.3.1-3: Summary of genotoxicity studies with laminarin

| Study type | Batch n°. purity | Results | References |
|---|---|---|---|
| In vitro Genotoxicity studies | | | |
| Ames test | Batch n°. 99S21 97.6% | Negative | Marzin, 2000 |
| In vivo Genotoxicity studies | | | |
| Mice MN | Batch n°. 99S10 99% | Negative | Haddouk, 2001 |

*Long-term toxicity studies:*

The nature of laminarin, an algae cell wand polysaccharide, rapidly and extensively fermented by intestinal bacteria, gives rise to fermentation products such as, butyrate, propionate, acetate, $CO_2$, $H_2S$ 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 polysaccharides/glucose, 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 for life. No long-term studies are necessary.

*Reproductive toxicity and teratogenicity*

No reproductive study was performed in rats. A developmental rat and rabbit study showed that Laminarin is not toxic for the development. Neither embrytoxicity nor maternal toxicity was observed. The NOAEL in this study is > 1000 mg/kg bw/d.

*Neurotoxicity*

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. From the different toxicological studies performed in rats and dogs, no symptoms of neurotoxicity were observed. Laminarin is a polysaccharide. No neurotoxic effects are expected for this kind of compound.

*Human toxicology information:*

Laminarin is not produced industrially yet, so no clinical case could have been observed. In the production and handling of the pilot batches, and during the experimental field applications, no incidence occurred.

**2.3.2 Acceptable daily intake (ADI)**

Laminarin is a polysaccharide, which is devoid of acute toxicity. No specific effects / target organs were identified from the short-term toxicity studies performed in rat and dog. No developmental toxicity was observed in rats. Laminarin is not genotoxic. As laminarin is degraded into glucose by plants, no residue of biological significance will occur in plants. Since there is no risk for consumers from the use of laminarin as plant protection product, no ADI has been allocated by the rapporteur.

Note: the applicant proposes to use the value of 1000 mg/kg bw/d for setting an ADI. Applying an assessment

factor of 100: ADI = 10 mg/kg bw/d

### 2.3.3 Acute reference dose

Not allocated, not necessary.

### 2.3.4 Acceptable operator exposure level (AOEL)

Laminarin is a polysaccharide and is devoid of acute toxicity. No specific effects/target organs were reported from the short-term toxicity studies performed in rat and dog. No developmental toxicity was observed in rats. Laminarin is not genotoxic. A significant percutaneous absorption is excluded. The preparation is a liquid suspension. An inhalation risk is not expected. The allocation of an AOEL is considered not necessary.

Note: the applicant proposes to use the value of 1000 mg/kg bw/d for setting an AOEL. Applying an assessment factor of 100: AOEL = 10mg/kg bw/d.

This value was used for assessment of operator exposure.

### 2.3.5 Drinking water limit

The maximum admissible concentration of an active substance is 0.1 µg/L, as established by the directive 89/778/EEC.

### 2.3.6 impact on human or animal health arising from exposure to the active substance or to impurities contained in it.

*Operators, bystanders and worker exposure*

The available data for laminarin do not support evidence of acute toxicity, short term-toxicity, genotoxicity and developmental toxicity. A significant percutaneous absorption is excluded. The product Phyliq LS is not volatile.
Using both UK model (table B.2.3.6-1) and the German model (table B.2.3.6-2), for operators, without protective equipment the estimated exposure reaches 0.047% of the AOEL according to the German model and 0.113% of the AOEL according to the UK POEM model.

Table 2.3.6-1: Summary of exposure as a proportion of the AOEL, UK model with Phyliq SL

| Plant protection product/application method Phyliq SL | Total systemic exposure 60 kg person (mg/kg bw/d) | % of AOEL |
|---|---|---|
| | No PPE worn | No PPE worn |
| Field crop/tractor mounted | 0.01136 | 0.113 |

Table 2.3.6-2 : Summary of exposure as a proportion of the AOEL German model with Phyliq SL

| Plant protection product/application method Phyliq SL | Total systemic exposure 70 kg person (mg/kg bw/d) | % of AOEL |
|---|---|---|
| | No PPE worn | No PPE worn |
| Field crop/tractor mounted | 0.00471 | 0.047 |

Under practical conditions of use, the potential exposure of a bystander, even standing for a 1 hour period nearby sprayer, represents 0.0036% of the AOEL.

Phyliq SL is applied to cereals once between full tailoring stage and 1 cm ear stage, which do not require manual operations. Operations involving humans in such crops are essentially performed mechanically (e.g. spraying and harvest). This provides only limited opportunity for workers to enter treated areas and be exposed to this non-volatile, low toxicity product. Because of these considerations, workers exposure was not estimated.

## 2.4 Residues

### 2.4.1 Definition of the residues relevant to MRLs

The formulation Phyliq SL (containing 37 g laminarin/l) is applied at the rate of 37 g a.s./l, on cereals (wheat, barley and rice) once a year, at an early growth stage between full tillering and the 1 cm-ear stage (BBCH 29-30) when the ear is not apparent.

The toxicological evaluation showed that Laminarin (B-1,3 linked glucans) is a polysaccharide which is devoid of acute toxicity. No specific effects/target organs were identified from the short-term toxicity studies performed in rat and dog. No developmental toxicity was observed in rats and no ADI has been allocated by the RMS.
In *plants*, laminarin may undergo degradation by polysaccharide and oligosaccharide hydrolases leading to production of glucose. In consequence, there is no possibility to define a residue as such, as it would have to be glucose itself and no plant metabolism studies are necessary to re-enforce that statement.
In *ruminants*, fermentative production of short chain of fatty acids (which are further metabolized before excretion into breath and flatus) is the principal mechanism of intestinal digestion and further livestock metabolism studies are not necessary.

Therefore, no residue of biological significance will occur in plants and in animals. It can be stated that there is no risk for consumers from the use of laminarin as plant protection product.

### 2.4.2 Residues relevant to consumer safety

See point 2.4.1

### 2.4.3 Residues relevant to worker safety

Not relevant

### 2.4.4 Proposed EU MRLs and compliance with existing MRLs

Not relevant

### 2.4.5 Proposed EU import tolerances and compliance with existing MRLs

Not relevant

### 2.4.6 Basis for differences, if any, in conclusions reached having regard to established or proposal CAC MRLs

Not relevant

## 2.5 Fate and behaviour in the environment

### 2.5.1 Definition of the residues relevant to the environment

The rapporteur considers that :
- The natural background level of mono-, di- or polysaccharides in soil and water is expected to be high and variable.
- Laminarin and its mono-, di- or polysaccharides metabolites have no (eco)toxicological significance.

Therefore, the establishment of residue definition in soil, water and air is not required.

### 2.5.2 Fate and behaviour in soil

No study has been conducted on the fate and behaviour of laminarin in soil (metabolism in soil, degradation rate, mobility in soil).

However, an extensive literature search which has been performed by the notifier shows that

- β-1,3- glucans are common plant polysaccharides
- β-1,3-glucanases do exist in soil
- β-1,3-glucanases are very common in bacteria, fungi, algae, higher plants, molluscs
- they are able to hydrolyze β-1,3-glucans like laminarin or callose
- The degradation of laminarin by soil micro-organisms would lead to smaller-sized oligosaccharides and monosaccharides (glucose). No other relevant metabolites degradation or reaction products is expected to appear.

### 2.5.3 Fate and behaviour in water

Laminarin is hydrolytically stable in sterile water at pH 4, 7 and 9.
Laminarin is photostable.

Laminarin is readily biodegradable (56-64% biodegradation at day 9-12 ; 76% biodegradation at day 28)

The degradation of laminarin which is a polysaccharide would lead to smaller-sized oligosaccharides and glucose. No other relevant metabolites degradation or reaction products is expected to appear.

**2.5.4 Fate and behaviour in air**

Laminarin having a very low vapour pressure (<2.6 $10^{-5}$ Pa at 25°C) and a very low Henry's law constant (< 1.5 10-6 $Pa.m^3.mol^{-1}$), no risk of volatilisation is to be expected in the recommended conditions of use.

**2.6 Effects on non-target species**

**2.6.1 Effects on terrestrial vertebrates**

An acute toxicity study and a dietary toxicity study of laminarin on birds have been conducted. The endpoints of these studies resulted in TER-values far above the Annex-VI trigger values. Also the endpoints of the rat-studies resulted in TER-values far above the Annex-VI trigger values. The risk for terrestrial vertebrates resulting from the exposure to the a.s. in crops treated with foliar spray formulation is therefore considered as negligible.

**2.6.2 Effects on aquatic species**

Acute toxicity tests with fish, Daphnia'a and an algae were performed. The endpoints of these studies resulted in TER-values far above the Annex-VI trigger values. The acute risk of laminarin to aquatic organisms is acceptable.

**2.6.3 Effects on bees and other arthropods**

An acute toxicity study with bees and 2 arthropod species have been conducted. The risk for honeybees is acceptable. The formulation Phyliq is harmless for *Aphidius rhopalosiphi* and *Typhlodromus pyri* at the recommended dose.

**2.6.4 Effects on earthworms and other soil macro-organisms**

No tests regarding acute toxicity or long term toxicity were performed. Laminarin is a natural polysaccharide of shorter size than cellulose, so no short or long term risk for earthworms and other soil macro-organisms is expected.

**2.6.5 Effects on soil micro-organisms**

A literature study performed by the notifier shows that :

- laminarase occurs in bacteria, fungi, algae, higher plants and molluscs.
- laminarase has been found in bacteria.

Laminarin is readily biodegradable. Continued or repeated exposure of soil micro-organisms is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g w.s./ha. No short or long term risk for soil micro-organisms is expected.

**2.6.6 Effects on other non-target organisms (flora and fauna)**

In the article by Bull T.A. (1967) on the enzymatic degradation of ß-glucans also their distribution in nature is discussed. According to this article ß-glucans are found in different species. The application of 37 g a.s./ha/year is not considered to form a risk to other flora and fauna as ß-glucans occur already naturally in different species.

**2.6.7 Effects on biological methods of sewage treatment**

In the study for the ready biodegradability of laminarin activated sewage micro-organisms were used. The test resulted that laminarin is readily biodegradable so no effect is expected on the micro-organisms of sewage

treatment plants.

**List of end points (based on doc 1654/VI/94, Rev. 7, 22 Apr 1998)**

**Identity, Physical and Chemical Properties, Details of Uses, Further Information, Methods of Analysis**

| Rapporteur Member State | Month and year | Active Substance (Name) |
|---|---|---|
| Belgium | May 2003 | Laminarin |

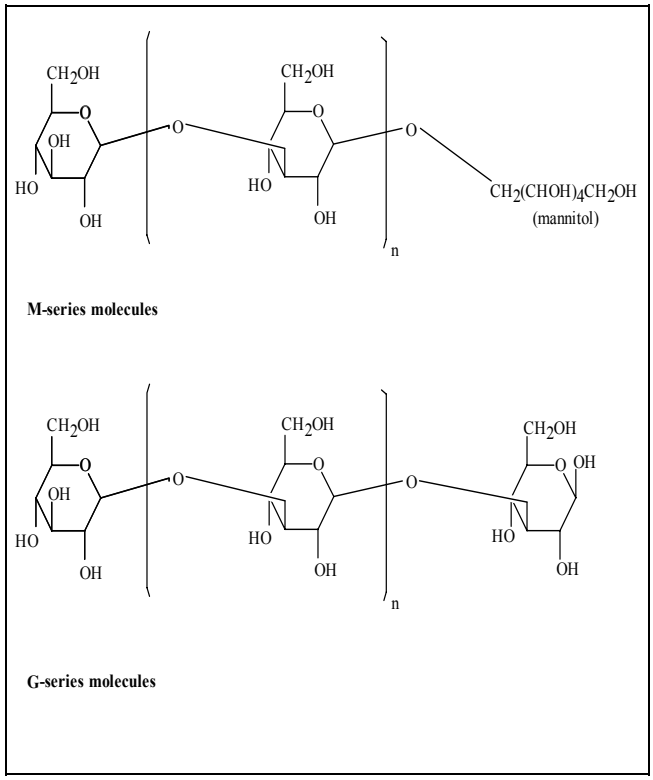
**Identity, Physical and Chemical Properties, Details of Uses, Further Information**

| | |
|---|---|
| Active substance (ISO Common Name) | Laminarin |
| Function (*e.g.* fungicide) | Elicitor of the crop's self-defence mechanisms |
| Rapporteur Member State | Belgium |

**Identity** (Annex IIA, point 1)

| | |
|---|---|
| Chemical name (IUPAC) | (1→3)-β-D-glucan<br>(according to IUPAC-IUB Joint Commission on Biochemical Nomenclature) |
| Chemical name (CA) | -<br>(notifier has submitted a description of the structure to CAS, in order to obtain a CA name) |
| CIPAC No | 671 |
| CAS No | 9008-22-4 |
| EEC No (EINECS or ELINCS) | 232-712-4 |
| FAO Specification (including year of publication) | Not available |
| Minimum purity of the active substance as manufactured (g/kg) | 860 g/kg on dry matter |
| Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg) | No relevant impurities are present in technical Laminarin |
| Molecular formula | $(C_6H_{12}O_6)_n$ n = 25 to 30 |
| Molecular mass | 4500 – 5000 g/mol |

Structural formula ‡



**Physical-chemical properties** (Annex IIA, point 2)

| | |
|---|---|
| Melting point (state purity) | could not be determined (decomposition) |
| Boiling point (state purity) | could not be determined (decomposition) |
| Temperature of decomposition | decomposition starting at ca. 216°C (98% on dry matter) |
| Appearance (state purity) | odourless white crystals (98% on dry matter)<br>odourless beige powder (89% on dry matter) |
| Relative density (state purity) | $D_4^{20}$ = 1.502 (98% on dry matter) |
| Surface tension | 72.2 mN/m at 20°C (1 g/L solution) |
| Vapour pressure (in Pa, state temperature) | < 2.6 x $10^{-5}$ Pa at 25°C |
| Henry's law constant (Pa $m^3$ $mol^{-1}$) | < 4.3 x $10^{-7}$ Pa.m³.$mol^{-1}$ at 23-25°C |
| Solubility in water (g/l or mg/l, state temperature) | 301.5 g/L at 23°C |
| | effect of pH : not relevant (a.s. is not acidic or basic) |
| Solubility in organic solvents (in g/l or mg/l, state temperature) | n-heptane : < 10 mg/L glucose<br>xylene : < 10 mg/L glucose<br>1,2-dichloroethane : < 10 mg/L glucose<br>methanol : 60 mg/L glucose<br>acetone : 21 mg/L glucose<br>ethylacetate : < 10 mg/L glucose<br>(at about 20°C) |
| Partition co-efficient (log $P_{OW}$) (state pH and temperature) | log $P_{ow}$ = -1.6 at 20°C |
| | effect of pH : not relevant (a.s. is not acidic or basic) |
| Hydrolytic stability ($DT_{50}$) (state pH and temperature) | at 50°C : hydrolytic stability at pH 4, 7 and 9 (= less than 10% degradation of Laminarin to glucose after 5 d) |
| Dissociation constant | no dissociation in water |
| UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength) | in neutral medium (pH 7.0) :<br>$\lambda_{max}$ = 260 nm; ε = 242 to 290 L.$mol^{-1}$.$cm^{-1}$<br>at 290 nm : ε-values in the range of 121 to 169 L.$mol^{-1}$.$cm^{-1}$ |
| Photostability ($DT_{50}$) (aqueous, sunlight, state pH)‡ | no good study could be conducted (due to the nature of the substance);<br>Laminarin is considered to be stable to the light |
| Quantum yield of direct phototransformation in water at Σ > 290 nm | not relevant |
| Flammability | not highly flammable;<br>no self-ignition temperature up to 420°C |
| Explosive properties | no explosive properties |

**Summary of uses supported by available data (active substance)**

| Crop and/ or situation | Member State or Country | Product name | F G or I | Pests or Group of pests controlled | Formulation | | Application | | | | Application rate per treatment | | | PHI (days) | Remarks: |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Type | Conc. of as | method kind | growth stage & season | number min max | interval between applications (min) | kg as/hL min max | water L/ha min max | kg as/ha min max | | |
| (a) | | | (b) | (c) | (d-f) | (i) | (f-h) | (j) | (k) | | | | | (l) | (m) |
| Wheat | FR, GB, BE, DE, NL | PHYLIQ | F | Foliar fungi | SL | 37 g/L | Foliar spraying | BBCH 29-30 | 1 | - | 0.0074 – 0.074 | 50-500 | 0.037 | - | - |
| Barley | FR, GB, BE, DE, NL | PHYLIQ | F | Foliar fungi | SL | 37 g/L | Foliar spraying | BBCH 29-30 | 1 | - | 0.0074 – 0.074 | 50-500 | 0.037 | - | - |

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

**Methods of Analysis**

**Analytical methods for the active substance** (Annex IIA, point 4.1)

| | |
|---|---|
| Technical as (principle of method) | - HPIC with amperometric detection for determination of Laminarin<br>- HPIC with amperometric detection (after total acid hydrolysis) for determination of Laminarin (as glucose)<br>- GC with FID (after total acid hydrolysis and derivatisation) for determination of Laminarin (as trimethylsilyl derivative of glucose) |
| Impurities in technical as (principle of method) | HPIC with amperometric detection (after total acid hydrolysis) for determination of impurity 1;<br>FAAS for determination of impurities 2-5;<br>Potentiometry for determination of impurity 6;<br>HPIC with conductivity detection for determination of impurity 7;<br>Titrimetry for determination of impurity 8 |
| Plant protection product (principle of method) | HPIC with amperometric detection for determination of Laminarin |

**Analytical methods for residues** (Annex IIA, point 4.2)

| | |
|---|---|
| Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes) | Not required (no residue is defined) |
| Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes) | Not required (no residue is defined) |
| Soil (principle of method and LOQ) | Not required (no residue is defined) |
| Water (principle of method and LOQ) | Not required (no residue is defined) |
| Air (principle of method and LOQ) | Not required (no residue is defined) |
| Body fluids and tissues (principle of method and LOQ) | Not required<br>(a.s. is not classified as toxic or highly toxic) |

**Classification and proposed labelling** (Annex IIA, point 10)

| | |
|---|---|
| with regard to physical/chemical data | No classification |

**Absorption, distribution, excretion and metabolism in mammals** (Annex IIA, point 5.1)

| | |
|---|---|
| Rate and extent of absorption | High bioavailability ( 90%) within 24 h |
| Distribution | Uniformly distributed |
| Potential for accumulation | No evidence for accumulation |
| Rate and extent of excretion | Rapid and extensive (approx. 90%) via breath and flatus |
| Metabolism in animals | Extensively metabolised via colonic microbiota fermentation : production of Short Chain Fatty Acids |
| Toxicologically significant compounds (animals, plants, and environment) | No toxicologically significant compound : laminarin is a polysaccharide of glucose and mannitol |

**Acute toxicity** (Annex IIA, point 5.2)

| | |
|---|---|
| Rat LD50 oral | > 2000 mg/kg bw |
| Rat LD50 dermal | > 5000 mg/kg bw |
| Rat LC50 inhalation aerosol | > 1.02 mg/L/4 h |
| Skin irritation | Not irritant |
| Eye irritation | Not irritant |
| Skin sensitisation (test method used and result) | Not sensitizer (M&K test) |

**Short term toxicity** (Annex IIA, point 5.3)

| | |
|---|---|
| Target/critical effect | No toxic effects |
| Lowest relevant oral NOAEL/NOEL | NOAEL > 1000 mg/kg bw/d |
| Lowest relevant dermal NOAEL/NOEL | No data, not relevant |
| Lowest relevant inhalation NOAEL/NOEL | No data, not relevant |

**Genotoxicity** (Annex IIA, point 5.4)

No genotoxic potential *in vitro* and *in vivo*

**Long term toxicity and carcinogenicity** (Annex IIA, point 5.5)

| | |
|---|---|
| Target/critical effect | No data, no studies required |
| Lowest relevant NOAEL/NOEL | Not applicable, no studies required |
| Carcinogenicity | Not applicable, no studies required |

**Reproductive toxicity** (Annex IIA, point 5.6)

| | |
|---|---|
| Reproduction target/critical effect | No data, no study required |
| Lowest relevant reproductive NOAEL/NOEL | Not applicable |
| Developmental target/critical effect | No toxic effects in rat, rabbit developmental study |
| Lowest relevant developmental NOAEL/NOEL | NOAEL > 1000 mg/kg bw/d |

**Neurotoxicity** (Annex IIA, point 5.7)

No neurotoxic potential

**Other toxicological studies** (Annex IIA, point 5.8)

No data, no study required

**Medical data** (Annex IIA, point 5.9)

No toxic effects are expected for this kind of compound

**Summary** (Annex IIA, point 5.10)

| | Value | Study | Safety factor |
|---|---|---|---|
| ADI | Not allocated | - | - |
| AOEL | 10 mg/kg bw/d | oral, rat, dog and rabbit studies | 100 |
| Drinking water limit | 0.1 µg/L | Dir 89/778/EEC | - |
| ArfD (acute reference dose) | - | - | - |

**Dermal absorption** (Annex IIIA, point 7.3)

Default value : 10% ; no relevant dermal absorption is expected

**Acceptable exposure scenarios** (including method of calculation)

| | |
|---|---|
| Operator | Acceptable without PPE (UK POEM and German model) |
| Workers | Acceptable |
| Bystanders | Acceptable |

**Classification and proposed labelling** (Annex IIA, point 10)

| | |
|---|---|
| with regard to toxicological data | No classification |

**Residues**

**Metabolism in plants** (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

| | |
|---|---|
| Plant groups covered | Not required |
| Rotational crops | Not required |
| Plant residue definition for monitoring | Not required |
| Plant residue definition for risk assessment | Not required |
| Conversion factor (monitoring to risk assessment) | Not required |

**Metabolism in livestock** (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

| | |
|---|---|
| Animals covered | Livestock metabolism studies are not required |
| Animal residue definition for monitoring | Not required |
| Animal residue definition for risk assessment | Not required |
| Conversion factor (monitoring to risk assessment) | Not required |
| Metabolism in rat and ruminant similar (yes/no) | No |
| Fat soluble residue: (yes/no) | No |

**Residues in succeeding crops** (Annex IIA, point 6.6, Annex IIIA, point 8.5)

| | |
|---|---|
| ..... | Not required |

**Stability of residues** (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

| | |
|---|---|
| ..... | Not required |

**Residues from livestock feeding studies** (Annex IIA, point 6.4, Annex IIIA, point 8.3)

| Intakes by livestock ≥ 0.1 mg/kg diet/day: | Ruminant: No | Poultry: no | Pig: no |
|---|---|---|---|
| Muscle | - | - | - |
| Liver | - | - | - |
| Kidney | - | - | - |
| Fat | - | - | - |
| Milk | - | - | - |
| Eggs | - | - | - |

**Summary of critical residues data** (Annex IIA, point 6.3, Annex IIIA, point 8.2)

| Crop | Northern or Mediterranean Region | Trials results relevant to the critical GAP<br><br>(a) | Recommendation/comments | MRL | STMR<br><br>(b) |
|---|---|---|---|---|---|

(a



**Appendix 6 Format For The Listing Of End Points to be Included in the Reasoned Statement of the Overall Conclusions Drawn by the Rapporteur Member State (Level 2)**

**Rapporteur Member State Month and year Active Substance (Name) page of**

**Consumer risk assessment** (Annex IIA, point 6.9, Annex IIIA, point 8.8)

| | |
|---|---|
| ADI | The RMS considers that an ADI is not required (ADI of 10 mg/kg bw/d has been proposed by the notifier) |
| TMDI (European Diet) (% ADI) | - |
| NEDI (% ADI) | - |
| Factors included in NEDI | - |
| ArfD | - |
| Acute exposure (% ARfD) | - |

**Processing factors** (Annex IIA, point 6.5, Annex IIIA, point 8.4)

| Crop/processed crop | Number of studies | Transfer factor | % Transference * |
|---|---|---|---|
| Not required | - | - | - |

* Calculated on the basis of distribution in the different portions, parts or products as determined through balance studies

**Proposed MRLs** (Annex IIA, point 6.7, Annex IIIA, point 8.6)

| | |
|---|---|
| Cereal grain | Not required |

Fate and Behaviour in the Environment

**Route of degradation (aerobic) in soil** (Annex IIA, point 7.1.1.1.1)

| | |
|---|---|
| Mineralization after 100 days | Not required.<br>The degradation of laminarin by soil micro-organisms would lead to smaller-sized oligosaccharides and monosaccharides (glucose). No other relevant metabolites degradation or reaction products is expected to appear. |
| Non-extractable residues after 100 days | Not required. |
| Relevant metabolites - name and/or code, % of applied (range and maximum) | Not required. |

**Route of degradation in soil - Supplemental studies** (Annex IIA, point 7.1.1.1.2)

| | |
|---|---|
| Anaerobic degradation | Not required. |
| Soil photolysis | Not required. |

**Rate of degradation in soil** (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

| | |
|---|---|
| Method of calculation | |
| Laboratory studies (range or median, with n value, with $r^2$ value) | $DT_{50lab}$ (20°C, aerobic): Not required. |
| | $DT_{90lab}$ (20°C, aerobic): Not required. |
| | $DT_{50lab}$ (10°C, aerobic): Not required. |
| | $DT_{50lab}$ (20°C, anaerobic): Not required. |
| | degradation in the saturated zone: Not required. |
| Field studies (state location, range or median with n value) ‡ | $DT_{50f}$: Not required. |
| | $DT_{90f}$: Not required. |
| Soil accumulation and plateau concentration ‡ | Not required |

**Soil adsorption/desorption** (Annex IIA, point 7.1.2)

| | |
|---|---|
| $K_f$ /$K_{oc}$<br>$K_d$<br>pH dependence (yes / no) (if yes type of dependence) | Not required |

**Mobility in soil** (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

| | |
|---|---|
| Column leaching | Not required |
| Aged residues leaching | Not required |
| Lysimeter/ field leaching studies | Not required |

**PEC (soil)** (Annex IIIA, point 9.1.3)

| | |
|---|---|
| Method of calculation | |
| Application rate | Cereals, 37 g a.s./ha, 1 application,<br>50% of applied dose reaching the soil |

| **PEC$_{(s)}$** | Single application<br>Actual | Single application<br>Time weighted average | Multiple application<br>Actual | Multiple application<br>Time weighted average |
|---|---|---|---|---|
| Initial | 0.025 mg a.s./kg soil | - | - | - |

**Route and rate of degradation in water** (Annex IIA, point 7.2.1)

| | |
|---|---|
| Hydrolysis of active substance and relevant metabolites ($DT_{50}$) (state pH and temperature) | at 50°C : hydrolytic stability at pH 4, 7 and 9 (= less than 10% degradation of Laminarin to glucose after 5 d) |
| Photolytic degradation of active substance and relevant metabolites | photostable |
| Readily biodegradable (yes/no) | yes |
| Degradation in water/sediment - $DT_{50}$ water<br>- $DT_{90}$ water<br>- $DT_{50}$ whole system<br>- $DT_{90}$ whole system | Not required.<br>The degradation of laminarin which is a polysaccharide would lead to smaller-sized oligosaccharides and glucose. No other relevant metabolites degradation or reaction products is expected to appear. |
| Mineralization | Not required. ($DT_{50}$ a.s. \| $CO_2$ ~ 8 days) |
| Non-extractable residues | Not required. |
| Distribution in water / sediment systems (active substance) | Not required. |
| Distribution in water / sediment systems (metabolites) | Not required. |

**PEC (surface water)** (Annex IIIA, point 9.2.3)

| | |
|---|---|
| Method of calculation | |
| Application rate | Cereals, 37 g a.s./ha, 1 application, |
| Main routes of entry | 1 m drift, 2.77% of applied dose reaching the waterbody |

| **PEC**$_{(sw)}$ | Single application<br>Actual | Single application<br>Time weighted average | Multiple application<br>Actual | Multiple application<br>Time weighted average |
|---|---|---|---|---|
| Initial | 0.342 µg a.s./l | - | - | - |

**PEC (sediment)**

| | |
|---|---|
| Method of calculation | Not required |
| Application rate | |

| **PEC**$_{(sed)}$ | Single application<br>Actual | Single application<br>Time weighted average | Multiple application<br>Actual | Multiple application<br>Time weighted average |
|---|---|---|---|---|
| Initial | - | - | - | - |
| Short term | - | - | - | - |
| Long term | - | - | - | - |

**PEC (ground water)** (Annex IIIA, point 9.2.1)

| | |
|---|---|
| Method of calculation and type of study (*e.g.* modelling, monitoring, lysimeter ) | Not required |
| Application rate | - |

**PEC**$_{(gw)}$

| | |
|---|---|
| Maximum concentration | - |
| Average annual concentration | - |

**Fate and behaviour in air** (Annex IIA, point 7.2.2, Annex III, point 9.3)

| | |
|---|---|
| Direct photolysis in air | - |
| Quantum yield of direct phototransformation | - |
| Photochemical oxidative degradation in air | Latitude: ..... Season: ..... $DT_{50}$ ..... |
| Volatilization | from plant surfaces: - |
| | from soil: - |

**PEC (air)**

| | |
|---|---|
| Method of calculation | - |

**PEC**$_{(a)}$

| | |
|---|---|
| Maximum concentration | - |

**Definition of the Residue** (Annex IIA, point 7.3)

| | |
|---|---|
| Relevant to the environment | The establishment of residue definitions in soil and water is not relevant |

**Monitoring data, if available** (Annex IIA, point 7.4)

| | |
|---|---|
| Soil (indicate location and type of study) | Not available |
| Surface water (indicate location and type of study) | Not available |
| Ground water (indicate location and type of study) | Not available |
| Air (indicate location and type of study) | Not available |

**Classification and proposed labelling** (Annex IIA, point 10)

| | |
|---|---|
| with regard to fate and behaviour data | Not classified |

**Effects on Non-target Species**

**Effects on terrestrial vertebrates** (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

| | |
|---|---|
| Acute toxicity to mammals | LD50 = 2000 mg/kg bw |
| Acute toxicity to birds | LD50 > 2000 mg /kg bw |
| Dietary toxicity to birds | LC50 > 5000 ppm |
| Reproductive toxicity to birds | Not required |

**Toxicity/exposure ratios for terrestrial vertebrates** (Annex IIIA, points 10.1 and 10.3)

| Application rate (g as/ha) | Crop | Category (*e.g.* insectivorous bird) | Time-scale | TER | Annex VI Trigger |
|---|---|---|---|---|---|
| 37 | Cereals | Herbivorous bird | Acute | 865 | 10 |
| | | Insectivorous bird | Acute | 3712 | 10 |
| | | Herbivorous bird | Short term | 792 | 10 |
| | | Insectivorous bird | Short term | 4994 | 10 |
| | | Herbivorous mammal | Acute | 331 | 10 |
| | | Insectivorous mammal | Acute | 7571 | 10 |
| | | Herbivorous mammal | Long term | 587 | 5 |
| | | Insectivorous mammal | Long term | 10391 | 5 |

**Toxicity data for aquatic species (most sensitive species of each group)** (Annex IIA, point 8.2, Annex IIIA, point 10.2) ‡

| Group | Test substance | Time-scale | Endpoint | Toxicity (mg/l) |
|---|---|---|---|---|
| Laboratory tests | | | | |
| *Oncorhynchus mykiss* | laminarine | Acute | LC50 | > 100 |
| *Daphnia magna* | laminarine | Acute | EC50 | > 100 |
| *Selenastrum capricornutum* | laminarine | Acute | EC50 | >100 |

| Microcosm or mesocosm tests |
|---|
| Not required |

**Toxicity/exposure ratios for the most sensitive aquatic organisms** (Annex IIIA, point 10.2)

| Application rate (g as/ha) | Crop | Organism | Time-scale | Distance (m) | TER | Annex VI Trigger |
|---|---|---|---|---|---|---|
| 37 | Cereals | *Oncorhynchus mykiss* | Acute | 1 | 292398 | 100 |
| | | *Daphnia magna* | Acute | 1 | 292398 | 100 |
| | | *Selenastrum capricornutum* | Acute | 1 | 292398 | 10 |

**Bioconcentration**

| | |
|---|---|
| Bioconcentration factor (BCF) ‡ | Not required |
| Annex VI Trigger:for the bioconcentration factor | |
| Clearance time ($CT_{50}$) ($CT_{90}$) | Not required |
| Level of residues (%) in organisms after the 14 day depuration phase | Not required |

**Effects on honeybees** (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

| | |
|---|---|
| Acute oral toxicity | LD50 > 118.64 µg a.s./bee |
| Acute contact toxicity | LC50 > 100 µg a.s./bee |

**Hazard quotients for honey bees** (Annex IIIA, point 10.4)

| Application rate (g as/ha) | Crop | Route | Hazard quotient | Annex VI Trigger |
|---|---|---|---|---|
| Laboratory tests | | | | |
| 37 | Cereals | Oral | 0.31 | 50 |
| | | Contact | 0.37 | 50 |

| |
|---|
| Field or semi-field tests<br>Not required |

**Effects on other arthropod species** (Annex IIA, point 8.3.2, Annex IIIA, point 10.5) ‡

| Species | Stage | Test Substance | Dose (L/ha) | Endpoint | Effect | Annex VI Trigger |
|---|---|---|---|---|---|---|
| Laboratory tests | | | | | | |
| *A. rhopalosiphi* | Adult | Phyliq | 3.0 | Beneficial capacity | 26% | 30% |
| *T. pyri* | Adult | Phyliq | 1.0 | Beneficial capacity | 29.41% | 30% |

| Field or semi-field tests |
|---|
| Not required |

**Effects on earthworms** (Annex IIA, point 8.4, Annex IIIA, point 10.6)

| | |
|---|---|
| Acute toxicity | Not required |
| Reproductive toxicity | Not required |

**Toxicity/exposure ratios for earthworms** (Annex IIIA, point 10.6)

| Application rate (kg as/ha) | Crop | Time-scale | TER | Annex VI Trigger |
|---|---|---|---|---|
| - | | | | |

**Effects on soil micro-organisms** (Annex IIA, point 8.5, Annex IIIA, point 10.7)

| | |
|---|---|
| Nitrogen mineralization | Not required |
| Carbon mineralization | Not required |

**Classification and proposed labelling** (Annex IIA, point 10)

| | |
|---|---|
| with regard to ecotoxicological data | / |

# LEVEL 3

# Laminarin

# Proposed decision with respect to the application for inclusion of the active substance in Annex I

### 3.1 Background to the proposed decision

Brown algae such as the *Laminaria* genus store carbohydrate as laminarans (or laminarin), a class of low-molecular weight β-1,3 glucans. The active substance is a polysaccharide of 25-30 glycosyl units. Laminaran are hydrolyzed by laminarase enzymes.

Laminarin induces natural defence reactions in the plants. It will be used on cereals.
Relevant residues of laminarin in food of plant and animal origin and in the environmental compartments are not expected to occur.

According to the toxicological properties of laminarin, harmful effects on the health of operators, bystanders, workers or consumers are not to be expected when the plant protection product is used in accordance with good plant protection practice.

The available data for laminarin do not support evidence of acute, short term, genotoxic, or development damaging properties of the active substance. It can be anticipated that no carcinogenic or reproductive effects will occur.

The RMS does not propose an ADI.

For operators, bystanders and workers, although no relevant exposure is expected, an exposure assessment was performed based on an AOEL of 10-mg/kg bw/d as proposed by the applicant.

The toxicological evaluation showed that Laminarin is a polysaccharide which is devoid of detrimental health effects (no ADI is proposed).
In plants, laminarin may undergo degradation by polysaccharide and oligosaccharide hydrolases leading to production of glucose.
In ruminants, fermentative production of short chain of fatty acids is the principal mechanism of intestinal digestion .

No residue of biological significance will occur in plants and in animals and it can be stated that there is no risk for consumers from the use of laminarin as plant protection product.

β-1,3- glucans are common plant polysaccharides which are hydrolyzed by β-1,3-glucanases. These enzymes are common in bacteria, fungi, algae, higher plants, molluscs
The degradation of laminarin by soil micro-organisms and water micro-organisms would lead to smaller-sized oligosaccharides and monosaccharides (glucose). No other relevant metabolites degradation or reaction products is expected to appear.

The evaluation which has been performed reveals that non-target organisms are not at risk.

### 3.2 Proposed decision concerning inclusion in Annex I

It is proposed to include the active substance laminarin in Annex I of Directive 91/414/EEC.

### 3.3 Rationale for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate

Not relevant

# LEVEL 4

# Laminarin

# Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in the Annex I

**4.1 Identity of the active substance**

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**4.2 Physical and chemical properties of the active substance**

- IIA 2.9.1 : Ongoing supplementary hydrolysis study, addressing eventual hydrolysis into smaller sized oligosaccharides (Huntingdon Life Sciences study protocol N° GOM/004), should be submitted as soon as the report becomes available.

- IIIA 2.7.1 : Justification is required for the fact that the difference between the determined a.s. content of PHYLIQ and that declared exceeds the FAO tolerance.

- IIIA 2.7.3 : Ongoing shelf life study on PHYLIQ at ambient temperature in commercial packaging (incl. assessment of resistance of packaging material to its contents – IIIA 4.1.3) should be submitted as soon as the report becomes available.

**4.3 Data on application and further information**

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**4.4 Classification, packaging and labelling**

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**4.5 Methods of analysis**

-

**4.6 Toxicology and metabolism**

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**4.7 Residue data**

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**4.8 Environmental fate and behaviour**

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**4.9 Ecotoxicology**

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