

ANNEX B

Laminarin

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.1 Acute oral toxicity (Annex IIA 8.1.1)

Laminarin – Acute oral toxicity (LD₅₀) to the bobwhite quail (Rodgers M.H., 2002)

Guidelines :

US EPA Subdivision E Guideline 71-1

GLP :

Yes

Material and Methods :

Test Substance: Laminarine, purity : 85%

Test species : Bobwhite quail (*Colinus virginianus*)

Sex, weight, age : 5 males and 5 females per concentration, 178-186 g, ± 14 months

Applied concentrations : untreated control, 500, 1000 and 2000 mg a.s. /kg body weight;

Type of application : oral intubation

Time of exposure : one single application, monitoring during 14 days

Findings :

Mortality : No mortalities were recorded.

Body weight : No evidence of a treatment related effect.

Clinical signs : No abnormalities were recorded.

Feed consumption : No evidence of a treatment related effect.

Endpoints :

LD₅₀ > 2000 mg a.s./kg bw

NOEL = 2000 mg a.s./kg bw

Conclusions :

Study is acceptable.

B.9.1.2 Avian dietary toxicity (5day) (Annex IIA 8.1.2)

Laminarin – Dietary toxicity (LC₅₀) to the bobwhite quail (Rodgers M.H., 2001).

Guidelines :

US EPA Subdivision F Guideline 71-2

GLP :

Yes

Material and Methods :

Test Substance: Laminarin, purity : 85%

Test species : Bobwhite quail (*Colinus virginianus*)

Sex, weight, age : the sex of the birds was not determined, ± 13 g, 10 days

Applied concentrations : untreated control, 156, 313, 625, 1250, 2500 and 5000 mg/kg feed;

Type of application : *ad libitum*, a.s. in food was not analysed

Time of exposure : Short-term feeding test (5 days with exposition by the feed + 3 days observations)

Findings :

Mortality : One bird from the control group and one bird from the 2500 ppm group died. These mortalities were considered as not treatment related.

Body weight : No evidence of a treatment related effect.

Clinical signs : No abnormalities were recorded.

Feed consumption : No evidence of a treatment related effect.

Endpoints :

LC₅₀ (5d) > 5000 ppm

NOEC (5d) = 5000 ppm

Conclusions :

Study is acceptable.

Laminarin is a natural polysaccharide which belongs to the group of β -glucans. β -glucan is a normal component of barley. Some references available in literature are related to the feeding of chickens with barley and on the effects of enzyme supplementation on the β -glucan transformation.

Natural influence of broiler chicken diets based on covered normal, waxy and high amylose barleys with or without enzyme supplementation (Bergh M.O. *et al.*, 1999)

A total of 384 day-old broiler chicken were fed unpelleted diets based on covered barley cultivars (696 g/kg) with normal, high amylose (HAG) and high amylopectin (waxy) starch for a total of 18 days. Diets were fed ad libitum without or with a supplementation of commercial enzyme preparation containing β -glucanase activity. Normal diets were found to contain approximately 30 g/kg total β -glucan while the HAG and waxy diets contained approximately 40 g/kg total β -glucan. Generally birds fed the normal diets weighed more, consumed more feed and had lower feed conversion ratios than animals fed the HAG and waxy diets, respectively at 13 days of age. Enzyme supplementation of diets generally increased chicken body weights and feed intakes, improved feed conversion ratios and increased total and HDL-cholesterol concentrations in comparison to birds provided diets without enzyme. Mortality for the duration of the experiment was 1% and was not influenced by individual diet barley type or enzyme supplementation. β -glucan increases the viscosity in the gastrointestinal tract and it is generally accepted that increased gastrointestinal viscosity may result in poorer utilisation of feed by chickens

Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens (Annison G., 1992)

Four commercial feed enzyme (Enzymes A, B, C and D) preparations possessing xylanase and β -glucanase activities were added to a wheat-based diet and fed to broiler chickens in a modified, classical apparent metabolisable energy trial. Elevated β -glucanase activity was detected in the ileal contents of birds fed diets supplemented with enzymes B and D. β -glucanase activity was detected in almost all the birds (except three on a total of 48 birds) including 7 of the 8 birds fed the control diet.

The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance (Vukic Vranjes M. *et al.*, 1995).

The experiment was conducted to study the effect of extruded vs. unprocessed barley in the diet on the response of broiler chickens to *Trichoderma viride* enzyme supplement. The four experimental diets were as follows : unprocessed barley, unprocessed barley + enzyme, extruded barley, extruded barley + enzyme. Factorial analysis confirmed the positive influence of barley extrusion and enzyme on β -glucan degradability.

Growth, viscosity and beta-glucanase activity of intestinal fluid in broiler chickens fed on barley-based diets with or without exogenous beta-glucanase (Philip J.S. *et al.*, 1995).

Three groups of thirty birds were fed diets containing 500 g barley/kg diet, with or without exogenous β -glucanase for up to 35 days. birds which received diets containing the exogenous enzymes grew faster for the first 21 days but after that there was no apparent difference in weight gain. the latter finding adds weight to the notion that either the birds adapt to the presence of β -glucanase in the diet or that the bigger size of the birds negates the adverse effects evident among younger birds. Viscosity of intestinal fluid in birds given only the basal diet decreased with age but there was no corresponding increase in β -glucanase activity. This discounts bacterial β -glucanase as a contributory factor in the adaptation to β -glucanase apparent in older birds.

B.9.1.3 Subchronic and reproductive toxicity (Annex IIA 8.1.3)

Continued or repeated exposure of adults or exposure of nest sites during the breeding season is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g/ha.

B.9.1.4 Acute oral toxicity of the preparations (Annex IIIA 10.1.1)

The preparation Phyliq contains only human food additives, except for 1.5% surfactant, in addition to the active substance. Testing of the acute oral toxicity of the preparations is not considered as necessary as it is very unlikely that the TER_a or the TER_{st} are below 100.

B.9.1.5 Supervised cage or field trials (Annex IIIA 10.1.2)

Not required.

B.9.1.6 Acceptance of bait, granules or treated seeds by birds (palatability test) (Annex IIIA 10.1.3)

Not required.

B.9.1.7 Effects of secondary poisoning (Annex IIIA 10.1.4)

Not required.

B.9.1.8 Summary of effects to birds - exposure and risk assessment for birds (Annex IIIA 10.1)

Tab. B.9.1.8-1 : Summary of effects of Laminarine to birds.

Test species	Test System	Results	References
Bobwhite quail	Acute oral toxicity	$LD_{50} > 2000$ mg a.s./kg bw	Rodgers M.H., 2002
Bobwhite quail	8 day dietary toxicity	LC_{50} (5d) > 5000 ppm	Rodgers M.H., 2001

The risk assessment for birds is based on the new Guidance Document for birds and Mammals Under Council Directive 91/414/EEC of November 2001. As a worst-case scenario it was assumed that the birds obtained 100% of their diet in the treated area.

The LC_{50} was recalculated from ppm into mg a.s./kg bw. Based on a mean bodyweight of 19.4 g and a mean food consumption of 3.8 g/bird/day, 5000 ppm becomes 980 mg a.s./kg bw.

Tab. B.9.1.8-2 : Estimated oral uptake of Laminarine by birds

Target crop	Bird type	Time scale	FIR/bw	RUD (90%)	MAF	ETE (mg a.s./kg bw/day)	Toxicity Exposure Ratio
Cereals 1 x 37 g a.s./ha	Herbivorous bird (early)	acute	0.44	142	1	2.31	865
	Insectivorous bird (late)	acute	1.04	14	n.a.	0.54	3712
	Herbivorous bird (early)	short term	0.44	76	1	1.24	792
	Insectivorous bird (late)	short term	1.04	5.1	n.a.	0.20	4994

The risk for birds resulting from the exposure to the a.s., sprayed in cereals, is negligible.

This conclusion is confirmed by references in literature :

Laminarin is a polysaccharide which belongs to the β -glucans. β -glucans are a natural compound of barley which contains approximately 40 g β -glucan per kilogram seeds (or 320 kg β glucan/ha, assuming a harvest of 8000 kg seeds/ha) (Bergh M.O. *et al.*, 1999 and Vukic Vranjes M. *et al.*, 1995).

References in literature do not mention any significant mortality due to the ingestion of β -glucans. Feed conversion in broiler chickens was ameliorated when β -glucanase was added to the diets (Bergh M.O. *et al.*, 1999). β -glucanase activity was also detected in birds fed a diet without enzyme preparations containing β -glucanase activity (Annison G., 1992). Birds seem to adopt to β -glucan-containing diets but the experiment provided no evidence to suggest that adaptation to dietary β -glucan involves the appearance of β -glucanase, whether of endogenous or bacterial origin, in the crop or small intestine of birds not receiving exogenous β -glucanase (Philip J.S. *et al.*, 1995).

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)**B.9.2.1 Acute toxicity of the active substance and metabolites, degradation or reaction products to fish (Annex IIA 8.2.1)**

Acute toxicity in freshwater fish (96 Hours) – *Oncorhynchus mykiss* – Semi-static system (Licata-Messana L., 2001a).

Guidelines :

EEC Directive 92/69 – C1 (1992)

OECD Guideline N° 203 (1992)

GLP :

Yes

Material and Methods :

Test substance : Laminarin, chemical purity : 91 %

Test species : Rainbow trout, *Oncorhynchus mykiss*

Number of organisms, length, loading : 7 fish per concentration, 68 - 95 mm, 1.3 g/L

Type of test : Semi-static system

Applied and measured concentrations :

nominal : control, 100 mg a.s./L. Nominal concentrations could not be verified as the limit of detection is 100 mg a.s./L.

Test conditions :

temperature : 15.4 – 16.3 °C

pH : 7.41 – 8.00

oxygen content : The dissolved oxygen concentration exceeded 60% of saturation.

total hardness : 90 – 105 mg of CaCO₃ per litre of test medium

Photoperiod : artificial, 12 hours out of 24.

Analytical methods : gas chromatography using a Flame Ionisation Detector.

Findings :

Mortality : No mortalities were observed during the study.

Behavioural observations : Nothing to report.

Endpoints :

LC₅₀ (96h) > 100 mg a.s./L

NOEC (96h) = 100 mg a.s./L

Conclusions :

The study is acceptable. In a preliminary test 5 concentrations (1-100 mg a.s./L) were tested beside the control. A microbial growth was observed in the test media. Therefore the main test was performed using a semi-static system with one concentration beside the control as no mortality was observed during the preliminary test.

Acute toxicity in freshwater fish (96 Hours) – *Danio rerio* – Semi-static system (Licata-Messana L., 2001b).

Guidelines :

EEC Directive 92/69 – C1 (1992)

OECD Guideline N° 203 (1992)

GLP :

Yes

Material and Methods :

Test substance : Laminarin, chemical purity : 91 %

Test species : Zebra fish, *Danio rerio*

Number of organisms, length, loading : 10 fish per concentration, 32 - 35 mm, 1.05 g/L

Type of test : Semi-static system

Applied and measured concentrations : nominal : control, 100 mg a.s./L. Nominal concentrations could not be verified as the limit of detection is 100 mg a.s./L.

Test conditions :

temperature : 20.3 – 22.0 °C

pH : 7.20 – 8.13

oxygen content : The dissolved oxygen concentration exceeded 60% of saturation.

total hardness : 90 – 100 mg of CaCO₃ per litre of test medium

Photoperiod : artificial, 12 hours out of 24.

Analytical methods : gas chromatography using a Flame Ionisation Detector.

Findings :

Mortality : No mortalities were observed during the study.

Behavioural observations : Nothing to report.

Endpoints :

LC₅₀ (96h) > 100 mg a.s./L

NOEC (96h) = 100 mg a.s./L

Conclusions :

The study is acceptable. In a preliminary test 5 concentrations (1-100 mg a.s./L) were tested beside the control. A microbial growth was observed in the test media. Therefore the main test was performed using a semi-static system with one concentration beside the control as no mortality was observed during the preliminary test.

B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2)

Continued or repeated exposure of fish is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g/ha. Therefore testing of the chronic toxicity of laminarin to fish is not considered as necessary.

References in literature mention the distribution of $\beta(1,3)$ -D-glucans in fish organs. This distribution was examined due to the immunostimulatory effects in fish.

B.9.2.3 Bioaccumulation potential in fish (Annex IIA 8.2.3)

Laminarin will only be applied once a year at a rate of 37 g/ha and the $\log P_{ow} < 3$ so no bioconcentration is expected. Therefore testing of the bioaccumulation potential of laminarin in fish is not considered as necessary

B.9.2.4 Acute toxicity to invertebrates (Annex IIA 8.2.4)

Acute toxicity of LAMINARIN to *Daphnia magna* in a 48-hour semi-static Immobilization Test (Hertl J., 2001).

Guidelines :

- Commission Directive 92/69/EEC, Annex Part C, C. 2.: "Acute Toxicity for *Daphnia*", December 29, 1992
- OECD Guideline 202 : "Daphnia sp. Acute Immobilisation Test and Reproduction Test", 1984

GLP :

yes

Material and Methods :

Test substance : Laminarin, chemical purity : 94 %

Test species : *Daphnia magna*

Number of organisms, age : 20 organisms per concentration, 6-21.5 hours old

Applied and measured concentrations : 10, 30 and 100 mg a.s./L. Measured concentrations ranging from 84 – 90 % of the nominal concentrations.

Test conditions :

temperature : 21°C

pH : 7.7-7.9

oxygen content : The dissolved oxygen concentration exceeded 60% of saturation.

total hardness : 2.5 mmol of CaCO₃ per litre of test medium

Photoperiod : 16h light : 8 h dark

Analytical methods : HPLC with amperometric detection

Findings :

Mortality : no immobilisation

Conclusions :

The study is in general in order with the guideline 92/69/EEG, method C2.

Endpoints :

EC₅₀ (48h) > 100 mg a.s./L

NOEC (48h) = 100 mg a.s./L

B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Continued or repeated exposure of aquatic invertebrates is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g/ha. Therefore testing of the chronic toxicity of laminarin to aquatic invertebrates is not considered as necessary.

B.9.2.6 Effects on algal growth (Annex IIA 8.2.6)

H11 – Algal growth inhibition study (Gnemi P., 2000).

Guidelines :

C3 EEC Guidelines

OECD 201 Guideline

GLP :

Yes

Material and Methods :

Test substance : Laminarin, chemical purity : not given

Test species : *Selenastrum capricornutum* CCAP 278/4

Number of organisms, length, loading : $3 \times \pm 1.0 \times 10^4$ cells/ml per test concentration, $6 \times \pm 1.0 \times 10^4$ cells/ml in the control.

Applied and measured concentrations : 0 and 100 mg/L; Measured concentrations ranging from 97.75 – 101.64 % of the nominal concentrations.

Test conditions :

temperature : 23 ± 2 ° C.

pH : 7.8 – 8.8.

total hardness : 40 mg of CaCO₃ per litre of test medium.

Photoperiod : continuous uniform illumination of approximately 6000 Lux in the spectral range 400 – 700 nm.

Analytical methods : HPLC.

Findings and conclusions:

The study is acceptable.

Endpoints :

E_bC₅₀ (72h) > 100 mg a.s./L

E_rC₅₀ (72h) > 100 mg a.s./L

NOEC (72h) = 100 mg a.s./L

B.9.2.7 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

Laminarin is readily biodegradable in the water phase (see point 8.4.3) so no risk for sediment dwelling organisms is expected. Therefore testing of the effects of laminarin on sediment dwelling organisms is not considered as necessary.

B.9.2.8 Effects on aquatic plants (Annex IIA 8.2.8)

Not required.

B.9.2.9 Acute toxicity of the preparations (Annex IIIA 10.2.1)

The preparation Phylig contains only human food additives, except for 1.5% surfactant, in addition to the active substance and will not be directly applied on water surfaces. Therefore acute toxicity studies with the formulation on water organisms are not considered necessary.

B.9.2.10 Microcosm and mesocosm study (Annex IIIA 10.2.2)

Not required.

B.9.2.11 Residue data in fish (Annex IIIA 10.2.3)

Laminarin will only be applied once a year at a rate of 37 g/ha and the $\log P_{ow} < 3$ so no bioconcentration and hence no residues are expected.

B.9.2.12 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)

Not required.

B.9.2.13 Summary of effects to water organisms (Annex IIA 8.2, Annex IIIA 10.2)

Table B.9.2.15-1 : Summary of effects of Laminarin to water organisms

Test species	Test system	Results	References
<i>Oncorhynchus mykiss</i>	semi-static (96 hours)	LC ₅₀ > 100 mg a.s./L NOEC = 100 mg a.s./L	Licata-Messana, 2001a
<i>Danio rerio</i>	semi-static (96 hours)	LC ₅₀ > 100 mg a.s./L NOEC = 100 mg a.s./L	Licata-Messana, 2001b
<i>Daphnia magna</i>	Semi-static (48 hours)	EC ₅₀ > 100 mg a.s./L NOEC = 100 mg a.s./L	Hertl, 2001
<i>Selenastrum capricornutum</i>	static (72 hours)	E _b C ₅₀ > 100 mg a.s./L E _r C ₅₀ > 100 mg a.s./L NOEC = 100 mg a.s./L	Gnemi, 2000

B.9.2.14 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

The details of the PEC calculations are presented on point B.8.6 but the following assumptions were made to assess the risks for water organisms :

- one application a year of 37 g a.s./ha
- depth of the waterlayer is 30 cm
- the toxicity endpoints were compared to the maximum concentrations just after the application of the active substance.

Table B.9.2.16-1 : Estimated initial concentrations of Laminarin in surface water

Target crop	Application rate (g a.s./ha)	Distance (m)	Drift (%)	Estimated initial concentration (mg a.s./l water)
Cereals	37	1	2.77	0.000342
		5	0.57	0.00007

Table B.9.2.16-2 : Theoretical Exposure Ratio for aquatic organisms exposed to Laminarin

Target crop	Test species	Time scale	Distance (m)	TER	Annex VI Trigger
Cereals	<i>Oncorhynchus mykiss</i>	acute	1	$> 100/0.000342 = 292398$	100
			5	$> 100/0.00007 = 1428571$	100
	<i>Daphnia magna</i>	acute	1	$> 100/0.000342 = 292398$	100
			5	$> 100/0.00007 = 1428571$	100
	<i>Selenastrum capricornutum</i>	acute	1	$> 100/0.000342 = 292398$	10
			5	$> 100/0.00007 = 1428571$	10

The acute risk of laminarin to aquatic organisms is acceptable.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3.1)

The risk assessment for small mammals is based on the new Guidance Document for birds and Mammals Under Council Directive 91/414/EEC of November 2001. As a worst-case scenario it was assumed that the mammals obtained 100% of their diet in the treated area.

Tab. B.9.3-1 : Summary of effects of Laminarin on terrestrial vertebrates

Test species	Test system	Effect concentration	References
rat	acute oral	LD ₅₀ > 2000 mg/kg bw	Delille, 1998a
	90 day feeding	NOAEL > 20000 mg/kg feed	Audeval, 2001a
	prenatal developmental study	NOAEL > 20000 mg/kg feed	Audeval, 2001c
	acute oral of the preparation	LD ₅₀ > 2000 mg/kg bw	Audeval, 2000a

The NOAEL values expressed in mg/kg feed were divided by 20 to obtain a NOAEL-value expressed in mg/kg bw for the TER calculations.

Tab. B.9.3-2 : Estimated oral uptake of Laminarin by small mammals

Target crop	Animal type	Time scale	FIR/bw	RUD (90%)	f _{twa}	MAF	ETE (mg a.i./kg bw/day)	Toxicity Exposure Ratio
Cereals 1 x 37 g a.s./ha	Herbivorous mammal (early)	acute	1.15	142	n.a.	1	6.04	331
	Insectivorous mammal (late)	acute	0.51	14	n.a.	n.a.	0.26	7571
	Herbivorous mammal (early)	long term	1.15	76	0.53	1	1.71	587
	Insectivorous mammal (late)	long term	0.51	5.1	n.a.	n.a.	0.096	10391

The acute and long term risk for mammals resulting from the exposure to the a.s. in crops treated with foliar spray formulation is negligible.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.3.2)

B.9.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)

Assessment of Side Effects of Laminarin to the Honey Bee, *Apis mellifera* L. in the Laboratory (Kling A., 2000).

Guidelines :

EPPO Guideline No. 170

GLP :

yes

Material and Methods :

Test substance : Laminarin, chemical purity : 91 %

Test species : Honeybees (*Apis mellifera* L.); foraging bees

Number of organisms : 10 bees x 5 replicates/ concentration

Type of test : oral and contact toxicity test (48 hours)

Applied concentrations : 100 µg a.s./bee (nominal); solvent control; positive control :

Exposure route :

The honeybees were exposed to a nominal dose of 100 µg a.s./bee, administered with a micro-applicator on the ventral side of the thorax of each bee.

The oral nominal test dose corresponded to an actual intake of 118.64 µg a.s./bee.

Feeding : during the oral exposure test bees were not fed 1 hour and 52 minutes prior to treatment. During the contact exposure test the bees were fed with a 50 % aqueous sucrose solution *ad libitum*. Also the bees in the oral test were fed with a 50 % sucrose solution *ad libitum* after they had consumed the test substance.

Test conditions :

Temperature 24.0 – 25.0 °C, relative humidity 62 – 80 %, the test animals were kept under constant darkness.

Findings and conclusions:

Study is acceptable.

LD₅₀ (48h) contact > 100 µg a.s./bee (nominal) (highest concentration tested)

NOEL (48h) contact = 100 µg a.s./bee (nominal)

LD₅₀ (48h) oral > 118.64 µg a.s./bee

NOEL (48h) oral = 118.64 µg a.s./bee

B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

Not required.

B.9.4.3 Acute toxicity of the preparations to bees (Annex IIIA 10.4.1)

The preparation Phylq contains only human food additives, except for 1.5% surfactant, in addition to the active substance and the active substance laminarin has a low acute toxicity to bees. Therefore acute toxicity studies with the formulation on bees are not considered necessary.

B.9.4.4 Effects on bees of residues on crops (Annex IIIA 10.4.2)

Not required.

B.9.4.5 Cage tests (Annex IIIA 10.4.3)

Not required.

B.9.4.6 Field tests to investigate special effects (Annex IIIA 10.4.4)

Not required.

B.9.4.7 Tunnel testing to investigate effects of feeding on contaminated honey (Annex IIIA 10.4.5)

Not required.

B.9.4.8 Exposure and risk assessment for bees (Annex IIIA 10.4)

Table B.9.4.8-1 : Toxicity and risk assessment for bees

Test species	Test system	Results	Risk Ratio	References
Honeybee (<i>Apis mellifera carnica</i>)	Acute oral toxicity test	$LD_{50} > 118.64 \mu\text{g a.s./bee.}$	$Q_{HO} = 0.31$	(Kling A., 2000)
Honeybee (<i>Apis mellifera carnica</i>)	Acute contact toxicity test	$LD_{50} > 100 \mu\text{g a.s./bee.}$	$Q_{HC} = 0.37$	(Kling A., 2000)

B.9.5 Effects on other arthropods species (Annex IIA 8.3.2; Annex IIIA 10.5)**B.9.5.1 Effects of the active substance on non-target terrestrial arthropods (Annex IIA 8.3.2)**

Studies with the formulation were carried out.

B.9.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) Annex IIIA 10.5.1)

The effects of Phylig (37 g/L Laminarin) on *Aphidius rhopalosiphii* (Hymenoptera, Braconidae) on artificial substrate in the laboratory : LR₅₀ estimation and reproduction assessment (Tessier C., 2001a).

Guidelines :

- ESCORT Guidance Document, Barett *et al.*, 1994)
- The method used was based on the method described by Mead-Briggs (1998), Mead-Briggs *et al.* (1998) and Mead-Briggs *et al.* (2000).

GLP :

Yes

Material and Methods :

Test substance : Phylig SL formulation containing 37 g/L laminarin

Test species : the parasitic wasp, *Aphidius rhopalosiphii*.

Number of organisms : 10 animals x 3 replicates/concentration.

Type of test : Lab test.

Applied and measured concentrations : water control, 0.1, 0.3, 1.0, 3.0 and 10.0 L Phylig/ha and a toxic standard (dimethoate SC 40, 0.85 mL/ha).

Exposure route : The glass plates were sprayed 59 min. to 1 h 06 min. before the wasps were introduced into each test chamber. After 48 hours of exposure, individual female wasps from the 3.0 and 10.0 L/ha groups were transferred into fecundity chambers. After a further 48 hours, the females were removed and fecundity was assessed for 14 more days.

Test conditions : Temperature : 20 °C; 70 % relative humidity; light regime : 16 hours light, ventilation with air pump

Findings :

Table 8.5.1-1 Biological observations during an acute toxicity test with *Aphidius rhopalosiphii*

Evaluation criteria	Control	Toxic standard	Treatment				
			0.1 L/ha	0.3 L/ha	1.0 L/ha	3.0 L/ha	10.0 L/ha
Mortality (%)	0	100*	0	6.7	0	3.3	0
Fecundity (mummies/female/24 h)	8.5	/	not assessed	not assessed	not assessed	6.3	4.6*
E (beneficial capacity)						26 %	46 %

* significant difference with the water control ($\alpha = 0.05$)

Conclusion :

Study is acceptable. Phylig is harmless to *Aphidius rhopalosiphii* at 3 times the recommended dose.

The effects of Phylig (37 g/L Laminarin) on *Typhlodromus pyri* (Acari, Phytoseiidae) on artificial substrate in the laboratory : LR₅₀ estimation and reproduction assessment (Tessier C., 2001b).

Guidelines :

- ESCORT Guidance Document, Barett *et al.*, 1994)
- The method used was based on the method described by Mead-Briggs (1998), Mead-Briggs *et al.* (1998) and Mead-Briggs *et al.* (2000).

GLP :

Yes

Material and Methods :*Test substance* : Phylig SL formulation containing 37 g/L laminarin*Test species* : the predatory mite, *Typhlodromus pyri*.*Number of organisms* : 20 animals x 3 replicates/concentration.*Type of test* : Lab test.*Applied and measured concentrations* : water control, 0.1, 0.3, 1.0, 3.0 and 10.0 L Phylig/ha and a toxic standard (fenpropathrin EC 100, 0.5 L/ha).*Exposure route* : The glass plates and shelters were sprayed 1 hour before the mites were introduced into each test chamber. Mortality assessments were carried out 1, 3 and 7 days after application. Fecundity assessments were carried out 10, 12 and 14 days after application.*Test conditions* : Temperature : 25 °C; 70 % relative humidity; light regime : 16 hours light.Findings :Table 8.5.1-1 Biological observations during an acute toxicity test with *Typhlodromus pyri*

Evaluation criteria	Control	Toxic standard	Treatment				
			0.1 L/ha	0.3 L/ha	1.0 L/ha	3.0 L/ha	10.0 L/ha
Mortality (%) at 7 days	15.0	100.0*	28.3	26.7	40.0*	38.3	88.3*
Fecundity at 14 days (eggs/female/24 h)	2.3	/	2.3	2.3	2.3	2.1	2.4
E (beneficial capacity)			15.65	13.76	29.41	33.7	85.64

* significant difference with the water control ($\alpha = 0.05$)Conclusion :Study is acceptable. Phylig is harmless to *Typhlodromus pyri* at the recommended dose.**B.9.5.3 Effects of the formulations on non-target terrestrial arthropods (field tests) Annex IIIA 10.5.2)**

Not required.

B.9.5.4 Summary of effects, exposure and risk assessment for non-target terrestrial arthropods

Table B.9.5.4-1 : Summary of effects of Laminarin to non-target terrestrial arthropods

Test species	Test system	Duration of exposure	Results	Risk Assessment	References
<i>Aphidius rhopalosiphii</i>	lab test	48 hours	E = 26 % 3 L/ha Phylig	harmless	Tessier C., 2001a
<i>Typhlodromus pyri</i>	lab test	14 days	E = 29.41 % 1 L/ha Phylig	harmless	Tessier C., 2001b

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.3.6)

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

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

Continued or repeated exposure of non-target macro-organisms is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g/ha. The a.s. is readily biodegradable (DT₅₀ a.s. | CO₂ ~ 8 days), see point B.8.4.3. There is no short or long term risk for soil macro-organisms expected.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.9.8.1 Impact of the active substance on soil microbial activity (Annex IIA 8.5)

Laminaran (Black W.A.P. *et al.*, date unknown)

This article states that laminarase occurs in bacteria, fungi, algae, higher plants and molluscs. These enzymes are involved in the intracellular mobilisation of food reserves and are also encountered in the extracellular breakdown of plant debris and in the digestive metabolism of invertebrates.

Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description (Ivanova E.P. *et al.*, 1998)

Four strains of marine, aerobic, agar-decomposing bacteria with one polar flagellum and with DNA G+C contents of 38.9-40.2 mol% were isolated from the Far-Eastern mussels *Crenomytilus grayanus* and *Patinopecten yessoensis*. These four strains are described in the article. One of the used properties is the enzyme activity. In strain KMM 188 laminarase has been found.

Purification and Some Properties of *p*-Nitrophenyl-β-D-glucoside-hydrolyzing Enzymes in Culture Filtrate of *Bacillus circulans* KA-304 Grown on Cell-wall Preparation of *Schizophyllum commune* (Mizuno K. *et al.*, 1998).

Hydrolysing activities toward *p*-nitrophenyl-β-D-glucoside and laminarin in a culture filtrate of *Bacillus circulans* KA-304, were observed. Laminarase activity increased in the medium with cell wall preparation (CWP) of *Schizophyllum commune*. Laminarin was as effective as the CWP to increase *p*-nitrophenyl-β-D-glucoside-hydrolyzing and laminarase activities.

B.9.8.2 Impact of the formulations on soil microbial activity (laboratory) (Annex IIIA 10.7.1)

Laminarin is readily biodegradable (DT_{50} a.s. | $CO_2 \sim 8$ days), see point B.8.4.3. Continued or repeated exposure of soil micro-organisms is unlikely to occur as laminarin will only be applied once a year at a rate of 37 g/ha. A study on the impact of the formulation on soil microbial activity is not considered necessary.

B.9.8.3 Further laboratory, glasshouse or field testing to investigate impact on soil microbial activity (Annex IIIA 10.7.2)

See Point 9.8.2.

B.9.8.4 Summary of studies on non-target micro-organisms - exposure and risk assessment for non-target micro-organisms

A literature search has been performed by the notifier which is described under point B.8.1..

No risk is expected for non-target micro-organisms.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6; Annex IIIA 10.8)

In the article by Bull T.A. (1967) on the enzymatic degradation of β -glucans also their distribution in nature is discussed. According to this article β -glucans are found in the crown gall of *Agrobacterium* species, in mycoplasma, *Euglena gracilis*, *Saccharomyces gracilis*, Angiosperms, dicotyledon seed coats, lichens and *Penicillium luteum*. The application of 37 g a.s./ha/year is not considered to form a risk to other flora and fauna as β -glucans occur already naturally in different species.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

In the study for the ready biodegradability of laminarin (see point B.8.4.3) activated sewage micro-organisms were used. The test resulted that laminarin is readily biodegradable so no effect is expected on the micro-organisms of sewage treatment plants.

B.9.11 References relied on

Author(s)	Annex Point / Reference number	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	Owner
ANNISON G.	IIA, 8.1/02	1992	Commercial enzyme supplementation of wheat-based diets raises ileal glycanase	N	-

			activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. Animal Feed Science and Technology, 38, 105-121 Non-GLP, published		
BERGH M.O., RAZDAN A., AMAN P.	IIA, 8.1/01	1999	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	-
BLACK W.A.P., DEWAR E.T.	IIA, 8.5/01	1954	Laminaran J.Sci.Food Agric., 5, 137-145 Non-GLP, published	N	-
GNEMI P.	IIA, 8.2/04	2000	H 11- Algal growth inhibition study CERB - Study N° 990705 EX GLP, unpublished	Y	GOËMAR
HERTL J.	IIA, 8.2/03	2001	Acute toxicity of LAMINARIN to <i>Daphnia magna</i> in a 48-hour immobilization test IBACON GmbH - Study N° 10041220 GLP, unpublished	Y	GOËMAR
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.	IIA, 8.5/02	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	-
KLING A.	IIA, 8.3/01	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	Y	GOËMAR
LICATA-MESSANA L.	IIA, 8.2/01	2001a	Laminarin – Acute toxicity in freshwater fish (96 hours) <i>Oncorhynchus mykiss</i> SEPC - Study N° 00-907005-022 GLP, unpublished	Y	GOËMAR
LICATA-MESSANA L.	IIA, 8.2/02	2001b	Laminarin – Acute toxicity in freshwater fish (96 hours) <i>Danio rerio</i> SEPC - Study N° 00-907005-021 GLP, unpublished	Y	GOËMAR
MIZUNO K., AWAZU N., TACHIKI T.	IIA, 8.5/03	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	N	-

			Non-GLP, published		
PHILIP J.S., GILBERT H.J., SMITHARD R.R.	IIA, 8.1/04	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	-
TESSIER C.	IIA, 8.3/02	2001a	Phyliq. The effects of Phyliq (37 g/L Laminarin) on <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) on artificial substrate in laboratory : LR50 estimation and reproduction assessment. PROMO-VERT- Study N° 01APGOL25 GLP, unpublished	Y	GOËMAR
TESSIER C.	IIA, 8.3/03	2001b	Phyliq. The effects of Phyliq (37 g/L Laminarin) on <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) on artificial substrate in laboratory : LR50 estimation and reproduction assessment. PROMO-VERT- Study N° 01TYGOL24 GLP, unpublished	Y	GOËMAR
VUKIC-VRANJES M., WENK C.	IIA, 8.1/03	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	-

Author(s)	Annex Point / Reference Number	Year	Title Testing facility, Report No., GLP or GEP Status published or not	Data Protection Claimed Y/N	Owner
TESSIER C.	IIIA, 10.5/01 (location : IIA, 8.3/02)	2001a	Phyliq - The effects of Phyliq (37 g/L Laminarin) on <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) on artificial substrate in laboratory : LR50 estimation and reproduction assessment. PROMO-VERT Report No. 01APGOL25 GLP, unpublished	Y	GOËMAR
TESSIER C.	IIIA, 10.5/02 (location : IIA, 8.3/03)	2001b	Phyliq - The effects of Phyliq (37 g/L Laminarin) on <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) on artificial substrate in laboratory : LR50 estimation and reproduction assessment. PROMO-VERT Report No. 01TYGOL24 GLP, unpublished	Y	GOËMAR