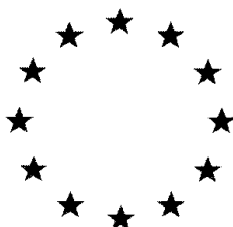


# **European Commission**



**VOLUME 3- Annex B (AS)**

**Laminarin**

**B.9 Ecotoxicology data**

**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
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## TABLE OF CONTENTS – VOLUME 3 B.9

B.9	Ecotoxicology data .....	4
B.9.1	Effects on birds and other terrestrial vertebrates .....	4
B.9.2	Effects on aquatic organisms .....	7
B.9.3	Effects on arthropods .....	13
B.9.4	Effects on non-target soil meso- and macrofauna.....	17
B.9.5	Effects on soil nitrogen transformation .....	21
B.9.6	Effects on terrestrial non-target higher plants .....	22
B.9.7	Effects on other terrestrial organisms (flora and fauna) .....	26
B.9.8	Effects on biological methods for sewage treatment.....	26
B.9.9	Monitoring data .....	26
B.9.10	Biological activity of metabolites potentially occurring in groundwater.....	26
B.9.11	References relied on .....	27

**B.9 Ecotoxicology data****B.9.1 Effects on birds and other terrestrial vertebrates****B.9.1.1 Effects on birds****B.9.1.1.1 Acute oral toxicity to birds**

Previous evaluation	In DAR (2003)
	The RMS has re-evaluated the study. It is acceptable for use in risk assessment. The doses were not adjusted for the purity of the test substance, which was stated as being > 85%. Since the purity is below the technical specification of >95%, the RMS has adjusted the dose to account for the purity of the test substance. Therefore, the LD <sub>50</sub> to be used in the risk assessment is <b>&gt;1700 mg/kg</b> . Since there were no effects at any dose, no LD <sub>10</sub> or LD <sub>20</sub> could be calculated.

Study CA 8.1.1/01

**Report:** (2002a); Laminarin – Acute oral toxicity (LD<sub>50</sub>) to the Bobwhite quail

Unpublished report N° GOM 001/022173, 2002

Dates of experimental work : 30/04/2001 to 28/12/2001

**Guidelines:** US-EPA 71-1 (1982), including draft revision of March 1988

Deviations: the analysis of the formulation administered has not been conducted due to the difficulty in analysing the test substance.

**GLP:** Yes (certified laboratory)**Material and** Test material: Laminarin; Batch N°S012000; Purity on dry matter: 85%**method :** The purpose of this test was to determine the acute oral toxicity of Laminarin to the Bobwhite quail: *Colinus virginianus*. Groups of 10 birds (5 males and 5 females) were administered the suspension of Laminarin in corn oil at respective doses of: 0-500-1000 and 2000 mg/kg, by gavage (oral intubation).

Birds were observed daily for 14 days post-treatment. Body-weights were recorded on Days 0-7-14. Food consumption was recorded weekly. Macroscopic post-mortem examination was conducted on all birds who died during the study, all control birds and 10 dosed birds.

**Findings:** There were no mortalities and there were no clinical signs of toxicity. Group mean bodyweights were unaffected by treatment. Food consumption was unaffected by treatment. No abnormalities were observed at *post-mortem* examination.**Conclusion:** Under the conditions of this study, the acute oral LD<sub>50</sub> value of Laminarin to the bobwhite quail must lie in excess of 2000 mg/kg, the maximum dose level used. The No Observed Effect Level (NOEL) was 2000 mg/kg.

**B.9.1.1.2 Short-term dietary toxicity to birds**

Previous evaluation	In DAR (2003)
	The RMS has re-evaluated the study and found it acceptable for use in risk assessment. There were no treatment-related mortalities, thus an $LC_{10}$ and $LC_{20}$ could not be calculated. The endpoint was not adjusted for the purity of the test substance, which was reported as being > 85%. Since this value is below the technical specification of > 95% purity, the RMS has adjusted the endpoint for use in risk assessment to <b><math>LC_{50} = &gt; 4250\text{ppm}</math></b> . Although the short-term toxicity values are no longer used in risk assessment, for the sake of comparison the RMS has also calculated the dietary intake in mg a.s./kg b.w./d using the actual bodyweight and food intake reported in the study report. The <b><math>LD_{50} = &gt; 1188\text{ mg a.s./kg b.w./d}</math></b> .

Study CA 8.1.1/02

**Report:** (2002b); Laminarin – Dietary toxicity ( $LC_{50}$ ) to the Bobwhite quail

Unpublished report N°GOM 002/014410, 23/04/2002

Dates of experimental work: 22/08/2001 to 08/09/2001

**Guidelines:** US-EPA 71-2 (1982), including draft revision of March 1988

Deviations: the analysis of the formulation administered has not been conducted due to the difficulty in analysing the test substance.

**GLP:** Yes (certified laboratory)**Material and** Test material: Laminarin; Batch N°S012000; Purity on dry matter: 85 %

**methods :** The purpose of this test was to assess the dietary toxicity of Laminarin to the Bobwhite quail: *Colinus virginianus*. Groups of 10 young birds were offered *ad libitum* for 5 days diets containing 0, 0, 156, 313, 625, 1250, or 5000 ppm Laminarin.

Birds were observed daily during the 5-day treatment period and during a 3-day post-treatment period. Mortalities, bird health and clinical signs, including the appearance of excreta, were recorded at each observation.

Group mean body-weights were recorded on Days 0-5 and 8.

Group mean food consumption was recorded daily during the treatment period and over Days 6 to 8.

Macroscopic *post-mortem* examination was conducted on all birds from the highest dose group and from one control group.

**Findings :** One bird from a control group and one bird from the group treated at 2500 ppm were found dead on Day 5. Both birds were subdued prior to death, although death of the treated bird was considered not to be related to treatment.

The excreta of each group of birds appeared normal. Bodyweight gains and group mean food consumptions were variable, and there was no evidence of a treatment-related effect.

No abnormalities were detected in any bird examined *post-mortem*.

**Conclusion :** The dietary LC<sub>50</sub> value of Laminarin was found to be in excess of 5000 ppm.  
The No Observed Effect Level was considered to be 5000 ppm.

#### B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

<i>Previous evaluation</i>	<i>In DAR (2003)</i>
	<i>The RMS has re-evaluated the literature studies presented and slightly adjusted the summary presented below.</i>

Literature data was submitted to address this point for the original Annex I inclusion of laminarin. These data remain the same, but the summary has been slightly adjusted by the RMS.

Due to the natural origin and molecular structure of the test substance and to the very low toxicity observed in the acute oral study and in the dietary study, as well as to the desire to minimize vertebrate testing, no sub-chronic or reproduction study was conducted.

Laminarin is a polysaccharide which belongs to the  $\beta$ -glucans.  $\beta$ -glucans are a major component of the soluble fibers fraction of cereals, including those fed to poultry. For example,  $\beta$ -glucans are a natural component of barley, which contains approximately 40 g  $\beta$ -glucan per kilogram seeds ( or 320 kg  $\beta$  glucan/ha, assuming a harvest of 8000 kg seeds/ha) (Bergh M.O. *et al.*, 1999, CA 8.1.1/03, and Vukic Vranjes M. *et al.*, 1995, CA 8.1.1/05).

References in literature do not mention any significant mortality due to the ingestion of  $\beta$ -glucans. In an 18 day study in chickens, higher  $\beta$ -glucan (soluble and non-soluble) in the diet was found to result in lower feed-conversion. This effect was ameliorated when  $\beta$ -glucanase was added to the diets. No other effects were noted in the study (Bergh an M.O. *et al.*, 1998, CA 8.1.1/03). Another study looked at the effect of commercial enzyme mixtures on the feed conversion, intestinal viscosity and natural enzyme expression of broilers from age 1 day to age 22 days (Annison G., 1992, CA 8.1.1/04). Again, no other effects or toxicity was noted. Male broilers seemed to adapt to  $\beta$ -glucan-containing diets in other experiments where they were fed high  $\beta$ -glucan diet (~50% barley) over 36 to 54 days (from 1 day old), but the experiments provided no evidence to suggest that adaptation to dietary  $\beta$ -glucan involves the appearance of  $\beta$ -glucanase, whether of endogenous or bacterial origin, in the crop or small intestine of birds not receiving exogenous  $\beta$ -glucanase (Vukic Vranjes M. and Wenk C., 1995, CA 8.1.1/05 and Philip J.S. *et al.*, 1995, CA 8.1.1/06).

Newer studies with  $\beta$ -glucans suggest that oral administration of  $\beta$ -glucan isolated from mushrooms (*Pleurotus florida*) may have a positive effect of immune up-regulation when challenged with virus (Paul *et. al.*, 2012, CA 8.1.1/07), which is similar to effects seen in mammalian systems (see section 9.1.2).

#### B.9.1.2 Effects on terrestrial vertebrates other than birds

#### **B.9.1.2.1 Acute oral toxicity to mammals**

Please refer to section 6.1.1 of the MCA dossier for summary and evaluation of acute toxicity to mammals.

#### **B.9.1.2.2 Long-term and reproduction toxicity to mammals**

Please refer to Section B.6 (MCA) of this dossier for study summaries and discussion.

Due to the structure of Laminarin, which is similar to starch or glycogen, to the very high NOAELs observed in the 90-day studies on rats and dogs (absence of any treatment-related effect in two species), to the favorable conclusions of both rat and rabbit developmental toxicity studies (see Point CA 5.6.2) (absence of any treatment-related maternal or foetal effects in two species) and to the recognition of *Laminaria digitata* as a human food, no reproduction studies were conducted.

In order to avoid unnecessary vertebrate testing, no further long-term testing were conducted.

The NOAELs observed in the 90-day studies on rats and dogs were 1000 mg a.s./kg b.w./day.

#### **B.9.1.3 Active substance bioconcentration in prey of birds and mammals**

Not relevant as Laminarin has no bioaccumulation potential ( $\text{Log } K_{ow} = -1.6$ ).

#### **B.9.1.4 Other data on effects on terrestrial vertebrate wildlife (reptiles and amphibians)**

There is no data submitted to address effects on terrestrial vertebrates other than birds and mammals, and no literature data was found for those groups. Considering the nature of laminarin, the RMS concludes that a risk to other terrestrial vertebrates is unlikely.

#### **B.9.1.5 Potential for endocrine disruption**

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

### **B.9.2 Effects on aquatic organisms**

#### **B.9.2.1 Acute toxicity to fish**

Study CA 8.2.1-1

Previous evaluation	In DAR (2003)
	<i>The RMS has re-evaluated the study. Analytics were performed at t=0, 24, and 96 hours. At 0 and 96 hours the measured concentration of laminarin (expressed as D-glucose) was estimated at 80 and 55 mg/L, respectively, in the 100 mg/L concentration. At 0 hours the measured concentration was 140 mg/L (or 143 mg/L). The author of the analytical report attributes this to the fact that the limit of quantification of laminarin (as D-glucose) is ~100 mg/L. Since there were no effects at any time point at any concentration, and in the interest of animal welfare, the RMS finds the test nonetheless acceptable for use in risk assessment. Since there were no effects at any level, no LC<sub>10</sub> or LC<sub>20</sub> could be calculated. The 96 hour LC<sub>50</sub> is &gt; 100 mg/L (nominal).</i>

**Report:** (2001a); Acute toxicity in freshwater fish (96 Hours)- *Oncorhynchus mykiss* – Semi-static system

Unpublished report N° 00-907005-022, 22/01/2001

Dates of experimental work: 16/10/2000 to 27/10/2000

**Guidelines:** OECD N°203 (1992)

Directive 92/69/EEC - C1(1992)

Deviations: the size of few fish was higher than 8 cm and the biological load was higher than 1 g/L (it as 1.3 g/L). These deviations were not considered to have affected the quality or the interpretation of the results obtained.

**GLP:** Yes (certified laboratory)

**Material and** Test material: Laminarin; Batch N°S012000; Purity on dry matter: 89 %

**methods:** The purpose of this test was to determine the acute lethal toxicity of Laminarin to freshwater fish: *Oncorhynchus mykiss*. The freshwater fish was exposed to the Laminarin dilution water at a sole concentration: 100 mg/L, using a semi-static system, for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours.

**Findings:** The following results were obtained: no mortality was observed at any reading. LC<sub>0</sub> at each time of exposure:

Period of exposure	24 hours	48 hours	72 hours	96 hours
LC <sub>0</sub> (mg/L)	> 100	> 100	> 100	> 100

**Conclusion:** The LC<sub>0</sub> - 96 hours (and the LC<sub>50</sub> - 96 hours), for the freshwater fish (*Oncorhynchus mykiss*) is higher than 100 mg/L.



## Study CA 8.2.1-2

Previous evaluation	In DAR (2003)
	The RMS has re-evaluated the study. Analytics were performed at $t=0$ , 24, and 96 hours. At 24 and 96 hours the measured concentration of laminarin (expressed as D-glucose) was estimated at 80 and 55 mg/L, respectively, in the 100 mg/L concentration. At 0 hours the measured concentration was 140 mg/L (or 143 mg/L). The author of the analytical report attributes this to the fact that the limit of quantification of laminarin (as D-glucose) is ~100 mg/L. Since there were no effects at any time point at any concentration, and in the interest of animal welfare, the RMS finds the test nonetheless acceptable for use in risk assessment. Since there were no effects at any level, no $LC_{10}$ or $LC_{20}$ could be calculated. The 96 hour $LC_{50}$ is > 100 mg/L (nominal).

**Report:** (2001b); Acute toxicity in freshwater fish (96 Hours) - *Danio rerio* – Semi-static system

Unpublished report N°00-907005-021, 22/01/2001

Dates of experimental work: 09/10/2000 to 27/10/2000

**Guidelines :** OECD N°203 (1992)

Directive 92/69/EEC - C1(1992)

Deviations : none

**GLP :** Yes (certified laboratory)

**Material and** Test material: Laminarin; Batch N°S210300; Purity on dry matter: 98 %

**methods :** The purpose of this test was to determine the acute lethal toxicity of Laminarin to freshwater fish: *Danio rerio*. The freshwater fish was exposed to the Laminarin dilution water at a sole concentration: 100 mg/L, using a semi-static system, for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours.

**Findings :** The following results were obtained: no mortality was observed at any reading.

$LC_0$  at each time of exposure:

Period of exposure	24 hours	48 hours	72 hours	96 hours
$LC_0$ (mg/L)	> 100	> 100	> 100	> 100

**Conclusion :** The  $LC_0$  - 96 hours (and the  $LC_{50}$  - 96 hours), for the freshwater fish (*Danio rerio*) is higher than 100 mg/L.

## B.9.2.2 Long-term and chronic toxicity to fish

Previous evaluation	In DAR (2003)
	The RMS has re-evaluated the available literature, which is acceptable.

Literature data was submitted to support this data point in the original DAR. The studies were considered acceptable for use in the risk assessment.

Numerous studies have been conducted on fish showing that there is no long term toxicity after repeated application (Ref. 8.2.2/01, 8.2.2/02, 8.2.2/03, 8.2.2/04, 8.2.2/05). These studies investigate the effects of laminarin administration orally, intraperitoneally and intravenously for periods of several days to several weeks. The purpose of each is to investigate the use of laminarin as an immune modulator in fish farming, including investigation of metabolism and storage of laminarin in fish. No toxicity was mentioned in any of the tests, though certainly it would have been considering the purpose of the research. The RMS has looked at the public literature on laminarin in fish, which has been updated significantly since the original inclusion. All of these data seem to indicate that laminarin has a beneficial effect on the fish immune system.

As laminarin acts as an elicitor of the crop's self defence mechanisms, aquatic plants and algae are the most sensitive species. A study on aquatic plants runs over 7 days whereas a toxicity study on algae runs over 72 hours. Thus, it was deemed more relevant to perform a toxicity study on aquatic plants to address the chronic risk to aquatic organisms. Consequently, a toxicity study on *Lemna gibba* exposed to Laminarin is being performed. However, at the time of the initial draft of this RAR, the final study report is not yet available (see CA 9.2.7).

#### B.9.2.3 Potential for endocrine disruption

Laminarin is a carbohydrate with similar structure as starch or glycogen. No toxicity is expected after repeat application, and no effects on post-natal development are expected. Laminarin is not expected to have endocrine disruption properties.

#### B.9.2.4 Acute toxicity to aquatic invertebrates

Study 8.2.4/01

Previous evaluation	In DAR (2003)
	<i>The RMS has re-evaluated the study and found it acceptable for use in risk assessment. The validity criteria were met. The analysed content at 0 and 24 hours was &gt; 80% of nominal. The purity of the tested substance was just under 95% (94%). Thus, adjusted for purity, the nominal highest concentration was 94 mg/L. However, considering that the purity is only slightly under 95%, the RMS considers the value of &gt; 100 mg a.s./L to be acceptable for use in risk assessment. No EC<sub>10</sub> or EC<sub>20</sub> could be calculated, since there were no effects at the highest tested concentration.</i>

**Report:** HERTL J., (2001)  
 Acute toxicity of Laminarin to *Daphnia magna* in a 48-hour immobilisation test.  
 Unpublished report N°10041220, 29/03/2001  
 Dates of experimental work : 12/12/2000 to 14/12/2000

**Guidelines:** OECD N°202 (1984)  
 Deviations: none

**GLP:** Yes (certified laboratory)

- Material and methods:** Test material: Laminarin; Batch N°99S10; Purity: 94 %
- methods:** In order to assess the effect of Laminarin on the mobility/mortality of *Daphnia magna*, young *Daphnia* were exposed in a semi-static test for 48 hours to three concentrations of Laminarin: 10, 30 and 100 mg/L (nominal).
- Findings:** No reduced mobility and no mortality were observed at any concentration at any reading.
- Conclusion:** The 48 hour EC<sub>50</sub> for *Daphnia magna* is higher than 94 mg/L.

#### B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates

Laminarin is of natural aquatic origin (it originates from a brown sea alga) and is a polysaccharide of twenty-five glucosyl units. The degradation of Laminarin in water will result in smaller-sized oligosaccharides and, ultimately, glucose. Besides smaller oligosaccharides and glucose, no relevant metabolite, degradation or reaction product is expected to appear under any circumstances. The most significant residue in the environment would therefore be glucose itself. The ready biodegradability test performed with Laminarin shows that Laminarin is readily biodegradable with 76% of biodegradation after 28 days, which corresponds to a DT<sub>50</sub> of 15 days according to the TGD.

As Laminarin is degraded by micro-organisms as soon as it applied, aquatic organisms are exposed rapidly to oligosaccharides and glucose. Thus, long-term exposure to aquatic insects is not expected.

Considering this, and the fact that laminarin is not an insecticide, no Guideline study has been conducted for the chronic toxicity of Laminarin to aquatic invertebrate species.

As Laminarin acts as an elicitor of the crop's self defence mechanisms, aquatic plants and algae are the most sensitive species. Consequently, a toxicity study on *Lemna gibba* exposed to Laminarin was performed and summarised under Point CA 8.2.7.

#### B.9.2.6 Effects on algal growth

Study 8.2.6/01

Previous evaluation	In DAR (2003)
	The RMS has re-evaluated the study. Nowhere in the report can the RMS find the purity of the test substance stated, however, the applicant has stated that the test substance in question was according to the technical specification (and therefore > 95%). This is considered acceptable. In addition, the test was performed under the old OECD 201 Guideline. Under the newest guideline (update 2011) this test does not meet the validity criteria that the mean CV for section by section growth rates in control cultures not exceed 35% (it was 47.6%, calculated by the RMS using ToxRatPro 3.1.0.). All other validity criteria were met. The applicant presented a re-calculation of the section by section growth rate, but used a value of 10 <sup>4</sup> as the starting cell concentration, whereas the test report indicates a starting concentration of 0.594 x 10 <sup>4</sup> cells. However, considering the fact that no effects at all were determined up to the highest test concentration, the test was performed before the newest guideline update, the fact



	<i>that the substance in question is a growth regulator and not expected to have phytotoxic effects, and the fact that all other validity criteria were met, the RMS considers the test acceptable for use in risk assessment.</i>
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**Report:** GNEMI P. (2000); H 11- Algal growth inhibition study; CERB Baugy; RBM Italy;  
Unpublished report N°990705 EX, 25/05/2000

Dates of experimental work: 07/03/2000 to 10/03/2000

**Guidelines:** EEC Guideline C3, Directive 92/69/EEC, Annex V

Directive 92/32, VII Amendment

OECD 201 (1993)

Deviations: none

**GLP:** Yes (certified laboratory)

**Material** Test material: H 11; Batch N°99521

**and** The test system was treated with the nominal concentration of 100 mg/L. One control  
**methods:** only with algal growing medium was also tested. Exposure period: 72 hours. The algae  
(in 100 mL silylated glass flasks) were kept in suspension by placing them on an  
orbital shaker (about 100 rpm speed).

Algae used were *Selenastrum capricornutum*.

Initial cell concentration:  $0.594 \times 10^4$  cells/mL. The cell concentration was determined at  
24, 48 and 72 hours after the start of the study. A microscope with counting chamber  
was used.

At 0 and 72 hours, pH of the test and control solutions was measured.

EbC<sub>50</sub>, ErC<sub>50</sub> and their statistical limits were calculated by the modified probit method  
set up by the Flemish Institute for Technological Research.

NOEC values for biomass and growth rate were determined by ANOVA calculation.

**Findings:** The following results were obtained:

	24 h	48 h	72 h
E <sub>b</sub> C <sub>50</sub> (mg/L)	> 100	> 100	> 100
NOEC <sub>b</sub> (mg/L)	> 100	> 100	> 100
E <sub>r</sub> C <sub>50</sub> (mg/L)	> 100	> 100	> 100
NOEC <sub>r</sub> (mg/L)	> 100	> 100	> 100

**Conclusion:** The E<sub>r</sub>C<sub>50</sub> or E<sub>b</sub>C<sub>50</sub> of > 100 mg/L can be used in risk assessment.

#### B.9.2.7 Effects on aquatic macrophytes

<i>Previous evaluation</i>	<i>None. New data submitted for this renewal dossier (2015).</i>
	<i>The RMS has not received a final report for this study. Preliminary data from the range-finding test do not indicate any toxicity, nor is any toxicity expected based on the working mechanism of laminarin and the fact that it is ubiquitous in the aquatic environment.</i>

The notifier has provided a study protocol and an initial (email) report from the study director stating that no effects on macrophytes were seen up to the highest tested dose in a range-finding study.

The results of the range-finding test are presented below (please see KCA 8.2.7/02).

**Results of the range-finding test with *Lemna* exposed to Laminarin**

Nominal concentration (mg/L)	Replicat number	Frond number			
		Day 0	Day 3	Day 5	Day 7
Control	V1	12	46	94	146
	V2	12	50	95	176
	Mean	12	48	95	161
1.0	V3	12	59	98	158
	V4	12	45	81	113
	Mean	12	52	90	136
10.0	V5	12	54	99	168
	V6	12	62	113	148
	Mean	12	58	106	158
50.0	V7	12	50	89	140
	V8	12	40	82	114
	Mean	12	45	86	127
100	V9	12	57	86	129
	V10	12	41	100	153
	Mean	12	49	93	141

From the available preliminary, data it appears that Laminarin is not toxic to non-target higher aquatic plants. In addition, toxicity is not expected, as laminarin is extracted from algae and elicits the immune response of plants.

#### B.9.2.8 Further testing on aquatic organisms

Laminarin is a natural product of aquatic origin. The toxicity profile shown in the tests presented in this dossier and in the public literature suggest that it is not toxic to aquatic organisms. No further testing was considered necessary.

### B.9.3 Effects on arthropods

#### B.9.3.1 Effects on bees

Study 8.3.1/01

Previous evaluation	In DAR (2003)
	<i>The RMS has re-evaluated the study. The validity criteria were met and the study is considered valid. The summary from the original DAR was adjusted (below) where necessary according to the new evaluation.</i>

KLING A. (2000); Assesment of Side Effects of Laminarin to the Honey Bee, *Apis mellifera* L. in the Laboratory; GAB Biotechnologie GmbH & IBU Umweltanalytik GmbH, Nierfern-Öschelbronn, Germany

Unpublished report N° 200011342/01-BLEU, 10/11/2000

Dates of experimental work : 05/09/2000 to 25/10/2000

**Guidelines:** EPPO Guideline N°170 (1992)

**GLP:** Yes (certified laboratory)

Deviations: none

**Material and** Test material: Laminarin; Batch N°S012000; Purity on dry matter: 91 %

**Methods:** The oral and contact toxicity of Laminarin to the honey bee (*Apis mellifera* L.) were determined in a limit test (EPPO Guideline n°170). In the laboratory, the bees were exposed to the highest recommended dose of 100 µg a.s. per bee of Laminarin by feeding and topical application. To guarantee a higher actual intake of the active substance the test substance and toxic standard solutions in the oral toxicity test were offered more concentrated than the intended nominal dose. Therefore the actual intake of active substance in the test substance group and the toxic standard was higher than the nominal dose.

**Findings:** Oral Toxicity Test : Results summarised in the following table:

Treatment (nominal doses)	Intake of test substance [µg a.s./bee]	Mortality [%]		Corrected for Control Mortality [%]	
		24 h	48 h	24 h	48 h
Control	---	2.0	2.0	-	-
Test substance: Laminarin					
100 µg a.s./bee	118.64	0.0	2.0	0.0	0.0
Toxic standard: "Perfekthion"					
0.17 µg a.s./bee	0.18	76.0	92.0	75.5	91.8

Contact Toxicity Test : Results summarised in the following table :

Treatment	Mortality [%]		Corrected for Control Mortality [%]	
	24 h	48 h	24 h	48 h
Control	10.0	10.0	--	--
Test substance: Laminarin				
100 µg a.s./bee	0.0	4.0	0.0	0.0
Toxic standard: "Perfekthion"				
0.30 µg a.s./bee	94.0	94.0	93.3	93.3

**Conclusion:** According to the results of this study it can be assumed that the oral LD<sub>50</sub>/ 48 h of Laminarin is above 118.64 µg a.s./bee and the contact LD<sub>50</sub>/ 48 h is above 100.00 µg a.s./bee. Regarding the behaviour, the treated bees did not differ from the control at any time during the test. Consequently, the oral and contact NOEC values are 118.64 and 100.00 µg a.s./bee, respectively.

#### **B.9.3.1.1 Chronic toxicity to bees**

Laminarin shows no acute oral and contact toxicity to bees and is quickly degraded in the environment, including by plants (See section 8.1.1.1). In addition, as previously stated, laminarin is an organic compound found in algae. It is a polysaccharide of twenty-five glucosyl units. The degradation of laminarin in soil will result in smaller-sized oligosaccharides and ultimately glucose. Beside smaller-sized oligosaccharides and glucose, no relevant metabolite, degradation or reaction product is expected to appear in any circumstances. The most significant residue in the environment will therefore be glucose itself.

In long-term effect studies in bees, and in commercial bee-keeping, the feeding of a sucrose solution as an additive is common. Indeed, in Appendix O of the EFSA Guidance Document on bees (2013), the suggested chronic study design states that *"pollen should be available throughout the study [...] Alternatively, commercial protein supply can be used. Sucrose solution should be prepared using demineralised water and a final concentration of 500 g/L should be achieved"*.

Considering that the degradation of laminarin leads to the formation of oligosaccharides and ultimately glucose, the chronic risk of bees exposed to laminarin is expected to be low. Consequently the chronic risk to bees can be considered acceptable and no chronic study is considered necessary.

#### **B.9.3.1.2 Effects on honeybee development and other honeybee life stages**

As stated above in Section 9.3.1.1, exposure to laminarin is expected to be very short, with the major break-down product being glucose. Certainly it is highly unlikely that early life stages of bees will be exposed to the active substance, but rather to the major metabolites. Sucrose is regularly used in commercial bee keeping, as well as in bee toxicity testing. Therefore, no effects on honey bee development are expected and no study is deemed necessary.

#### **B.9.3.1.3 Sub-lethal effects**

As stated above in Section 9.3.1.1, exposure to laminarin is expected to be very short, with the major break-down product being glucose. Sucrose is regularly used in commercial bee keeping, as well as in bee toxicity testing. Thus, no sub-lethal effects of laminarin on bees are expected and no further testing is considered necessary.

#### **B.9.3.2 Effects on non-target arthropods other than bees**

The following studies were performed with PHYLIQ, the old representative formulation for laminarin. They were submitted in support of the Laminarin inclusion in Annex I of the Directive 91/414/EEC. They were already assessed and considered as valid and acceptable for the risk assessment. The RMS has re-evaluated the studies and re-calculated the endpoints using ToxRatPro 3.1.0.

The study on *Typhlodromus pyri* was performed according the "Open" method described by Overmeer (1988). New guidelines are now available for laboratory tests in arthropods. The RMS checked

whether the validity criteria for the new test methods (IOBR) are met for the tests below (see RMS evaluation in the grey boxes). The RMS has adjusted the study summaries from the original DAR (2003), to include new information and new endpoints.

#### CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

Previous evaluation	In DAR (2003)
	<i>The RMS has re-evaluated the study. The study was appropriately performed and the validity criteria were met, thus the study is considered appropriate for use in risk assessment. The RMS has adjusted the original summary (below) as necessary, to reflect the re-evaluation and re-calculated endpoints.</i>

- Report:** TESSIER C. (2001a)  
Phyliq. The effects of Phyliq (37 g Laminarin/L) on *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) on artificial substrate in laboratory: LR<sub>50</sub> estimation and reproduction assessment.  
Unpublished report PROMO-VERT N° 01APGOL25, March 15, 2001  
Dates of experimental work : 04/01/2001 to 23/01/2001
- Guidelines:** IOBC ring-test method as described by Mead-Briggs (1998), Mead-Briggs *et al.* (1998, 2000).  
Deviation: several rates are used in order to calculate a LR<sub>50</sub>.
- GLP:** Yes (certified laboratory)
- Material and methods:** The test was conducted with Phyliq (37 g Laminarin/L) at 5 rates of 0.1-0.3-1-3-10 L/ha, the medium rate corresponding to field rate. The test item was compared to a water control and to a toxic reference (dimethoate). Three replicates were used for each treatment. Mortality was assessed 24 and 48 hours after application. Surviving females from the rates resulting in less than 50% corrected mortality were transferred into fecundity chambers for testing fecundity on *Rhopalosiphum padi*.
- Findings:** Mortality at 48 hours was < 10% in all groups treated with Laminarin and in the control. Mortality of 100% was observed in the toxic reference.  
Fecundity was reduced by 26% (not significant) at 3 L/ha and by 46% (significant) at 10 L/ha.
- Conclusion:** Laminarin is of very low toxicity to the sensitive species *Aphidius rhopalosiphi*. The LR<sub>50</sub> was > 10L phyliq/ha. The reproductive ER<sub>50</sub> was also > 10 L product/ha. The ER<sub>10</sub> and ER<sub>20</sub> were calculated to be 0.8 L product/ha and 2.0 L product/ha, respectively.

#### Study CA 8.3.2.2

Previous evaluation	In DAR (2003)
	<i>The RMS has re-evaluated the study. The validity criteria are met and it is acceptable for use in risk assessment. The LR<sub>50</sub>, LR<sub>20</sub> and LR<sub>10</sub>, and the NOER for reproduction were calculated by the RMS using ToxRatPro 3.1.0. Due to the lower number of control replicates (three</i>



	<i>rather than five), the statistical significance (p) of the lethal rate calculations was &gt;0.05 (0.08). The RMS finds that considering the level of effects and the doses, this is acceptable and the calculated LR<sub>50</sub> (seen below) can be used in risk assessment.</i>
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**Report:** TESSIER C. (2001b)

PHYLIQ. The effects of Phyliq (37 g Laminarin/L) on *Typhlodromus pyri* (Acari, Phytoseiidae) on artificial substrate in laboratory: LR<sub>50</sub> estimation and reproduction assessment.

Unpublished report PROMO-VERT N° 01TYGOL24, March 15, 2001

Dates of experimental work : 15/01/2001 to 02/02/2001

**Guidelines:** "Open" method described by Overmeer (1988)

Deviation: several rates are used in order to calculate a LR<sub>50</sub>.

**GLP:** Yes (certified laboratory)

**Material and methods:** The test was conducted with Phyliq (37 g Laminarin/L) at 5 rates of 0.1-0.3-1-3-10 L/ha, the medium rate corresponding to field rate. The test item was compared to a water control and to a toxic reference (fenpropathrin). Three replicates were used for each treatment. Mortality was assessed 1, 3 and 7 days after application. Test units from the rates resulting in less than 50% corrected mortality were assessed for fecundity.

**Findings:** Corrected mortality after 7 days was significant (86%) only at the highest rate (10 L/ha). The LR<sub>50</sub> was calculated as 3.1 L/ha. Mortality of 100% was observed in the toxic reference.

Fecundity was initially strongly reduced at 10 L/ha (by 84% at Day 10), but recovered rapidly from Day 12 onwards and no significant difference was seen anymore when considering the fecundity at Day 12 and Day 14. Consequently, it can be concluded that the fecundity of *T. pyri* was not significantly affected when the test item Phyliq was applied at rates equal to or below 10.0 L/ha under laboratory "worst case" conditions.

**Conclusion:** Laminarin is of moderate toxicity to the sensitive species *Typhlodromus pyri*. High mortality was seen and transient effect on fecundity was seen at 10 L/ha. The LR<sub>50</sub> value was determined to be 3.1 L/ha and the NOER for fecundity was > 10.0 L/ha. The LR<sub>10</sub> and LR<sub>20</sub> were 0.1 and 0.4 L/ha, respectively.

#### **B.9.4 Effects on non-target soil meso- and macrofauna**

##### **B.9.4.1 Earthworm – sub-lethal effects**

##### **B.9.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)**

###### **B.9.4.2.1 Earthworms – sub-lethal effects**

<i>Previous evaluation</i>	<i>New study, submitted for this renewal dossier (2015); see RMS evaluation below (grey box).</i>
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The study summary as presented by the notifier is followed by comments of the RMS in a grey commenting box.

Data point addressed	CA.8.4.1
Author(s) (year)	Winkelmann G., 2015
Title	Laminarin – Earthworm ( <i>Eisenia fetida</i> ), Effects on Reproduction
Report number (Doc. No.)	RBN16029
Testing facility	D <sub>R</sub> . U. Noack-Laboratorien, Sarstedt, Germany
Published	No
Test guideline used	OECD 222 (2004), ISO 11268, part 2 (1998) and BBA Guideline (1994)
Deviations	<p><u>From the guideline:</u> Food was provided on day 0 (test start) instead of day 1 due to good experience with this procedure. The soil moisture deviated by more than 10 % from the initial value in the control and all test item concentrations.</p> <p><u>From the study plan:</u></p> <ul style="list-style-type: none"> <li>- The soil moisture deviated by more than 10 % from the initial value at the control and all test item concentrations.</li> <li>- The test item concentrations were based on mg test item/kg soil dry weight (corresponding to technical active substance) and on mg a.s./kg soil dry weight (based on purity of the active substance mentioned in the certificate of analysis).</li> <li>- Determination of pH-value, soil moisture and offspring was done on day 55 instead on day 56.</li> </ul> <p>These deviations are considered to have no impact on quality and integrity of the study.</p>
GLP	Yes (certified laboratory)

### Executive Summary

In a 56-day reproduction laboratory study, adult earthworms (*Eisenia fetida*) were exposed in groups of 10 (8 and 4 replicates for the control and for the tested groups respectively) to OECD 222 artificial soil containing 10% peat and treated with 0, 25.0, 45.0, 81.0, 146 and 262 mg a.s./kg soil d.w. (based on technical content) corresponding to 23.8, 42.8, 77.0, 139 and 249 mg a.s./kg soil d.w. (based on purity of Laminarin of 95%).

Assessment of adult worm mortality, behavioral effects and biomass development was carried out after 28 days exposure. Reproduction rate (number of offspring) was assessed after an additional 28-day period.

No mortality was observed in any treatment group. The body weight change of the earthworms after 4 weeks exposure to Laminarin was not statistically significantly reduced compared to the control up to and including the highest test concentration of 262 mg a.s./kg soil. No pathological symptoms and no evident changes in the behaviour of the earthworms were observed in the control or in any of the application rates

The reproduction rates were not significantly different compared to the control up to and including the highest test concentration of 262 mg a.s./kg soil.

Therefore, the NOEC of Laminarin concerning mortality, biomass and reproduction of earthworms was determined to be 262 mg/kg soil dry weight (technical active substance), corresponding to 249 mg a.s./kg soil dry weight (based on purity of tested Laminarin from the certificate of analysis). The EC<sub>50</sub> - value for reproduction was not determined since no significant reduction of reproduction occurred.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:** Laminarin  
**Description:** light beige powder, solid  
**Lot/Batch #:** 55927  
**Purity:** > 95 % according to Certificate of Analysis  
**Stability of test compound:** stable under test conditions
2. **Vehicle and/or positive control:** deionised water / Carbendazim
3. **Test animals**  
**Species:** *Eisenia fetida* (Annelida, Lumbricidae)  
**Age:** 2 to 12 months, with well-developed clitellum  
**Body weight:** 310 mg - 570 mg (wet mass)  
**Source:** Bred under standardised conditions by IBACON  
**Diet:** dried litter of stinging nettle and porridge oats  
**Test units:** plastic boxes (bottom surface area of 177 cm<sup>2</sup> and a height of 14 cm) with perforated transparent lids, filled with about 600 g OECD 222 soil. Ten worms per unit.  
**Acclimation period:** 2 days, in artificial soil, under test conditions

### Environmental conditions

- Temperature:** 18.0°C - 22.0°C  
**Photoperiod:** 16 h light (400 - 800 lux); 8 hours dark  
**Water content:** At experimental start: 32.2% to 34.2%  
At experimental end: 38.7% to 40.5%  
**Soil pH:** 5.77 – 5.93 (initiation); 6.23 - 6.36 (end)

### B. STUDY DESIGN AND METHODS:

1. **In life dates:** 07/08/2014 to 02/10/2014
2. **Experimental treatments**

Adult earthworms (*Eisenia fetida*) were exposed to Laminarin in an artificial soil (OECD 222 containing 10% peat) in a 56-day reproduction test. The test units consisted of plastic boxes with perforated transparent lids filled with about 600 g soil dry weight. The earthworms were exposed to 0, 25.0, 45.0, 81.0, 146 and 262 mg a.s./kg soil d.w. (based on technical content) corresponding to 23.8, 42.8, 77.0, 139 and 249 mg a.s./kg soil d.w. (based on purity of Laminarin of 95%). The test item was incorporated into the soil by mixing. Four replicates of 10 animals were performed for each treatment group and 8 for the control. Mortality was assessed after 28 days of exposure. Then, worms were observed for a further 28-day period to assess effects on reproduction.

### 3. Observations

Mortality, behavioral abnormalities and biomass development were recorded after 28 days of exposure. Reproduction rate (number of juveniles) was assessed after an additional 28-day period.

### 4. Statistics

One Way Analysis of Variance (ANOVA) was carried out for the determination of statistically significant differences compared to the control. A Normality Test and an Equal Variance Test were conducted prior to running the ANOVA. P-values for both Normality and Equal Variance Test are 0.05. The  $\alpha$ -value for ANOVA test (acceptable probability of incorrectly concluding that there is a difference) is  $\alpha = 0.05$ . For the endpoint of reproduction the arithmetic mean and the variance (coefficient of variation) per treatment and control were calculated.

## II. RESULTS AND DISCUSSION

Control mortality was 0% after 28 days. The number of juvenile worms per replicate in control was from 216 to 325. The coefficient of variance of reproduction in control was 13.2%. Therefore, all study validity criteria were met.

No mortality was observed in any treatment group.

The body weight change of the earthworms after 4 weeks exposure to Laminarin was not statistically significantly reduced compared to the control up to and including the highest test concentration of 262 mg test item/kg soil (ANOVA,  $\alpha = 0.05$ ).

The reproduction rates were not significantly different compared to the control up to and including the highest test concentration of 262 mg test item/kg soil (ANOVA,  $\alpha = 0.05$ ). The NOEC for reproduction was determined to be 262 mg a.s./kg soil (based on technical content) and 249 mg a.s./kg soil (based on purity of 95%). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control (see Table CA 8.4.1-1).

**Table CA 8.4.1-1: Effect of Laminarin on earthworms (*Eisenia fetida*) in a 56-day reproduction study**

Laminarin (technical) [mg/kg soil]	Control	25.0	45.0	81.0	146	262
Laminarin (pure) [mg/kg soil]	Control	23.8	42.8	77.0	139	249
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%] <sup>1)</sup>	30.0	29.6 (n.s.)	32.4 (n.s.)	33.0 (n.s.)	30.8 (n.s.)	32.9 (n.s.)
No. of juveniles (day 56) <sup>1)</sup>	267	273 (n.s.)	241 (n.s.)	279 (n.s.)	271 (n.s.)	287 (n.s.)
Reproduction in [%] of control (day 56)	-	- 2.25	9.74	- 4.49	- 1.50	- 7.49

- = not applicable

n.s. = not significantly different compared to the control

<sup>1)</sup> ANOVA,  $\alpha = 0.05$

The following endpoint was determined:

NOEC<sub>mortality,biomass,reproduction</sub> = 262 mg a.s./kg soil (based on technical content)

NOEC<sub>mortality,biomass,reproduction</sub> = 249 mg a.s./kg soil mg a.s./kg (based on purity > 95%)

### III. CONCLUSIONS

In an earthworm reproduction and growth study with Laminarin the overall No Observed Effect Concentration (NOEC) for mortality, biomass and reproduction of the earthworm *Eisenia fetida* was determined to be 262 mg test item/kg soil, i.e. the highest concentration tested, equivalent to 249 mg a.s./kg soil ( based on purity > 95%)

(Winkelmann G., 2015)

Study Comments: IIIA 8.4.1	<p>The laboratory which performed the test is GLP certified.</p> <p>The earthworms were fed with cattle manure during the first 4 weeks. At test start and at day 28 the food was carefully mixed into the soil, on the other days the food was placed on the soil surface. The temperature was recorded continuously via hygrothermometer.</p> <p>All test conditions remained within the ranges prescribed by the protocol. All validity criteria were met. The test is therefore considered reliable for use in risk assessment.</p>
Agreed endpoint/s: IIIA 8.4.1	<p>NOEC = 262 mg a.s./kg soil d.w. (based on technical content); 249 mg a.s./kg soil d.w. based on purity &gt; 95%. The log Kow of laminarin is -1.6, thus, this value is not adjusted. There were no significant dose-related effects in the study, thus EC<sub>10</sub> and EC<sub>20</sub> values could not be calculated.</p>

#### B.9.4.2.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

#### B.9.4.2.3 Species level testing

According to the Guidance Document SANCO/10329/2002, the risk for macro-organisms particularly deals with the problem of persistent active substances (DT<sub>90 field</sub> > 100 days). Testing is required where contamination of soil is possible and DT<sub>90 field</sub> is between 100 and 365 days and the standard HQ for arthropods (*Typhlodromus* and *Aphidius*) > 2.

After application, Laminarin will be rapidly degraded to smaller sized oligosaccharides and ultimately to glucose which is naturally present in soil (see Section 8.1.1.1 for discussion). Thus, very low soil persistence is expected for Laminarin.

#### B.9.5 Effects on soil nitrogen transformation

Previous evaluation	In DAR (2003)
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As presented by Black and Dewar (Ref CA 8.5/01), most bacteria and fungi possess laminarinase (or laminarase) activity (p. 142). For instance in a study on the phenotypic diversity of 6 strains of *Pseudoalteromonas citrea*, Ivanova *et al.* (Ref CA 8.5/02) showed that they all produce gelatinase and amylase, and each of them was analysed positively for laminarinase activity.

Here again, it has been demonstrated by Mizuno *et al.* (Ref CA 8.5/03) that laminarinase is present in *Bacillus circulans*, and that expression was induced by the presence of a cell-wall preparation from a basidiomycete fungus.

Considering this, no risk to soil non-target micro-organisms are to be expected from the application of Laminarin as proposed in the GAP and no specific study is considered necessary.

## **B.9.6 Effects on terrestrial non-target higher plants**

### **B.9.6.1 Summary of screening data**

The representative formulation is currently registered in several Member States for use on a variety of crops. Another formulation, Vacciplant Grandes Cultures, is registered in several Member States for use in cereals. The following Table reports the existing uses on different families of crops and their GAPs.

**Table CA 9.6.1-1: Existing uses of Vacciplant Grandes Cultures and Vacciplant Fruits et Légumes.**

Crops	Product	BBCH stages	Maximum number of applications	Minimum interval between applications	Application rate	
					L f.p./ha	g a.s./ha
Wheat	Vacciplant Grandes Cultures	BBCH 30-89	3	7	1.0	37.0
Barley	Vacciplant Grandes Cultures	BBCH 30-89	3	7	0.75	27.8
Lettuce	Vacciplant Fruits et Légumes	BBCH 13-49	16	7	2.5	113
Tomato	Vacciplant Fruits et Légumes	BBCH 10-89	7	7	2.0	90.0
Zucchini, Pumpkins	Vacciplants Fruits et Légumes	BBCH 10-89	6	5	0.75	33.8

f.p.: formulated product

Considering the fact that Vacciplant Grandes Cultures is registered on two monocotyledonous species of the same family: wheat and barley (*Graminae*), and that the product Vacciplant Fruits et Légumes is registered on at least 4 different dicotyledonous species: lettuce (*Asteraceae*), tomato (*Solanacea*), zucchini and pumpkins (*Cucurbitaceae*), it is clear that phytotoxic effects are not seen in a variety of plant species.

Further, an efficacy trial of PHYSPE 1 (formulation based on laminarin with a content of 45 g a.s./L) on leek (monocotyledonous species, *Allium*) in the field is summarized below by the notifier. However, this study was presented to the RMS in French, and therefore could not be evaluated. The notifier should address this point.

The uses are reported for 2 monocotyledonous families (*Graminae* with wheat and barley and *Allium* with leek) and for 4 dicotyledonous species (*Asteraceae* with lettuce, *Solanacea* with tomato, *Cucurbitaceae* with zucchini and pumpkins). The above presented and already registered uses and the results of the efficacy trial on leek cover the majority of non-target plants. Consequently, for at least 4 dicotyledonous species and 2 monocotyledonous families, the product does not present adverse phytotoxic effects at the field rate, which can be extrapolated for all non-target plants.

**Report:** LETOUZE P. and DUBOIS J. (2006)  
Essai d'efficacité d'une spécialité GOEMAR, nommée PHYSPE1 contre la rouille (*Puccinia porri*) sur poireau de plein champ  
Unpublished report coded N°FGOE062131 for COLEOR and VFPCPI06SL1 for GOEMAR

**Guidelines:** Standards EPPO 1/120(2) and 1/124(2)

**GEP:** Yes

**Material and methods:** The trial was conducted with Physpé 1 (45 g Laminarin/L) (coded GO1 in this trial) at 2 rates of 1.0 and 1.5 L/ha in a spray volume of 600 L/ha on leek (variety: CEZANNE (Rijk Zwaan)). The trial was located at Montfarville (50 760 - France - Maritime climatic zone).

Physpé 1 was integrated in a program: first 2 preventive applications with Physpé 1 at 1.0 or 1.5 L/ha and then curative applications with tebuconazole at 250 g/ha.

Programs with Physpé 1 were compared to an untreated control (water) and a reference program (first preventive application with azoxystrobin at 250 g/ha, second preventive application with chlorothalonil at 1440 g/ha and then curative applications with tebuconazole at 250 g/ha).

Four replicates were used for each treatment.

Leeks were planted on 29/06/2006. Applications started on 29/09/2006. The other applications were further performed on 10/10/2006, 27/10/2006 and 10/11/2006. At these dates, temperature and hygrometry were measured. The assessments were done on 26/10/2006, 09/11/2006, 28/11/2006 and 13/12/2006 for all treatments.

Table CA 8.6.1-2 summarized the environmental conditions during applications.

**Table CA 8.6.1-2: Environmental conditions during applications**

Application date	Soil temperature	Dew	Speed of wind	Nebulosity
29/09/2006	19.8°C	none	low	sunny
10/10/2006	15.6°C	none	none	overcast
27/10/2006	7.4°C	none	none	overcast
10/11/2006	8.4°C	none	moderate	sunny

**Findings:** During the trial, the crop always presented a uniform appearance without vegetation delay or depressive appearance whatever the treatment or replicate considered. No phytotoxic effect (changing of color, vegetation delay) was observed in comparison with the control. That means that up to 1.5 L of Physpé 1, no phytotoxic effects are expected on leek. Thus it can be concluded that the ER<sub>50</sub> of Physpé 1 for leek is > 1.5 L/ha.

**Conclusion:** In the conditions of this trial, Physpé 1 (45 g/L Laminarin) had no phytotoxic effects on leek up to the maximum tested rate of 1.5 L/ha. So it can be concluded that the ER<sub>50</sub> for this species is > 1.5 L/ha.

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<p><b>Study</b></p> <p><b>Comments:</b></p> <p><b>IIIA 8.6.1</b></p>	<p>The study was submitted to the RMS in French, and therefore could not be evaluated by the RMS, however, the co-RMS, France, has evaluated the study:</p> <p>The co-RMS (FR) checked the consistency of the summary provided by the applicant with the study report which is available only in French. The information reported in the study summary are in accordance with the one of the study report.</p> <p>The trial is used in the efficacy section to support the effectiveness of Physpé 1 (45 g/L Laminarin) for the use on leeks against <i>Puccinia porri</i>.</p> <p><u>History of the nursery and crop (page 4-5 of the report):</u></p> <ul style="list-style-type: none"> <li>• Previous crop : winter cauliflower</li> <li>• Sowing of the nursery : 5 april 2006</li> <li>• Fertilizer: 500 kg /ha ENTEC (14/8/17) performed on 4 April</li> <li>• Pesticide applications to protect the nursery: <ul style="list-style-type: none"> <li>Herbicide applied on 6 april 2004 : RAMROD 9 L/ha</li> <li>Herbicide applied on 31 may 2006 : PRESKILL at 0.5L/ha</li> <li>Protection against <i>Thrips tabaci</i> on 15 june 2006: MESUROL at 1.5 kg/ha</li> <li>Fungicide on 15 june 2006 : ORZIN legume at 2L/ha</li> </ul> </li> <li>• Ploughing : 27-28 june 2006</li> <li>• Fertilizer before the planting of leek : 20 t/ha of green waste compost 600 kg/ha of ENTEC (14/8/17) on 28 june 2006</li> <li>• Leek plantation on 29 june 2006</li> <li>• Protection of the crop: <ul style="list-style-type: none"> <li>Herbicide : GESAGARDE at 2 kg/ha on 21 July 2006</li> <li>Protection against <i>Thrips tabaci</i>: <ul style="list-style-type: none"> <li>MESUROL at 1.5 kg/ha on 26 july 2006</li> <li>CURATER at 20 kg/ha on 3 August 2006</li> <li>MAGEOS at 0.33 kg/ha on 4 August 2006</li> <li>DICARZOL 200 at 2.5 kg/ha + HELIOSOL at 1.2 L/ha in 600L/ha on 16 august 2006</li> <li>MESUROL at 1.5 kg/ha + HELIOSOL at 1.2 L/ha in 600L/ha on 6 september 2006</li> <li>MAGEOS at 0.33 kg/ha + HELIOSOL at 1.2 L:ha in 600L/ha on 26 september 2006</li> </ul> </li> <li>Protection against rust : TABOU at 1 L/ha on 7 September 2006</li> </ul> </li> <li>• Hoeing on 1 august and hilling on 11 September 2006</li> <li>• Irrigation : None</li> </ul> <p>The study report does not contain the details of the observations of the leek regarding symptoms other than those linked to effects of <i>Puccinia porri</i> to leek.</p> <p>The observations performed on 26/10/2006, 09/11/2006, 28/11/2006 and 13/12/2006 reported in the study report (tables 1, 5, 9 and 13) are related to the mean percentage of plants attacked in each class. The classes are percentage of foliar surface affected by <i>Puccinia porri</i> : 0, 1, 5, 10, 25, 50 and 75% of foliar surface affected. Thus, the class 0% is the one for plants that are not affected by <i>Puccinia porri</i>.</p> <p>In the findings/conclusion paragraphs proposed above by the notifier (underlined in red), the sentence “No phytotoxic effect (changing of color, vegetation delay) was observed in comparison with the control.” Seems to be based on the paragraph A on page 10 of the study report. The translation made by co-RMS of this paragraph A is as follows:</p> <p>A. <i>Effect of products on the aspect of the crop</i>  <i>The crop has always presented an uniform aspect, without delay in growth, nor depressed aspect, at any of the modality and replicates.</i></p> <p>No raw data is available.</p>
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<b>Agreed endpoint/s:</b> <b>IIIA 8.6.1</b>	<p>No phytotoxicity was seen in this efficacy trial, but regardless, as laminarin is an elicitor of plant defence mechanisms, no phytotoxic effects are expected.</p> <p><b>Co-RMS opinion:</b>  It is always difficult to use efficacy trials to demonstrate the lack of phytotoxicity of a compound for non target plants in ecotox risk assessment. In the present case, the leeks have been intensively protected as shown is the history of the nursery and crop above. Therefore, the use of this study may be considered with caution. However, even with this intensive protection of the crop by plant protection products, no phytotoxicity is reported.  However, we agree with the RMS that laminarin is used as an elicitor of the crop's self-defence mechanisms and is considered beneficial for plants. Thus, as indicated by RMS, no adverse effects on non-target plants following applications of laminarin according to the GAP are expected.</p>
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#### **B.9.6.2 Testing on non-target plants**

The screening data confirm the absence of phytotoxic effects of laminarin, although only one family of monocotyledonous plants were considered. In addition, laminarin acts as an elicitor of the crop's self defence mechanisms rather than as an herbicide. Thus, it is considered beneficial for plants. Consequently, no further studies on non-target plants were considered necessary.

#### **B.9.7 Effects on other terrestrial organisms (flora and fauna)**

Laminarin is a polysaccharide of relatively small size ( $M \# = 5000 \text{ g.mol}^{-1}$ ), no effect is expected on non-target organisms, all of which have  $\beta$ -glucanase activity in order to hydrolyse their own storage polysaccharide(s) (which is (are) usually much larger).

#### **B.9.8 Effects on biological methods for sewage treatment**

As explained under § 9.5, no effect is to be expected on the micro-organisms of sewage treatment plants. Quite the opposite, actually, the active substance laminarin can be a food-source and support the growth of the micro-organisms of these treatment plants.

#### **B.9.9 Monitoring data**

Due to the favorable profile of laminarin and its low toxicity for all non-target organisms, no monitoring data were deemed necessary.

#### **B.9.10 Biological activity of metabolites potentially occurring in groundwater**

No relevant metabolites.



**B.9.11 References relied on****New Public Literature Review**

A literature review was performed by the notifier. The search criteria included all relevant metabolites, substance names and product names, and that all organisms were considered relevant, unless they had already been addressed in the mammalian toxicology section. Review papers and studies with mixtures were eliminated. From this literature search the applicant found only one relevant study:

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
8.2.1.2	Babíček, K. Čechová, I. Simon, R. R. Harwood, M. Cox, D. J.	2007	Toxicological assessment of a particulate yeast (1,3/1,6)- $\beta$ -d-glucan in rats <a href="#">Food Chem Toxicol.</a> 2007 Sep;45(9):1719-30. Epub 2007 Mar 23. Non-GLP Published	Y	N	NA	NA

A number of studies were eliminated as “mixture” studies, since they pertained to diet and thus were typically performed using a mixed diet. However, the relevant studies have been discussed in the risk assessment, as they directly pertain to the nature of the substance in question and the need for chronic toxicity testing. Therefore, this is considered acceptable.

**New studies submitted for this dossier**

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
CA 8.2.7/01	Allen R.	2015	<b>Protocol :</b> Laminarin : Higher Plant ( <i>Lemna</i> ) Growth Inhibition Semi-static Test according to OECD 221 (2006) Huntingdon LifeSciences, England Report No.DQU0009 GLP Unpublished	N	Y	A 10-year data protection period is claimed as this study : - is necessary to show that Laminarin has a low toxicity to aquatic higher plants - has not been	Laboratoires Goëmar SAS

						submitted in the past - has been made in accordance with the GLP principles.	
CA 8.2.7/02	Allen R.	2015	E-mail results of range-finding test on <i>Lemna</i> exposed to Laminarin (for report No.DQU0009)	N	Y	A 10-year data protection period is claimed as this study : - is necessary to show that Laminarin has a low toxicity to aquatic higher plants - has not been submitted in the past	Laboratoires Goëmar SAS
CA 8.4.1/01	Winkelmann G.	2015	Laminarin – Earthworm ( <i>Eisenia fetida</i> ), Effects on Reproduction Dr. U. Noack-Laboratorien, Sarstedt, Germany Report No.RBN16029 GLP Unpublished	N	Y	A 10-year data protection period is claimed as this study : - is necessary to show that Laminarin has a low toxicity to earthworm - has not been submitted in the past - has been made in accordance with the GLP principles.	Laboratoires Goëmar SAS
CA 8.6.1/01	Letouze P. and Dubois J.	2006	Essai d'efficacité d'une spécialité GOEMAR, nommée PHYSPE1 contre la rouille ( <i>Puccinia porri</i> ) sur poireau de plein champ SILEBAN, France Report No.VFPCPI06SL1	N	Y	A 10-year data protection period is claimed as this study : - is necessary to show that Laminarin based product has no phytotoxic effect - has not been submitted in	Laboratoires Goëmar SAS

						the past - has been made in accordance with the GEP principles.	
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### Studies from the original Annex I inclusion

Data point	Author(s)	Year	Title Source Company, Report No. GLP or GEP status Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection is claimed	Owner
CA 8.1.1/01	██████████	2002a	Laminarin – Acute oral toxicity (LD <sub>50</sub> ) to the Bobwhite quail ██████████ Study N°GOM 001/022173 GLP, unpublished	Y	N		Laboratoires Goëmar SAS
CA 8.1.1/02	██████████	2002b	Laminarin – Dietary toxicity (LC <sub>50</sub> ) to the Bobwhite quail ██████████ Study N°GOM 002/014410 GLP, unpublished	Y	N		Laboratoires Goëmar SAS
CA 8.1.1/03	Bergh M.O., Razdan A., Aman P.	1998	Nutritional influence of broiler chicken diets based on covered normal, waxy and high amylose barleys with or without enzyme supplementation. Animal Feed Science and Technology, 78, 215-226 Non-GLP, published	N	N		-

CA 8.1.1/04	Annison G.	1992	Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. Animal Feed Science and Technology, 38, 105-121 Non-GLP, published	N	N	-
CA 8.1.1/05	Vukic-Vranjes M., Wenk C.	1995	The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. Animal Feed Science and Technology, 54, 21-32 Non-GLP, published	N	N	-
CA 8.1.1/06	Philip J.S., Gilbert H.J., Smithard R.R.	1995	Growth, viscosity and beta-glucanase activity of intestinal fluid in broiler chickens fed on barley-based diets with or without exogenous beta-glucanase. British Poultry Science, 36, 599-603 Non-GLP, published	N	N	-

CA 8.2.1/01	██████████ ██████████	2001a	Laminarin – Acute toxicity in freshwater fish (96 hours) <i>Oncorhynchus mykiss</i> ██████████ - Study N° 00-907005-022 GLP, unpublished	Y	N	-
CA 8.2.1/02	██████████ ██████████	2001b	Laminarin – Acute toxicity in freshwater fish (96 hours) <i>Danio rerio</i> ██████████ - Study N° 00-907005-021 GLP, unpublished	Y	N	-
CA 8.2.2/01	Ingebrigsten K., Horsberg T.E., Dalmo R., Seljelid R.	1993	Tissue distribution of the immunomodulator aminated $\beta$ 1-3 polyglucose in Atlantic salmon ( <i>Salmo salar</i> ) after intravenous, intraperitoneal and peroral administration. Aquaculture, 117, 29-35 Non-GLP, published	N	N	-
CA 8.2.2/02	Dalmo R.A., Ingebrigsten K., Bogwald J., Horsberg T.E., Seljelid R.	1995	Accumulation of immunomodulatory laminaran [ $\beta$ 1-3-D-glucan] in the spleen and kidney of Atlantic salmon, <i>Salmo salar</i> L. Journal of Fish Diseases, 18, 545-553 Non-GLP, published	N	N	-
CA 8.2.2/03	Dalmo R.A., Ingebrigsten K., Sveinbjornsson B., Seljelid R.	1996	Accumulation of immunomodulatory laminaran [ $\beta$ 1-3-D-glucan] in the heart, spleen and kidney of Atlantic cod, <i>Gadus morhua</i> L. Journal of Fish Diseases, 19, 129-136 Non-GLP, published	N	N	-

CA 8.2.2/04	Black W.A.P., Dewar E.T.	1954	Laminaran J.Sci. Food Agri., 5, 137-145 Non-GLP, published	N	N	-
CA 8.2.2/05	Sturmbauer C.	1991	Different enzymes for laminarine digestion in <i>Chondrostoma nasus</i> (Cyprinidae) and <i>Oreochromis</i> sp. (Cichlidae). Comp. Biochem. Physiol ,Vol.100A, No.1, 199-202 Non-GLP, published	N	N	-
CA 8.2.4/01	Hertl J.	2001	Acute toxicity of LAMINARIN to <i>Daphnia magna</i> in a 48-hour immobilization test IBACON GmbH - Study N° 10041220 GLP, unpublished	N	N	Laboratoires Goëmar SAS
CA 8.2.6/01	Gnemi P.	2000	H 11- Algal growth inhibition study CERB - Study N° 990705 EX GLP, unpublished	N	N	Laboratoires Goëmar SAS
CA 8.3.1/01	Kling A.	2000	Assesment of Side Effects of Laminarin to the Honey Bee, <i>Apis mellifera</i> L. in the Laboratory. GAB Biotechnologie GmbH Study N° 20001342/01-BLEU GLP, unpublished	N	N	Laboratoires Goëmar SAS
CA 8.3.2/01	Tessier C.	2001a	Phyliq. The effects of Phyliq (37 g/L Laminarin) on <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) on artificial substrate in laboratory : LR <sub>50</sub> estimation and reproduction assessment. PROMO-VERT- Study N° 01APGOL25	N	N	Laboratoires Goëmar SAS



			GLP, unpublished				
CA 8.3.2/02	Tessier C.	2001b	Phyliq. The effects of Phyliq (37 g/L Laminarin) on <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) on artificial substrate in laboratory : LR <sub>50</sub> estimation and reproduction assessment. PROMO-VERT-Study N° 01TYGOL24 GLP, unpublished	N	N		Laboratoires Goëmar SAS
CA 8.5/01	Black W.A.P., Dewar E.T.	1954	Laminaran J.Sci.Food Agric., 5, 137-145 Non-GLP, published	N	N		-
CA 8.5/02	Ivanova E.P., Kiprianova E.A., Mikhailov V.V., Levanova G.F., Garagulya A.D., Gorshkova N.M., Vysotskii M.V., Nicolau D.V., Yumoto N., Taguchi T., Yoshikawa S.	1998	Phenotypic diversity of <i>Pseudoalteromonas citrea</i> from different marine habitats and emendation of the description. International Journal of Systematic Bacteriology 48, 247-256 Non-GLP, published	N	N		-
CA 8.5/03	Mizuno K., Awazu N., Tachiki T.	1998	Purification and some properties of p-nitrophenyl-β-D-glucoside-hydrolyzing enzymes in culture filtrate of <i>Bacillus circulans</i> KA-304 grown on cell-wall preparation of <i>Schizophyllum commune</i> Biosci. Biotechnol. Biochem., 62(1), 39-43 Non-GLP, published	N	N		-