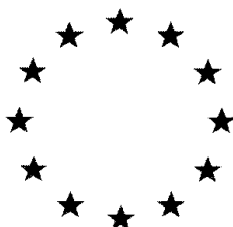


# **European Commission**



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

**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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### B.9 Ecotoxicology data and assessment of risks for non-target species

Vacciplant Fruits et Legumes was not a representative formulation during the previous EU review process of laminarin, resulting in the Annex I approval of this active substance. Therefore, all data and information on Vacciplant Fruits et Legumes are considered as new in the context of this renewal application. Study summaries and evaluations and their respective conclusions were written by RMS, except where indicated otherwise. Where the study evaluation or conclusion from RMS deviated from the applicant's, this is reported in grey boxes under the summaries. Studies from the old dossier were not re-evaluated, and summary/evaluations from the old DAR/Addendum were copied, except where indicated otherwise. If on top of the study summary/evaluation no reference is made to the old dossier, then it concerns a new study submitted for this Annex I renewal. In the endpoint overviews in the risk assessment sections, it is also clearly indicated whether it concerns old or new studies.

Studies performed with the representative formulation from the old dossier (PHYLIQ) are included in this DRAR when used by the applicant or considered necessary by RMS. The former formulation (PHYLIQ) was modified by replacement of several co-formulants (see Document J). Vacciplant Fruits et Légumes contains also a higher amount of non-classified active substance: 45 g/L laminarin instead of 37 g/L (nominal concentration).

#### Intended representative uses

Vacciplant Fruits et Légumes is a soluble concentrate (SL) formulation containing 45 g/L Laminarin. This product is intended for professional and amateur use as an elicitor of the crop's own self-defence mechanisms on fruits and vegetables, as shown in Table CP 9.1-1.

**Table 9.1-1 Intended uses of Vacciplant Fruits et Légumes**

Crop	Max. number of Applications	Minimum interval between applications (days)	Application Rate		Time of application
			[L product/ha]	[g a.s./ha]	
Apple	20	7	1.0	45.0	BBCH 11-89
Apple, Pear	7	10	0.75	33.8	BBCH 56-89
Vine	10	10	2.0	90.0	BBCH 11-89
Lettuce	16	7	2.5	113	BBCH 13-49
Lettuce	7	7	3.0	135	BBCH 16-49
Strawberry	7	5	2.0	90.0	BBCH 12-92
Tomato	7	7	3.0	135	BBCH 10-89
Zucchini, Pumpkins	6	5	0.75	33.8	BBCH 10-89
Aubergine, Pepper, Green bean, Cucumber,	7	7	3.0	135	BBCH 60-89 (BBCH 51-89 for Green bean and Cucumber)

Zucchini					
Kiwi	7	10	2.0	90.0	BBCH 11-95

### B.9.1 Effects on birds and other terrestrial vertebrates

#### B.9.1.1 Effects on birds

There were no studies with the formulated product submitted.

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

There were no studies with the formulated product submitted which are applicable for the terrestrial vertebrates risk assessment. For other mammalian studies with the formulated product, please see section B.6.

### B.9.2 Risk assessment for birds and other terrestrial vertebrates

In Volume 1, section 2.9.1 an overview of the available endpoints for birds and mammals is given.

#### Acute Risk Assessment - Birds

The risk assessment is performed according to the Guidance for Risk Assessment for Birds and Mammals (EFSA, 2009). Depending on the crop category, different bird indicator species are chosen. Acute “shortcut values” (based on 90th percentile residues) according to Table 6 of the Birds and Mammals Guidance document (2009) were considered for the screening assessment. These are shown in Table 9.2-1.

**Table 9.2-1: Indicator species and default values for the acute risk assessment, screening step**

Use	Crop	Indicator species	SV
Orchards	Apple, pear, kiwi	Small insectivorous bird	46.8
Vine	Vineyard	Small omnivorous bird	95.3
Leafy vegetables, fruiting vegetables, strawberry, pulses	Lettuce, tomato, zucchini, pumpkin, eggplant, cucumber, strawberry, green bean	Small omnivorous bird	158.8

The “Daily dietary dose” (DDD) values are calculated by multiplying respective “shortcut values” with the corresponding highest single application rates in kg/ha. “Multiple application factors” for 90th percentile residue data (MAF<sub>90</sub>) are selected from Table 7 of the guidance document. The DDD values were calculated according to the following equation: DDD = application rate [kg /ha] × shortcut value × MAF<sub>90</sub>. The TER value = LD<sub>50</sub> / DDD. TER values for birds for the worst-case uses, considering default values, are shown in Table 9.2-2.

**Table 9.2-2: Acute Screening Step, Birds**

Indicator species	Small insectivorous	Small omnivorous	Small omnivorous
Worst case Crop/scenario	kiwi	vineyard	vegetables
Max. single application rate [kg a.s./ha]	0.090	0.090	0.135
Shortcut value	46.8	95.3	158.8
frequency	7	10	7
Interval [d]	10	10	7
Multiple application factor for 90 <sup>th</sup> percentile residue data (DT <sub>50</sub> = 10)	1.6	1.6	1.9
Daily dietary dose	6.7392	13.7232	40.7322
Endpoint (LD <sub>50</sub> ) [mg a.s./kg b.w.]	> 1700	> 1700	> 1700
TER	> 252	> 124	> 42
Trigger value [TER]	10	10	10
Refinement required	No	No	No

As shown in Table 9.2-2, the acute risk assessment for birds passes in the screening step.

### Reproductive Risk Assessment – Birds

After application, Laminarin is rapidly degraded into smaller-sized oligosaccharides and ultimately to glucose. Considering this relatively quick degradation to natural food components long-term exposure of birds to laminarin is expected, and no risk from the degradation products is expected.

This conclusion is confirmed by references in literature: The absence of risk is confirmed by numerous papers published on the digestibility of cereal-based diets for chicken (see for instance Ref CA 8.1.1/03 to CA 8.1.1/06). These papers have been submitted previously in support of laminarin inscription in Annex I of the Directive 91/414/EEC. They were assessed at that time, and considered as useful for the risk assessment. These papers study the growth of chicken fed with diets containing  $\beta$ -glucans and the effect of adding  $\beta$ -glucanases to the diet for several weeks (up to 54 days). Further discussion can be found in Section CA 9.1. In none of these papers was any sub-chronic effect or reproduction effect mentioned. The RMS does not consider a reproductive risk assessment for laminarin necessary.

That said, it is possible to perform a reproductive risk assessment using the surrogate chronic toxicity value of the LD<sub>50</sub>/10, or > 170 mg/kg bw/(d). The RMS will show this risk assessment below as further weight of evidence.

**Table 9.2-3: Indicator species and default values for the reproductive risk assessment, screening step**

Use	Crop	Indicator species	SV
Orchards	Apple, pear, kiwi	Small insectivorous bird	18.2
Vine	Vineyard	Small omnivorous bird	38.9

Use	Crop	Indicator species	SV
Leafy vegetables, fruiting vegetables, strawberry, pulses	Lettuce, tomato, zucchini, pumpkin, eggplant, cucumber, strawberry, green bean	Small omnivorous bird	64.8

The “Daily dietary dose” (DDD) values are calculated by multiplying respective “shortcut values” with the corresponding highest single application rates in kg/ha. “Multiple application factors” for mean residue data (MAF<sub>m</sub>) are selected from Table 11 of the guidance document. The DDD values were calculated according to the following equation: DDD = application rate [kg /ha] × shortcut value × MAF<sub>m</sub>. The TER value = (LD<sub>50</sub>/10) / DDD. TER values for birds for the worst-case uses, considering default values, are shown in Table 9.2-4.

**Table 9.2-4: Reproductive Screening Step, Birds**

Indicator species	Small insectivorous	Small omnivorous	Small omnivorous
Worst case Crop/scenario	kiwi	vineyard	vegetables
Max. single application rate [kg a.s./ha]	0.090	0.090	0.135
Shortcut value	18.2	38.9	64.8
frequency	7	10	7
Interval [d]	10	10	7
Multiple application factor for mean residue data (DT <sub>50</sub> = 10)	2.0	2.0	2.5
Daily dietary dose	1.73628	3.71106	11.5911
Endpoint (LD <sub>50</sub> /10) [mg a.s./kg b.w.]	> 170	> 170	> 170
TER	> 97.9	> 45.8	> 14.7
Trigger value [TER]	5	5	5
Refinement required	No	No	No

As shown in Table 9.2-4, the reproductive risk assessment using the surrogate value of the LD<sub>50</sub>/10 passes in the screening step.

### Risk for birds through drinking water

#### Leaf Scenario

Since lettuce is a proposed use for Vacciplant Fruits et Legumes, the leaf scenario for small birds is applicable. The exposure from leaf whorls is calculated using the concentration in the spray (C<sub>spray</sub>) divided by 5.

The minimum water use for lettuce at 135 g a.s./ha is 750 L/ha. Thus the C<sub>spray</sub> = 0.18 g a.s./L. This value is divided by 5 to calculate the PEC<sub>pool</sub> of 0.036 g a.s./L. This value is then multiplied by the drinking water intake for a small bird (0.46 L/kg bw/d), resulting in the PEC<sub>dw</sub> of 0.017. Comparing this to the acute toxicity value of > 2000, results in a TER of > 100000. This is significantly greater than the trigger of 10. The risk to birds from leaf whorls is considered negligible.

#### Puddle Scenario

According to the Guidance Document of EFSA (2009)<sup>1</sup>, due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by birds, no specific calculations of exposure or TERs are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances ( $K_{oc} < 500$  L/kg) or 3 000 in the case of more sorptive substances ( $K_{oc} \geq 500$  L/kg).

The worst-case scenario is to consider the maximum number of application and the maximum application rate to determine the worst-case effective application rate (AREff). The AREff is calculated with the following formula:

$$AREff = AR * MAF_m$$

with:

AR: application rate (g a.s./ha)

MAF<sub>m</sub>: MAF mean (= 2.6; 20 applications with a 7 d-minimal interval)

For the intended uses of Vacciplant Fruits et Légumes, the worst case scenario is to consider 20 applications at the maximum rate of 135 g a.s./ha. This leads to calculate an AREff of 351 g a.s./ha. The ratio of the effective application rate (351 g a.s./ha) to relevant endpoint (acute LD<sub>50</sub> of > 1500 mg a.s./kg b.w./d) is 0.176. Since this ratio is below 50 and then below 3 000, no specific calculation of exposure of birds through drinking water is necessary.

### Risk for birds from secondary poisoning

Laminarin has a low Log Pow (Log Pow = -1.6) indicating low bioaccumulation potential (Log Pow < 3). Therefore, no risk of secondary poisoning is expected.

### Acute Risk Assessment – Mammals

Depending on the crop category, different mammalian indicator species are chosen. Acute “shortcut values” (based on 90th percentile residues) according to Table 8 of the Birds and Mammals Guidance document (2009) were considered for the screening assessment. These are shown in Table 9.2-5.

**Table 9.2-5: Indicator species and default values for the acute and reproductive risk assessments, screening step**

Use	Crop	Indicator species	Shortcut value
Orchards, leafy vegetables, fruiting vegetables	Lettuce, tomato, zucchini, pumpkin, eggplant, cucumber,	Small herbivorous mammal	136.4
pulses, vineyard strawberries	green bean, vine strawberry	Small herbivorous mammal	118.4

The “Daily dietary dose” (DDD) values are calculated by multiplying respective “shortcut values” with the corresponding highest single application rates in kg/ha. “Multiple application factors” for 90th percentile residue data (MAF<sub>90</sub>) are selected from Table 9 of the guidance document. The DDD values were calculated according to the following equation: DDD = application rate [kg /ha] × shortcut value ×

<sup>1</sup> EFSA Journal 2009; 7(12):1438. Risk assessment for birds and mammals.



MAF<sub>90</sub>. The TER value = LD<sub>50</sub> / DDD. TER values for birds for the worst-case uses, considering default values, are shown in Table 9.2-6.

**Table 9.2-6: Acute Screening Step, mammals**

Indicator species	Small herbivorous	Small herbivorous
Crop /scenario	Orchards/vineyard/vegetables	Strawberry
Max. single application rate [kg a.s./ha]	0.135	0.090
Shortcut value	136.4	118.4
frequency	7	7
Interval [d]	7	5
Multiple application factor for 90 <sup>th</sup> percentile residue data (DT <sub>50</sub> = 10)	1.9	1.9
Daily dietary dose	34.9866	20.2464
Endpoint (LD <sub>50</sub> ) [mg a.s./kg b.w.]	> 2000	> 2000
TER	> 57.2	> 98.8
Trigger value [TER]	10	10
Refinement required	No	No

As shown in Table 9.2-4, the acute risk assessment for mammals passes in the screening step. All proposed uses of Vacciplant Fruits et Legumes show an acceptable acute risk to mammals.

#### Reproductive risk assessment – Mammals

Depending on the crop category, different mammalian indicator species are chosen. Shortcut values (based on mean residues) according to Table 11 of the Birds and Mammals Guidance document (2009) were considered for the screening assessment.

**Table 9.2-7: Indicator species and default values for the reproductive risk assessment, screening step**

Use	Crop	Indicator species	Shortcut value
Orchards, leafy vegetables, fruiting vegetables, pulses, vineyard strawberries	Lettuce, tomato, zucchini, pumpkin, eggplant, cucumber, green bean, vine strawberry	Small herbivorous mammal	72.3
		Small herbivorous mammal	48.3

The “daily dietary dose” (DDD) values were calculated by multiplying the “shortcut values” with the respective highest single application rates of the active ingredient in kg/ha and a time-weighted average factor (TWA) of 0.53 – this factor estimates time weighted exposure over 21 days assuming a default DT<sub>50</sub> of 10 days. “Multiple application factors” for mean residue data (MAF<sub>m</sub>) were selected from Table 12 of the guidance document.

The DDD values were calculated according to the following equation:  $DDD = \text{application rate [kg / ha]} \times \text{shortcut value} \times f_{TWA} \times MAF_m$ . The TER value is NOEL or NOAEL / DDD. TER values for mammals for the worst-case uses, considering default values, are shown in Table 9.2-8.

**Table 9.2-8: Reproductive risk assessment screening step, wild mammals**

Indicator species	Small herbivorous	Small herbivorous
Crop /scenario	Ochards/vine/vegetables	strawberry
Max. single application rate [kg a.s./ha]	0.135	0.090
Shortcut value	72.3	48.3
frequency	7	7
Interval [d]	7	5
Multiple application factor for mean residue data (DT <sub>50</sub> = 10)	2.5	3.1*
f <sub>TWA</sub>	0.53	0.53
Daily dietary dose	12.9327	7.1421
Endpoint (NOAEL) [mg a.s./kg b.w./d]	1000	1000
TER	77.3	140
Trigger value [TER]	5	5
Refinement required	No	No

\* calculated by the RMS based on the equation presented in Appendix H of EFSA, 2009.

As shown in Table 9.2-8, the reproductive risk assessment for the worst-case proposed uses of Vacciplant Fruits et Legumes passes in the screening step. No reproductive risk to mammals is expected from the use of the product according to the proposed GAP.

### Risks to mammals through drinking water

According to the Guidance Document of EFSA (2009)<sup>2</sup>, due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by mammals, no specific calculations of exposure or TERs are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3 000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

The worst-case scenario is to consider the maximum number of application and the maximum application rate to determine the worst-case effective application rate (AR<sub>eff</sub>). The AR<sub>eff</sub> is calculated with the following formula:

$$AR_{eff} = AR * MAF_m$$

with:

AR: application rate (g a.s./ha)

MAF<sub>m</sub>: MAF mean (= 2.6; 20 applications with a 7 d-minimal interval)

For the intended uses of Vacciplant Fruits et Légumes, the worst case scenario is to consider 20 applications at the maximum rate of 135 g a.s./ha. This leads to calculate an AR<sub>eff</sub> of 351 g a.s./ha. The ratio of the effective application rate (351 g a.s./ha) to relevant endpoints (acute LD<sub>50</sub> of > 2 000 mg a.s./kg b.w./d. and NOAEL of 1000 mg a.s./kg b.w./d.) are 0.0176 and 0.351, respectively. Since

<sup>2</sup> EFSA Journal 2009; 7(12):1438. Risk assessment for birds and mammals.

these ratios are below 50 (and 3 000), no specific calculation of exposure of mammals through drinking water is necessary.

### Risk of secondary poisoning

Laminarin has a low Log Pow (Log Pow = -1.6) indicating low bioaccumulation potential (Log Pow < 3). Therefore, no risk of secondary poisoning is expected.

## B.9.3 Effects on aquatic organisms

### B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

#### B.9.3.1.1 Acute toxicity to fish

Data point addressed:	CP 10.2.1/01
Author(s) (year):	(2008a)
Title:	Fish, Acute toxicity test (OECD 203, July 1992)
Report number (Doc. No.):	report No.08-29-010-ES
Testing facility:	
Published:	No
Test guideline used:	OECD 203 (July 1992)
Deviations:	The stability of the test item in water was checked by appropriate analytical verification of the test solutions at test initiation and at test completion. This deviation was not considered to have affected the integrity of the study.
GLP:	Yes

#### Executive Summary:

In a static acute toxicity test, fish (*Brachidanio rerio*) were exposed for 96 hours to the limit nominal concentration of 103.5 mg product/L water.

Due to the weak concentration of Laminarin in the solution, the analysis method can detect the laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

No mortality occurred in the test item treated unit and the fish appeared healthy, without abnormal behaviour.

The 96 hours LC<sub>50</sub> of Vacciplant Fruits et Légumes to the fish *Brachidanio rerio* was > 103.5 mg/L test item/L, based on the limit nominal concentration.

## I. MATERIALS AND METHODS

### A. MATERIALS:

<b>1. Test Material:</b>	Vacciplant Fruits et Légumes = Iodus 2 Cultures spécialisées
<b>Description:</b>	beige opaque liquid
<b>Lot/Batch #:</b>	040308
<b>Purity:</b>	Nominal: Laminarin: 45 g/L Analytical: Laminarin: 46.5 g/L

<b>Stability of test compound:</b>	stable in test conditions
<b>2. System:</b>	static
<b>3. Test organism</b>	
<b>Species:</b>	<i>Brachidanio rerio</i>
<b>Weight, length:</b>	2.7 ± 0.2 cm (mean length)
<b>Source:</b>	Jardiland, Pau, France
<b>Acclimation period:</b>	They were maintained at 23 ± 1°C, 16 h light, in the same dilution water as used for testing.
<b>Diet:</b>	no feeding during the test
<b>Holding conditions:</b>	18 L capacity glass containers filled with 5 L of reconstituted water
<b>Environmental conditions</b>	
<b>Temperature:</b>	22.1°C – 23.1°C
<b>Concentration in oxygen:</b>	64.2% to 78.4% (dissolved oxygen)
<b>pH:</b>	6.47 – 6.90
<b>Photoperiod:</b>	Alternating 16-hour light (290-620 lux) and 8-hour dark cycles

## B. STUDY DESIGN AND METHODS:

- 1. In life dates:** from 25/03/2008 to 19/04/2008
- 2. Organisms assignment and treatment**  
Fish were exposed by group of 7 for 96 hours to the limit nominal concentration of 100 mg product/L (one replicate for the test item and for the control).
- 3. Test item analysis**  
The analyses were performed with HPLC method by Goëmar. After dilution of Vacciplant Fruits et Légumes in the reconstituted water (100 g product/L) laminarin was analysed at the beginning of the study and at the end of the study.
- 4. Observations**  
Fish were observed after 24, 48, 72 and 96 hours of exposure for sublethal effects (abnormal behavior or appearance) and mortality.
- 5. Statistics**  
No statistics were performed because at the tested limit concentration, no mortality neither sublethal effect occurred.

## II. RESULTS AND DISCUSSION

### A. ANALYTICAL DETERMINATION

Due to the weak concentration of Laminarin in the solution, the analysis method can detect the Laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

**B. MORTALITY, BEHAVIOUR AND CLINICAL SIGNS**

No mortality occurred in the test item treated unit and the fish appeared healthy, without abnormal behaviour.

**Table CP 10.2.1-1 Mortality of fish exposed for 96 hours to Vacciplant Fruits et Légumes**

Nominal concentration (mg/L)	Mortality (number of fish)				% of mortality at 96 h
	24 h	48 h	72 h	96 h	
control	0/7	0/7	0/7	0/7	0
103.5	0/7	0/7	0/7	0/7	0

**III. CONCLUSIONS**

In the conditions of the test, the 96 hours LC<sub>50</sub> of Vacciplant Fruits et Légumes to the fish *Brachidanio rerio* was > 103.5 mg test item/L (actual concentration) based on the limit nominal concentration of 100 mg/L.

Study Comments: IIIA 10.2.1./01	<p>The analytical method used for the analysis of the test concentrations to which the fish were exposed is discussed in part B5. The method was considered valid.</p> <p>The age of the fish at test start was not reported. No mortality occurred in any of the test item treated units, except the 10 mg/L treated unit (1 specimen out of 7), but the phenomenon was not confirmed in the 100 mg/L treated unit. In every case, the fish appeared healthy, and exhibited normal behaviour.</p> <p>The study met the validity criteria according to OECD 203. All test conditions remained within the ranges prescribed by the protocol.</p> <p>As the measured concentration of the test item was &gt; 80% of the nominal value, the nominal value was used.</p> <p>The RMS considers the study reliable without restrictions.</p>
Agreed endpoint/s: IIIA 10.2.1./01	<p>The 96-hour LC<sub>50</sub> of Vacciplant Fruits et Légumes to the fish <i>Brachidanio rerio</i> was &gt; 103.5 mg/L test item/L, equivalent to &gt; 4.81 mg laminarin/L at 96 hours after application (nominal concentration).</p>

**B.9.3.1.2 Acute toxicity to aquatic invertebrates**

Data point addressed:	CP 10.2.1/02
Author(s) (year):	Servajean E. (2008b)
Title:	Daphnia sp., Acute immobilisation test (OECD 202, April 2004)
Report number (Doc. No.):	Report No.08-29-004-ES
Testing facility:	Phytosafe s.a.r.l., Pau, France
Published:	No
Test guideline used:	OECD No.202 (2004)
Deviations:	<p>1. The stability of the test item in water was checked by appropriate analytical verification of the test solutions at test initiation and at test completion.</p> <p>2. In the range-finding test, the control group involved 4 replicates units instead of one.</p> <p>These deviations were not considered to have affected the integrity of the study.</p>
GLP:	Yes

**Executive Summary:**

In a static acute toxicity test, daphnids (*Daphnia magna*) were exposed for 48 hours to the limit nominal concentration of 105.1 mg Vacciplant Fruits et Légumes/L in reconstituted water.

Due to the weak concentration of laminarin in the solution, the analysis method can detect the laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

After 24 and 48 hours of exposure, no immobilisation was observed in the test item treated group. Consequently, the 48 hours EC<sub>50</sub> of Vacciplant Fruits et Légumes to *Daphnia magna* was determined to be > 105.1 mg test item/L, based on nominal concentration (dose limit test).

**I. MATERIALS AND METHODS****A. MATERIALS:**

1. **Test Material:** Vacciplant Fruits et Légumes = Iodus 2 Cultures spécialisées
  - Description:** beige opaque liquid
  - Lot/Batch #:** 040308
  - Purity:**
    - Nominal: Laminarin: 45 g/L
    - Analytical: Laminarin: 46.5 g/L
  - Stability of test compound:** stable in test conditions
2. **System:** static
3. **Test organism**
  - Species:** daphnid (*Daphnia magna*)
  - Age:** less than 24 hours at test initiation

<b>Source:</b>	the strain was received from Aqsualiment (France), then they were regularly sub-cultures at the Phytosafe site in the same dilution water as used for testing
<b>Acclimation period:</b>	1 day
<b>Diet:</b>	Daphnids were not feed during the test
<b>Holding conditions:</b>	15 mL capacity glass tubes filled with 9.9 mL of reconstituted water
<b>Environmental conditions</b>	
<b>Temperature:</b>	19.5°C
<b>Concentration in oxygen:</b>	dissolved oxygen: 75.5% - 81.7%
<b>pH:</b>	6.80 to 6.92
<b>Photoperiod:</b>	darkness

## **B. STUDY DESIGN AND METHODS:**

1. **In life dates:** between 31/03/2008 and 05/04/2008

2. **Organisms assignment and treatment**

Daphnids were exposed by groups of 5 for 48 hours to the limit nominal concentration of 105.1 mg product/L (four replicates for the test item and for the control).

3. **Test item analysis**

The analyses were performed with HPLC method by Goëmar. After dilution of Vacciplant Fruits et Légumes in the reconstituted water (100 g product/L) Laminarin was analysed at the beginning of the study and at the end of the study.

4. **Observations**

Immobilization was observed by visual control after 24 and 48 hours of exposure. Any abnormal behavior or appearance was reported.

5. **Statistics**

For determination of the NOEC, F-variance analysis at 5% confidence level served to judge upon significant deviation of mean percentage of immobilization within the treatment group as compared to the control.

## **II. RESULTS AND DISCUSSION**

### **A. ANALYTICAL DETERMINATION**

Due to the weak concentration of Laminarin in the solution, the analysis method can detect the Laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

### **B. IMMOBILISATION**

**Table CP 10.2.1-2 Immobilisation of daphnids exposed for 48 hours to Vacciplant Fruits et Légumes**

Nominal concentration of test item (mg/L)	% immobilization at 24 hours				% immobilization at 48 hours			
	Rep.1	Rep.2	Rep.3	Rep.4	Rep.1	Rep.2	Rep.3	Rep.4
0	0	0	20	0	0	0	20	0
105.1	0	0	0	0	0	0	0	0
Dichromate 0.6 mg/L	0	0	20	0				
Dichromate 1.0 mg/L	80	60	60	40				
Dichromate 1.7 mg/L	80	100	100	100				

### III. CONCLUSIONS

In the conditions of the test, the 48 hours NOEC and EC<sub>50</sub> of Vacciplant Fruits et Légumes to *Daphnia magna* was determined to be 105.1 mg test item/L and > 105.1 mg test item/L, respectively, based on nominal concentration (dose limit test).

(Servajean E., 2008b)



Study Comments: IIIA 10.2.1/02	<p>The analytical method used for the analysis of the test concentrations to which the fish were exposed is discussed in part B5. The method was considered valid.</p> <p>No mortality occurred in any of the test item treated units, except in replicate number 3 of the control (20%). As the average mortality in the control was 5% the study met this validity criterion as the mortality was &lt;10%. The study also met the other validity criteria according to OECD 202. All test conditions remained within the ranges prescribed by the protocol.</p> <p>As the measured concentration of the test item was &gt; 80% of the nominal value, the nominal value was used.</p> <p>the RMS considers the study reliable without restrictions.</p>
Agreed endpoint/s: IIIA 10.2.1/02	The 48-hour LC50 of Vacciplant Fruits et Légumes to <i>Daphnia magna</i> was > 105.1 mg/L test item/L, equivalent to > 4.89 mg laminarin/L at 48 hours after application (nominal concentration).

#### B.9.3.1.3 Toxicity to algae

Data point addressed	CP 10.2.1/03
Author(s) (year)	Servajean E. (2008c)
Title	Freshwater alga, Growth inhibition test (OECD 201, March 2006)
Report number (Doc. No.)	Report No.08-29-009-ES
Testing facility	Phytosafe s.a.r.l, Pau, France
Published	No
Test guideline used	OECD No.201 (2006)
Deviations	<p>1. The stability of the test item in water was checked by appropriate analytical verification of the test solutions at test initiation and at test completion.</p> <p>2. In the range-finding test, the control group involved 2 replicates units instead of one.</p> <p>These deviations were not considered to have affected the integrity of the study.</p>
GLP	Yes

**Executive Summary:**

In a static toxicity test, the algal species *Desmodesmus subspicatus* was exposed for 72 hours to the limit nominal concentration of 103.3 mg/L of Vacciplant Fruits et Légumes. The initial cell concentration was approximately 2.03 E+06 cells/mL.

Due to the weak concentration of Laminarin in the solution, the analysis method can detect the laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

The specific growth rate was slightly affected within the first day of testing. On the days later, the growth was restored but the overall specific growth rate remained slightly reduced as compared to the control: percent inhibition throughout the 72 h-testing period was 3.1%.

The yield of biomass after 72 h of testing was significantly reduced (14.6%) as compared to the control. In the light of the analysis of specific growth rate, the result was taken as a consequence of the initial affected period (first 24 h of testing), but the conclusion was that the exponential growth after this initial period was not affected.

$E_rC_{50}$  (72 h) > 103.3 mg test item/L

$E_bC_{50}$  (72 h) > 103.3 mg test item/L

**I. MATERIALS AND METHODS****A. MATERIALS:**

- |                                    |  |
|------------------------------------|--|
| <b>1. Test Material:</b>           | Vacciplant Fruits et Légumes = Iodur 2 Cultures spécialisées   |
| <b>Description:</b>                | beige opaque liquid  |
| <b>Lot/Batch #:</b>                | 040308   |
| <b>Purity:</b>                     | Nominal: Laminarin: 45 g/L<br>Analytical: Laminarin: 46.5 g/L  |
| <b>Stability of test compound:</b> | stable in test conditions  |
| <b>2. System:</b>                  | static   |
| <b>3. Test organism</b>            |  |
| <b>Species:</b>                    | <i>Desmodesmus subspicatus</i>   |
| <b>Initial cell count:</b>         | 2.03 E+06 cells/mL, in exponential growth phase.   |
| <b>Source:</b>                     | The strain was provided by the Muséum National d'Histoire Naturelle (Paris, France) and regularly sub-cultures in the OECD medium at the Phytosafe site. |
| <b>Acclimation period:</b>         | pre-culture prepared 2-4 days prior to the test start under the same conditions as in the test.  |
| <b>Holding conditions:</b>         | Glass Erlenmeyer's of 250 mL volume filled with 100 mL of culture.   |
| <b>Environmental conditions</b>    |  |

<b>Water Temperature:</b>	21°C – 24°C
<b>pH:</b>	6.69 – 7.28 (start); 7.51 – 7.61 (end)
<b>Photoperiod:</b>	continuous illumination (4400 – 8880 lux)

## B. STUDY DESIGN AND METHODS:

1. **In life dates:** from 04/04/2008 to 24/04/2008

2. **Organisms assignment and treatment**

The alga *Desmodesmus subspicatus* was exposed for 72 hours to the limit nominal concentration of 103.3 mg/L of Vacciplant Fruits et Légumes in six replicates. There was six replicate units for the control and three replicate units for the reference toxic (Potassium dichromate). The initial cell concentration was approximately 2.03 E+06 cells/mL.

3. **Test item analysis**

The analyses were performed with HPLC method by Goëmar. After dilution of Vacciplant Fruits et Légumes in the reconstituted water (100 g product/L) Laminarin was analysed at the beginning of the study and at the end of the study.

4. **Observations**

The algal biomass in each flask was determined daily during the test period, using small volumes removed from the test solution by pipette. These volumes were not replaced. The numeration was done by manual cell counting by microscope.

Microscopic observation was performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae at the end of the test.

5. **Statistics**

The NOEC was derived from F-variance analysis at 5%-confidence level of the response variable (specific growth rate or yield) in each treated group as compared to the control group. Classically the EC<sub>50</sub> values and 95%-confidence intervals were derived from regression analysis of the concentration response-curves. In this case where no dose-related effect was observed, the EC<sub>50</sub> values were not calculated.

## II. RESULTS AND DISCUSSION

Due to the weak concentration of Laminarin in the solution, the analysis method can detect the Laminarin without giving the precise quantity because it is outside the concentration range. In the chromatogram in the report, the peak 1 shows the presence of Laminarin in the solution at the beginning of the study.

Cell concentrations are summarized in CP 10.2.1-3. The inhibition of growth rate and yield are provided in Table CP 10.2.1-4.

**Table CP 10.2.1-3: Cell concentration of *Desmodesmus subspicatus* exposed to Vacciplant Fruits et Légumes for 72 hours**

Replicate	Cell concentration (*10 <sup>6</sup> cells/mL)
-----------	--

	0 hour	24 hours	48 hours	72 hours
<b>Control</b>				
Replicate 1	2.03	15.5	81.0	360.0
Replicate 2	2.03	12.0	74.5	309.5
Replicate 3	2.03	9.5	63.5	287.0
Replicate 4	2.03	11.0	60.5	279.5
Replicate 5	2.03	11.5	82.5	309.0
Replicate 6	2.03	14.0	81.0	322.0
<b>Test item 103.3 mg/L</b>				
Replicate 1	2.03	8.0	65.5	260.5
Replicate 2	2.03	9.5	56.5	241.0
Replicate 3	2.03	8.5	60.5	269.0
Replicate 4	2.03	12.0	67.5	294.5
Replicate 5	2.03	8.5	60.5	257.5
Replicate 6	2.03	9.0	66.0	273.0
<b>Potassium dichromate 0.2 mg/L</b>				
Replicate 1	2.03	6.5	36.5	206.5
Replicate 2	2.03	8.5	35.0	222.5
Replicate 3	2.03	9.0	36.5	214.5
<b>Potassium dichromate 0.6 mg/L</b>				
Replicate 1	2.03	3.5	17.5	65.0
Replicate 2	2.03	3.0	14.5	69.0
Replicate 3	2.03	4.5	14.5	70.5
<b>Potassium dichromate 0.75 mg/L</b>				
Replicate 1	2.03	3.5	7.5	39.0
Replicate 2	2.03	2.0	8.0	53.5
Replicate 3	2.03	2.0	7.5	47.0
<b>Potassium dichromate 1.0 mg/L</b>				
Replicate 1	2.03	1.0	4.0	21.0
Replicate 2	2.03	1.0	2.5	14.5
Replicate 3	2.03	1.5	2.5	14.0

**Table CP 10.2.1-4 Inhibition of growth of *Desmodesmus subspicatus* exposed to Vacciplant Fruits et Légumes for 72 hours**

Nominal concentration	Growth rate (0 -72 hours)	Yield (0 - 72 hours)	0-72 h inhibition (%) in	
			Growth rate	Yield
Control	1.68	3.09 E+08	-	-
Test item at 103.3 mg/L	1.62	2.64 E+08	3.0	14.6
Potassium dichromate 0.2 mg/L	1.55	2.12 E+08	7.34	31.27
Potassium dichromate 0.6 mg/L	1.17	6.61 E+07	30.14	78.61
Potassium dichromate 0.75 mg/L	1.04	4.45 E+07	37.90	85.61
Potassium dichromate 1.0 mg/L	0.69	1.45 E+07	58.68	95.32

#### Effects of the test item

The specific growth rate was slightly affected within the first day of testing. On the days later, the growth was restored but the overall specific growth rate remained slightly reduced as compared to the control: percent inhibition throughout the 72 h-testing period was 3.1%.

The yield of biomass after 72 h of testing was significantly reduced (14.6%) as compared to the control. In the light of the analysis of specific growth rate, the result was taken as a consequence of the initial affected period (first 24 h of testing), but the conclusion was that the exponential growth after this initial period was not affected.

The 72-hour  $E_rC_{50}$  and  $E_yC_{50}$  were determined to be:

	$E_rC_{50}$	$E_yC_{50}$
$EC_{50}$ (mg/L)	> 103.3	> 103.3

### III. CONCLUSIONS

The influence of Vacciplant Fruits et Légumes on the growth of the algae *Desmodesmus subspicatus* was assessed in a static dose limit test. The 72-hour  $E_rC_{50}$  and the 72-hour  $E_yC_{50}$  values were determined to be > 103.3 mg test item/L.

(Servajeau E., 2008c)

Study Comments: IIIA 10.2.1/03	<p>The analytical method used for the analysis of the test concentrations to which the fish were exposed is discussed in part B5. The method was considered valid.</p> <p>The study met the validity criteria according to OECD 201. All test conditions remained within the ranges prescribed by the protocol.</p> <p>As the measured concentration of the test item was &gt; 80% of the nominal value, the nominal value was used for endpoint determination.</p> <p>The 72-hour <math>E_vC_{50}</math> and <math>E_rC_{50}</math> are both greater than the maximum tested dose of 103.3 mg product/L (&gt; 4.8 mg a.s./L). Since the effects on growth rate at 72 hours were &lt; 10%, no <math>E_rC_{10}</math> or <math>E_rC_{20}</math> could be calculated. In addition the effect on yield at 72-hours was less than 20%, however, an <math>E_vC_{10}</math> of 81 mg product/L (or 3.77 mg a.s./L) could be calculated.</p>
Agreed endpoint/s: IIIA 10.2.1/03	72-hour $E_rC_{50}$ = > 103.3 mg product/L (> 4.8 mg a.s./L).

### B.9.3.2 Additional long-term and chronic toxicity to fish, aquatic invertebrates and sediment dwelling organisms

Laminarin is readily biodegradable, so it is not expected to be persistent in surface water. In addition, laminarin shows low acute toxicity to aquatic organisms.

In addition,  $\beta$ -1,3-glucan has been regularly investigated for use in aquaculture due to positive immune regulating properties. Please see Section CA 9.3 for more information. Considering this, no chronic studies with the product were considered necessary.

#### Co-formulant

Vacciplant Fruits et Légumes contains [REDACTED], which is classified H412 (chronic 3) for aquatic. Since no chronic studies for aquatic organisms were performed with the product, the toxicity data for [REDACTED] from the SDS is presented.

**Table CP 9.3.2-1 Toxicity of [REDACTED] to aquatic organisms**

Group	Test Species	Test duration	Toxicity endpoints	Results (mg/L)	Reference
Fish	<i>Oryzias latipes</i>	96 h	LC <sub>50</sub>	59.5	SDS (see Doc J Point CP 7.4)
	<i>Leuciscus idus</i>	48 h	NOEC	50	
Invertebrates	<i>Daphnia magna</i>	48 h	EC <sub>50</sub>	11.2	
		21 d	NOEC	0.2	
Algae	<i>Pseudokirchneriella subcapitata</i>	72 h	EC <sub>50</sub>	91	

### B.9.4 Risk assessment for aquatic organisms

#### B.9.4.1 Aquatic risk assessment

In Volume 1, section 2.9.2.1 an overview of the available endpoints for aquatic organisms is given and the relevant endpoints for the risk assessment are determined.

The risk assessment for aquatic organisms has been conducted in line with the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001).

*Metabolites*: No relevant metabolites of laminarin in surface water were identified in Section CP B.9.2.5.

The acute risk assessments were carried out using the initial  $PEC_{SW}$  calculated with FOCUS Surface Water, Step 2 and the lowest available toxicity endpoints for algae, *Daphnia* and fish. There is no final aquatic higher plant endpoint available at this time, so the risk assessment will again change once the endpoint becomes available. The resulting TER-values are presented for the different crops for laminarin. TERs that do not meet the trigger of 10 for algae and the trigger of 100 for *Daphnia* and fish for the acute risk are presented in bold.  $PEC_{SW}$  estimations may be found in Section CP.B.8.5.

**Table B.9.4-01 Toxicity-Exposure Ratios for Aquatic organisms based on FOCUS Step 2 PECs**

Species	L(E)C <sub>50</sub> or NOEC	Pome fruit, early application Step 2	Vines, early application Step 2	Leafy and fruting vegetables Step 2
	[µg as/L]	(PEC <sub>SW</sub> 38.2 µg/L)	(PEC <sub>SW</sub> 30.2 µg/L)	(PEC <sub>SW</sub> 71.7 µg/L)
<b>Acute</b>				
Algae	> 4800	> 125.7	> 158.9	> 66.9
Invertebrates	> 100000	> 2617	> 3311	> 1394
Fish	> 100000	> 2617	> 3311	> 1394

### Chronic Risk Assessment

No chronic studies were performed with laminarin, considering the fact that plants are the most sensitive species, and considering the nature of the active substance. The chronic risk to aquatic life can be considered low.

Further, considering the fact that the co-formulant [REDACTED] is present only at < 1% in the formulated product (0.22%), and the fact that the most sensitive species is invertebrates (and therefore different from the most sensitive species for laminarin) the RMS finds it unlikely that the product would present a chronic risk to aquatic life.

### B.9.4.2 Bioconcentration

The log Pow for laminarin is -1.16. Thus, no fish bioconcentration study was performed and a risk assessment for bioconcentration (in fish) is not necessary.

## **B.9.5 Effects on arthropods**

### **B.9.5.1 Effects on bees**

#### **B.9.5.1.1 Acute toxicity to bees**

Since the formulation Vacciplant Fruits et Légumes contains only one active substance, no toxicity test with this product is required. The toxicity is predicted with the active substance data.

#### **B.9.5.1.2 Chronic toxicity to bees**

It is not expected that the product will be more toxic than the active substance for a chronic exposure. Thus, no chronic toxicity study on bees was conducted with the product and is not required.

#### **B.9.5.1.3 Effects on honeybee development and other honeybee life stages**

It is not expected that the product will be more toxic than the active substance for a chronic exposure. Therefore, no larval toxicity study was conducted or required.

#### **B.9.5.1.4 Sub-lethal effects**

No sub-lethal effects were observed in toxicity studies with the active substance, thus no toxicity study to investigate sub-lethal effects on bees was conducted with the product nor is one required.

#### **B.9.5.1.5 Cage and tunnel tests**

The risk of the product, Vacciplant Fruits et Légumes, to bees is acceptable when applied according to the Good Agricultural Practice, as shown by the HQ calculations. Therefore, no cage or tunnel tests are required.

#### **B.9.5.1.6 Field tests with honeybees**

The risk of the product, Vacciplant Fruits et Légumes, to bees is acceptable when applied according to the Good Agricultural Practice, as shown by the HQ calculations. Therefore, no field tests are required.

### **B.9.5.2 Effects on non-target arthropods other than bees**

#### **B.9.5.2.1 Standard laboratory tests for non-target arthropods**

Two laboratory studies were performed with PHYLIQ, the old representative formulation. They were submitted previously in support of Laminarin inscription in Annex I of the Directive 91/414/EEC. The RMS has re-evaluated the studies (see CA 9.3.2), and recalculated the endpoints derived. These have been adjusted in the summaries below. Although the lead formulation for this dRAR contains slightly more laminarin than the formulation Phylq (45 g laminarin/L vs 37 g laminarin/L), the RMS considers the studies acceptable to assess the toxicity of laminarin to non-target arthropods since the co-formulants are equivalent or less toxic and the main ingredients are the a.s. and water. To address the



differing levels of a.s., the risk assessment will be performed considering the g a.s./ha, rather than L formulation/ha.

#### **Study 9.5.2.1-1 Acute toxicity to *Aphidius rhopalosiphi***

- 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 (> 370 g a.s./ha). The reproductive ER<sub>50</sub> was also > 10 L product/ha (> 370 g a.s./ha). The ER<sub>10</sub> and ER<sub>20</sub> were calculated to be 0.8 L product/ha (29.6 g a.s./ha) and 2.0 L product/ha (74 g a.s./ha), respectively.

#### **Study 9.5.2.1-2 Acute toxicity to *Typhlodromus pyri***

- 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 Phylq (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 Phylq 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 (114.7 g a.s./ha) and the NOER for fecundity was > 10.0 L/ha (> 370 g a.s./ha). The LR<sub>10</sub> and LR<sub>20</sub> were 0.1 and 0.4 L/ha (3.7 and 14.8 g a.s./ha), respectively.

#### **B.9.5.2.2 Extended laboratory testing, aged residue studies with non-target arthropods**

Since the risk for non-target arthropods is acceptable by considering the results of laboratory studies, no extended laboratory studies are required.

#### **B.9.5.2.3 Semi-field studies with non-target arthropods**

Semi-field tests with non-target arthropods are not required because the risk for non-target arthropods from exposure to Vacciplant Fruits et Légumes is considered low when recommended use instructions are respected.

#### **B.9.5.2.4 Field studies with non-target arthropods**

Field tests with non-target arthropods are not required because the risk for non-target arthropods from exposure to Vacciplant Fruits et Légumes is considered low when recommended use instructions are respected.

### **B.9.6 Risk assessment for arthropods**

#### **B.9.6.1 Risk assessment for bees**

Procedures for risk assessment are conducted in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to EPPO (EPPO 170 and EPPO

2002). EPPO 2002 was updated with EPPO 2010<sup>3</sup>, an update focussing especially on systemic substances.

Exposure to bees is relevant for the proposed uses based on the following considerations: the crops themselves are attractive to bees for nectar and/or pollen collection.

**In Volume 1, section 2.9.3.1 an overview of the available endpoints for bees is given.**

#### **Acute risk to honey bees**

An indication of hazard (Hazard Quotient or HQ) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the single application rate (expressed in g/ha) and the lowest laboratory contact and oral LD<sub>50</sub> (expressed in µg/bee).

$$\text{HQ} = \text{Application rate [g/ha]} / \text{LD}_{50} \text{ oral or LD}_{50} \text{ contact [\mu g/bee]}$$

HQ values are calculated using data from the studies performed with the formulated product. HQ values higher than 50 indicate the need of higher tiered tests to clarify the actual risk to honey bees. For the acute risk assessment the lowest LD<sub>50</sub> (expressed in a.s.) of > 100 µg a.i./bee is used.

**Table 9.6.1-1 Hazard quotients for bees – acute exposure**

<b>Crop</b>	<b>Exposure route</b>	<b>LD<sub>50</sub> [µg a.i./bee]</b>	<b>Application rate [g a.s./ha]</b>	<b>Hazard quotient HQ</b>	<b>Trigger</b>	<b>Refined risk assessment</b>
Leafy vegetables	Contact and oral	>100	135	< 1.35	50	No

As shown in Table 9.6.1-1, the acute risk to honey bees from the use of Vacciplant Fruits et Legumes according to the GAP is acceptable.

No other toxicity data for honey bees are available, and no further risk assessment is deemed necessary. Laminarin is rapidly broken down into smaller carbohydrate strands and eventually glucose. Chronic exposure to parent laminarin is not expected and the breakdown products are not bee toxic.

#### **B.9.6.2 Risk assessment for non-target arthropods other than bees**

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002, referring to ESCORT 2). The recommendations of ESCORT 3, a follow-up workshop, will be taken into account for this DAR for issues where guidance is lacking.

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<sup>3</sup> EPPO 2010: Environmental risk assessment scheme for plant protection products. Chapter 10: Honeybees. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 40, 323–331

**In Volume 1, section 2.9.3.2 an overview of the available endpoints for non-target arthropods is given.**

Predicted Environmental Rates (PERs) are estimated according to ESCORT 2 (Candolfi *et al.*, 2001). The field rate (corresponding to the in-field rate) and the drift rate (corresponding to the off-field rate) are calculated with the following formulae:

Field rate ( $PER_{in-field}$ ) = Application rate \* MAF

Drift rate ( $PER_{off-field}$ ) = Application rate \* MAF \* (drift factor / vegetation distribution factor)

where:

Application rate: expressed as L product/ha

MAF: Multiple Application Factor from ESCORT 2 Guidance Document

Drift factor: at 1 m for field crops, at 3 m for orchards and vineyards according to overall corresponding percentile drift data of Ganzelmeier *et al.* (1995, recently recalculated by German BBA and UBA and published by BBA, 2000).

Vegetation distribution factor (VDF): drift interception by the vegetation (default value: 10)

**Table 9.6.2-1 In-field PER values for the intended uses of Vacciplant Fruits et Légumes**

Crop	Scenario	Max. number of Applications	MAF	GAP Application Rate [g a.s./ha]	$PER_{in-field}$ (g a.s./ha)
Apple	Fruit crops (early)	20	3.5	45	157,5
Apple, Pear	Fruit crops (early)	7	3.4	33.75	114,75
Vine	Grapevine (late)	10	3.5	90	315
Lettuce	Field crops	16	3.5	112,5	393,75
Lettuce	Field crops	7	3.4	135	459
Strawberry	Field crops	7	3.4	90	306
Tomato	Field crops	7	3.4	135	459
Zucchini, Pumpkins	Field crops	6	3.2	33.75	108
Aubergine, Pepper, Greenbean, Cucumber, Zucchini	Field crops	7	3.4	135	459
Kiwi	Fruit crops (early)	7	3.4	90	306

**Table 9.6.2-2 Off-field PER values for the intended uses of Vacciplant Fruits et Légumes**

Crop	Scenario	PER <sub>in-field</sub> (g a.s./ha)	Drift factor (%)	VDF	PER <sub>off-field</sub> (g a.s./ha)
Apple	Fruit crops (early)	157,5	22.24	10	3.50
Apple, Pear	Fruit crops (early)	114,75	22.69	10	2.60
Vine	Grapevine (late)	315	6.26	10	1.97
Lettuce	Field crops	393,75	1.52	10	0.599
Lettuce	Field crops	459	1.61	10	0.739
Strawberry	Field crops	306	1.61	10	0.493
Tomato	Field crops	459	1.61	10	0.739
Zucchini, Pumpkins	Field crops	108	1.64	10	0.177
Aubergine, Pepper, Greenbean, Cucumber, Zucchini	Field crops	459	1.61	10	0.739
Kiwi	Fruit crops (early)	157,5	22.69	10	3.56

**Hazard quotients calculations**

Hazard quotients are calculated in-field and off-field according to the formulae below:

$$HQ_{in-field} = PER_{in-field} / LR_{50}$$

$$HQ_{off-field} = (PER_{off-field} / LR_{50}) * \text{correction factor}$$

where:

LR<sub>50</sub>: expressed L Vacciplant Fruits et Légumes/ha

Correction factor: applied to cover the inter-species variability in sensitivity of off-field non-target arthropod species to plant protection product (default value: 10)

In-field HQ and off-field HQ values are calculated in Table 9.6.2-3 and 9.6.2-4, below. Only the worst-case uses are presented. These cover all other proposed uses.

**Table 9.6.2-3: In-field HQ values for *Aphidius rhopalosiphi* and *Typhlodromus pyri***

Crop	Max. number of Applications	GAP Application Rate [g a.s./ha]	PER <sub>in-field</sub> (g a.s./ha)	LR <sub>50</sub> (g a.s./ha)	In-field HQ	Trigger
<b><i>Aphidius rhopalosiphi</i></b>						
Lettuce, Tomato, Aubergine, Pepper, Greenbean,	7	135	459	> 370	< 1.24	2

Crop	Max. number of Applications	GAP Application Rate [g a.s./ha]	PER <sub>in-field</sub> (g a.s./ha)	LR <sub>50</sub> (g a.s./ha)	In-field HQ	Trigger
Cucumber, Zucchini						
<b><i>Typhlodromus pyri</i></b>						
Lettuce, Tomato, Aubergine, Pepper, Greenbean, Cucumber, Zucchini	7	135	459	114.7	<b>4.0</b>	2

Since this resulted in an in-field risk to *T. pyri*, other uses were assessed:

**Table 9.6.2-4: In-field HQ values for *Aphidius rhopalosiphi* and *Typhlodromus pyri***

Crop	Max. number of Applications	GAP Application Rate [g a.s./ha]	PER <sub>in-field</sub> (g a.s./ha)	LR <sub>50</sub> (L PP/ha)	In-field HQ	Trigger
<i>Aphidius rhopalosiphi</i>						
Vine	10	90	315	> 370	< 0.85	2
Kiwi, strawberry	7	90	306		< 0.83	
Apple, early	20	45	157.5		< 0.43	
<i>Typhlodromus pyri</i>						
Vine	10	90	315	114.7	<b>2.75</b>	2
Kiwi, strawberry	7	90	306		<b>2.67</b>	
Apple, early	20	45	157.5		1.37	

The in-field risk from uses in apple, pear, zucchini and pumpkin show an acceptable risk to non-target arthropods. For all other uses a refined in-field risk assessment is required. Since the toxicity values for *T. pyri* were re-calculated from the test from the original Annex I inclusion by the RMS at the time of the initial review, the notifier did not expect the issue with *T. pyri*. To address this, the notifier has begun an extended laboratory test with *T. pyri* (expected completion mid-2016), but the RMS notes that in the event that the Tier I trigger is not met, testing with two additional species is required. However, it might also be considered that laminarin is readily biodegradable (see CP B8), and subject to quick degradation by ubiquitously present laminarases (see section CA B7). Therefore, although no measured DT<sub>50</sub> value in plants (or other matrices) is available, the MAF values used in the risk assessment represent a worst-case, and it is unlikely that the true in-field PERs are at the levels presented in the risk assessment above. For example, if a MAF of 1.7 is assumed, all uses would be acceptable (use in lettuce, tomato, aubergine, pepper, green bean, cucumber and zucchini would show an HQ of 2.0). The RMS would appreciate the opinion of the MSs as to whether they consider further testing in additional species necessary.

Table 9.6.2-5: Off-field HQ values for *Typhlodromus pyri* and *Aphidius rhopalosiphi*

Crop	Max. number of Applications	GAP Application Rate [g a.s./ha]	PER <sub>off-field</sub> (g a.s./ha)	Correction factor	LR <sub>50</sub> (g a.s./ha)	Off-field HQ	Trigger
<b><i>Aphidius rhopalosiphi</i></b>							
Apple	20	45	3.50	10	> 370	< 0.09	2
Apple, Pear	7	33.75	2.60			< 0.09	2
Vine	10	90	1.97			< 0.05	2
Lettuce	16	112,5	0.599			< 0.02	2
Lettuce	7	135	0.739			< 0.02	2
Strawberry	7	90	0.493			< 0.01	2
Tomato	7	135	0.739			< 0.02	2
Zucchini, Pumpkins	6	33.75	0.177			< 0.005	2
Aubergine, Pepper, Greenbean, Cucumber, Zucchini	7	135	0.739			< 0.02	2
Kiwi	7	90	3.56			< 0.91	2
<b><i>Typhlodromus pyri</i></b>							
Apple	20	45	3.50	10	114.7	0.31	2
Apple, Pear	7	33.75	2.60			0.31	2
Vine	10	90	1.97			0.17	2
Lettuce	16	112,5	0.599			0.05	2
Lettuce	7	135	0.739			0.07	2
Strawberry	7	90	0.493			0.04	2
Tomato	7	135	0.739			0.07	2
Zucchini, Pumpkins	6	33.75	0.177			0.02	2
Aubergine, Pepper, Greenbean, Cucumber, Zucchini	7	135	0.739			0.07	2
Kiwi	7	90	3.56			0.61	2

The off-field HQs are below the trigger of 2, indicating an acceptable risk off-field for both standard non-target arthropod species when Vacciplant Fruits et Légumes is applied according to the Good Agricultural Practice.

### **B.9.7 Effects on non-target soil meso- and macrofauna**

#### **B.9.7.1 Effects on earthworm**

As laminarin is expected to have a very short soil persistence, as laminarinase is found in many micro-organisms (for more details, please see Point CA 8.5). Therefore, no studies were performed with the formulation. An earthworm reproduction study was performed with the active substance and is summarized in Volume 3CA. The risk assessment may be found below.

#### **B.9.7.2 Effects on non-target soil meso- and macrofauna (other than earthworms)**

#### **B.9.7.3 Risk assessment for earthworms**

##### **Toxicity data**

No data on acute and chronic toxicity were deemed required in the review report of Laminarin. In the framework of the renewal of the active substance laminarin, a chronic (reproduction) study in earthworms was submitted by the notifier and summarized in section CA B.9.4.2.1.

##### Acute risk assessment

According to Regulation 283/2013, an acute toxicity study on earthworms is no longer required. Consequently, no study on the acute toxicity of laminarin to earthworms was conducted.

##### Long-term risk assessment

The potential chronic risk to earthworms was assessed by comparing the  $PEC_{\text{Soil, initial}}$  with the NOEC value to generate chronic TER values. The  $TER_{LT}$  was calculated as follows:

$$TER_{LT} = \frac{NOEC(mg/kg)}{PEC_{\text{Soil}}(mg/kg)}$$

The worst-case  $TER_{LT}$  value is shown in Table CP 9.4-4 based on the maximum initial  $PEC_{\text{soil}}$  (found in volume B.8 CP, **Table B.10.1.3-3**).

**Table CP 9.4-4: Chronic risk assessment for earthworms exposed to Vacciplant Fruits et Légumes**

Active substance	Worst-case use	Number of applications	Maximum use rate [g a.s. /ha]	Initial $PEC_s$ [mg a.s./kg]	NOEC [mg a.s./kg]	$TER_{LT}$	Trigger
Laminarin	Lettuce	16	113	0.700	249	356	5

The worst-case long-term TER value is significantly greater than the trigger value of 5, therefore the chronic risk to earthworms exposed to Vacciplant Fruits et Légumes is considered acceptable.



**B.9.7.4 Risk assessment for soil meso- and macrofauna (other than earthworms)**

According to the Guidance Document (SANCO/10329/2002) the risk for macro-organisms particularly addresses persistent active substances ( $DT_{90 \text{ field}} > 100$  days). Testing is required where contamination of soil is possible, the  $DT_{90 \text{ field}}$  is between 100 and 365 days and the HQ for the standard arthropod species (*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. Thus, a very low persistence is expected for laminarin. Although the HQ for *T. pyri* was greater than 2, this is considered highly conservative, as it does not take into account the rapid dissipation of laminarin from plant surfaces. With this, and the rapid dissipation in soil in mind, the RMS does not consider further testing with soil macrofauna necessary.

**B.9.8 Risk assessment for soil nitrogen transformation**

As laminarin is expected to have a very short soil persistence, and as laminarinase is found in many micro-organisms (for more details, please see Point CA 8.5), no risk to soil non-target micro-organisms are to be expected from the applications of Vacciplant Fruits et Légumes in the field.

**B.9.9 Effects on soil nitrogen transformation**

No studies were submitted, but considering the ubiquitous presence of laminarinase in soil microorganisms and fungi, no effect on nitrogen transformation is expected.

**B.9.10 Effects on terrestrial non-target higher plants****B.9.10.1 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, Pumkins	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 (*Solanaceae*), zucchini and pumkins (*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, *Solanaceae* with tomato, *Cucurbitaceae* with zucchini and pumkins). 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.9.10.2 Testing on non-target plants**

##### **B.9.10.3 Extended laboratory studies on non-target plants**

None performed, nor considered necessary.

##### **B.9.10.4 Semi-field and field tests on non-target plants**

None performed, nor considered necessary.

#### **B.9.11 Risk assessment for terrestrial non-target higher plants**

The screening data showed no phytotoxicity to leek, but using efficacy trials to prove a lack of phytotoxicity is difficult (see full summary above). RMS notes that laminarin is used as an elicitor of the crop's self-defence mechanisms and is considered beneficial for plants. The RMS does not expect adverse effects on non-target plants following applications of laminarin according to the GAP, and the co-RMS concurs.

#### **B.9.12 Effects on other terrestrial organisms (flora and fauna)**

Laminarin is a polysaccharide of relatively small size ( $M_n = 5000 \text{ g.mol}^{-1}$ ), so 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.9.13 Risk assessment for other terrestrial organisms (flora and fauna)**

No effects on other terrestrial organisms are expected, considering the relatively low toxicity profile of laminarin.

**B.9.14 References relied on**

A literature search was carried out. In the search strategy search terms related to product were included in the first place. In table CP9.5-1 the general search terms are reported.

**Table B.10.5-1: Search terms for all sections**

Database: PubMed Search restrictions: all fields	Search terms	Number of summary records retrieved
Active substance common and ISO name	1. Laminarin	308
Active substance chemical name (CA)	2. Laminaran	24
Active substance other names or codes	3. H11	308 – not relevant denomination – will not be used further.
CAS No.	4. 9008-22-4	0
Chemical Name (IUPAC)	5. (1→3)-β-D-glucan	514
EC No	6. 232-712-4	0
CIPAC No.:	7. 671	not relevant denomination – will not be used further.
Trade names	8. IODUS 2 9. VACCIPLANT 10. VAXIPLANT	0 1 0 In view of the very low number of matches, the trade names will not be used further.

As reported in the table above hardly any records were retrieved and trade names were not used for section specific searching.

**Data submitted for the renewal**

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
CP 10.2/01	██████ █	2008a	Fish, Acute toxicity test (OECD 203, July 1992), GLP, Unpublished	Y	N		Laboratoires Goëmar SAS
CP 10.2/02	Servajeau E	2008b	Daphnia sp., Acute immobilisation test (OECD 202, April 2004) GLP, Unpublished	N	N		Laboratoires Goëmar SAS
CP 10.2/03	Servajeau E	2008c	Freshwater alga, Growth inhibition test (OECD 201, March 2006) GLP, Unpublished	N	N		Laboratoires Goëmar SAS