

European Commission



VOLUME 3 – Annex B (AS)

Laminarin

B.8 Environmental fate and behaviour

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.8

B.8	Environmental fate and behaviour.....	4
B.8.1.	Fate and behaviour in soil	4
B.8.2.	Fate and Behaviour in Water and Sediment (CA 7.2)	7
B.8.3.	Fate and Behaviour in Air (CA 7.3).....	9
B.8.4.	Monitoring Data	10
B.8.5.	Definition of the Residue (CA 7.4)	10
B.8.6.	References relied on	11

B.8 Environmental fate and behaviour

B.8.1. Fate and behaviour in soil

No study has been conducted on the behaviour of Laminarin in soil. The active substance is a polysaccharide of # 25 glucosyl units ($M \# 5\,000\text{ g.mol}^{-1}$), so can be compared to cellulose for its fate and behaviour in soil, which is well known in the literature.

B.8.2.1. Route and rate of degradation in soil (CA.7.1.1)

CA 7.1.1.1 Aerobic degradation

No study was submitted in support of the Laminarin inscription in Annex I of the Directive 91/414/EEC. In the original assessment a scientific reasoning was presented to address the fate and behavior of laminarin in soil. This reasoning is considered still acceptable and presented in the current dossier.

Previous evaluation:	In DAR (2003)
----------------------	---------------

An extensive literature search has been performed by the notifier on:

- the distribution of β -1,3 glucans in various plants :

Brown algae such as the *Laminaria* genus store carbohydrate as laminarans, 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.

- β -1,3 glucans are also a major constituents of cell walls in yeast, filamentous fungi.

- Callose is a β -1,3 glucan which is found in a variety of situations in the tissues of higher plants : sieve tubes, young tracheids, lactifers, anthers and pollen mother cells, pollen grain and pollen tubes, root hairs, leaf and stem hairs, cystoliths, pericarp, abscission tissue, lenticels, root endodermis, pit canals and wound tissue.

- Lichenin, oat glucan and barley glucan are closely related and have a β -1,3 β -1,4 glucan structure. (review by Clarke *et al*, 1963)

- the occurrence of various types of glucanases and more specifically laminarases (also known as β -1,3-glucanases)

Various soil microorganisms (eubacteria, streptomycetes, fungi, algae, protozoa), soil macroorganisms (annelids, arthropods, molluscs) and higher plants possess β -1,3-glucanases. Laminarinase activity is the result of a multi-enzyme system (review by Clarke *et al*, 1963 ; review by Bull *et al*, 1966) and appear to be implicated in the intracellular mobilization of food reserve; extracellular depolymerisation of plant debris by microorganisms; and the digestive metabolism of vertebrates.

- the β -1,3-glucanase activity in soil

The effect of various physical and chemical treatments on accumulated β -1,3-glucanase activity in soil was investigated in the laboratory. Enzyme activity was remarkably constant during long term storage of unamended soil irrespective of incubations conditions. The enzyme system was unaffected

by flooding and NPK fertilizer, but was stimulated by pig-slurry and glucose and inhibited by ground limestone. (Lethbridge *et al*, 1980). In this study laminarin breakdown products were analysed for. The absence of laminaridextrins suggests that this soil either possesses very active β -glucosidase(s) which hydrolyse any laminaridextrins as fast as they are produced by an endo- 1,3- β -glucanase, or that exo- 1,3- β -glucanase releasing glucose was the only laminarin hydrolysing activity present. Glucose was the only detectable breakdown product of laminarin in soil.

- the role of laminarase in the biological control of pathogenic fungi

'A number of bacteria lysing *Fusarium oxysporum* were isolated from soil. One of these, a strain of *Bacillus cereus* was studied in detail. Lysis of *Pythium*, *Streptomyces*, *Agrobacterium* and *Pseudomonas* species appeared to be associated with chitinase and laminarase activity of *B. cereus*' (Mitchell, 1962)

- 'Soil treatment with either of 2 fungal cell-wall constituents, chitin or laminarin, resulted in decline in severity of diseases caused by several soil-borne fungal pathogens (*Fusarium* species). The evidence indicated that adding chitin or laminarin to soil stimulated a microbial flora capable of digesting mycelium of specific fungi' (Mitchell, 1963),

- the analytical determination of β -1,3-glucanase activity in soil (Hayano, 1973) (Lethbridge *et al*, 1978)

The main conclusions which can be drawn from this literature survey is that :

- β -1,3- glucanases 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.
- The DT50soil of laminarin cannot be determined easily. Nevertheless, soil persistence should not exceed a few weeks.

CA 7.1.1.2 Anaerobic degradation

The following literature data has been submitted previously in support of Laminarin inscription in Annex I of the Directive 91/414/EEC. It was already assessed and considered as valid and acceptable for the risk assessment. Therefore, reference data are not submitted again in the present dossier.

In anaerobic conditions, this easier degradation due to the smaller size has been demonstrated. Mountfort and Rhodes (1991) studied the anaerobic degradation of mono-, di- or poly-saccharides by *Paecilomyces lilacinus*, a fungus found in mullet's gut, but also widely reported in nature, including many soils and in sediments from estuarine habitats. It appeared that, beside glucose itself, the best

growth of the fungus was supported by Laminarin, followed by monosaccharides or disaccharides ; no growth occurred with cellulose.

CA 7.1.1.3 Soil photolysis

Laminarin having a low molar decadic absorption coefficient ($\epsilon = 160 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$), no photodegradation is expected to occur on the ground after application.

B.8.2.1. Rate of degradation in soil (CA 7.1.2)

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

No study is available. In the point CA 7.2.2.1, a study demonstrates that Laminarin is readily biodegradable. In the Technical Guidance Document on Risk Assessment¹, it is expected that for a substance readily biodegradable a DT_{50} in soil of 30 days can be used as default.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

No data, not required. Laminarin is a polysaccharide which leads to smaller-sized oligosaccharides and monosaccharides (glucose) after degradation. No other relevant metabolites, degradation or reaction products are expected.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

No data, not required.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

No data, not required.

CA 7.1.2.2.2 Soil accumulation studies

No data, not required.

B.8.2.2. Absorption and desorption in soil (CA 7.1.3)

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

No data is submitted nor required. No risk assessment for groundwater is required as no exposure to groundwater is expected.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

¹ Technical Guidance Document on Risk Assessment, Part II, European Chemicals Bureau, 2003

No data, not required. Laminarin is a polysaccharide which leads to smaller-sized oligosaccharides and monosaccharides (glucose) after degradation. No other relevant metabolites, degradation or reaction products are expected.

B.8.2.3. Mobility in soil (CA 7.1.4)

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No data provided, not required.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No metabolite is expected for Laminarin therefore no study is conducted. No study required.

CA 7.1.4.2 Lysimeter studies

No data provided, not required.

CA 7.1.4.3 Field leaching studies

No data provided, not required.

B.8.2. Fate and Behaviour in Water and Sediment (CA 7.2)

B.8.2.1. Route and rate of degradation in aquatic systems (chemical and photochemical degradation) (CA 7.2.1)

CA 7.2.1.1 Hydrolytic degradation

In Point CA 2.8, it has been shown that Laminarin is stable in sterile water at ambient temperature at pHs 4, 7 and 9. Beside smaller-sized oligosaccharides and glucose, no relevant metabolite, degradation or reaction product is expected to appear in any circumstances.

CA 7.2.1.2 Direct photochemical degradation

Laminarin being considered as stable to light due to its low molar decadic absorption coefficient, smaller sized oligosaccharides will have even lower coefficients, so will be even less subject to photochemical degradation.

CA 7.2.1.3 Indirect photochemical degradation

Not required.

B.8.2.2. Route and rate of biological degradation in aquatic systems (CA 7.2.2)

CA 7.2.2.1 "Ready biodegradability"

The following study was submitted in support of the Laminarin inscription in Annex I of the Directive 91/414/EEC. It was already assessed and considered as valid and acceptable for the risk assessment. Therefore, only the summary of the study is submitted in the present dossier.

Report :	LICATA-MESSANA L. (2000) ; Ready Biodegradability – Modified Sturm test – SEPC, Andrézieux-Bouthéon, France Unpublished report N°00-907005-024, 25/10/2000 Dates of experimental work : 25/07/2000 to 24/08/2000
Guidelines :	OECD No.301B (1992) – EEC Directive 92/69 C4C (1992) Deviation: Temperature of the media; for # 1 day, the temperature of the media was higher than the maximum requested (28.6°C maximum instead of 24°C maximum).
GLP :	Yes (certified laboratory)

Previous evaluation:	In DAR (2003)
----------------------	---------------

Material and Methods :

Test material : laminarin code H11; batch S012000, purity on dry matter 88.9%

Test conditions : 37.49 mg a.s./l has been incubated in a water medium inoculated with activated sewage micro-organisms at 20.3-28.6°C, pH 7.31-7.49. The biodegradation was monitored over a 28-day period by means of CO₂ analysis, CO₂ being released and incorporated to BaCO₃. Control assay without any test substance and assay using sodium acetate as reference substance were run in simultaneously.

Findings :

- Biodegradation of laminarin :

Biodegradation of the test item after 28 days	:	76 %
Biodegradation of the test item at the end of the 10-day window	:	65 %
Biodegradation of the reference item after 14 days	:	71 %
Biodegradation of the test item in the toxicity control after 14 days	:	65 %

Conclusions :

Laminarin is readily biodegradable in the conditions of the test.

CA 7.2.2.2 Aerobic mineralization in surface water

No study is presented nor required, please refer to CA 7.2.2.3, below.

CA 7.2.2.3 Water/sediment studies

The following information was submitted in support of the Laminarin inscription in Annex I of the Directive 91/414/EEC. It was already assessed and considered as valid and acceptable for the risk assessment. Therefore, the information is submitted in the present dossier.

No study required :

- The a.s. is readily biodegradable (DT₅₀ a.s. | CO₂ ~ 8 days)
- The degradation of laminarin by micro-organisms would lead to smaller-sized oligosaccharides and glucose.

For the current dossier no study was submitted. The following information is provided instead to address the data point.

In point CA 7.2.2.1, a study demonstrates that Laminarin is readily biodegradable. It is expected that Laminarin (which is stable in sterile water) will be relatively stable in this non-sterile water, but will be readily degraded by the micro-organisms. Therefore no study has been conducted as the outcome would only depend on the equilibrium between the water phase and the soil phase.

In the Technical Guidance Document on Risk Assessment², it is expected that for a substance readily biodegradable a DT₅₀ in water of 15 days can be used as default.

CA 7.2.2.4 Irradiated water/sediment study

Laminarin being considered as stable to light due to its low molar decadic absorption coefficient, smaller sized oligosaccharides will have even lower coefficients, so will be even less subject to photochemical degradation. No study is required.

B.8.2.3. Degradation in the Saturated Zone CA 7.2.3)

Laminarin being stable in sterile water, it is anticipated that limited degradation would occur in the saturated zone. Therefore any product reaching the groundwater would hardly be degraded. However due to the ready biodegradability of the compound and to its sensitivity to the attack from many bacteria strains, it will be degraded in smaller sized oligosaccharides and ultimately to glucose before reaching the water table.

B.8.3. Fate and Behaviour in Air (CA 7.3)

B.8.3.1. Route and rate of degradation in air (CA 7.3.1)

The following information was submitted in support of the Laminarin inscription in Annex I of the Directive 91/414/EEC. It was already assessed and considered as valid and acceptable for the risk assessment. Therefore, the information is submitted in the present dossier.

Laminarin having a very low vapour pressure ($< 2.6 \cdot 10^{-5}$ Pa at 25°C) and a very low Henry's law constant ($< 1.5 \cdot 10^{-6}$ Pa.m³.mol⁻¹), no risk of volatilization is to be expected in the recommended conditions of use. In addition, the vapour pressure is expected to be $< 10^{-5}$ Pa at 20°C, laminarin can be considered non-volatile according to FOCUS air guidance.

Previous evaluation:	In DAR (2003)
----------------------	---------------

B.8.3.2. Transport via air (CA 7.3.2)

Not required.

B.8.3.3. Local and global effects (CA 7.3.3)

Not required.

² Technical Guidance Document on Risk Assessment, Part II, European Chemicals Bureau, 2003

B.8.4. Monitoring Data

No monitoring data is available. Indeed, monitoring appears to be extremely difficult as no analytical method sensitive enough is available at this moment: the most sensitive method today hardly reaches the 100 mg/L level. With the quantity of Laminarin applied by year, no contamination at the above LOQ level is conceivable.

B.8.5. Definition of the Residue (CA 7.4)

CA 7.4.1 Definition of the residue for risk assessment

The following information was submitted in support of the Laminarin inscription in Annex I of the Directive 91/414/EEC. It was already assessed and considered as valid and acceptable for the risk assessment. Therefore, the information is submitted in the present dossier.

The notifier proposes this residue definition : 'Laminarin being a polysaccharide, its decomposition in the environment will give rise to oligosaccharides and to glucose. The most significant residue in soil would therefore be glucose itself, what is in fact the most accurate way to quantify laminarin after total acid hydrolysis.'

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.

For the current assessment the following information was provided:

Laminarin being a polysaccharide, its decomposition in the environment will give rise to oligosaccharides and to glucose. The most significant residue in the environment would therefore be glucose itself, what is in fact the most accurate way to quantify Laminarin after total acid hydrolysis. This will be better achieved in water and air where the background level should be rather low. In soil however it is expected that background will be much higher and a high variability must be expected, due to the abundance of mono-, di- or poly-saccharides in soil from arable zones.

CA 7.4.2 Definition of the residue for monitoring

No residue is expected for monitoring.

B.8.6. References relied on

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:	submitted for the purpose of renewal, essential
----------------------	---

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

Table B.8.6-01: 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

Table B.8.6-02: 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	2. Laminaran	24

name (CA)		
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

- soil OR degradation OR metabolites OR photolysis OR soil residues
- soil accumulation OR soil contamination
- mobility OR adsorption OR desorption OR lysimeter
- modelling OR PEC OR Focus modelling
- groundwater OR leaching
- *water OR water* OR sediment OR dissipation OR saturated zone OR hydrolysis OR photo transformation OR biodegradability
- drift OR run-off OR drainage
- air OR volat* OR atmosphere OR long-range transport OR short-range transport

Table B.8.6-3:0 Search process for fate and behaviour

Data requirements captured by the search	Search: PubMed and ScienceDirect
All data requirements together	<p>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.</p> <p>ScienceDirect is a leading full-text scientific database offering journal articles and book chapters from more than 2,500 journals and almost 20,000 books.</p> <p>Date of the search: 11/03/2014 (PubMed), 03/10/2014 (ScienceDirect)</p> <p>Date span of the search: 11/03/2004 – 11/03/2014 (PubMed) 2004 to present (ScienceDirect)</p> <p>Date of the latest database update included in the search: 11/03/2014 (PubMed) 03/10/2014 (Science Direct)</p> <p>Search strategies used for this data requirement (including any limits):</p> <p>Total number of summary records retrieved: 1081</p> <p>Total number of <u>not relevant</u> summary records: 1081</p> <p>Total number of <u>not clearly relevant</u> summary records: 0</p> <p>Total number of relevant summary records: 0</p>

Table B.8.6-04: Search terms for fate and behaviour

Database: PubMed and ScienceDirect Search restrictions: PubMed: all fields ScienceDirect: Agriculture and environmental fate	Search terms		Number of summary records retrieved in Pubmed database	Number of summary records retrieved in ScienceDirect database
Active substance common name and ISO name	1.	Laminarin	308	71
Active substance chemical name (CA)	2.	Laminaran	24	21
Active substance Chemical Name (IUPAC)	3.	(1→3)-β-D-glucan	514	175
Without trade names	4.	Soil OR degradation OR photolysis OR soil residues	14	10
	5.	soil accumulation OR soil contamination	0	0
	6.	mobility OR adsorption OR desorption OR lysimeter	6	4
	7.	modeling OR PEC OR Focus modelling	4	2
	8.	groundwater OR leaching	0	0
	9.	*water OR water* OR sediment OR dissipation OR saturated zone OR hydrolysis OR photo transformation OR biodegradability	51	9
	10.	drift OR run-off OR drainage	1	0
	11.	air OR volat* OR atmosphere OR long-range transport OR short-range transport	17	22

Table B.8.6-05: Relevance criteria for the section fate and behaviour

Data requirement (indicated by the corresponding OECD data point number)	Criteria for relevance
Environmental Fate and Behaviour in soil (OECD IIA 7.1 - 7.4)	<ul style="list-style-type: none"> • Content of the papers addresses data requirements for environmental fate and behaviour in soil • Well defined test material applied as active substance solution or plant protection product (not as a by-product/ingredient of a soil amendment) • Substrate: representative agriculture used soils with well-defined soil properties (e.g. pH, organic carbon content, microbial biomass etc.) also field studies • No previous contamination of the soil • Exposure through active substance applied as a solution or through a commercial formulation, no mixtures with other active substances

Data requirement (indicated by the corresponding OECD data point number)	Criteria for relevance
Environmental Fate and Behaviour in water and sediment (OECD IIA 7.5 - 7.9)	<ul style="list-style-type: none"> •Content of the papers addresses data requirements for environmental fate and behaviour in water •Well defined test material (including its purity and impurity profile) •Test material used are samples from representative European aquatic resources (no contamination) •Exposure through active substance solution or plant protection product, no mixtures with other active substances
Environmental Fate and Behaviour in air (OECD IIA 7.10)	<ul style="list-style-type: none"> •Content of the papers addresses data requirements for environmental fate and behaviour in air •Well defined test material (including its purity and impurity profile) •No analytical papers • Representative sampling in Europe

Lists of relevant and non-relevant studies

Table B.8.6-06: List of studies considered as relevant or of unclear relevance, classified by author(s) – fate and behaviour

Authors	Data requirement (indicated by the corresponding OECD data point number)	Year	Title	Source
None of the hits retrieved (table 7.6-04) complies with the criteria for relevance (table 7.6-05). No information on the active substance in the environment is available.				

Table B.8.6-07: List of studies considered as relevant or of unclear relevance, classified by data requirement(s) - fate and behaviour

Data requirement (indicated by the corresponding OECD data point number)	Authors	Year	Title	Source
None of the hits retrieved (table 7.6-04) complies with the criteria for relevance (table 7.6-05). No information on the active substance in the environment is available.				

Table B.8.6-08: List of the studies excluded from the risk assessment after detailed assessment of the full-text documents, classified by author(s) - fate and behaviour

Authors	Year	Title	Source	Reason(s) for not including this study in the dossier
None of the hits retrieved (table 7.6-04) complies with the criteria for relevance (table 7.6-05). No information on the active substance in the environment is available therefore no detailed assessment was performed.				

RMS conclusion:

The literature search was well performed according to the guidance document. The search criteria as well as the relevance criteria are adequate. No relevant public literature was retrieved for the section fate and behaviour.

Reference list – Studies previously submitted for the original Annex I inclusion of laminarin.

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
CA 7.1.1.1.	Clarke et al.	1963	Chemistry and Biochemistry of β 1-3- glucan. A review. Non-GLP Published	N	N		/
CA 7.1.1.1.	Bull and Chesters	1966	The biochemistry of laminarin and the nature of laminarinase Non-GLP Published	N	N		/
CA 7.1.1.1.	Lethbridge et al	1980	Glucanase activity in soil Non-GLP Published	N	N		/
CA 7.1.1.1.	Micthell	1963	Lysis of soil fungi by bacteria Non-GLP Published	N	N		/
CA 7.1.1.1.	Hayano	1972	A method for the determination of β - glucosidase activity in soil Non-GLP Published	N	N		/
CA 7.2.2	LICATA- MESSANA L	2000	Ready Biodegradability – Modified Sturm test SEPC Report n°00-907005- 024 GLP Unpublished	N	N		Laboratoires Goëmar S.A.S