

ANNEX B

Laminarin

B.3 Further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 to 3.6)**B.3.1.1 Function (Annex IIA 3.1)**

“Elicitor of the crop’s self-defence mechanisms.”

B.3.1.2 Effects on harmful organisms (Annex IIA 3.2.1)

“Laminarin has no direct effect on harmful organisms. It stimulates the natural defences of the plant against pathogens. The a.s. enhances defence reactions which inhibit the development of the pathogens”

Phyliq (SL containing 37 g/L laminarin) will be used alone or in combination with other synthesis fungicides for the protection of cereals against pathogens. It protects the cereals with lasting effects for 45 days.

Alone, its efficacy is about 50%, therefore it will be used in a fungicidal program which will allow to reduce the amount of synthesis fungicide applied. Field trials were performed with laminarin application (1 cm-ear) followed by a normal fungicide application program.

B.3.1.3 Translocation in plants (Annex IIA 3.2.2)

“Laminarin is a natural oligosaccharide with a molecular weight of # 5 000 g.mol⁻¹. As such it is probably not transported in the plants. 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.”

B.3.1.4 Fields of use (Annex IIA 3.3)

Agriculture

B.3.1.5 Pests controlled and crops protected (Annex IIA 3.4.1, 3.4.2)

Crop	Crop code	Disease - <i>pathogen</i>	Disease code
Winter wheat	TRZAW	eyespot <i>Pseudocercospora herpotrichoides</i>	PSDCHE
		septoria leaf spot <i>Septoria tritici</i>	SEPTTR
		powdery mildew <i>Erysiphe graminis f. sp. tritici</i>	ERYSGT
Barley, spring and winter	HORVS HORVW	powdery mildew <i>Erysiphe graminis f. sp. tritici</i>	ERYSGT

Crop	Crop code	Disease - <i>pathogen</i>	Disease code
		Helminthosporium leaf spot <i>Helminthosporium spp.</i>	HELMSP
		rhynchosporium leaf spot <i>Rhynchosporium secalis</i>	RHYNSE

B.3.1.6 Effects achieved - mode of action (Annex IIA 3.4.3, 3.5.1)

“ Treatment of plant cell cultures as well as treatment of whole plant with Laminarin induces metabolic changes typical of defence responses : early events (such as ionic fluxes, phosphorylations, oxidative burst), followed by stimulation of the phenylpropanoic- and lipid-derived pathways (leading to signals like salicylic acid and jasmonates respectively), and production of PR-proteins (known for their high antimicrobial potential).

While Laminarin has no direct antifungal activity, spraying with Laminarin at low rates (37 g.ha⁻¹) efficiently protects cereals against infection by fungi, viruses, bacteria.

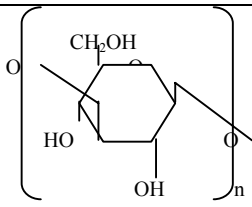
It is believed today that smaller-sized oligosaccharides are the "active" parts of the molecule, that they are recognised by the cell-wall membranes and are therefore responsible for the stimulation of the natural defences of the plant. (Klarzynski et al, 2000)

β-1,3-1,6-oligosaccharides were recognized to be actively involved in plant-pathogens interactions. The structure of an active β-glucoside was determined. (Albersheim et al, 1992)”

B.3.1.7 Information relative to the formation of active metabolites and degradation products (Annex IIA 3.5.2, 3.5.3)

“Beside an active hepta-β-glucoside, the most probable other smaller-sized oligosaccharides are the following :

Table : Information on putative active degradation products

Chemical name	ISO common name	CAS Number	EINECS Number	Empirical formula	Molecular Mass g/mol	Structural formula
Oligolaminarans	-	-	-	(C ₆ H ₁₂ O ₆) _n 7 < n < 25	> 1153 < 4500	 <p>n = 1 to 25</p>
Laminariheptaose	-	-	-	(C ₆ H ₁₂ O ₆) ₇	1153	
Laminarihexaose	-	-	-	(C ₆ H ₁₂ O ₆) ₆	990	
Laminaripentaose	-	-	-	(C ₆ H ₁₂ O ₆) ₅	828	
Laminaritetraose	-	-	-	(C ₆ H ₁₂ O ₆) ₄	666	
Laminaritriose	-	-	-	(C ₆ H ₁₂ O ₆) ₃	504	
Laminaribiose	-	-	-	(C ₆ H ₁₂ O ₆) ₂	342	
Glucose	D-glucose	5996-10-1	200-075-1	C ₆ H ₁₂ O ₆	180	

In plants, the occurrence of natural β -glucans and consequently of β -glucanases has been known for a long time and suggests that the main role of such enzymes is in the mobilisation of intracellular food reserves in germination and growth processes, and in the extracellular digestion of polysaccharides to provide carbon compounds. (Bull, 1967)”

Plants have evolved signalling mechanisms to regulate the expression of genes that are essential for their growth, development and defence against pests. Some of these signals or regulatory molecules (elicitors) are oligosaccharides (oligosaccharides with regulatory activities are also called "oligosaccharins"). (Côté et al., 1994)

The enzymatic degradation of Laminarin by natural β -1,3-glucanases affords oligolaminarans, glucose and intermediary oligosaccharides : di- to hepta-saccharides, which are recognized to play an essential role as biological signals in the plant-pathogen interaction, beside their role as a carbon source. (Shevchenko et al., 1986)

In presence of Laminarin the naturally occurring β -1,3-glucanases of the plants recognize this substrate and hydrolyse it in smaller-sized oligosaccharides and ultimately in glucose. »

B.3.1.8 Information on the possible occurrence of the development of resistance or cross-resistance (Annex IIA 3.6)

“Laminarin being a natural product and having no direct effect on the pathogens (it stimulates several pathways in the plant cell), there is no chance that resistance or cross-resistance could develop.”

B.3.2 Data on application relevant to the plant protection products (Annex IIIA 3)

B.3.2.1 Fields of use (Annex IIIA 3.1)

See B.3.1.4

B.3.2.2 Nature of the effects on harmful organisms (Annex IIIA 3.2)

See B.3.1.2

B.3.2.3 Pests controlled and crops protected (Annex IIIA 3.3)

Rate of application (Annex IIIA 3.4)

Concentration of active substance in material used (Annex IIIA 3.5)

Description of the method of application, type of equipment used and type and volume of diluent per unit of area or volume (Annex IIIA 3.6)

Number and timing of applications and duration of protection afforded (Annex IIIA 3.7)

Details of the intended uses are given in the table below.

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	-	

B.3.2.4 Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops - Limitations on choice of succeeding crops (Annex IIIA 3.8)

No waiting period is needed due to the easy biodegradability of the active substance in soil.

No limitation on choice of succeeding crops

B.3.2.5 Proposed instructions for use as printed, or to be printed, on labels (Document C)

A draft label was proposed for the formulation

B.3.3 Summary of data on application

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B.3.4 Further information on the active substance (Annex IIA 3.7 to 3.9)

B.3.4.1 Recommended methods and precautions relating to handling, storage, transport, fire (Annex IIA 3.7)

This information is presented under the form of a safety data sheet pursuant to Article 27 of Council Directive 67/548/EEC.

Hazards identification : Not a dangerous product

Handling : Handle carefully to avoid accidental dispersion.
 Wash hands with water after handling the product.
 Do not eat or drink during the handling.

Personal protection : no specific recommendations

Storage : Keep the product into original container.
 Containers must be labelled and kept closed.
 Store away from food, drink, farm animals and animal feedingstuffs.

Transport : • Classification Rail / Road RID / ADR : not regulated
 • Classification Maritime : not regulated
 • Classification Air : not regulated

Fire : *Extinguishing media* : Water, foam, CO₂, multi-purpose dry powder extinguishers. Class A fire.

Precautions : Evacuate the place.
 Provide for holding contaminated water.
 Avoid dust formation.

Protective equipment : Professionals : use a self contained breathing apparatus.

B.3.4.2 Procedures for destruction or decontamination of the active substance, contaminated packaging and contaminated materials

B.3.4.2.1 Controlled incineration - Pyrolytic behaviour under controlled conditions at 800°C (Annex IIA 3.8.1)

The active substance and contaminated material or packaging can be destroyed by controlled incineration.

The halogen content of Laminarin is less than 60%; information on the pyrolytic behaviour of the active substance is thus not required.

B.3.4.2.2 Methods other than controlled incineration for disposal of the active substance, contaminated packaging and contaminated materials (Annex IIA 3.8.2)

No other methods are proposed. Recommended method is controlled incineration

B.3.4.3 Methods for decontamination of water in the case of an accident (Annex IIA 3.9)

Laminarin being a natural product extracted from sea algae, no special procedure is necessary for decontamination of water. In case of an accident, Laminarin will be rapidly diluted in water and further hydrolysed to smaller oligosaccharides and ultimately to glucose.

B.3.5 Further information on the plant protection product PHYLIQ (Annex IIIA 4)

B 3.5.1 Packagings, suitability of the packaging material to its content (Annex IIIA 4.1)

B.3.5.1.1 Description and specification of the packaging and materials used in packaging, size, capacity, size of openings, type of closure and seals (Annex IIIA 4.1.1)

Table B.3.5.1.1-1 : Description of receptacle for PHYLIQ

Type :	5 L polyethylene container
Material :	HDPE (density = 1.4)
Manner of construction :	Extrusion
Opening :	63 mm diameter
Shape/Size :	square, handle isolated from the content L * l * h = 192 mm * 140 mm * 302 mm
Capacity :	5 L
Closure :	screw cap with additional tamper evident, e.g. sealing disk
Combined outer packaging :	cardboard box for 4x5L dimensions : 380 mm x 279 mm x 305 mm

B.3.5.1.2 Suitability of the packaging and closures (Annex IIIA 4.1.2)

Packaging and closure are very common ones, widely used for soluble concentrates.

UN registration N° : 3H1/Y 1,4/100/-
F/BVT 222523/AP

Copy of certificate N° 3078 dated 31/08/98 was submitted

B.3.5.1.3 Resistance of the packaging material to its contents (Annex IIIA 4.1.3)

Packaging material is widely used for soluble concentrates

Shelf life study demonstrating the resistance of the packaging material to its contents according to GIFAP Monograph N° 17 is ongoing (report to be submitted, once available). Until now, the storage test at ambient temperature has shown no apparent transformation of the packaging.

B.3.5.2 Procedures for cleaning application equipment and protective clothing (Annex IIIA 4.2)

Rinsing with water and detergent.

Effectiveness of the cleaning procedures :

The product is easily soluble in water. It can be removed from surfaces with water. The addition of detergent enhances the cleaning process.

B.3.5.3 Re-entry intervals, waiting periods and other precautions to protect man, livestock and the environment

B.3.5.3.1 Pre-harvest intervals, re-entry intervals or withholding periods to minimize residues in crops, plants, plant products, treated areas or spaces (Annex IIIA 4.3.1)

Pre-harvest and re-entry periods of PHYLIQ :

Pre-harvest interval (in days) for each relevant crop :	No PHI is required as there will be only one application at BBCH 29-30 for each cereal crop
Re-entry period (in days) for livestock to areas to be grazed :	Not relevant : no grazing of cereals
Re-entry period (in hours or days) for man to crops, buildings or spaces treated :	PHYLIQ will only be applied outdoor. According to GAP, re-entry is not recommended before the treatment has dried. A specific re-entry period is not required
Withholding period (in days) for animal feedingstuffs :	As no significant residues are expected in crop parts used as animal feed, and hence no expected residues in food of animal origin, a withholding period before milking or slaughter is not necessary
Waiting period (in days) between application and handling treated products :	Not relevant : crop is not handled before harvest
Waiting period (in days) between last application and sowing or planting succeeding crops :	Waiting period is not needed due to the easy biodegradability of the active substance in soil

B.3.5.3.2 Information on any specific agricultural, plant health or environmental conditions under which the preparation may or may not be used (Annex IIIA 4.3.2)

None of the test results obtained or observations made were such that restrictions should be imposed.

B.3.5.4 Recommended methods, precautions and handling procedures to minimize the risks relating to warehouse storage, user level storage, transport, fire - Detailed procedures for use in the event of an accident during transport, storage or use (Annexes IIIA 4.4 and 4.5)

This information is presented under the form of a safety data sheet pursuant to Article 27 of Council Directive 67/548/EEC.

Handling : Handle carefully to avoid accidental dispersion and spatter.

Do not eat, drink or smoke when handling the product.

Wash hands with water after handling.

Personal protection : No protective equipment specifically required, as PHYLIQ is of very low acute toxicity

Gloves and coverall are recommended.

Wash hands before breaks and at the end of work.

Storage : Store in a dark and temperate place, to maintain quality.

Keep the product in its original container. Containers must be labelled and kept closed.

Store away from children, domestic animals, food, drink and animal feedingstuffs.

Transport : • RID / ADR regulation : not concerned

• OMI regulation : not concerned

• IATA regulation : not concerned

Fire :

Extinguishing media : water, CO₂, foam or multi-purpose dry powder extinguishers

Combustion products : no special danger from combustion products

Precautions : Fight fire in early stages only if safe to do so.

Provide for holding contaminated water.

Protective equipment : Wear respiratory protection.

Procedures to minimize the generation of waste :

Rinse the container and pour rinsing water into spraying tank.

Only purchase and store quantities of product required in the short term.

Do not open larger containers than is necessary for immediate requirements.

Do not mix a volume of spray solution greater than is required for immediate use.

Accidental release measures :

Containment of spillages : Prevent entry into drains, waters or soil. Use a neutral absorbent material to collect spillage and sweep it as much as possible. Flush residues with water.

Decontamination of areas, vehicles and buildings : Use a damp cloth to clean floors and other objects after removal of contaminated absorbent.

Disposal of damaged packaging, adsorbents and other materials : Do not reintroduce into original container. Place contaminated absorbent and used cleaning materials into closable and identified containers. Treat as waste.

Protection of emergency workers and bystanders : Use the personal protective equipment proposed above.

First aid measures :

General information : Remove victims from the danger zone. Do not induce vomiting; do not give anything to drink or eat.

Upon inhalation : Bring accident victims out into the fresh air

Upon swallowing : Wash out mouth with water, keep the victim at rest and take medical advice.

Following skin contact : Wash skin with water. Remove soiled or soaked clothing.

Following eye contact : Rinse eyes thoroughly with water. Seek specialist medical advice if irritation develops.

B.3.5.5 Procedures for destruction or decontamination of the formulation and its packaging

B.3.5.5.1 Neutralization procedures for use in the event of accidental spillages (Annex IIIA 4.6.1)

Not relevant as PHYLIQ is of limited acidity (pH # 4), and is anyway of very low acute toxicity.

B.3.5.5.2 Controlled incineration - Pyrolytic behaviour of the active substance under controlled conditions at 800E C (Annex IIIA 4.6.2)

Detailed instructions for safe disposal :

Package product wastes. Close and label waste receptacles and, likewise, any uncleaned empty containers. Dispose of them at a suitable waste incineration plant, in accordance with the official regulations. Where large quantities are concerned, consult the supplier.

The halogen content of Laminarin is less than 60%; information on the pyrolytic behaviour of the active substance is thus not required.

B.3.5.5.3 Methods other than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated materials (Annex IIIA 4.6.3)

No other methods are currently available.

B.3.6 References relied on

Annex Point / Reference number	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	Owner
IIA 3.5/01	KLARZYNSKI O., PLESSE B., JOUBERT J.M., YVIN J.C., KOPP M., KLOAREG B., FRITIG B.	2000	Linear β -1,3 glucans are elicitors of defense responses in Tobacco. Plant Physiology, vol 124, 1027-1037 Non-GLP, published	N	-
IIA 3.5/02	ALBERSHEIM P., DARVILL A., AUGUR C., CHEONG J.J., EBERHARD S., HAHAN M.G., MARFA V., MOHNEN D., O'NEILL M.A., SPIRO M.D., YORK W.S.	1992	Oligosaccharins : oligosaccharide regulatory molecules Acc. Chem. Res. 25, 77-83 Non-GLP, published	N	-
IIA 3.5/03	BULL A.T.	1967	The enzymatic degradation of β -glucans. Int. Biodetn Bull., 3, (1), 3-12 Non-GLP, published	N	-
IIA 3.5/04	CÔTE F., HAHN M.G.	1994	Oligosaccharins : structures and signal transduction. Plant Molecular Biology 26 , 1379-1411 Non-GLP, published	N	-
IIA 3.5/05	SHEVCHENKO N.M., ZVYAGINTSEVA T.N., ELYAKOVA L.A.	1986	Mode of action of endo-(1 \rightarrow 3)- β -D-glucanases from marine molluscs on the laminarin from <i>Laminaria cichorioides</i> : the structure and teh inhibitory effect of the resulting (1 \rightarrow 3;1 \rightarrow 6)- β -D-gluco-oligosaccharides. Carbohydrate Research, 148, 57-62 Non-GLP, published	N	-