Petition to Include Laminarin on the National List

Item A—Please indicate which section or sections the petitioned substance will be included on and/or removed from the National List.

• Synthetic substances allowed for use in organic crop production, § 205.601.

The substance for which this petition is submitted is Laminarin (CAS No. 9008-22-4), an extract of a brown seaweed, *Laminaria digitata*.

Item B—Please provide concise and comprehensive responses in providing all of the following information items on the substance being petitioned:
1. The substance's chemical or material common name.

Laminarin

2. The manufacturer's or producer's name, address and telephone number and other contact information of the manufacturer/producer of the substance listed in the petition.

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3. The intended or current use of the substance such as use as a pesticide, animal feed additive, processing aid, nonagricultural ingredient, sanitizer or disinfectant. If the substance is an agricultural ingredient, the petition must provide a list of the types of product(s) (e.g., cereals, salad dressings) for which the substance will be used and a description of the substance's function in the product(s) (e.g., ingredient, flavoring agent, emulsifier, processing aid).

Pesticide for pre-harvest use on various fruits and vegetables to stimulate the plant's natural disease-defense mechanism.

4. A list of the crop, livestock or handling activities for which the substance will be used. If used for crops or livestock, the substance's rate and method of application must be described. If used for handling (including processing), the substance's mode of action must be described.

Laminarin is registered by EPA for application to:
Fruiting Vegetables: Tomato, Eggplant, Pepper
Cucurbits: Zucchini, Cucumber, Watermelon, Cantaloupe, Squash, Pumpkin, Muskmelon
Bulb vegetables: Onion, Leek

Head and leafy vegetables: Lettuce, Spinach, Celery
Cole crops (brassicas)
Stone fruit: Peach
Pome fruit: Apple, Pear
Strawberries
Table grapes
Wine grapes
Blueberries

Rate: 0.52 - 1.04 fl. oz. laminarin per acre via spray.

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product. Petitioners with concerns for confidential business information may follow the guidelines in the Instructions for Submitting CBI listed in #13.

Stage 1: Fresh *Laminaria digitata* seaweed, harvested on the North Brittany coast of France, undergoes extraction in tap water that has a pH adjusted to 2 by addition of sulfuric acid. At this stage sulphuric acid is a processing aid. Laminarin can be extracted at neutral pH or in acidic conditions. The described acidic conditions do not modify the chemical structure of laminarin. The addition of sulphuric acid avoids the co-extraction of other compounds such as alginates (which occurs at neutral pH). When alginates are extracted, the solution has a higher viscosity; purification and filtration steps for laminarin then become much more difficult. This is the reason why sulphuric acid is used to lower the pH and to facilitate the manufacturing process.

Stage 2: The extract is then filtered using a Seitz filter.

Stage 3: The solution then undergoes tangential filtration (membrane technology – physical process) to remove impurities from the solution. The filtrate containing laminarin is kept for the next purification step and the retentate is removed.

Stage 4: The filtrate (see above) then undergoes a second tangential filtration to remove any remaining impurities (filtrate), thereby resulting in a purified solution of laminarin in water (retentate).

Stage 5: The pH is adjusted between 6 and 7 by adding sodium hydroxide to neutralize the acidic solution, resulting in a solution of laminarin at neutral pH for formulation purposes (i.e., Vacciplant formulation). The addition of dilute sodium hydroxide does not modify the chemical structure of laminarin.

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance. If this information is not available, the petitioner should state so in the petition.

Laminarin is an EPA-registered active ingredient for use in formulating the end-use product, Vacciplant (EPA Reg. No. 83941-2). A summary of EPA's scientific review of the laminarin data is shown below.

Acute Toxicity Testing

Acute Oral Toxicity: An acute oral toxicity study shows that the active ingredient Laminarin has an LD_{50} of greater than 2000 mg/Kg in rats. This was the maximum dose rate. There were no observed toxicological effects on the test subjects at the maximum dose. The study supports the finding that this active ingredient poses no significant human health risk with regard to food uses.

Acute Dermal Toxicity: An acute oral toxicity study shows that the active ingredient Laminarin has an LD_{50} of greater than 5000 mg/Kg in rats, which is considered to be virtually non-toxic. Data substantiate the active ingredient's relative dermal non-toxicity to both occupational users and the general public.

Acute Inhalation Toxicity: An acute oral inhalation study shows that the active ingredient Laminarin has an LC_{50} of greater than 1.02 mg/L in rats, which shows no significant inhalation toxicity. This was the maximum dose rate, and no toxicological effects were observed on the test subjects.

Primary Eye Irritation: A primary eye irritation study on rabbits demonstrated Laminarin to be non-irritating. There were no observed effects for this route of exposure relative to the use of Laminarin.

Primary Dermal Irritation: A skin irritation study on rabbits demonstrated that Laminarin was not irritating to the skin. The findings are consistent with the other dermal studies and confirm that Laminarin is not toxic through this route of exposure.

Skin Sensitization: Data indicate Laminarin is not a dermal sensitizer.

Subchronic Testing: Three subchronic oral tests were conducted. Laminarin has no subchronic toxicological effect through the oral route of exposure.

A 28-day oral toxicity study in rats found no toxicological effects regarding mortality, clinical observations, neurotoxicity assessment, body weight, food consumption, hematology, clinical chemistry, organ weights, and macroscopic or microscopic observations. The NOEL was determined to be 1,000 mg/kg/day.

A 90-day oral toxicity study in rats found no statistical difference in hematology, clinical chemistry, or urinalysis between test subjects and the control. The NOEL was determined to be 1,000 mg/kg/day.

Another 90-day oral toxicity study in dogs also found no statistical difference in hematology, clinical chemistry, or urinalysis between test subjects and the control. The NOEL was again determined to be 1,000 mg/kg/day.

Developmental Toxicity: Two studies were conducted – one with rats and one with rabbits.

The prenatal developmental toxicity study in rats found no significant reproductive effects or fetal abnormalities, and established a NOAEL of 1,000 mg/kg/day. The findings suggest negligible risk with regard to developmental toxicity.

The prenatal developmental toxicity study in rabbits found no significant treatment-related reproductive effects or fetal abnormalities, and confirmed a NOAEL of 1,000 mg/kg/day. Laminarin poses negligible risk with regard to developmental toxicity.

***Mutagenicity Testing*:** Three genotoxicity studies (Bacterial Reverse Mutation Test; *In Vitro* Mammalian Cells in Culture Assay; and Bone Marrow Micronucleus) were performed on Laminarin. These mutagenicity studies confirm that there are no expected dietary, occupational, or non-occupational risks of mutagenicity with regard to Laminarin.

The Reverse Mutation Assay showed that Laminarin did not induce mutant colonies over expected background levels.

The *In Vitro* Mammalian Cells in Culture Assay demonstrated that Laminarin did not damage chromosomes or the mitotic apparatus of bone marrow cells.

A Bone Marrow Micronucleus Assay indicated that no toxicity was noted in either sex at any dose up to the limit dose of 2000 mg/kg. There are no expected dietary, occupational, or non-occupational risks of mutagenicity with regard to Laminarin.

Immunotoxicity Testing: For the following reasons, laminarin is not expected to be immunotoxic. 1) The potential for any immunotoxic effect is precluded by Laminarin's biodegradability. 2) Laminarin is not structurally related to any known immunotoxic chemical. 3) There is a long history of the consumption of Laminarin without known immunotoxicological incident. 4) The toxicological profile in acute toxicological studies, subchronic studies and developmental studies does not suggest any immunotoxicity. All information points to the lack of dietary risk posed by the immunotoxicity of Laminarin residues, and supports the exemption from the requirement of a tolerance.

***Avian Testing*:** In an acute oral toxicity study, groups of bobwhite quail were administered single oral doses ranging up to 2000 mg/kg body weight Laminarin. They were observed for 14 days. There were no mortalities and no signs of adverse effects; all birds appeared healthy during the test, and macroscopic examination revealed no abnormalities in any birds. The acute oral LD_{50} was >2000 mg/kg, the highest dose tested.

In a dietary toxicity study on bobwhite quail, groups of chicks were provided a Laminarin-dosed diet for 5 days, at concentrations ranging up to 5000 ppm per feeding. The diet was maintained for 5 days. There were no treatment-related effects on mortality, body weight, or feed consumption, and no clinical signs of toxicity. The dietary LD_{50} was determined to be >5000 ppm.

***Aquatic Organism Testing*:** In an acute toxicity test, groups of *Daphnia magna* were exposed to concentrations of Laminarin up to 100 mg/L Laminarin. No daphnid mortality or immobility was seen in any of the test groups after 24 or 48 hours.

In this study, the 48-hr NOEC and EC_0 were each ≥100 mg/L, and the LOEC and EC_{50} were >100 mg/L.

In an acute toxicity test, groups of zebrafish were exposed to a nominal concentration of 0 or 100 mg/L Laminarin for 96 hours. No mortality or adverse clinical signs were seen at any intervals or in any of the test groups. The 96-hr LCD_{50} for Laminarin in Zebra Fish was >100 mg/L.

In a second toxicity test, groups of Rainbow Trout fry were exposed to a nominal concentration of 0 or 100 mg/L Laminarin for 96 hours. No mortality or adverse clinical signs were seen in any of the test groups. The 96-hr LCD_{50} for Laminarin in Rainbow Trout was >100 mg/L.

A 72-hour laboratory study was conducted to determine the effects of Laminarin (100 mg/L, nominal) on the growth of the unicellular freshwater green algae. An untreated control was also included in the test. At test end, cell growth and density were similar in the test material and control group. The 72-hour EbC_{50} and ErC_{50} for H11 were >100 mg/L, and the NOECb and NOECr were >100 mg/L.

***Non-Target Plant Testing*:** Laminarin has been shown to have a non-toxic mode of action relative to plants. As an SAR inducer, Laminarin bolsters plant health. Accordingly, Laminarin would actually be expected to have a strengthening effect on non-target plants.

***Non-Target Insect Testing*:** In a laboratory study, groups of male and female adult parasitic wasps were exposed for 48 hours to 37 g/L Laminarin sprayed on glass plates at varying rates up to 10 L/ha. Some issues of loss of fecundity were observed at the highest dose; but none were observed at the doses that were in line with the expected applications of the active ingredient. (The dose at which there was a loss of fecundity was 10x greater than expected residues of Laminarin at the time of pesticidal application.) There was no statistically significant difference in mortality between the treated wasps and the untreated control groups.

Limit tests were conducted to determine the acute oral and acute contact toxicity of Laminarin to the honey bee. Both tests used a nominal dose of 100 µg Laminarin/bee.

In the oral toxicity test, groups of caged bees were provided the test material in a 50% w/v sucrose solution for six hours, and then monitored for mortality at intervals up to 48 hours. After 48 hours, there was no difference in mortality of the untreated control and test material groups. In this test, the 48-hr oral toxicity LD_{50} for Laminarin was >118.64 µg/bee.

In the contact toxicity test, bees were anesthetized with carbon dioxide and received an individual application of Laminarin to the ventral thorax. In this test, the 48-hr contact toxicity LD_{50} was >100.00 µg/bee.

Physical/Chemical Properties for Laminarin

Property	Results
Color	White
Physical State	Powder
Odor	Low odor
Stability	Stable after 14 days at 54°C in the presence of aluminum acetate or aluminum
Oxidation/Reduction: Chemical Incompatibility	No oxidizing properties
Flammability	None flammable; neither development nor ignition of gas were observed after contact with water. An exothermic reaction was observed at 236°C±3°C (mean value). No self-ignition temperature was recorded up to 420°C.
Explodability	Not explosive
Miscibility	Not applicable, product is not an emulsifiable liquid and will not be diluted with petroleum solvents.
pH	6.25±0.02 at 23.2°C (1% w/v)
Melting Range	No melting point could be determined. The test material became yellow at 204-215C, then it turned brown at 216-225.2C. At about 310.6-316.2C, the test material was completely retracted and blacked colored. The test material probably degraded during the test.
Relative Density	D_4^{20} = 1.515±0.04 - 1.502±0.06
Partition Coefficient	Log P = -1.6
Water Solubility	> 88.6 g/L at 20°C; < 10 mg/L (n-heptane); < 10 mg/L at 20°C (xylene, 1,2-dichloroethane, and ethyl acetate); 60 mg/L (methanol); 21 mg/L at 20°C (acetone)
Vapor Pressure	< 2.6 x 10^{-5} Pa at 25°C

Toxicity Data Summary

Test	Results
Acute Oral Toxicity	LD_{50}>2,000 mg/kg
Acute Dermal Toxicity	LD_{50}>5,000 mg/kg
Acute Inhalation Toxicity	>1.02 mg/L
Primary Eye Irritation	Non irritating
Primary Dermal Irritation	Non- irritating
Dermal Sensitization	Not a sensitizer
Acute Subcutaneous Toxicity	LD_{50}>1,000 mg/kg
28 day Oral Toxicity - Rat	NOEL=1,000 mg/kg/day
90 day Oral Toxicity - Rat	NOEL=1,000 mg/kg/day
Subchronic Oral Toxicity (gavage) - Dog	NOEL=1,000 mg/kg/day
Prenatal Developmental Toxicity - Rat	Maternal NOEL $\geq$ 1,000 mg/kg/day Developmental NOEL > 1,000 mg/kg/day
Prenatal Developmental Toxicity – Rabbit	Maternal LOAEL = 1,000 mg/kg/day Developmental LOAEL = 1,000 mg/kg/day
Bacterial Reverse Mutation	No evidence of induced mutant colonies over background.
In Vitro Mammalian Cells in Culture Gene Mutation Assay	No evidence of induced mutant colonies over background.
Bone Marrow Micronucleus in mice	No toxicity was noted in either sex at any dose up to the limit dose of 2,000 mg/kg bw
Immunotoxicity	Not expected to be immunotoxic.

Ecotoxicity Data Summary

Test	Results
Acute Toxicity Test, Daphnids	EC_{50} >100 mg/L
Acute Toxicity Freshwater Fish *Danio rerio*	96 hr LC_{50} >100 mg/L
Acute Toxicity Freshwater Fish Rainbow Trout (*Oncorhynchus mykiss*)	96-hr LC_{50} >100 mg/L
Avian Acute Oral Toxicity Bobwhite (*Colinus virginianus*)	LD_{50} >2000 mg/kg
Avian Dietary Toxicity Bobwhite (*Colinus virginianus*)	LC_{50} >5000 ppm
Acute Contact Toxicity Honey bee (*Apis mellifera)*	48-hr LD_{50} >100 µg/bee
Acute Oral Toxicity Honey bee (*Apis mellifera)*	LD_{50} >118.64 µg/bee
Algal Toxicity Green alga *Selenastrum capricornutum*.	E_bC_{50}, E_rC_{50}, NOECb, and NOECr for the test material at 24, 48, and 72 hours were each >100 mg/L
Biodegradability	Biodegradation in the reference material and toxicity

Test	Results
	controls was 71% and 65%, respectively, after 14 days. Laminarin was concluded to be readily biodegradable under the test conditions.
Nontarget Insect Testing	Exposure to 10 L/ha of the test material did significantly lower the fecundity of *A. rhopalosiphi* females compared to the untreated control. However, the product label for Vacciplant recommends an application rate of 14 fl. oz./A, which is equivalent to 0.7 to 1.05 L/ha, well below the 10 L/ha rate at which fecundity was affected.

7. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers. If this information does not exist, the petitioner should state so in the petition.

Laminarin itself is not EPA registered; however, the end-use product that contains laminarin, Vacciplant, is registered by EPA. Vacciplant was initially registered on February 15, 2010 (EPA Reg. No. 83941-2; attached). The label was subsequently amended on June 21, 2011 (attached).

Laminarin is exempt from the requirement of a tolerance (40 CFR 180.1295) on all food commodities when applied pre-harvest. See attached.

Laminarin is not registered in any state; Vacciplant, however, is currently registered in the following 32 states:

Alabama
Arizona
Arkansas
Connecticut
Delaware
Florida
Georgia
Idaho
Indiana
Kentucky
Louisiana
Maryland
Massachusetts
Michigan
Mississippi
Missouri
New Hampshire
New Jersey
New Mexico
North Carolina
Ohio
Oklahoma
Oregon
Pennsylvania
Rhode Island
South Carolina
Tennessee
Texas
Vermont
Virginia
Washington
Wisconsin

8. The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance. If the substance does not have an assigned product number, the petitioner should state so in the petition.

CAS No. 9008-22-4

The most recent EPA stamped-accepted Vacciplant label is attached. There are no other EPA-registered products that contain laminarin as the active ingredient.

9. The substance's physical properties and chemical mode of action including (a) Chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts from its use and/or manufacture; (d) effects on human health; and, (e) effects on soil organisms, crops, or livestock.

- Color: white
- Physical State: powder
- Odor: practically none
- Stability to Normal and Elevated Temperatures, Metals, and Metal Ions: In the presence of Aluminum acetate: Stable after 14 days at 54 °C. In the presence of Aluminum: Stable after 14 days at 54 °C.
- Oxidation/Reduction: Chemical Incompatibility: No oxidizing properties.
- Flammability: Non-flammable
- Explodability: Not explosive
- pH: 6.25 ± 0.02 at 23.2°C (1% w/v)
- UV/Visible Absorption: Under acidic, neutral, and alkaline conditions, the test material showed significant absorbance maxima at ~ 260 nm. Absorptivity data are shown below. Values of molar absorption coefficient are quoted as a range due to the molecular weight range of the test material.

Matrix	Wavelength (nm)	Molar absorption coefficient ($dm^3.mol^{-1}.cm^{-1}$)
Acidic (pH 1.9)	264	245 to 294
Neutral (pH 7.0)	260	242 to 290
Basic (pH 11.8)	258	264 to 317

- Melting Point/Melting Range: No melting point could be determined.
- Density/Relative Density/Bulk Density: $D_{(+20°C / +4°C)} = 1.5$
- Dissociation Constant: No dissociation in water.

- Partition Coefficient (*n*-octanol/water), shake flask method:

Sample	Volume of Water (in mL)	Volume of n-Octanol (in mL)	Volume of Stock Solution (in mL)	Concentration of D-glucose in the Water Phase (in g/L)	Concentration of D-glucose in the n-Octanol* Phase (in g/L)	Partition Coefficient (log 10)	Partition Coefficient (mean value) (log 10)
1.1	100	60	20	6.12	0.157	- 1.59	- 1.6
1.2	100	60	20	5.90	0.236	- 1.40	
2.1	80	60	20	7.54	0.192	- 1.59	
2.2	80	60	20	7.46	0.156	- 1.68	
3.1	70	180	20	8.73	0.170	- 1.71	
3.2	70	180	20	7.85	0.156	- 1.70	

- Water Solubility: column elution method; shake flask method: >88.6 g/L at about 20°C
- n-Heptane Solubility: column elution method; shake flask method: < 10 mg/L
- Xylene Solubility: column elution method; shake flask method: < 10 mg/L at about 20°C
- 1,2 Dichloroethane Solubility: column elution method; shake flask method: < 10 mg/L at about 20°C
- Methanol Solubility: column elution method; shake flask method: 60 mg/L (s.d. 4 mg/L)
- Acetone Solubility: column elution method; shake flask method: 21 mg/L (s.d. 7 mg/L) at about 20°C
- Ethyl Acetate Solubility: column elution method; shake flask method: < 10 mg/L at about 20°C
- Vapor Pressure: $< 2.6 \times 10^{-5}$ Pa @ 25°C
- Surface Tension: 72.2 mN/m ± 0.9 mN/m at 1000 mg/L

Chemical mode of action: Elicits plants' natural defense mechanism (SAR; Systemic Acquired Resistance).

Chemical interactions with other substances, especially substances used in organic production: None known.

Toxicity and environmental persistence: Laminarin is of low toxicity and breaks down quickly in the environment. See the attached EPA Biopesticide Registration Action Document and underlying Data Evaluation Records (DERs).

Environmental impacts from its use and/or manufacture: Laminarin and Vacciplant are manufactured solely in France. There are no known negative environmental impacts from the manufacture or use of these substances.

Effects on human health: See the attached DERs and above data summaries. Both laminarin and Vacciplant are of low toxicity; EPA has exempted laminarin from the requirement of a tolerance on all food commodities when applied to growing crops (40 CFR 180.1295).

Effects on soil organisms, crops, or livestock: No data have been generated regarding laminarin's effects on soil organisms. When applied to crops, laminarin induces an SAR response. Based on

the numerous mammalian toxicity, irritation, and mutagenicity studies conducted with laminarin, no adverse effects are anticipated if livestock are exposed to laminarin.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies. If this information does not exist, the petitioner should state so in the petition.

An MSDS for laminarin is attached.

A substance report from the National Institute of Environmental Health Studies does not exist.

11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List. For petitions to include non-organic agricultural substances onto the National List, this information item should include research concerning why the substance should be permitted in the production or handling of an organic product, including the availability of organic alternatives. Commercial availability does not depend upon geographic location or local market conditions. If research information does not exist for the petitioned substance, the petitioner should state so in the petition.

EPA Vacciplant Registration Notice and stamped-accepted label, Feb. 15, 2010.

Laminarin tolerance exemption, Title 40 Code of Federal Regulations, Part 180.1295, July 1, 2011.

EPA Vacciplant label amendment approval and new stamped-accepted label, June 14, 2012.

EPA Laminarin Biopesticide Registration Action Document (BRAD), Feb. 13, 2010.

EPA Data Evaluation Record: Baudet, L. (2002) Phycarine 96S51: Acute Toxicity Study Safety Test in the Rat by the Oral Route. Project Number: 20010618/ST. Unpublished study prepared by Centre de Recherches Biologiques. 27 p.

EPA Data Evaluation Record: Audeval Gerald, C. (2001) H11 (Batch 99S24) Acute Dermal Toxicity Study in the Rat. Project Number: 20000698/ST, 20000698/ST/TAUVD/H11. Unpublished study prepared by Centre de Recherches Biologiques. 30 p.

Muller, W. (1999) Evaluation of Acute Inhalation Toxicity with Phycarine in Rats. Project Number: 980001/EX, TOXLABS/1998/7019/INH. Unpublished study prepared by Centre de Recherches Biologiques. 19 p.

EPA Data Evaluation Record: Baudet, L. (2002) Phycarine 96S51: Ocular Primary Irritation in the Rabbit. Project Number: 20010615/ST. Unpublished study prepared by Centre de

Recherches Biologiques. 27 p.

EPA Data Evaluation Record: Baudet, L. (2002) Phycarine 96S51: Cutaneous Primary Irritation in the Rabbit: Final Report. Project Number: 20010617/ST/PSI/RABBIT/PHYCARIINE. Unpublished study prepared by Centre de Recherches Biologiques. 25 p.

EPA Data Evaluation Record: Baudet, L. (2002) Phycarine 96S51: Study of Cutaneous Sensitization Using the Magnusson and Kligman Maximisation Test in the Guinea Pig: Final Report. Project Number: 20010616/ST. Unpublished study prepared by Centre de Recherches Biologiques. 39 p.

EPA Data Evaluation Record: Delille, M. (1998) Acute Toxicity Study: Safety Test in the Rat by the Subcutaneous Route: (Phycarine 96S51). Project Number: 970353/ST. Unpublished study prepared by Centre de Recherches Biologiques. 24 p.

EPA Data Evaluation Record: Longobardi, C. (2000) 4489-1 (product H 11): 4 Week Oral Toxicity Study in Rats: Final Report. Project Number: 7286, 7286/T/240/99. Unpublished study prepared by Research Toxicology Centre, S.p.A.. 178 p.

EPA Data Evaluation Record: Audeval Gerard, C. (2001) H11 (Batch 99S24): 90-Day Repeated Dose Oral Toxicity Study in the Rat. Project Number: 20000389/T. Unpublished study prepared by Centre de Recherches Biologiques. 237 p.

EPA Data Evaluation Record: Audeval Gerard, C. (2001) 90-Day Repeated Dose Oral Toxicity Study in the Dog. Project Number: 20000390/T. Unpublished study prepared by Centre de Recherches Biologiques. 257 p.

EPA Data Evaluation Record: Audeval Gerard, C. (2001) H11 (Batch 99S24): Study for the Effects on Embryo-Foetal Development in the Rat by the Oral Route. Project Number: 20000387/T. Unpublished study prepared by Centre de Recherches Biologiques. 127 p.

EPA Data Evaluation Record: Gerard, C. (2001) Study for the Effects on Embryo-Foetal Development in the Rabbit by the Oral Route: H11 (Batch 99S24). Project Number: NO/20000388/T. Unpublished study prepared by CERB (Centre de Recherches Biologiques). 127 p.

EPA Data Evaluation Record: Marzin, D. (2000) Mutagenicity Test on Bacteria (Salmonella typhimurium his and Escherichia coli trp) Using B.N. Ames's Technique with H11. Project Number: IPL/R/991011/H11/GOEMAR/LABORATORY. Unpublished study prepared by Institut Pasteur de Lille. 38 p.

EPA Data Evaluation Record: Haddouk, H. (2002) In Vitro Mammalian Cell Gene Mutation Test in L5178Y TK Mouse Lymphoma Cells: Laminarin. Project Number: 22626/MLY. Unpublished study prepared by Centre International de Toxicologie. 47 p.

EPA Data Evaluation Record: Haddouk, H. (2001) Bone Marrow Micronucleus Test by Oral Route in Mice: Laminarin. Project Number: 21149/MAS, 21149/MAS/LAMINARIN/LABORATOIRES/GOEMAR/SA. Unpublished study prepared by Centre International de Toxicologie. 37 p.

EPA Data Evaluation Record: Smith, F. (2007) Toxicology Waiver Request - Immunotoxicity (Laminarin). Unpublished study prepared by SciReg, Inc. 11 p.

EPA Data Evaluation Record: Herti, J. (2001) Acute Toxicity of Laminarin to Daphnia magna in a 48-Hour Semi-Static Immobilization Test: Final Report. Project Number: PROJECT/10041220. Unpublished study prepared by Institut fuer Biologische Analytik und Consulting IBACON. 61 p.

EPA Data Evaluation Record: Luc, L. (2001) Acute Toxicity in Freshwater Fish (96h): Oncorhynchus mykiss (Laminarin). Project Number: 00/907005/021. Unpublished study prepared by SEPC. 50 p.

EPA Data Evaluation Record: Luc, L. (2001) Acute Toxicity in Freshwater Fish (96h): Danio rerio: (Laminarin). Project Number: 00/907005/022. Unpublished study prepared by SEPC. 49 p.

EPA Data Evaluation Record: Rodgers, M. (2002) Laminarin: Acute Oral Toxicity (LD50) to the Bobwhite Quail. Project Number: GOM/001, GOM/001/022173. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 23 p.

EPA Data Evaluation Record: Rodgers, M. (2002) Laminarin: Dietary Toxicity (LC50) to the Bobwhite Quail. Project Number: GOM/002, GOM/002/014410. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 21 p.

EPA Data Evaluation Record: Kling, A. (2000) Assessment of Side Effects of Laminarin to the Honey Bee, Apis mellifera L. in the Laboratory: Final Report. Project Number: 20001342/01/BLEU. Unpublished study prepared by Arbeitsgemeinschaft GAB Biotechnologie. 33 p.

EPA Data Evaluation Record: Gnemi, P. (2000) H11: Algal Growth Inhibition Study. Project Number: 990714. Unpublished study prepared by Istituto di Ricerche Biomediche Antoine Marxer RBM S.p.A. 27 p.

EPA Data Evaluation Record: Luc, L. (2000) Ready Biodegradability Modified Strum Test: (Laminarin). Project Number: 00/907005/024. Unpublished study prepared by SEPC. 24 p.

EPA Data Evaluation Record: Smith, F. (2008) Toxicology Waiver Request- Acute Inhalation Toxicity: (VacciPlant). Unpublished study prepared by SciReg, Inc. 65 p.

EPA Data Evaluation Record: Tessier, C. (2001) The Effects of Phyliq (37 g/L Laminarin) on Aphidius rhopalosphi (Hymenoptera, Braconidae) on Artificial Substrate in Laboratory; LR_{50} Estimation and Reproduction Assessment. Project Number: 01APGOL25. Unpublished study prepared by PROMO-VERT S.A.

EPA Data Evaluation Record: Tessier, C. (2001) The Effects of Phyliq (37 g/L Laminarin) on Typhlodromus pyri (Acari, Phytoseiidae) on Artificial Substrate in Laboratory; LR_{50} Estimation and Reproduction Assessment. Project Number: 01TYGOL24. Unpublished study prepared by PROMO-VERT S.A.

Quintelas, G. (2001) Abiotic Degradation of Laminarin pH Dependent Hydrolysis (Test C7). Project Number: SEP/00/075, SEPC/00/907005/025, 00/907005/025. Unpublished study prepared by Defitraces. 30 p.

Bernardon, Mery A. (2012) Laminarin controls the disease, *Venturia inaequalis*, of apples and gloeosporium of apples, *Gloeosporium album* and *G. perenans*). CIMA Tours, France.

Van Hemelrijck, W. (2013) Efficacy of a new oligosaccharide active against scab on apple. Colloque, France.

12. A ‘‘Petition Justification Statement’’ which provides justification for any of the following actions requested in the petition:
A. Inclusion of a Synthetic on the National List, §§ 205.601, 205.603, 205.605(b)
• Explain why the synthetic substance is necessary for the production or handling of an organic product.

The petitioned substance, laminarin, is a naturally-occurring substance extracted from seaweed. It stimulates a plant’s natural defense mechanisms against disease. Laminarin is similar to other naturally-occurring substances that are used in organic crop production, such as cytokinin. Unlike laminarin, however, cytokinin is a plant growth regulator. Laminarin promotes pesticidal activity within the plant. EPA has determined that a tolerance for residues of laminarin on food or feed crops is not required when applied pre-harvest and, as such, issued a tolerance exemption (40 CFR 180.1295). Laminarin gives the organic crop grower the ability to use a natural pesticide that is exempt from the requirement of tolerance without limitation to effectively combat a variety of bacterial and fungal diseases such as blights, mildews, and scabs.

• Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.

The petitioner is not aware of any non-synthetic substances, synthetic substances on the National List, or alternative cultural methods that could be used in place of laminarin.

• Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural methods.

Laminarin is non-toxic to mammals, birds, insects, and plants, does not bio-accumulate, and is readily degraded in the environment to constituents that can be utilized by plants for nutritional purposes.

B. Removal of a Synthetic From the National List, §§ 205.601, 205.603, 205.605(b)
• Explain why the synthetic substance is no longer necessary or appropriate for the production or handling of an organic product.

Not applicable.

• Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.

Not applicable.

C. Inclusion of a Prohibition of a Non-Synthetic, §§ 205.602 and 205.604
• Explain why the non-synthetic substance should not be permitted in the production of an organic product.

Not applicable.

• Describe other non-synthetic substances or synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned substance.

Not applicable.

D. Removal of a Prohibited Non-Synthetic From the National List, §§ 205.602 and 205.604
• Explain why the non-synthetic substance should be permitted in the production of an organic product.

Not applicable.

• Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the non-synthetic substance that supports its use instead of the use of other non-synthetic or synthetic substances on the National List or alternative cultural methods.

Not applicable.

E. Inclusion of a Non-Synthetic, Non-Agricultural Substance Onto the National List, § 205.605(a)
• Explain why the substance is necessary for use in organic handling.

Not applicable.

• Describe non-synthetic or synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.

Not applicable.

• Describe any beneficial effects on the environment, or human health from the use of the substance that support its use instead of the use of non-synthetic or synthetic substances on the National List or alternative cultural methods.

Not applicable.

F. Removal of a Non-Synthetic, Non-Agricultural Substance From the National List, § 205.605(a)
• Explain why the substance is no longer necessary for use in organic handling.

Not applicable.

• Describe any non-synthetic or synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned substance.

Not applicable.

G. Inclusion of a Non-Organically Produced Agricultural Substance Onto the National List, § 205.606
• Provide a comparative description on why the non-organic form of the substance is necessary for use in organic handling.

Not applicable.

• Provide current and historical industry information/research/evidence that explains how or why the substance cannot be obtained organically in the appropriate form, appropriate quality, and appropriate quantity to fulfill an essential function in a system of organic handling.

Not applicable.

• Describe industry information on substance non-availability of organic sources including but not limited to the following guidance regarding commercial availability evaluation criteria: (1) Regions of production, including factors such as climate and number of regions; (2) Number of suppliers and amount produced; (3) Current and historical supplies related to weather events such as hurricanes, floods, and droughts that may temporarily halt production or destroy crops or supplies; (4) Trade related issues such as evidence of hoarding, war, trade barriers, or civil unrest that may temporarily restrict supplies, and (5) Other issues which may present a challenge to a consistent supply.

Not applicable.

H. Removal of a Non-Organically Produced Agricultural Substance From the National List, § 205.606
• Provide a comparative description as to why the non-organic form of the substance is not necessary for use in organic handling.

Not applicable.

• Provide current and historical industry information/research/evidence that explains how or why the substance can be obtained organically in the appropriate form, appropriate quality, and appropriate quantity to fulfill an essential function in a system of organic handling.

Not applicable.

• Provide new industry information on substance availability of organic sources including but not limited to the following guidance commercial availability evaluation criteria: (1) Region of production, including factors such as climate and number of regions; (2) Number of suppliers and amount produced; (3) Current and historical supplies related to weather events such as hurricanes, floods, or droughts that temporarily halt production or destroy crops or supplies; (4) Trade related issues such as evidence of hoarding, war, trade barriers, and civil unrest that may temporarily restrict supplies and; (5) Any other issues which may present a challenge to a consistent supply.

Not applicable.

13. A Confidential Business Information Statement which describes the specific required information contained in the petition that is considered to be Confidential Business Information (CBI) or confidential commercial information and the basis for that

determination. Petitioners should limit their submission of confidential information to that needed to address the areas for which this notice requests information. Final determination regarding whether to afford CBI treatment to submitted petitions will be made by USDA pursuant to 7 CFR 1.27(d). Instructions for submitting CBI to the National List Petition process are presented in the instructions below:
(a) Financial or commercial information the petitioner does not want disclosed for competitive reasons may be claimed as CBI. Applicants must submit a written justification to support each claim. (b) ‘‘Trade secrets’’ (information relating to the production process, such as formulas, processes, quality control tests and data, and research methodology) may be claimed as CBI. This information must be (1) commercially valuable, (2) used in the applicant’s business, and (3) maintained in secrecy. (c) Each page containing CBI material must have ‘‘CBI Copy’’ marked in the upper right corner of the page. In the right margin, mark the CBI information with a bracket and ‘‘CBI.’’ (d) The CBI-deleted copy should be a facsimile of the CBI copy, except for spaces occurring in the text where CBI has been deleted. Be sure that the CBI deleted copy is paginated the same as the CBI copy (The CBI-deleted copy of the application should be made from the same copy of the application which originally contained CBI). Additional material (transitions, paraphrasing, or generic substitutions, etc.) should not be included in the CBI-deleted copy. (e) Each page with CBI-deletions should be marked ‘‘CBI-deleted’’ at the upper right corner of the page. In the right margin, mark the place where the CBI material has been deleted with a bracket and ‘‘CBI-deleted.’’ (f) If several pages are CBI-deleted, a single page designating the numbers of deleted pages may be substituted for blank pages. (For example, ‘‘pages 7 through 10 have been CBI-deleted.’’) (g) All published references that appear in the CBI copy should be included in the reference list of the CBI deleted copy. Published information cannot be claimed as confidential.

Not applicable.

83941-2 C 02-15-2010 C

1/6

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
(7511P)
1200 Pennsylvania Avenue NW
Washington, DC 20460

EPA Reg. Number:	Date of Issuance:
83941-2	15 Feb 2010
Term of Issuance:	Unconditional

NOTICE OF PESTICIDE:

X Registration ____ Re-registration
(under FIFRA, as amended)

Name of Pesticide Product:
Vacciplant

Name and Address of Registrant (include ZIP Code):
Laboratoires Goemar SA,
c/o SciReg, Inc.
12733 Director's Loop,
Woodbridge, VA 22192

12A 01

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Biopesticides and Pollution Prevention Division prior to use of the label in commerce. In any correspondence on this product, always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This registration does not eliminate the need for continual reassessment of the pesticide. If the EPA determines at any time, that additional data are required to maintain in effect an existing registration, the Agency will require submission of such data under section 3(c)(2)(B) of FIFRA.

The product is registered in accordance with FIFRA section 3(c)(5) and is subject to the following terms and conditions:

1. Submit and/or cite all data required for registration of your product under FIFRA section 3(c)(5) and section 4 when the Agency requires all registrants of similar products to submit such data.
2. Make the following label change before you release the product for shipment: Revise the EPA Registration Number to read, "EPA Reg. No. 83941-2."
3. Submit two (2) copies of the revised final printed labeling before you release the product for shipment. Refer to the A-79 enclosure for a further description of the final printed label.

A stamped copy of the label is enclosed for your record.

Signature of Approving Official:

Keith A. Matthews, Acting Director
Biopesticides and Pollution Prevention Division

Date:
15 Feb 2010

EPA Form 8570-6

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VACCIPLANT

STIMULANT OF PLANT DEFENSE REACTIONS

Active Ingredient:
Laminarin 3.51%
Other Ingredients: 96.49%
Total 100.00%

KEEP OUT OF REACH OF CHILDREN
CAUTION

FIRST AID	
IF SWALLOWED	• Call a poison control center or doctor immediately for treatment advice. • Have person sip a glass of water if able to swallow. • Do not induce vomiting unless told to do so by a poison control center or doctor. • Do not give anything by mouth to an unconscious person.
IF ON SKIN OR CLOTHING	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
IF INHALED	• Move person to fresh air. • If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. • Call a poison control center or doctor for further treatment advice.
HOT LINE NUMBER Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may also contact 1-800-858-7378 (National Pesticide Information Center) for emergency medical treatment information.	

EPA Reg. No. 83941-
EPA Est. No. 83941-FRA-001

NET CONTENTS:

ACCEPTED

FEB 15 2010

Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 83941-2

Laboratoires Goëmar SA
Z.A.C. La Madeleine
Avenue General Patton
35400 Saint-Malo
France

Page 1
Feb. 9. 2010

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

Caution. Harmful if swallowed, absorbed through skin, or inhaled. Avoid contact with skin, eyes, or clothing. Avoid breathing spray mist. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

ENVIRONMENTAL HAZARDS

For terrestrial uses: Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment wash water or rinsate.

PHYSICAL AND CHEMICAL HAZARDS

FOR CHEMICAL EMERGENCY: spill, leak, fire, exposure, or accident call CHEMTREC 1-800-424-9300.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear long pants, long-sleeved shirt, shoes plus socks, and chemical-resistant gloves. Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS

Users should:

- Remove PPE clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read entire label before using. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the State or Tribal agency responsible for pesticide regulation. Do not apply this product through any type of irrigation system. Do not apply this product when raining.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 4 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Shoes plus socks
- Chemical-resistant gloves made of any waterproof material

PREPARATION OF THE MIXTURE

Shake the container and pour the required amount of VacciPlant into the sprayer tank while filling with the appropriate amount of water. Maintain agitation. Do not store the mixture overnight.

GENERAL APPLICATION INSTRUCTIONS

Apply the required amount of VacciPlant in 5-100 gallons of water per acre. To optimize the efficiency, use 10-40 gallons of water per acre. Do not apply in volumes greater than 100 gallons of water per acre.

Crops	Disease	Number of Applications/Season	Application Interval	Rate/Acre/ Application	Application Instructions
Fruiting Vegetables	Bacterial spot Bacterial speck	2 - 7	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. Repeat applications until disease conditions end. Add a copper product to VacciPlant if the disease developing symptoms appear.
Tomato	Gray mold Powdery mildew Phytophthora blight	2 - 10			Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.
	Early blight Anthracnose	2 - 10			
Eggplant Pepper	Powdery mildew Phytophthora blight	2 - 10	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.
Cucurbits - Zucchini - Cucumber - Watermelon - Melon	Powdery mildew Phytophthora blight	1 - 8	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.
Leafy vegetables - Lettuce - Spinach	Downy mildew Gray mold	1 - 6	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.

Brassicas - Cabbage	Downy mildew Gray mold	1 - 6	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.
Peach	Monilia	3	10 days	9.7-14.4 fl oz	Apply VacciPlant every 10 days with an appropriate fungicide. Make the first application 30 days before harvest.
Apple/pear	Fire blight	2 - 6	10 days	9.7-14.4 fl oz	Apply VacciPlant every 10 days from green bud stage. Apply the last application at petal fall stage. Continue the VacciPlant applications if disease symptoms occur after bloom.
Strawberry	Gray mold	1 - 6	10 days	9.7-14.4 fl oz	Start applications at the beginning of flowering, then continue with successive applications until harvest. In case of weather conditions favorable to disease development (high humidity, temperature greater than 68°F), apply an appropriate fungicide.
	Leather rot Leaf scorch Powdery mildew	1 - 10	10 days	9.7-14.4 fl oz	Start VacciPlant applications preventively, when weather conditions become favorable for disease development. When the first symptoms occur, apply an appropriate fungicide. Then : - if the new leaves or new fruits are free of any symptom, continue VacciPlant applications. - if the new leaves or new fruits show symptoms, continue using a fungicide as long as new leaves or new fruits are affected. Follow the same instructions at each new phase of disease onset.

COMPATIBILITY

VacciPlant is compatible with many commonly used crop protection products, but has not been fully evaluated with all. Evaluate tank-mix compatibility prior to use. Observe the most restrictive of the labeling limitations and precautions of all products used in mixtures. Apply to a small area of the crop to ensure there are no phytotoxic effects. Contact your VacciPlant technical service representative for additional details.

- VacciPlant acts early against specified plant diseases inducing plant defense reactions.
- VacciPlant induces systemic resistance, which allows protection during growth.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Keep container tightly closed when not in use.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Nonrefillable container. Do not reuse or refill this container. Triple rinse (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into the application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Then offer for recycling, if available, or puncture or dispose of in a sanitary landfill, or by incineration. Do not burn unless allowed by state and local ordinances.

CONDITIONS OF SALE AND WARRANTY

LABORATOIRES GOËMAR SA warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes stated on the label when used according to the directions under normal use conditions.

To the extent consistent with applicable law, neither this warranty nor any other warranty of merchantability or fitness for a particular purpose, expressed or implied, extends to the use of this product contrary to the label instructions, the buyer assumes the risk of any such uses.

§ 180.1290 *Pasteuria usgae;* exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of *Pasteuria usgae* in or on all food commodities when applied preharvest and used as a nematicide in accordance with good agricultural practices.

[75 FR 37737, June 30, 2010]

§ 180.1291 Cold pressed neem oil; exemption from the requirement of a tolerance.

Residues of the biochemical pesticide cold pressed neem oil are exempt from the requirement of a tolerance in or on all food commodities.

[74 FR 55463, Oct. 28, 2009]

§ 180.1292 Ulocladium oudemansii (U3 Strain); exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established in/on all food commodities for residues of *Ulocladium oudemansii* (U3 Strain), when applied or used pre-harvest-only, excluding applications made post-harvest or to processed commodities, as a microbial fungicide in accordance with good agricultural practices.

[74 FR 55458, Oct. 28, 2009]

§ 180.1293 Trichoderma gamsii strain ICC 080; exemption from the requirement of a tolerance.

Trichoderma gamsii strain ICC 080 is exempted from the requirement of a tolerance in or on all food and feed commodities when applied preharvest and used in accordance with good agricultural practices.

[75 FR 8507, Feb. 25, 2010]

§ 180.1294 Trichoderma asperellum strain ICC 012; exemption from the requirement of a tolerance.

Trichoderma asperellum strain ICC 012 is exempted from the requirement of a tolerance in or on all food and feed commodities when applied pre-harvest and used in accordance with good agricultural practices.

[75 FR 9530, Mar. 3, 2010]

§ 180.1295 Laminarin; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of laminarin in or on all food commodities when laminarin is applied preharvest.

[75 FR 8256, Feb. 24, 2010]

§ 180.1296 Terpene Constituents α-terpinene, d-limonene and p-cymene, of the Extract of Chenopodium *ambrosioides* near ambrosioides as Synthetically Manufactured; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for the residues of the biochemical pesticide Terpene Constituents α-terpinene, d-limonene and p-cymene, of the Extract of Chenopodium *ambrosioides* near *ambrosioides* as Synthetically Manufactured when used as an insecticide/acaricide in or on all food commodities.

[75 FR 39455, July 9, 2010]

§ 180.1297 Homobrassinolide; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for the residues of homobrassinolide in or on all food commodities when applied/used as a plant growth regulator in accordance with good agricultural practices.

[75 FR 39459, July 9, 2010]

§ 180.1298 Trichoderma hamatum isolate 382; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of *Trichoderma hamatum* isolate 382 in or on all food commodities when applied as a fungicide and used in accordance with good agricultural practices.

[75 FR 43076, July 23, 2010]

§ 180.1299 Prohydrojasmon; temporary exemption from the requirement of a tolerance.

A temporary exemption from the requirement of a tolerance is established for residues of prohydrojasmon, propyl-3-oxo-2-pentylcyclo-pentylacetate, when used on red apples varieties preharvest and when used in accordance

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Mr. Frederick T. Smith
Laboratoires Goëmar SA
c/o SciReg, Inc.
12733 Director's Loop
Woodbridge, VA 22192

JUN 14 2012

Subject: Laboratoires Goëmar SA; Vacciplant, EPA Registration No. 83941-2;
Label Amendment to Add More Specific Directions and Caneberries;
D# 463802, Application Dated 4/17/12

Dear Mr. Smith:

The amendment referred to above, submitted in connection with registration under FIFRA section 3(c)(7)(A), is acceptable provided that you:

1. Submit and/or cite all data required for registration/reregistration of your product under FIFRA section 3(c)(5) when the Agency requires all registrants of similar products to submit such data.

2. Submit two (2) copies of your final printed labeling before you release the product for shipment. Final printed labeling means the label or labeling of the product when distributed or sold. Clearly legible reproductions or photo reductions will be accepted for unusual labels, such as those silk-screened directly onto glass or metal containers or large bags or drum labels.

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA section 6(b). Your release for shipment of the product bearing the amended labeling constitutes acceptance of these conditions.

If you have any questions contact Chris Pfeifer at 703-308-0031 or by email at: pfeifer.chris@epa.gov. A stamped copy of the label is enclosed for your records.

Sincerely,

Linda A. Hollis, Chief
Biochemical Pesticides Branch
Biopesticides and Pollution
Prevention Division (7511P)

Enclosure

STIMULANT OF PLANT DEFENSE REACTIONS

Vacciplant®

Active Ingredient:	
Laminarin	3.51%
Other Ingredients:	96.49%
Total	100.00%

ACCEPTED
JUN 14 2012
Under the Federal Insecticide, Fungicide and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 83941-2

KEEP OUT OF REACH OF CHILDREN

CAUTION

See side panel for first aid.

EPA Reg. No. 83941-2 EPA Est. No. 83941-FRA-001

NET CONTENTS: 1.3 gal (5L)

Produced by:

Laboratoires Goëmar S.A.
Parc Technopolitain Atalante St Malo
CS41908 St Jouan des Guérets
35419 Saint Malo CEDEX - FRANCE
Tel.: +33 2 99191919
http://www.goemar.com

GOËMAR

CETI USVACSR - 03/12

FIRST AID
IF SWALLOWED • Call a poison control center or doctor immediately for treatment advice. • Have person sip a glass of water if able to swallow. • Do not induce vomiting unless told to do so by a poison control center or doctor. • Do not give anything by mouth to an unconscious person.
IF ON SKIN OR CLOTHING • Take off contaminated clothing. • Rinse skin immediately with plenty of water for 15-20 minutes. • Call a poison control center or doctor for treatment advice.
IF INHALED • Move person to fresh air. • If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. • Call a poison control center or doctor for further treatment advice.
HOT LINE NUMBER Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may also contact 1-800-858-7378 (National Pesticide Information Center) for emergency medical treatment information.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

Caution. Harmful if swallowed, absorbed through skin or inhaled. Avoid contact with skin, eyes, or clothing. Avoid breathing spray mist. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

ENVIRONMENTAL HAZARDS

For terrestrial uses: Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment wash water or rinsate.

PHYSICAL AND CHEMICAL HAZARDS

FOR CHEMICAL EMERGENCY: spill, leak, fire, exposure, or accident call CHEMTREC 1-800-424-9300.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear long pants, long-sleeved shirt, shoes plus socks, and chemical-resistant gloves. Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS

Users should:

- Remove PPE clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read entire label before using. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your State or Tribe, consult the State or Tribal agency responsible for pesticide regulation. Do not apply this product through any type of irrigation system. Do not apply this product when raining.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 4 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Shoes plus socks
- Chemical-resistant gloves made of any waterproof material

PREPARATION OF THE MIXTURE

Shake the container and pour the required amount of Vacciplant into the sprayer tank partially filled with water while filling with the appropriate amount of water. Maintain good agitation. Do not store the mixture overnight.

GENERAL APPLICATION INSTRUCTIONS

Apply the required amount of Vacciplant in combination with a registered fungicide in an appropriate volume of water. If Vacciplant is sprayed alone, apply at 0.15% volume/volume in applications exceeding 70 gallons per acre. At volumes under 70 gallons per acre, apply Vacciplant at 14 fluid ounces per acre. Do not apply this product post-harvest. Apply the required amount of Vacciplant according to the table below.

Crops	Disease	No. of Applications/Season	Application Interval	Rate/Acre/ Application	Application Instructions
Fruiting Vegetables **Tomato**	Bacterial spot Bacterial speck Early blight Phytophthora blight Botrytis Powdery mildew Anthracnose	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Eggplant **Pepper**	Phytophthora blight Bacterial spot Downy mildew Powdery mildew	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Cucurbits **- Zucchini** **- Cucumber** **- Watermelon** **- Cantaloupe** **- Squash** **- Pumpkin** **- Muskmelon**	Phytophthora blight Angular leaf spot Powdery mildew Downy mildew	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Bulb vegetables **- Onion** **- Leek**	Bacterial leaf streak Yellow soft virus	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Head and leafy vegetables **- Lettuce** **- Spinach** **- Celery**	Downy mildew Botrytis	2 - 6	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water Repeat applications until conditions favorable for disease prevalence have diminished.
Cole Crops **Brassicas** **- Cabbage** **- Broccoli** **- Brussel sprouts** **- Chinese cabbage** **- Cauliflower**	Downy mildew Powdery mildew	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or planting in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Stone fruits **- Peach**	Monilia Fruit brown rot	2 - 4	7 - 10 days	14 fl oz	Start Vacciplant preventative applications in combination with an appropriate registered fungicide in an appropriate volume of water. Begin application 30 days before harvest. Repeat applications until conditions favorable for disease prevalence have diminished. Do not apply post-harvest.
Pome fruits **- Apple** **- Pear**	Fire blight	2 - 7	7 - 10 days	14 fl oz	Apply Vacciplant in an appropriate volume of water in combination with an appropriate registered bactericide from green bud stage to petal fall stage on one year old wood. After blooming time, continue Vacciplant applications if weather conditions are favorable for disease.
	Scab	2 - 10	7 - 10 days	14 fl oz	After primary contamination of scab, apply Vacciplant in an appropriate volume of water. In situations that may cause high disease pressure (rain showers for 18h and over 1 inch), apply in combination with an appropriate registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished..
	Gloeosporium rot	2 - 10	7 - 10 days	14 fl oz	Apply Vacciplant in combination with an appropriate registered fungicide in an appropriate volume of water every 7-10 days from 3 weeks before harvest. Repeat application until conditions favorable for disease prevalence have diminished. Do not apply post-harvest.
Strawberries	Botrytis	2 - 6	7 - 10 days	14 fl oz	Start Vacciplant preventative applications immediately after transplant or prior to bloom in combination with a registered fungicide in an appropriate amount of water. Repeat applications until conditions favorable for disease prevalence have diminished.
	Leaf spot, Leather rot, Leaf scorch, Powdery mildew, Anthracnose	2 - 10			
Table grapes **Wine grapes**	Powdery mildew	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications in combination with a registered fungicide in an appropriate amount of water. Repeat applications until conditions favorable for disease prevalence have diminished.
Berries **- Blueberry** **- Cane Berries**	Botrytis blight	2 - 10	7 - 10 days	14 fl oz	Start Vacciplant preventative applications prior to early bloom in combination with a registered fungicide in an appropriate volume of water. Repeat applications until conditions favorable for disease prevalence have diminished.

COMPATIBILITY

Vacciplant is compatible with many commonly used crop protection products, but has not been fully evaluated with all. Evaluate tank-mix compatibility prior to use. Observe the most restrictive of the labeling limitations and precautions of all product used in mixtures. Apply to a small area of the crop to ensure there are no phytotoxic effects. Contact your Vacciplant technical service representative for additional details.

- Vacciplant acts early *against* specified plant diseases inducing plant defense reactions.
- Vacciplant reduces systemic resistance, which allows protection during growth.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Keep container tightly closed when not in use.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Handling: Nonrefillable container. Do not reuse or refill this container. Triple rinse (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into the application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Then offer for recycling, if available, or puncture or dispose of in a sanitary landfill, or by incineration. Do not burn unless allowed by state and local ordinances.

CONDITIONS OF SALE AND WARRANTY

Laboratoires GOËMAR S.A. warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes stated on the label when used according to the directions under normal use conditions.

To the extent consistent with applicable law, neither this warranty nor any other warranty of merchantability or fitness for a particular purpose, expressed or implied, extends to the use of this product contrary to the label instructions; the buyer assumes the risk of any such uses.

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BIOPESTICIDE REGISTRATION ACTION DOCUMENT

Laminarin

PC Code: 123200

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

Last updated- February 13, 2010

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BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

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I. EXECUTIVE SUMMARY

Laminarin is a common component of the human diet. It is a naturally occurring polysaccharide carbohydrate (oliggosaccharide) found in all edible plants. It is a major constituent in cereal grains, and is consumed as an intentionally added ingredient in many dietary supplements and texturing agents. Laminarin is typically extracted from brown algae, where it is present in great quantity as a storage glucan – a carbohydrate food reserve that can be converted to glucose when necessary. The extraction results in a low-odor white powder, which is extremely nutrient rich. Besides being the source for this biochemical pesticide, this algal extract is also a regular ingredient in the Japanese diet. As a biochemical pesticide active ingredient, Laminarin stimulates the natural defense reactions of agricultural crops such as fruiting vegetables, tomato, eggplant, pepper, zucchini, cucurbits, watermelon, melons, grape, apple, pear, and strawberries against such disease organisms as gray mold, powdery mildew, downy mildew, fire blight, and bacterial spot. As a naturally occurring oligosaccharide, residues of the active ingredient are indistinguishable from other naturally occurring plant oligosaccharides. In addition to the long history of human consumption of Laminarin without known toxicological effect, data and information submitted to the Agency in conjunction with the petition for the exemption from a requirement of tolerance confirm that Laminarin is virtually non-toxic and poses no dietary risks to humans.

Acute, subchronic and developmental studies submitted in the application for registration provided sufficient information to satisfy all mammalian toxicology data requirements. No toxicological endpoints were established, and no adverse effects were observed with regard to mammalian health.

Because Laminarin is considered to be "toxicologically innocuous," no residue studies are required to support an exemption from the requirement of a tolerance. Laminarin's low toxicity profile notwithstanding, another justification for an exemption from the requirement of a tolerance is the minimal likelihood of residues for this biochemical pesticide. Laminarin is intended for application as a Systemic Acquired Resistance (SAR) inducer – a preventative mode of action. As such, it is applied early in a crop's life cycle – in its growing stages - to help build immunity to disease organisms such as mold and bacterial infection. And as a biochemical, it biodegrades rapidly. Data indicate that the active ingredient is more than 65% biodegraded after two weeks (MRID 47264954). Calculations indicate that it would be largely biodegraded long before any final application would be practicable. Accordingly, no significant exposures are expected at the time of harvest.

No dietary risks are expected with regard to the use of the active ingredient Laminarin. Significant dietary exposures (including exposures via drinking water) are not expected for the active ingredient Laminarin. In the event of dietary exposure, the toxicological data demonstrate that Laminarin is not toxic or pathogenic to mammals. It is a regular constituent of the human diet; and there have been no health effects associated with its consumption by people. Furthermore, all data demonstrate that no acute, sub-chronic, chronic, immune, or endocrine-

disrupting effects are associated with the use of the active ingredient. Accordingly, no harm to infants, children, and the general U.S. population is anticipated with regard to dietary exposure. Because of the nontoxic profile and the lack of expected residue of the active ingredient, the risks associated with the proposed food uses of this active ingredient are expected to be negligible.

The potential for aggregate, non-occupational exposure is expected to be insignificant as the active ingredient is largely biodegraded within two weeks, and applications of Laminarin occur early in the growing season. Moreover, given a lack of acute toxicological endpoints and because Laminarin is not known to share any structural similarity to any chemicals with common mechanisms of toxicity, the likelihood of risks resulting from such de minimis exposures is negligible.

Non-target organism and environmental fate data requirements were satisfied by valid studies. Laminarin occurs naturally in the terrestrial environment, and is not associated with any known detrimental effect. All information available to the Agency validates a non-toxic mode of action, and a lack of adverse effect relative to non-target organisms.

In accordance with T-REX Model, the Individual Effects Chance Model Version 1.1 and the non-target data submitted, the Agency has made a "No Effect" (NE) determination for direct and indirect effects to any listed threatened and endangered species and their habitat as a result of the proposed uses of Laminarin.

On October 1, 2009, EPA announced a new policy to provide a more meaningful opportunity for the public to participate on major registration decisions before they occur. According to this new policy, EPA will provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients, first food use, first outdoor use, first residential use, and other actions for which we anticipate significant public interest. The registration application for Laminarin is for a "new active ingredient" whose registration would result in a "first outdoor use" and a "first food use." Therefore, consistent with the new policy of making registration actions more transparent, the Agency provided a 30-day comment period on the Laminarin application and EPA's preliminary risk assessment. EPA did not receive any comments during the comment period.

EPA believes, based on the risk assessment and information submitted in support of the registration of Laminarin, that it is in the best interests of the public and the environment to issue the registration for Laminarin. The basis for this preliminary decision can be found in the risk assessment for Laminarin, which is characterized in this BRAD. As discussed above, acute toxicity data for Laminarin indicate a nontoxic profile. Laminarin does not demonstrate subchronic or developmental toxicity, and it is not mutagenic or genotoxic. EPA has no concerns for any non-target organisms exposed to Laminarin in accordance with approved label directions. EPA has not identified any toxic endpoints for non-target mammals, birds, plants, aquatic, or soil organisms. Nor are there concerns for any threatened and endangered species.

Thus, given that Laminarin has very low toxicity, and presents little if any risk to non-target organisms, and data confirm its effectiveness as a Systemic Acquired Response (SAR) Inducer, EPA concludes that it is in the best interests of the public and the environment to issue the registration for Laminarin.

EPA reviewed data requirements for granting registration under Section 3(c)(5) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). It was determined that the data/information submitted fulfilled current data requirements (refer to 40 CFR Subpart U § 158.2000).

II. ACTIVE INGREDIENT OVERVIEW

Common Name: Laminarin

Chemical Names: Oligosaccharide, Polysaccharide Carbohydrate, Glucan

Trade & Other Names: Laminarin

CAS Registry Number: 9008-22-4

OPP Chemical Code: 123200

Type of Pesticide: SAR Inducer

III. REGULATORY BACKGROUND

On July 31, 2008, EPA published in the Federal Register (Volume 74, Number 49) a notice announcing that Laboratoires Goemar SA, Z.AC La Madeline, Avenue General Patton, 35400 Saint-Malo, France c/o SciReg, Inc. 12733 Director's Loop, Woodbridge, VA 22192, submitted an application proposing to establish an exemption from the requirement of a tolerance for residues of the biochemical pesticide, Laminarin, in or on all food commodities (PP# 7E7276). On March 16, 2009, EPA published in the Federal Register (Volume 74, Number 49) a notice announcing that Laboratoires Goemar SA, Z.AC La Madeline, Avenue General Patton, 35400 Saint-Malo, France c/o SciReg, Inc. 12733 Director's Loop, Woodbridge, VA 22192, submitted an application to register a pesticide product (EPA File Symbol 83941-E) containing a new active ingredient (Laminarin) not included in any currently registered products. No comments were received following the publication of either notice. In addition, on January 5, 2010, EPA provided the opportunity for a 30-day comment period on the Agency's draft risk assessment and intention to register this pesticide product. EPA has not received any comment on this proposed action.

A. CLASSIFICATION

Laminarin is a naturally occurring polysaccharide carbohydrate present in most plants. With regard to its active properties, it is considered to be a SAR inducer, bolstering plant defense mechanisms, protecting plants against attacks from mold, bacteria and fungi. It does not work directly against pests, and is not associated with any toxic mode of action. Accordingly, Laminarin is considered to be a biochemical pesticide due to its nontoxic mode of action to the target pest, its natural occurrence in the environment, and its history of exposure to humans and the environment without known toxicity.

B. FOOD CLEARANCES/TOLERANCES

This active ingredient is being supported for food use. The Agency's risk assessment finds no evidence of dietary risk and supports Pesticide Petition# 7E7276, which proposes to establish an exemption from the requirement of a tolerance for residues of the biochemical pesticide, Laminarin, in or on all food commodities

IV. RISK ASSESSMENT

On October 26, 2007, the Agency issued a Final Rule in the Federal Register on the data requirements to support registration of biochemical and microbial pesticides, and updated the definitions for biochemical and microbial pesticides (72 FR 61002). The rule became effective on December 26, 2007. The data and information evaluated for this Biopesticide Registration Action Document (BRAD) were considered in light of these requirements.

Classifications for each data submission are assigned by EPA science reviewers and are an indication of the usefulness of the information contained in the documents for risk assessment. A rating of "ACCEPTABLE" indicates the study is scientifically sound and is useful for risk assessment. A "SUPPLEMENTAL" rating indicates the data provide some information that can be useful for risk assessment. The studies may have certain aspects determined not to be scientifically acceptable ("SUPPLEMENTAL: UPGRADABLE"). If a study is rated as "SUPPLEMENTAL: UPGRADABLE," the Environmental Protection Agency always provides an indication of what is lacking or what can be provided to change the rating to "ACCEPTABLE." If there is simply a "SUPPLEMENTAL" rating, the reviewer will often state that the study is not required by the current 40 CFR Part 158. Both "ACCEPTABLE" and "SUPPLEMENTAL" studies may be used in the risk assessment process as appropriate. An "UNACCEPTABLE" rating indicates that new data need to be submitted.

For the acute toxicity data requirements, toxicity categories are assigned for providing the appropriate precautionary labeling statement, based on the hazard(s) identified from studies and/or other information submitted to the Agency in support of a pesticide registration. The active ingredient or particular product is classified into Toxicity Category I, II, III, or IV, where

Toxicity Category I indicates the highest toxicity and Toxicity Category IV indicates the lowest toxicity.

A. PRODUCT ANALYSIS ASESSMENT

1. Product Chemistry and Composition

Laminarin is a naturally occurring oligosaccharide that can be found in most plants. It serves plants as a storage glucan - a carbohydrate food reserve - which breaks down into glucose and provides energy when plants are stressed. Laminarin extract is derived from brown algae (Laminara *digitata*), and is a low-odor white powder. Because of its nutrient richness and starchy character, Laminarin extract is commonly used as a dietary supplement, a food texturing agent and an ingredient in some ethnic cuisines.

All product chemistry data requirements for Laminarin have been satisfied. As an active ingredient, Laminarin extract is indistinguishable from the oligosaccharide that is produced naturally in pants. The extract has a high degree of purity and contains no impurities of toxicological significance. All data requirements for physical and chemical characteristics have been adequately addressed.

2. Analysis and Certification of Limits

The submitted data satisfied the requirement for Analysis and Certification of Limits. Five batch analyses and the analytical method used to determine the purity of Laminarin were examined and determined to be acceptable by the Agency. The certified limits for the active and inert ingredients fall within the ranges specified by OPPTS Guideline 830-1750.

3. Physical and Chemical Characteristics

The Agency has determined that the submitted data adequately describe the physical and chemical characteristics of Laminarin. Refer to Table 1 in Appendix A for The Series 830 physical and chemical properties.

B. HUMAN HEALTH ASSESSMENT

1. Toxicological Hazard Assessment

Adequate mammalian toxicology studies were provided in support of the registration of Laminarin for each data requirement. Acute toxicology data for Laminarin indicates that the active ingredient is virtually non-toxic to mammals, and that there are no toxicological endpoints relative to the use of Lamanarin as a SAR inducer. Accordingly, the data submitted demonstrate that the proposed uses of Laminarin pose no significant risks to human health and support a

finding of reasonable certainty that no harm to the general U.S. population, including infants and children, will result from exposure to this active ingredient.

Refer to Table 2 in Appendix A for a summary of the Toxicity Data Requirements for this food use active ingredient.

a. Acute Toxicity – Tier I (40 CFR § 158.2050)

Acute Oral Toxicity – Rat [OPPTS Guideline 870.1100; Master Record Identification (MRID) Numbers (Nos.) 47264930 and 47264943]: An acute oral toxicity study shows that the active ingredient Laminarin has an LD_{50} of greater than 2000 mg/Kg in rats. This was the maximum dose rate. There were no observed toxicological effects on the test subjects at the maximum dose. The study supports the finding that this active ingredient poses no significant human health risk with regard to food uses. The study was found "ACCEPTABLE" and Laminarin was classified as TOXICITY CATEGORY III for this route of exposure when used as a SAR inducer.

Acute Dermal Toxicity– Rabbits (OPPTS Guideline 870.1200; MRID Nos. 47264931 and 47264974): An acute oral toxicity study shows that the active ingredient Laminarin has an LD_{50} of greater than 5000 mg/Kg in rats, which is considered to be virtually non-toxic. Data substantiate the active ingredient's relative dermal non-toxicity to both occupational users and the general public. The study was found "ACCEPTABLE" and Laminarin was classified as TOXICITY CATEGORY IV for this route of exposure when used as a SAR inducer.

Acute Inhalation Toxicity (OPPTS Guideline 870.1300; MRID Number (No.). 47264932): An acute oral inhalation study shows that the active ingredient Laminarin has an LC_{50} of greater than 1.02 mg/L in rats, which shows no significant inhalation toxicity. This was the maximum dose rate, and no toxicological effects were observed on the test subjects. The study was found "ACCEPTABLE" and Laminarin was classified as TOXICITY CATEGORY III for this route of exposure when used as a SAR inducer.

Primary Eye Irritation (OPPTS Guideline 870.2400; MRID Nos. 47264933 and 47264976): A primary eye irritation study on rabbits demonstrated Laminarin to be non-irritating. There were no observed effects for this route of exposure relative to the use of Laminarin. The study was found "ACCEPTABLE" and Laminarin was classified as TOXICITY CATEGORY IV for this route of exposure when used as a SAR inducer.

Primary Dermal Irritation (OPPTS Guideline 870.2500; MRID No. 47264934): A skin irritation study on rabbits demonstrated that Laminarin was not irritating to the skin. The findings are consistent with the other dermal studies and confirm that Laminarin is not toxic through this route of exposure. The study was found "ACCEPTABLE" and Laminarin was classified as TOXICITY CATEGORY IV for this route of exposure when used as a SAR inducer.

Skin Sensitization (OPPTS Guideline 870.2600; MRID Nos. 47264935 and 47264978): Data indicate Laminarin is not a dermal sensitizer. However, any reported incidents may cause this position to be reconsidered.

Subchronic Testing (OPPTS Guideline 870.3100, 870.3250, 870.3465; MRID Nos. 47264937, 47264938 and 47264939): In accordance with footnote seven and eight in the Biochemical Pesticides Human Health Assessment Data Requirements table in 40 CFR § 158.2050, subchronic dermal and subchronic inhalation testing were not required for a lack of exposure. Three subchronic oral tests were submitted in support of Laminarin's food use. These studies satisfy the data requirement for subchronic oral testing and indicate that Laminarin has no subchronic toxicological effect through the oral route of exposure. A 28-day oral toxicity study found no toxicological effects regarding mortality, clinical observations, neurotoxicity assessment, body weight, food consumption, hematology, clinical chemistry, organ weights, and macroscopic or microscopic observations. The NOEL was determined to be 1,000 mg/kg/day. A 90-day oral toxicity study found no statistical difference in hematology, clinical chemistry, or urinalysis between test subjects and the control. The NOEL was determined to be 1,000 mg/kg/day. Another 90-day oral toxicity study also found no statistical difference in hematology, clinical chemistry, or urinalysis between test subjects and the control. And the NOEL was again determined to be 1,000 mg/kg/day. All subchronic oral toxicity studies indicate that Laminarin is not subchronically toxic through the oral route of exposure.

Developmental Toxicity (OPPTS Guideline 870.3700; MRID Nos. 47264940 and 47264941): Data submitted to the Agency satisfy the data requirement and support the Agency's conclusion that there is no risk of developmental toxicity associated with the new food uses. A prenatal developmental toxicity study on rats found no significant reproductive effects or fetal abnormalities, and established a NOAEL of 1,000 mg/kg/day. The findings suggest negligible risk with regard to developmental toxicity.

Mutagenicity Testing (OPPTS Guidelines 870.5100, 870.5300, 870.5375; MRID Nos. 47264942, 4726493 and 47264944): Three genotoxicity studies (a Bacterial Reverse Mutation Test, and an *In Vitro* Mammalian Cells in Culture Assay) were performed on the active ingredient Laminarin and satisfy the data requirement. The Reverse Mutation Assay showed that Laminarin did not induce mutant colonies over expected background levels. The *In Vitro* Mammalian Cells in Culture Assay demonstrated that Laminarin did not damage chromosomes or the mitotic apparatus of bone marrow cells. These mutagenicity studies are sufficient to confirm that there are no expected dietary, occupational, or non-occupational risks of mutagenicity with regard to Laminarin.

b. Acute Toxicity – Tier II and Tier III (40 CFR § 158.2050)

The Tier II studies listed below were the only higher Tier studies required for Laminarin. No other studies were required based on a lack of acute toxicity in the Tier I studies and a lack of exposure relative to its use pattern as a SAR inducer

Developmental Toxicity (OPPTS Guideline 870.3700; MRID No. 47264941): Tier II data are required on the active ingredient for developmental toxicity if there might be regular exposure to women. In that case, a second prenatal study using a different test subject is required. A second prenatal developmental toxicity study on rabbits found no significant treatment-related reproductive effects or fetal abnormalities, and confirmed a NOAEL of 1,000 mg/kg/day. Data submitted to the Agency confirm that Laminarin poses negligible risk with regard to developmental toxicity.

Mutagenicity Testing (OPPTS Guidelines 870.5385; MRID No. 47264944): Tier II data are required on the active ingredient for mutagenicity testing for the active ingredient. A bone Marrow Micronucleus Assay indicated that no toxicity was noted in either sex at any dose up to the limit dose of 2000 mg/kg. This mutagenicity study, in conjunction with the Tier I mutagenicity studies, satisfies the data requirement for mutagenicity testing and is sufficient to confirm that there are no expected dietary, occupational, or non-occupational risks of mutagenicity with regard to Laminarin.

Immunotoxicity Testing (OPPTS Guidelines 880.3550; MRID No. 47264945): A waiver request was accepted for immunotoxicity for the following reasons: 1) The potential for any immunotoxic effect is precluded by the Laminarin's biodegradability. 2) Laminarin is not structurally related to any known immunotoxic chemical. 3) There is a long history of the consumption of Laminarin without known immunotoxicological incident. 4) The toxicological profile in acute toxicological studies, subchronic studies and developmental studies does not suggest any immunotoxicity. All information points to the lack of dietary risk posed by the immunotoxicity of Laminarin residues, and supports the exemption from the requirement of a tolerance.

c. Effects on the Endocrine System

EPA is in the process of issuing test orders for endocrine effects. The schedule for issuance of test orders, and details regarding status is available at http://www.epa.gov/endo/. EPA has also established a docket for the test orders in www.regulations.gov under docket number EPA-HQ-OPP-2009-0634.

Data required under the test orders will provide information to help EPA identify whether chemicals have the potential to interact with the estrogen, androgen, and/or thyroid hormone systems, which regulate growth, metabolism, development, and reproduction. The data generated from the screens will provide robust and systematic scientific information that will help EPA identify whether additional testing is necessary.

Laminarin is a naturally occurring carbohydrate present in our fruits and vegetables. To date, there is no evidence to suggest that our natural exposure to Laminarin affects the immune system, functions in a manner similar to any known hormone, or that it acts as an endocrine

disruptor. Moreover, the use of Laminarin is not expected to result in any significant exposures, effectively obviating any opportunity for negative effects on humans or the environment. Therefore, it is unlikely that Laminarin will have estrogenic or endocrine effects.
Because there is no available evidence demonstrating that Laminarin is an endocrine disruptor, the Agency is not requiring information on the endocrine effects of Laminarin at this time. However, the Endocrine Disruption Screening Program (EDSP) has established a protocol, which guides the Agency in selecting suspect ingredients for review; and the Agency reserves the right to require new information, should the program require it. Presently, based on the lack of exposure and the negligible toxicity profile of the extract, no adverse effects to the endocrine or immune systems are known or expected.

2. Dose Response Assessment

No toxicological endpoints were identified; therefore, a dose response assessment was not required.

3. Dietary Exposure and Risk Characterization

Exposure to residues of Laminarin on foods is expected to be negligible; and even in the event of dietary exposure, no dietary risks are anticipated. Data submitted to the Agency show that Laminarin is 65% to 71% biodegraded within two weeks, and that it hydrolyzes very rapidly into glucose. Because applications tend to occur earlier in the growing season (due to its mode of action as a SAR inducer), and given its short-lived presence on crops, no significant pesticidal residues are anticipated for harvested foods. Even in the event of exposure to residues, however, no dietary risks are anticipated. Acute, subchronic, and teratogenicity studies support its non-toxic profile. It is already present in the human diet – especially in cereal grains - without any known detrimental effect. Furthermore, it is approved by US FDA as a food additive, a dietary supplement and a texturing agent in processed foods in quantities greater than any expected pesticidal residues. There is no information in the public literature suggesting any health issues to either animals or plants relative to this compound. In sum, no dietary exposure is expected; and any potential dietary exposures would not be expected to pose any quantifiable risk, due to its nontoxic profile.

4. Drinking Water Exposure Risk Characterization

Residues of Laminarin are not expected to be present in drinking water. Applications of Laminarin are made directly to terrestrial crops. These residues biodegrade rapidly, and are not expected to percolate through soil. Even in the event of an errant spray drift or an extraordinary rainfall event, Laminarin does not persist in water due to its rapid hydrolyzation into glucose. Moreover, risks from a miniscule aquatic exposure would be negligible, given Laminarin's nontoxic profile. Altogether, drinking water exposure is not expected to pose any quantifiable risk due to a lack of residues, and the nontoxicity of Laminarin.

5. Acute and Chronic Dietary Exposure and Risks for Sensitive Subpopulations, Particularly Infants and Children

Dietary exposure to humans, including infants and children, are considered negligible with regard to the pesticidal use of Laminarin. Because Laminarin is mostly biodegraded within two weeks and applications of Laminarin tend to occur earlier in the growing season with a minimum of ten day intervals between applications, no significant residues of Laminarin are expected on foods. Additionally, Laminarin is known to hydrolyze in water relatively rapidly, and so what few residues might exist at harvest would be degraded into glucose during processing. In the event that there are any residues, acute toxicity studies indicate that Laminarin has negligible toxicity. It is ubiquitous in nature and present in all edible plants, and there is no history of toxicological incident involving its consumption. Its use is approved by FDA as a food additive, a dietary supplement and as a texturing agent for processed foods. While no dietary exposures are expected, the Agency has determined there is a reasonable certainty of no harm to the general US population, including infants and children, from exposure to this active ingredient.

6. Occupational, Residential, School and Day Care Exposure and Risk Characterization

As an agricultural pesticide, some occupational exposure to Laminarin can reasonably be expected. Such occupational exposures are expected to be insignificant because - by virtue of Laminarin's mode of action as a SAR inducer - applications are directed and infrequent. The insignificance of any exposure is even more pronounced for residential, school, or daycare areas. Even in the event of incidental exposure, health risks to humans, including infants and children, are considered negligible, given Laminarin's nontoxic profile.

a. Occupational Exposure and Risk Characterization

Occupational exposures are expected to be minimal. As a biochemical with a preventative mode of action, applications of Laminarin will tend to be limited to early in the growing season when it can best bolster plant defenses; and applications are expected to occur at more infrequent intervals than most other pesticides, as SAR induction requires time to be actualized in the plants. Also, foliar applications are expected for the substance to be most effective, further diminishing the chance of spray drift associated with area-wide sprays. Regardless, requirements for the use of appropriate personal protective equipment and precautionary statements are required on product labels to mitigate any potential risks to pesticide handlers due to prolonged exposure. But, even in the event of occupational exposure, any health risks associated with regular exposure seem unlikely. Humans have long consumed Laminarin in fruits and vegetables with no history of detrimental effects. Moreover, Laminarin has been approved by FDA as a food additive and for use in ointments, suggesting a lack of risks for both the oral and the dermal routes of exposure. And with regard to pesticidal applications, all acute, subchronic, and developmental toxicity data submitted in support of this application for Laminarin confirm its lack of toxicity through all routes of exposure. Because of a lack of likely exposure to

residues and a well established nontoxic profile for Laminarin, no occupational risks are expected with regard to the use of this active ingredient.

b. Residential, School, and Daycare Exposure and Risk Characterization

The Agency does not expect any risks to children (or adults) in any of these environments. Due to the agricultural use pattern of Laminarin, the potential for significant exposure is negligible. Even in the remote event of incidental residue, the active ingredient has a nontoxic profile for all routes of exposure and a long history of consumption without incident. Due to limited exposure scenarios and negligible toxicity hazards, no risks are expected relative to these exposure scenarios.

7. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

The potential for aggregate exposure is expected to be insignificant. Directed Laminarin spray is not expected to result in significant amounts of respirable mist, and is not anticipated to be in non-occupational environments at all. Likewise directed foliar applications, are not likely to result in dermal exposures, especially in non-occupational environments. The chance of significant incidental residues, which could be consumed are also slight. Given a lack of any significant non-occupational exposure, a lack of concern regarding its naturally occurring background levels, and a lack of any acute toxicological endpoints for Laminarin, the aggregate exposure scenario presents no significant concerns for risk.

8. Cumulative Effects

Pursuant to FFDCA section 408(b)(2)(D)(v), EPA has considered available information concerning the cumulative effects of Laminarin residues and other substances that have a common mechanism of toxicity. These considerations include the potential for cumulative effects on infants and children of Laminarin residues and other substances with a common mechanism of toxicity. Because Laminarin has a long history of dietary consumption without incident, and because no toxicological endpoints have been established, the Agency concludes that Laminarin does not share a common mechanism of toxicity, and that there are no cumulative effects arising from Laminarin residues in or on food commodities.

9. Risk Characterization

The Agency considered human exposure to Laminarin in light of the relevant safety factors in FQPA and FIFRA. A determination has been made that no unreasonable adverse effects to the U.S. population in general, and to infants and children in particular, will result from the use of Laminarin when label instructions are followed.

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Hazards (Relative to the Biochemical Pesticides Nontarget Organisms and Environmental Fate Data Requirements - 40 CFR § 158.2060)

Non-target organism and environmental fate data requirements were satisfied by submission of studies. Laminarin is known to occur naturally in the terrestrial and aquatic environment, and is not associated with any known detrimental effect. All information available to the Agency validates a non-toxic mode of action, a lack of persistence in the environment, and a lack of adverse effects relative to non-target organisms.

In accordance with T-REX Model and the non-target data submitted, the Agency has made a "No Effect" (NE) determination for direct and indirect effects to any listed threatened and endangered species and their habitat as a result of the proposed uses of Laminarin.

Avian Testing (OPPTS Guidelines 850.2100, 850.2200; MRID Nos. 47264950 and 47264951): No avian toxicity is expected with regard to the pesticidal use of Laminarin. In an acute oral toxicity study, groups of bobwhites were administered single oral doses ranging up to 2000 mg/kg body weight Laminarin. They were observed for 14 days. There were no mortalities and no signs of adverse effects - all birds appeared healthy during the test, and macroscopic examination revealed no abnormalities in any birds. The acute oral LD_{50} was >2000 mg/kg, the highest dose tested. In a dietary toxicity study on bobwhites, groups of chicks were provided a Laminarin-dosed diet for 5 days, at concentrations ranging up to 5000 ppm per feeding. The diet was maintained for 5 days. There were no treatment-related effects on mortality, body weight, or feed consumption, and no clinical signs of toxicity. The dietary LD_{50} was determined to be >5000 ppm. A lack of toxicological endpoints supports the conclusion that Laminarin is nontoxic to birds.

Aquatic Organism Testing (OPPTS Guidelines 850.1010, 850.1075, 850.5400; MRID Nos. 47264947, 47264948, 47264949, and 47264953): No risks are expected with regard to the exposure of aquatic organisms to Laminarin. Aquatic exposure is unlikely due to the rapid biodegradation and hydrolysis of Laminarin. But in the event of aquatic exposures, no hazards are expected for aquatic organisms. In an acute toxicity test, groups of *Daphnia magna* were exposed to concentrations of Laminarin up to 100 mg/L Laminarin. No daphnid mortality or immobility was seen in any of the test groups after 24 or 48 hours. In this study, the 48-hr NOEC and EC_0 were each ≥100 mg/L, and the LOEC and EC_{50} were >100 mg/L. In an acute toxicity test, groups of zebrafish were exposed to a nominal concentration of 0 or 100 mg/L Laminarin for 96 hours. No mortality or adverse clinical signs were seen at any intervals or in any of the test groups. The 96-hr LC_{50} for Laminarin in Zebra Fish was >100 mg/L. In a second toxicity test, groups of Rainbow Trout fry were exposed to a nominal concentration of 0 or 100 mg/L Laminarin for 96 hours. No mortality or adverse clinical signs were seen in any of the test groups. The 96-hr LC_{50} for Laminarin in Rainbow Trout was >100 mg/L. A 72-hour laboratory

study was conducted to determine the effects of Laminarin (100 mg/L, nominal) on the growth of the unicellular freshwater green algae. An untreated control was also included in the test. At test end, cell growth and density were similar in the test material and control group. The 72-hour EbC_{50} and ErC_{50} for H11 were >100 mg/L, and the NOECb and NOECr were >100 mg/L. The 4 studies altogether confirm a lack of toxicological endpoints, and indicate that Laminarin is nontoxic to aquatic organisms

Non-Target Plant Testing (OPPTS Guidelines 850.4100, 850.4150): The data requirement was satisfied by information demonstrating a lack of hazard to non-target plants relative to the active ingredient's mode of action. The active ingredient is to be directed at agricultural crops; incidental residues would be negligible. To the degree that there is incidental exposure, Laminarin has been shown to have a non-toxic mode of action relative to plants. As a SAR inducer, Laminarin bolsters plant health. Accordingly, Laminarin would actually be expected to have a strengthening effect on non-target plants.

Non-Target Insect Testing (OPPTS Guideline 880.4350, 850.3020); MRID Nos. 47264979 and 47264952): Data indicate that the residues of Laminarin pose no risks of toxicity to non-target insects. In a laboratory study, groups of male and female adult parasitic wasps were exposed for 48 hours to 37 g/L Laminarin sprayed on glass plates at varying rates up to10.0 L/ha. Some issues of loss of fecundity were observed at the highest dose; but none were observed at the doses that were in line with the expected applications of the active ingredient. (The dose at which there was a loss of fecundity was 10x greater than expected residues of Laminarin at the time of pesticidal application.) There was no statistically significant difference in mortality between the treated wasps and the untreated control groups. Limit tests were conducted to determine the acute oral and acute contact toxicity of Laminarin to the Honey Bee. Both tests used a nominal dose of 100.00 µg Laminarin/bee. In the oral toxicity test, groups of caged bees were provided the test material in a 50% w/v sucrose solution for six hours, and then monitored for mortality at intervals up to 48 hours. After 48 hours, there was no difference in mortality of the untreated control and test material groups. In the contact toxicity test, bees were anesthetized with carbon dioxide and received an individual application of Laminarin to the ventral thorax. In this test, the 48-hr oral toxicity LD_{50} for Laminarin was >118.64 µg/bee, and the 48-hr contact toxicity LD_{50} was >100.00 µg/bee. Data indicate that exposures to Laminarin are not expected to result in any adverse effects to non-target insects.

2. Environmental Fate and Ground Water Data

The need for environmental fate and groundwater data was not triggered because results of the acute toxicity assessment did not trigger any additional Tier I studies.

3. Ecological Exposure and Risk Characterization

The use of Laminarin is not expected to result in significant ecological exposures; and to the degree that there are any incidental exposures, all data on file with the Agency demonstrate that

Laminarin is nontoxic to non-target organisms. Laminarin is intended to be applied directly to crops early in the growing season at bi-monthly intervals. Biodegradability data show that it does not persist, and that it hydrolyzes rapidly. (Laminarin is 65-71% biodegraded within two weeks.) When used according to the proposed label directions, no direct exposures are expected for non-target organisms. Laminarin is a naturally occurring carbohydrate. Its presence in the environment has no known toxicological effect on animals or plants. Data submitted to satisfy the non-target organism data requirements confirm Laminarin's lack of ecotoxicity. No adverse effects were observed on plants, insects, mammals, avian species and aquatic organisms; and no toxicological endpoints were identified for any of these organisms. No risks are expected to the environment with regard to the pesticidal use of Laminarin.

4. Threatened and Endangered Species Assessment

Based on the available data, a **No Effects (NE)** determination was made for Laminarin on threatened and endangered species when Laminarin is applied to crops as a SAR inducer. The Agency notes that all non-target organism data indicate no toxicity to non-target organisms. Laminarin has a non-toxic mode of action, which precludes toxic effects on plants. And Laminarin is intended to be applied as an agricultural product; accordingly exposures to threatened and endangered species are expected to be negligible. The Agency used its T-REX Model and its Individual Effects Chance Model Version 1.1, in conjunction with data submitted on non-target organisms to quantifiably estimate the chance of risk to endangered/threatened avian and aquatic species from exposure to Laminarin. These values (avian risk at 1 in 294,000 and aquatic risk at 1 in 418,000,000) suggest that Laminarin should not cause toxic risk to endangered/threatened species. The calculated RQ values for endangered/threatened terrestrial (RQ = 0.00) and aquatic species (RQ = 0.0045) are below the Agency's LOCs of 0.1 and 0.5, respectively, supporting the Agency's finding that exposure to Laminarin will have **No Effect (NE)** on threatened and endangered species.

V. ENVIRONMENTAL JUSTICE

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical, unusually high exposure to Laminarin, compared to the general population. Please comment if you are aware of any sub-populations that may have atypical, unusually high exposure compared to the general population.

VI. RISK MANAGEMENT AND REGISTRATION DECISIONS

A. Determination of Eligibility

Section 3(c)(5) of FIFRA provides for the registration of new active ingredients if it is determined that (A) its composition is such as to warrant the proposed claims for it; (B) its labeling and other materials required to be submitted comply with the requirements of FIFRA; (C) it will perform its intended function without unreasonable adverse effects on the environment; and (D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

The four criteria of the Eligibility Determination for Pesticidal Active Ingredients are satisfied by the science assessments supporting products containing Laminarin. Such products are not expected to cause unreasonable adverse effects, and are likely to provide protection as claimed when used according to label instructions. Therefore, EPA concludes that Laminarin is eligible for registration for the labeled uses.

B. Regulatory Decision

On October 1, 2009, EPA announced a new policy to provide a more meaningful opportunity for the public to participate on major registration decisions before they occur. According to this new policy, EPA intends to provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients; first food use; first outdoor use; first residential use; and other actions for which the Agency anticipates significant public interest. Accordingly, this pesticide was subject to a 30-day comment period as a new active ingredient with both food uses and outdoor uses. No comments were received during that comment period.

At this time, EPA believes, the data submitted fulfill the requirements of registration for products containing Laminarin for use as a SAR inducer. Acute toxicity data for Laminarin demonstrate that it is toxicity category III and IV for all routes of exposure. (No toxicological endpoints were established.) Data confirm that Laminarin does not demonstrate subchronic or developmental toxicity, and it is not mutagenic or genotoxic. EPA has no concerns for any non-target organisms exposed to Laminarin in accordance with its approved uses. EPA has not identified any toxic endpoints for non-target mammals, birds, plants, aquatic, or soil organisms; nor are there concerns for any threatened and endangered species. Given, the non-toxic character of Laminarin, EPA supports its registration under Section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Refer to Appendix B for product-specific information.

1. Conditional/Unconditional Registration

All data requirements are fulfilled and EPA has determined that an unconditional registration for Laminarin is warranted under Section 3(c)(5) of FIFRA.

C. Labeling

Before releasing pesticide products containing Laminarin for shipment, the applicant is required to provide appropriate labels.

VII. ACTIONS REQUIRED BY THE REGISTRANT

The Agency evaluated the data submitted in connection with the initial registration of Laminarin and determined that these data fulfill current registration guideline requirements. No additional data are required to be submitted to the Agency at this time. Additional data may be required for new uses and/or changes to existing uses.

Not withstanding the information stated in the previous paragraph, it should be clearly understood that certain, specific, data are required to be reported to the Agency as a requirement for maintaining the Federal registration for a pesticide product. A brief summary of these types of data are listed below.

A. Reporting of Adverse Effects and Hypersensitivity Incidents

Reports of all incidents of adverse effects to the environment must be submitted to the Agency under the provisions stated in FIFRA, Section 6(a)(2).

Additionally, all incidents of hypersensitivity (including both suspected and confirmed incidents) must be reported to the Agency under the provisions of 40 CFR Part 158.2140 OPPTS Guideline reference number 885.3400.

VIII. GLOSSARY OF ACRONYMS AND ABBREVIATIONS

BPPD	Biopesticides and Pollution Prevention Division
BRAD	Biopesticide Registration Action Document
CFR	Code of Federal Regulations
cm^3	cubic centimeter
CSF	Confidential Statement of Formula
°C	degrees Celsius
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EPA	Environmental Protection Agency (the "Agency")
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act

FR	Federal Register
g	gram
kg	kilogram
L	liter
LD_{50}	median lethal dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, or inhalation). It is expressed as a weight of substance per unit weight of animal (e.g., mg/kg).
MRID No.	Master Record Identification Number
mg	milligram
mL	milliliter
MP	manufacturing-use product
MPCA	microbial pest control agent
NE	"No Effect"
NIOSH	National Institute for Occupational Safety and Health
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
PCR	polymerase chain reaction
PPE	personal protective equipment
TGAI	technical grade of the active ingredient

IX. BIBLIOGRAPHY STUDIES SUBMITTED IN SUPPORT OF THIS REGISTRATION

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47391100	Laboratoires Goemar SA (2008) Submission of Product Chemistry and Toxicity Data in Support of the Application for Registration of VacciPlant and the Petition for Tolerance of Laminarin for Use on Food Crops.	02-Apr-2008
47391101	Smith, F. (2008) Product Identity and Composition, Description of Beginning Materials and Description of Formulation Process: (VacciPlant). Unpublished study prepared by SciReg, Inc. 52 p.	02-Apr-2008
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B. EPA Risk Assessment Memoranda

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APPENDIX A – BIOCHEMICAL PESTICIDE DATA REQUIREMENTS

TABLE 1. Physical/Chemical Properties for Laminarin

TABLE 1.0 Physical and Chemical Properties for pure TGAI Laminarin[a]			
Guideline Reference No.	**Property**	**Description of Result**	**Methods**
830.6302	Color	White	MRID 47264908
830.6303	Physical State	Powder	MRID 47264908
830.6304	Odor	Low odor	MRID 47264908
830.6313	Stability	Stable after 14 days at 54°C in the presence of aluminum acetate or aluminum	MRID 47264909
830.6314	Oxidation/Reduction: Chemical Incompatibility	No oxidizing properties	MRID 47264911
830.6315	Flammability	None flammable; neither development nor ignition of gas were observed after contact with water. An exothermic reaction was observed at 236°C±3°C (mean value). No self-ignition temperature was recorded up to 420°C.	MRID 47264912 MRID 47264913 MRID 47264914
830.6316	Explodability	Not explosive	MRID 47264915, checking heat, friction, and shock sensitivity
830.6317	Storage Stability	Not required for TGAI	
830.6319	Miscibility	Not applicable, product is not an emulsifiable liquid and will not be diluted with petroleum solvents.	
830.6320	Corrosion Characteristics	Not required for TGAI	
830.6321	Dielectric Breakdown Voltage	Not required for TGAI	

TABLE 1.0 Physical and Chemical Properties for pure TGAI Laminarin[a]			
Guideline Reference No.	**Property**	**Description of Result**	**Methods**
830.7000	pH	6.25±0.02 at 23.2°C (1% w/v)	MRID 47264920, by pH meter and a glass electrode
830.7100	Viscosity	Not required for TGAI	
830.7200	Melting Range	No melting point could be determined. The test material became yellow at 204-215C, then it turned brown at 216-225.2C. At about 310.6-316.2C, the test material was completely retracted and blacked colored. The test material probably degraded during the test.	MRID 47264916, by using an Electrothermal 8103 apparatus
830.7220	Boiling Range	The test material is a powder.	
830.7300	Relative Density	D_4^{20} = 1.515±0.04 - 1.502±0.06	MRID 47264917 and 47264918, by pycnometric method
830.7370	Dissociation Constant in Water	Can not be determined.	
830.7550	Partition Coefficient	Log P = -1.6	MRID 47264928, by shake flask method
830.7840	Water Solubility	> 88.6 g/L at 20°C; < 10 mg/L (n-heptane); < 10 mg/L at 20°C (xylene, 1,2-dichloroethane, and ethyl acetate); 60 mg/L (methanol); 21 mg/L at 20°C (acetone)	MRID 47264919 MRID 47264921 MRID 47264922 MRID 47264923 MRID 47264924 MRID 47264925 MRID 47264926 by flask method
830.7950	Vapor Pressure	< 2.6 x 10^{-5} Pa at 25°C	MRID 47264927, using a vapor pressure balance system

TABLE 2. Toxicity Data Requirements Summary

Table 2. 0 Toxicological Results/ Category			
Guideline # Test	**Results/Toxicology Category**	**MRID**	**Study Conclusion**
870.1100 Acute Oral	LD_{50}>2,000 mg/kg III	47264930 47264943	Acceptable
870.1200 Acute Dermal	LD_{50}>5,000 mg/kg IV	47264931 47264974	Acceptable
870.1300	> 1.02 mg/L	47264932	Acceptable

Table 2. 0 Toxicological Results/ Category			
Guideline # Test	**Results/Toxicology Category**	**MRID**	**Study Conclusion**
Acute Inhalation	III		
870.2400 Primary Eye Irritation	Non irritating IV	47264933 47264976	Acceptable
870.2500 Primary Dermal Irritation-Rabbits	Non- irritating IV	47264934	Acceptable
870.2600 Dermal Sensitization	Not a sensitizer IV	47264935 47264978	Acceptable
Acute Subcutaneous	LD_{50}>1,000 mg/kg	47264936	Acceptable
870.3050 28 day Oral Toxicity-Rat	NOEL=1,000 mg/kg/day	47264937	Acceptable
870.3100 90 day Oral Toxicity-Rat	NOEL=1,000 mg/kg/day	47264938	Acceptable
870.3150 Subchronic Oral Toxicity (gavage) - Dog	NOEL=1,000 mg/kg/day	47264939	Acceptable
870.3700a Prenatal Developmental Toxicity Study - Rat	Maternal NOEL ≥ 1,000 mg/kg/day Developmental NOEL > 1,000 mg/kg/day	47264940	Acceptable
870.3700b Prenatal Developmental Toxicity Study - Rabbit	Maternal LOAEL = 1,000 mg/kg/day Developmental LOAEL = 1,000 mg/kg/day	47264941	Acceptable
870.5100 Bacterial Reverse Mutation Test	There was no evidence of induced mutant colonies over background	47264942	Acceptable
870.5300 *In Vitro* Mammalian Cells in Culture Gene Mutation Assay	There was no evidence of induced mutant colonies over background.	47264943	Acceptable
870.5395 Bone Marrow Micronucleus assay in mouse	No toxicity was noted in either sex at any dose up to the limit dose of 2000 mg/kg bw	47264944	Acceptable
870.7800 Immunotoxicity	**Waiver Request**	47264945	Acceptable

TABLE 3. EcoToxicity Data Requirements Summary

Table 3.0 Ecotoxicity Data			
Guideline # Test	**Results/Toxicology Category**	**MRID**	**Study Conclusion**
850.1010	EC_{50} >100 mg/L.	47264947	Acceptable

Table 3.0 Ecotoxicity Data

Guideline # Test	Results/Toxicology Category	MRID	Study Conclusion
Acute Toxicity Test, Daphnids			
850.1075 Acute Toxicity Freshwater Fish *Danio rerio*	96 hr LC_{50} >100 mg/L.	47264948	Acceptable
850.1075 Fish Acute Freshwater Rainbow Trout *Oncorhynchus mykiss*	96-hr LC_{50} >100 mg/L.	47264949	Acceptable
850.2100 Avian Acute Oral Toxicity Bobwhite (*Colinus virginianus*)	LD_{50} >2000 mg/kg	47264950	Acceptable
850.2200 Avian Dietary Toxicity Bobwhite (*Colinus virginianus*)	LC_{50} >5000 ppm	47264951	Acceptable
850.3020 Acute Contact Toxicity Honey bee (*Apis mellifera)*	LD_{50} >118.64 µg/bee 48-hr contact LD_{50} >100.00 µg/bee.	47264952	Acceptable
850.5400 Algal Toxicity, Tiers I and II Green alga *Selenastrum capricornutum.*	E_bC_{50}, E_rC_{50}, NOECb, and NOECr for the test material at 24, 48, and 72 hours were each >100 mg/L	47264953	Acceptable
835.3110 Biodegradability	Biodegradation in the reference material and toxicity controls was 71% and 65%, respectively, after 14 days. Laminarin was concluded to be readily biodegradable under the test conditions.	47264954	Acceptable
880.4350 Nontarget Insect Testing	Exposure to 10.0 L/ha of the test material did significantly lower the fecundity of *A. rhopalosiphi* females compared to the untreated control. However, the product label for Vacciplant recommends an application rate of 9.7 to 14.4 oz/A, which the reviewer calculates to be equivalent to 0.7 to 1.05 L/ha, well below the 10.0 L/ha rate at which fecundity was affected.	47264979	Acceptable

DATA EVALUATION RECORD

LAMINARIN
(PHYCARINE® 96S51)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT (870.1100)
MRID 47264930

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
Susan Chang, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Oral Toxicity - Rats (OPPTS 870.1100)
MRID NO:	47264930
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	Phycarine® 96S51 (EPA Reg. No. 83941-R)
PROJECT NO:	20010618 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Acute Toxicity Study, Safety Test in the Rat by the Oral Route
AUTHOR:	L. Baudet
STUDY COMPLETED:	January 9, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The oral LD_{50} for male, female, and combined rats was greater than 2000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material**: Phycarine® 96S51

2. **Test Animals:** Ten male and ten female Sprague-Dawley (SPF) rats were received from IFFA CREDO specialized breeding establishment (Domaine des Oncins, St Germain Sur L'arbresle, BP 0109, 69592 L'arbresle cedex, France and weighed 146.7-167.2 g (males) and 118.8-130.0 g (females) on the day of dosing. The young adult animals, 6-7 weeks old, were housed (5/sex/group) in standard size cages. The animals were fed UAR A 04C10 foodstuff (Usine d'Alimentation Rationelle -7, rue Gallieni – Villemoisson – 91360 Epinay-Sur-Orge – France). Filtered tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19-23°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rats were identified by ear clip: Males: Nos. 972350 to 972354 (control group) and 972355 to 972359 (test group) and Females: Nos. 972360 to 972364 (control group) and 972365 to 972369 (test group) and were acclimated for six days and fasted overnight prior to dosing. The test material (2000 mg/kg body weight) as a suspension in water was dosed by gavage (Table 1). The control animals received water (10 mL/kg) under the same condition as treated animals. Body weight was recorded day of randomization, prior to dosing, and on days 3, 7, and 14. The test animals were observed for mortality and clinical signs of toxicity during the first several hours post-dosing and at least daily for 14 days. All animals were necropsied.

II. RESULTS:

1. **Mortality:** All rats survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
0	0/5	0/5	0/10
2000	0/5	0/5	0/10

Data taken from p. 14, MRID 47264930.

2. **Body Weight:** All rats gained weight throughout the study.

3. **Clinical Observations:** No clinical signs were noted from any animal throughout the study.

4. **Gross Necropsy:** No macroscopic organ or tissue abnormality was noted at necropsy.

III. DISCUSSION:

The oral LD_{50} for male, female, and combined rats was greater than 2000 mg/kg. This places Phycarine® 96S51 in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN
(H11, BATCH NO. 99S24)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT (870.1200)
MRID 47264931

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
Susan Chang, M.S. Signature: _____
Date: _____

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Dermal Toxicity - Rats (OPPTS 870.1200)
MRID NO:	47264931
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	H11, Batch No. 99S24
PROJECT NO:	20000698 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Acute Dermal Toxicity Study in the Rat
AUTHOR:	Caudeval Gerard
STUDY COMPLETED:	February 8, 2001
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The dermal LD_{50} for males, females, and combined was greater than 5000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material:** H11, Batch No. 99S24 (Identification β1-3 glucan from laminaria digitata; purity 90%).

2. **Test Animals:** Five male and five female Sprague-Dawley (SPF) rats were received from Centre d'Elevage Depre (Z.I. Malitorne – B.P. 70 -3, rue Joliot-Curie – 18230 Saint Doulchard - France were assigned, and weighed 182.4-192.1 g (males) and 161.3-169.9 g (females) on the day of treatment. The young adult animals, over 6 weeks old, were housed (5/sex/group) in standard size cages. The animals were fed UAR A 04-C10 Food product (Usine d'Alimentation Rationelle -7, rue Gallieni – Villemoisson – 91360 Epinay-Sur-Orge – France) and tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19-23°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rats were identified by tattooing: Male – Nos. 200001277 to 200001281; Female – Nos. 200001282 to 1286. The rats were acclimated for six days. The test material (5000 mg/kg body weight) in water was applied on a piece of absorbent gauze (6 cm x 5 cm) and placed on an area of the clipped dorsal trunk (at least 10% of the total body surface) and secured with an elastic band. The coverings were removed after 24 hours and excess test material removed. The test animals were observed during the first several hours after treatment for mortality, signs of gross toxicity, and behavior changes and at least once daily thereafter for 14 days. The rats were weighed prior to treatment and on days 7 and 14. The rats were euthanized on day 14 and necropsied.

II. RESULTS:

1. **Mortality:** All rats survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
5000	0/5	0/5	0/10

Data taken from p. 15, MRID 47264931.

2. **Clinical Observations:** No clinical signs or dermal reactions were noted from any animal during the study.

3. **Body Weight:** All rats gained weight during the study.

4. **Gross Necropsy:** No macroscopic organ or tissue abnormality was noted at necropsy.

III. DISCUSSION:

The acute dermal LD_{50} for males, females, and combined was greater than 5000 mg/kg. This places H11, Batch No. 99S24 in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN
(PHYCARINE® 96S51)

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT (870.2400)
MRID 47264933

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
Susan Chang, M.S. Signature: _____
Date: _____

Secondary Reviewers:
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Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Eye Irritation - Rabbits (OPPTS 870.2400)
MRID NO:	47264933
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	Phycarine® 96S51
PROJECT NO:	20010615 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Ocular Primary Irritation in the Rabbit
AUTHOR:	L. Baudet
STUDY COMPLETED:	January 9, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	No corneal opacity or iritis was noted on any rabbit during the study. Positive conjunctival irritation (score 2) was noted on 2/3 rabbits one hour after test material instillation with clearance by 24 hours. The maximum average score was 4.7 at one hour after test material instillation. Phycarine® 96S51 was minimally irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material:** Phycarine® 96S51

2. **Test Animals:** Three male or three female young adult New Zealand White rabbits were received from CEGAV specialized breeding establishment (Les Hautes Noës, Saint Mars d'Egrenne, 61350 Passais Conception, France). The animals were kept in cages of standard size. The animals were fed UAR 112 foodstuff (Usine d'Alimentation Rationelle -7, rue Gallieni – Villemoisson – 91360 Epinay-Sur-Orge – France). Tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 17-21°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rabbits were identified by an ear clip: Nos. 970376, 970377, and 970368. The rabbits were acclimated for at least five days. The test material (0.1 g/eye/animal) was applied in the conjunctival sac of the left eye, and the eye held closed for approximately 10 seconds. The right eye served as control. The eyes were examined and scored 1, 24, 48 and 72 hours after test material instillation.

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Ocular Lesions:** No corneal opacity or iritis was noted on any rabbit during the study. Slight to moderate chemosis was noted on 3/3 rabbits one hour after test material instillation with clearance by 24 hours. A slight redness was noted on 2/3 rabbits by 1-24 hours after test material instillation with clearance by 48 hours. Positive conjunctival irritation (score 2) was noted on 2/3 rabbits one hour after test material instillation with clearance by 24 hours (Table 1). The maximum average score was 4.7 at one hour after test material instillation (Table 2).

TABLE 1. Summary of Eye Irritation Scores with Time: Conjunctiva and Iris

Score Conditions	1 hour	24 hours	48 hours	72 hours
Conjunctiva				
Erythema	0 to 1	0 to 1	0	0
Chemosis	1 to 2	0	0	0
Discharge	-	-	-	-
Iris	0	0	0	0

Irritation score is based on Draize Method

Scale for Scoring Ocular Lesions

Scale for Scoring Ocular Lesions

Conjunctival Lesions:

Chemosis (swelling) of the eyelids and/or nictating membranes:

- No swelling 0
- Any swelling above normal (including of the nictitating membrane) 1
- Obvious swelling with partial eversion of lids 2
- Swelling with lids about half-closed 3
- Swelling with lids more than half-closed 4

Redness:

- Blood vessels normal 0
- Marked hyperemia of certain blood vessels (eye injected) 1
- Diffuse purple coloration, difficulty making out blood vessels individually 2
- Diffuse sustained red coloration 3

Iris Lesions:

- Normal 0

Markedly more folded than normal, congestion, hyperemia, moderate pericorneal change or swelling, any one of these signs or any combination of several of them, iris still reacting to light (one slow reaction is positive) 1

No reaction to light, hemorrhage, extensive destruction (one or more of these characteristics) 2

Cornea Lesions:

Taking into account only the zone with the most severe lesions.
The grading of the surface area of opacity is mentioned as an exponent of the degree of opacification on the result data sheet.

Degree of opacification:

- Neither ulceration, nor opacity 0

Areas opacity (other than slight tarnishing of normal gleam) focal or diffuse, details of iris clearly visible 1

- Presence of easily identifiable translucent zone, details of iris slightly masked 2
- Presence of opalescent zone, no details of iris visible, outline of pupil scarcely visible 3
- Presence of total corneal opacity rendering iris invisible 4

Surface area of opacity:

- A quarter (or less but not nil) 1
- Between quarter and half 2
- Between half and three-quarters 3
- From three-quarters to the whole surface 4

TABLE 2. Summary of Total[a] and Primary Eye Irritation Scores with Time				
Animal #	1 h	24 h	48 h	72 h
270376	6	2	0	0
270377	6	2	0	0
270368	2	0	0	0
Average scores[b]	4.7	1.3	0.0	0.0

[a]Formula: Total Irritation Score = I + II + III, where,
I = Corneal Score = [Density (A) x Area (B)] x 5
II = Iris Score = Severity x 5
III = Conjunctival Score = [Erythema (A) + Chemosis (B) + Discharge (C)] x 2
[b]Average Primary Irritation = Sum of Total Irritation Scores ÷ 3

III. DISCUSSION:

No corneal opacity or iritis was noted on any rabbit during the study. Positive conjunctival irritation (score 2) was noted on 2/3 rabbits one hour after test material instillation with clearance by 24 hours. The maximum average score was 4.7 at 24 hours after test material instillation. Phycarine® 96S51 was minimally irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN
(PHYCARINE® 96S51)

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT (870.2500)
MRID 47264934

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
Susan Chang, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Primary Dermal Irritation - Rabbits (OPPTS 870.2500)
MRID NO:	47264934
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	Phycarine® 96S51
PROJECT NO:	20010617 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Cutaneous Primary Irritation in the Rabbit
AUTHOR:	L. Baudet
STUDY COMPLETED:	January 9, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Very slight erythema was noted on 1/3 rabbits one through 72 hours after patch removal with clearance by day 8. The primary irritation index was 0.3. Phycarine® 96S51 was slightly irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material:** Phycarine® 96S51

2. **Test Animals:** Three male young adult New Zealand White rabbits were received from CEGAV specialized breeding establishment (Les Hautes Noës, Saint Mars d'Egrenne, 61350 Passais Conception, France). The animals were kept in cages of standard size. The animals were fed UAR 112 foodstuff. Tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 17-21°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rabbits were identified by an ear clip: Nos. 970367, 970388, and 970389. The rabbits were acclimated for at least five days. The fur on the flank of each rabbit was clipped on the day prior to treatment. The rabbits were treated with 0.5 g of test material moistened with 0.5 mL water applied on a gauze square of approximately 6 cm^2 and placed on the clipped intact dose site. The gauze was covered with a semi-occlusive non-allergenic dressing and secured with elastic tape. The covering was removed 4 hours later and the site rinsed using waterlogged compress to remove any coloration from the test material. Dermal examination was recorded at 1, 24, 48, and 72 hours and on day 8 after removal of the patch.

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Dermal responses:** Very slight erythema was noted on 1/3 rabbits one through 72 hours after patch removal with clearance by day 8. The primary irritation index was 0.3.

Irritation Scores:

TABLE 1. Summary of individual rabbit's dermal irritation scores with time

	Hours				Days
Animal Nos.	1	24	48	72	8
970367	0/0[a]	0/0	0/0	0/0	0/0
970388	0/0	0/0	0/0	0/0	0/0
970389	1/0	1/0	1/0	1/0	0/0

Data taken from p. 15, MRID 47264934.

[a]Erythema/Edema

Description of score system:

Erythema and eschar formation:

No erythema 0
Very slight erythema (scarcely visible) 1
Clearly visible erythema 2
Moderate to marked erythema 3
Severe erythema (redish purple) or eschar formation (deep lesions) preventing the grading of the erythema 4

Formation of Edema:

No edema 0

Very slight edema (scarcely visible) 1
Slight edema (contours well defined, visible swelling) 2
Moderate edema (thickness approximately 1 mm) 3
Severe edema (thickness more than 1 mm and area greater than that of the application) 4

III. DISCUSSION:

Very slight erythema was noted on 1/3 rabbits one through 72 hours after patch removal with clearance by day 8. The primary irritation index was 0.3. Phycarine® 96S51was slightly irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN
(PHYCARINE® 96S51)

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG (870.2600)
MRID 47264935

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
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Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Skin Sensitization - Guinea Pigs (OPPTS 870.2600)
MRID NO:	47264935
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	Phycarine® 96S51
PROJECT NO:	20010616 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Study of Cutaneous Sensitization Using the Magnusson and Kligman Maximization Test in the Guinea Pig
AUTHOR:	L. Baudet
STUDY COMPLETED:	January 9, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	After intradermal injection and epicutaneous inductions, the test and negative control animals showed no positive signs of reactivity at 24 and 48 hours after challenge. The study included a DNCB positive control study which was carried out within six months of the study and the results were appropriate. Phycarine® 96S51 was not a dermal sensitizer.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN:

1. **Test material:** Phycarine® 96S51

2. **Test animals:** Twenty-one male and 21 female Hartley guinea pigs received from Charles River specialized breeding station, 76410 Saint Aubin les Elbeuf, France were assigned to groups and weighed 331-377 g (males for main study) and 305-352 g (females for main study) at experiment start. The young adult animals were housed in group of five per sex in cages of standard size. The animals were fed UAR 106 special Guinea Pig foodstuff. Tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 17-21°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Male and Female guinea pigs were marked with picric acid tagging: Preliminary irritation testing – Nos. not reported; Negative Control – Nos. 971712 to 971716 (males) and Nos. 971767 to 971771 (females); Test: Nos. 971717 to 971726 (males) and Nos. 971772 to 971781 (females). The guinea pigs were acclimated for at least 7 days. The animals were induced and challenged according to the Magnusson and Kligman Maximization Test. The back, shoulder, or flanks of 20 test guinea pigs and 10 negative control animals were clipped prior to each treatment. Two males and two females were used to determine the maximum concentration causing slight to moderate irritation by intradermal injection (for primary induction), two males and two females were used to determine the maximum concentration causing a slight to moderate irritation by epicutaneous application (for topical induction); and two males and two females were used to determine the maximum non-irritant concentration by epicutaneous application (for challenge).

   Three pairs of intradermal injections (0.1 mL/site) were made into a 4 cm x 6 cm clipped area of skin of the guinea pigs on day 1. The injectables were Freund's complete adjuvant (diluted with equal volume of sterile and pyrogen-free isotonic sodium chloride solution, 25% test material in water, and 25% test material in a 1:1 emulsion of Freund's complete adjuvant with sterile saline. On day 8, the same region was clipped and pretreated with a 10% mixture of sodium lauryl sulfate in paraffin. On day 9, 0.5 mL of 25% test material in water, absorbed onto a piece of absorbent gauze (8 cm^2), was applied to the intradermal injection area under semi-occlusion for 48 hours. The vehicle control animals were treated similarly to the test animals with the exception that the test material was omitted from the intradermal injections and topical application. On day 22, the animals were topically challenged with 0.5 mL of 25% test material in water at naive sites for 24 hours. The sites were evaluated 24 and 48 hours post exposure.

II. RESULTS:

1. **Mortality:** All animals survived the study.

2. **Body Weight:** All animals gained weight during the study.

3. **Skin Effects:** Dermal reactions after intradermal injection induction and epicutaneous application induction were not reported. No irritation reaction was noted on any animal after challenge.

TABLE 1. Summary of Individual Erythema Challenge Scores with Time *								
	24 hours				48 hours			
Erythema Score	0	1	2	3	0	1	2	3
Treated	20	0	0	0	20	0	0	0
Negative Control	10	0	0	0	10	0	0	0

*Number of animals affected
Evaluation score is based on Buehler Grading Scale.

Scale for Scoring Skin Reaction

Buehler sensitization scoring scale

Erythema	Score
No visible modification	0
Slight erythema or patches	1
Moderate and confluent erythema	2
Intense erythema and tumefaction	3

III. DISCUSSION:

After intradermal injection and epicutaneous inductions, the test and negative control animals showed no positive signs of reactivity at 24 and 48 hours after challenge. The study included a DNCB positive control study which was carried out within six months of the study and the results were appropriate. Phycarine® 96S51 was not a dermal sensitizer. The packet is classified as **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN
(PHYCARINE® 96S51)

STUDY TYPE: ACUTE SUBCUTANEOUS TOXICITY - RAT
MRID 47264936

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
Susan Chang, M.S. Signature: _____
Date: _____

Secondary Reviewers:
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Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Subcutaneous Toxicity – Rats
MRID NO:	47264936
DP BARCODE NO:	DP 352311
CASE NO:	Not reported
DECISION NO:	385527
TEST MATERIAL:	Phycarine® 96S51
PROJECT NO:	970353 ST
SPONSOR:	Laboratories GOËMAR S.A., Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	CERB, Chemin de Montifault, 18800 Baugy, France
TITLE OF REPORT:	Acute Toxicity Study Safety Test in the Rat by the Subcutaneous Route
AUTHOR:	M. Delille
STUDY COMPLETED:	March 17, 1998
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The subcutaneous LD_{50} for males, females, and combined was greater than 1000 mg/kg.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN:

1. **Test Material:** Phycarine® 96S51

2. **Test Animals:** Ten male and ten female Sprague-Dawley (SPF) rats were received from IFFA CREDO specialized breeding establishment (Domaine des Oncins, St Germain Sur L'arbresle, BP 0109, 69592 L'arbresle cedex, France and weighed 170.1-179.6 g (males) and 130.9-139.2 g (females) on the day of dosing. The young adult animals, 6-7 weeks old, were housed (5/sex/group) in standard size cages. The animals were fed UAR A 04C10 foodstuff. Filtered tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 19-23°C; relative humidity, 45-65%; air changes, 10 per hour; and photoperiod, 12 hour light/dark.

3. **Methods:** Rats were identified by tattooing: Male – Nos. 972370 to 972374 (control group) and 972375 to 972379 (test group) and Females: Nos. 972380 to 972384 (control group) and 972385 to 972389 (test group). The rats were acclimated for six days. After over night fasting, the test material (1000 mg/kg body weight) as a suspension in water was injected subcutaneously into the animals. The control animals were injected subcutaneously with water (5 mL/kg). The test animals were observed during the first several hours after treatment and at least once daily thereafter for 14 days. Examination of the injection site was made daily. The rats were weighed prior to treatment and on days 7 and 14. The rats were euthanized on day 15 and necropsied.

II. RESULTS:

1. **Mortality:** All rats survived the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
0	0/5	0/5	0/10
1000	0/5	0/5	0/10

Data taken from p. 14, MRID 47264936.

2. **Clinical Observations:** No clinical signs were noted from any animal during the study. No abnormalities were observed at the injection site of any animal.

3. **Body Weight:** All rats gained weight during the study.

4. **Gross Necropsy:** No macroscopic organ or tissue abnormality was noted at necropsy.

III. DISCUSSION:

The acute subcutaneous LD_{50} for males, females, and combined was greater than 1000 mg/kg. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

LAMINARIN (4489-1; PRODUCT H 11; VACCIPLANT
OPPTS 870.3050
STUDY TYPE: FOUR WEEK ORAL TOXICITY - RAT

MRID 47264937

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

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Kimberly Slusher, M.S. Signature: _____
Date: _____

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EPA Reviewer: _____ **Signature:** _____

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity (Feeding) - Rat
OPPTS 870.33050 (rodent); OECD 407

PC CODE: 123200 **DP BARCODE:** 352311

TEST MATERIAL (PURITY): 4489-1 (Product H 11; purity >95%)

SYNONYMS: Vacciplant, *Laminaria digitata* powder (Kelp powder); Laminarin; β-1-3 Glucan

CITATION: Nunziata, A. (2000) 4489-4 (product H11); 4-week oral toxicity study in rats. Research Toxicology Centre – Rome, Via Tito Speri, 12, 00040 Rome, Italy. Report No. 7286/T/240/99. January 19, 2000. MRID 47264937. Unpublished.

SPONSOR: Biopredic, 14-18 rue Jean Pecker, 35000 Rennes, France

EXECUTIVE SUMMARY:

In a repeat oral toxicity study, (MRID 47264937) 4489-1 (product H 11, purity ≥95%, Batch No. 99S21) was administered by daily gavage to groups of five Sprague Dawley rats/sex/dose at concentrations of 0 or 1000 mg/kg bw/day for 28 days.

No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, organ weight, or gross or microscopic pathology were found. **The NOAEL for 4489-1 (product H 11) for male and female Sprague Dawley rats was 1000 mg/kg bw/day. A LOAEL was not identified.**

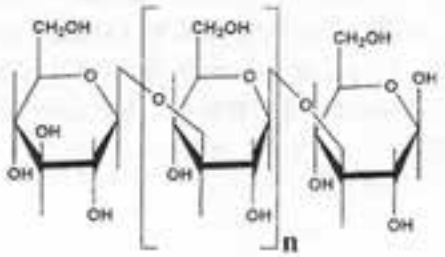
This 28-day repeat oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 28-day repeat oral toxicity study (OPPTS 870.3050; OECD 407) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** 4489-1 (product H 11)

Description:	White/beige powder
Lot/batch #:	99S21
Purity:	≥95%
Compound stability:	Not reported
CAS # of TGAI:	9008-22-4
Structure:	

2. **Vehicle:** 0.5% aqueous carboxymethylcellulose

3. **Test animals:**

Species:	Rat	
Strain:	Sprague Dawley	
Age/weight at study initiation:	51-53 days	
Source:	Harlan Nossan S.r.l., Correzzana (MI), Italy	
Housing:	five/sex/dose in polycarbonate cages	
Diet:	Altromin MT Pelleted diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2°C
	Humidity:	55 ± 10%
	Air changes:	15-20/hr
	Photoperiod:	12 hrs light/dark
Acclimation period:	24 days	

B. STUDY DESIGN:

1. **In life dates:** Start: August 9, 1999; End: September 6, 1999

2. **Animal assignment:** Animals were assigned to the groups in Table 1 by computer stratified randomization.

Table 1. Study design

Test group	Dose (mg/kg bw/day)	# Male	# Female
Control	0	5	5
High	1000	5	5

3. **Dose selection rationale:** Dose selection was the responsibility of the Sponsor. No other rationale was provided. The limit dose was tested.

4. **Dose preparation:** All rats were treated by gavage with the vehicle (control) or 1000 mg/kg bw/day 4489-1 daily for four weeks. The dosing solution was prepared daily and analyzed for concentration during weeks 1 and 4. The animals were gavaged at a volume of 10 mL/kg based on the most recent body weight.

   Results: Dose concentration, 24-hour stability, and homogeneity studies on solutions prepared during weeks 1 and 4 were done by the Sponsor and were not included in the Study report.

5. **Statistics:** Bartlett's test was done on all continuous data to determine homogeneity. Parametric data were analyzed by ANOVA followed by Dunnett's test. Nonparametric data were analyzed by a Modified t test. Because only one treatment group was used in the study, the reviewer suggests that Student t tests for equal or non-equal variances were more appropriate for analysis than ANOVA tests.

C. METHODS:

1. **Observations:**

   1a. **Cage-side observations:** Cage-side observations were done daily before dosing, immediately after dosing, and approximately one to two hours after dosing.

   1b. **Clinical examinations:** Detailed clinical examinations were given to each animal before treatment and once a week during treatment.

   1c. **Neurological evaluations:** During the detailed weekly clinical examinations, the animals were observed for absence of clonic movements, tremors, convulsions, repetitive mouth/jaw movement, myoclonic spasms, wet-dog shakes, absence of tonic movements, limb contractions, opisthotonus, emprosthotonus, jumps, dyspnea, ataxia, hunched posture, pronation, fore-limb drag, and hind-limb drag. The motor activity of each rat was measured during week 4 by an automatic recording device.

2. **Body weight:** Animals were weighed before treatment and weekly thereafter.

3. **Food consumption and compound intake:** The weight of food consumed by each cage of rats was recorded weekly and the average daily food intake/rat calculated.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were not done.

5. **Hematology and clinical chemistry:** Blood was drawn from the retro-orbital sinus of all rats into tubes containing EDTA (hematology), heparin (clinical chemistry), or citrate (coagulation studies) during week 4. The study report does not state whether the animals were fasting. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 28-day repeat dose oral rodent studies based on Guideline 870.3050

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES* (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 28-day repeat oral rodent studies based on Guideline 870.3050

6. **Urinalysis:** Urinalysis was not done.

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected and examined microscopically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM
	Tongue
	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
	Pancreas*
X	**RESPIRATORY**
X	Trachea*
X	Lung*
	Nose*
	Pharynx*
	Larynx*

X	CARDIOVASC./HEMAT.
	Aorta*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes (cervical & mesenteric)*
XX	Spleen*+
XX	Thymus*+
X	**UROGENITAL**
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
X	Uterus*+
	Mammary gland*
X	Cervix
X	Vagina

X	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
	Pituitary*
	Eyes (optic nerve)*
X	**GLANDULAR**
XX	Adrenal gland*+
	Lacrimal gland
	Parathyroid*
X	Thyroid*
X	**OTHER**
	Bone (sternum and/or femur)
	Skeletal muscle
	Skin*
X	All gross lesions and masses*

* Recommended for 28-day repeat oral rodent studies based on Guideline 870.3050
\+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

2. **Mortality:** All rats survived until scheduled sacrifice.

3. **Neurological evaluations:** No neurological effects were observed. Neurotoxicity and motor activity measurements did not show treatment-related changes.

B. BODY WEIGHT AND WEIGHT GAIN:

No treatment-related effects on body weight were found (Table 2).

TABLE 2. Body weight (g) and body weight gain (g) of male and female rats treated with 4489-1 (product H 11) for 28 days.

Group	Day				Body weight gain[a] (g)
	1	8	15	29	
Males					
Control	211.51 ± 6.02	289.97 ± 6.39	317.02 ± 5.32	349.01 ± 7.37	88.34 ± 12.81
1000 mg/kg bw/day	208.73 ± 11.27	299.23 ± 18.66	328.96 ± 21.57	362.94 ± 21.94	101.86 ± 9.61
Females					
Control	183.13 ± 6.48	197.34 ± 2.73	210.03 ± 4.63	231.33 ± 3.61	48.14 ± 7.61
1000 mg/kg bw/day	181.67 ± 7.54	197.77 ± 6.61	210.46 ± 13.61	228.74 ± 12.33	47.08 ± 5.46

Data from pages 48, 49, 105, and 106 of MRID 47264937
[a]Calculated by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No treatment-related effects were found.

2. **Compound intake:** The average daily intake of the test material is shown in Table 1.

D. BLOOD ANALYSES:

1. **Hematology:** No treatment-related effects were found. The RBC count of treated females was 3% greater than control but was well within historical limits and not of biological or toxicological concern.

2. **Clinical chemistry:** No treatment-related effects were found. The glucose was slightly decreased 7% and the potassium of treated male rats was slightly increased 6%, but both were well within control limits and not of biological or toxicological concern.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No treatment-related effects were found

2. **Gross pathology:** No treatment-related effects were found.

3. **Microscopic pathology:** No treatment-related effects were found.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that no toxicologically significant findings were observed in any of the parameters investigated and suggested a NOAEL of 1000 mg/kg bw/day for male and female rats.

B. REVIEWER COMMENTS:

In this study, male and female rats were treated by gavage with 0 or 1000 mg/kg bw/day 4489-1 (product H 11) for 28 days. No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, organ weight, or gross or microscopic pathology were found. The NOAEL for 4489-1 (product H 11) for male and female Sprague Dawley rats was 1000 mg/kg bw/day.

C. STUDY DEFICIENCIES:

No significant deficiencies that would affect interpretation of the study results were found.

DATA EVALUATION RECORD

LAMINARIN (H11, VACCIPLANT)
OPPTS 870.3100
STUDY TYPE: 90-DAY ORAL TOXICITY

MRID 47264938

Prepared for

Biopesticide and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
H.T. Borges, Ph.D., MT(ASCP), D.A.B.T. Signature: _____
Date: _____

Secondary Reviewers:
R.A. Young, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Kimberly Slusher, M.S. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: _____ **Signature:** _____

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity (Gavage) - Rat;
OPPTS 870.3100 (rodent); OECD 408

PC CODE: 123200 **DP BARCODE:** 352311

TEST MATERIAL (PURITY): H11 (purity 90%)

SYNONYMS: Vacciplant, *Laminaria digitata* powder (Kelp powder); Laminarin; β-1-3 Glucan

CITATION: Gerard, C.A. (2001) 90-day repeated dose oral toxicity study in the rat. Centre De Recherches Biologiques. Chemin De Montifault, 1880 Baugy, France. Report No. 20000389 T/90D.TOX.RAT/H11. March 16, 2001. MRID 47264938. Unpublished.

SPONSOR: Laboratoires GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 47264938), H11 (95% a.i., Batch No. 99S24) was administered daily to groups of 10 Sprague Dawley rats/sex at concentrations of 0 or 1000 mg/kg bw/day by gavage.

No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, organ weight, or gross or microscopic pathology were found. **The NOAEL for H11 in male and female Sprague Dawley rats was 1000 mg/kg bw/day. A LOAEL was not identified.**

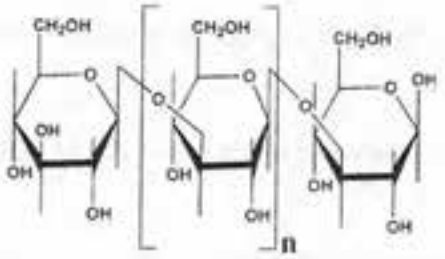
This 90-day subchronic toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day subchronic toxicity study (OPPTS 870.3100; OECD 408) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** H11

Description:	Light beige powder
Lot/batch #:	99S24
Purity:	90% a.i.
Compound stability:	June 1, 2003
CAS # of TGAI:	9008-22-4
Structure:	

2. **Vehicle:** Water

3. **Test animals:**

Species:	Rat
Strain:	Sprague Dawley
Age/weight at study initiation:	6 – 9 weeks; Males 142.3 – 162.2 g; Females 118.1 – 147.1 g
Source:	Charles River Laboratories, IFFA Credo, Domaine des Oncins, St. Germain sur L'Arbresle, BP 0109, 69592 L'Arbresle Cedex, France
Housing:	Groups of five/sex/dose
Diet:	UAR A04-C10 Food Product, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Environmental conditions:	**Temperature:** 19-23°C **Humidity:** 45-65% **Air changes:** 10/hr **Photoperiod:** 12 hours light/dark
Acclimation period:	≥5 days

B. STUDY DESIGN:

1. **In life dates:** Start: July 28, 2000; End: October 26, 2000

2. **Animal assignment:** Animals were randomly assigned based on body weight to the test groups noted in Table 1.

TABLE 1. Study design

Test group	Dose to animal (mg/kg bw/day	# Male	# Female
Control	0	10	10
High	1000	10	10

One additional rat/sex/group was added at the beginning of the study in case of gavage error death

3. **Dose selection rationale:** The dose was selected by the study Sponsor. The test material was reported free of toxic effect, so a limit dose of 1000 mg/kg bw/day was chosen.

4. **Dose preparation and analysis:** Dose solutions were prepared daily. Samples of solutions prepared during the first week and the last week were retained and frozen for future analysis. The dosing volume was 5 mL/kg based on the most recent body weight.

Results:

Homogeneity analysis: No data on dose homogeneity was included in the study report.

Stability analysis: Doses were prepared daily, however no data on dose stability were included in the study report.

Concentration analysis: No data on the concentration of dose solutions were found in the study report.

5. **Statistics:** Incidence data were analyzed by Fisher's test. Body weights, clinical chemistry, and hematology results were analyzed for each sex by two-way ANOVA for repeated measurements taking time and treatment into consideration. Continuous clinical pathology results were analyzed by one-way ANOVA. If one result from a data set was missing, an estimate based on the group mean was used. If more than one result was missing from a data set, the animal was excluded from statistical calculation. Daily clinical results, food and water consumption, ophthalmology, urinalysis and macroscopic results at necropsy were not statistically analyzed.

C. **METHODS:**

1. **Observations:**

   1a. **Cageside observations:** The rats were examined daily approximately one hour after dosing for clinical signs of toxicity and twice daily for moribundity and mortality.

   1b. **Clinical examinations:** Detailed clinical examinations were done weekly.

   1c. **Neurological evaluations:** During the 13th week of the study, the rats were individually observed for awareness, mood, motor activity, motor in-coordination, excitation, muscle tone, body posture, and reflexes according to the method described by Irwin (1968 Comprehensive observational assessment: 1A. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 13:222-257).

2. **Body weight:** All rats were weighed weekly.

3. **Food and water consumption:** Food and water consumption of all rats was determined weekly for each cage.

4. **Ophthalmoscopic examination:** Ophthalmologic examinations were done on all rats pre-treatment and on the day of necropsy. The external ocular adnexa and the anterior segment were examined macroscopically. If no abnormalities were present, the pupils were dilated with 0.5% tropicamide and photographs of the optic fundus and abnormalities taken.

5. **Hematology and clinical chemistry:** Blood samples were collected from all rats following an overnight fast prior to necropsy from the retro-orbital sinus into tubes containing EDTA (hematology), heparin (clinical chemistry), or citrate (coagulation studies). Blood samples collected pretreatment for hematological analyses were from unfasted animals. The report does not state whether samples for clinical chemistry analyses done pretreatment were from fasted animals. The CHECKED (X) parameters were examined at both intervals. Clinical chemistry parameters marked with an "A" were measured at necropsy only.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
A	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
A	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	A	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	A	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
A	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis*:** Urine was collected pretreatment and prior to necropsy from animals fasted for 16 hours. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)	X	Nitrate
X	Protein*	X	Urobilinogen

* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected and examined microscopically from all animals. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+	X	GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	OTHER
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
X	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Harderian Gland
X	Lung*	X	Mammary gland*		
	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were noted.

2. **Mortality:** No treatment-related deaths occurred.

3. **Neurological evaluations:** Reduced spontaneous locomotor activity was noted from one high-dose male rat and a decrease in grid-gripping performance was noted from one control female. Neither of these affects was attributed to treatment. No treatment-related neurobehavioral effects were noted.

B. BODY WEIGHT AND WEIGHT GAIN:

No treatment-related effects were found on body weight or body weight gain (Table 2).

TABLE 2. Average body weight and body weight gain during 90 days of treatment

Dose (mg/kg bw/day)	Body weights (g ± SD)				Total weight gain	
	Day -1	Day 8	Day 49	Day 90	g	% difference from control
Male						
0	150.8 ± 2.7	206.5 ± 14.4	414.8 ± 23.3	483.0 ± 33.4	332.2	
High	150.8 ± 6.1	212.6 ± 10.1	432.7 ± 44.1	498.4 ± 45.1	347.6	4.6
Female						
0	132.2 ± 9.8	168.1 ± 11.3	262.0 ± 23.2	282.7 ± 29.5	150.5	
High	130.3 ± 4.7	167.2 ± 8.2	264.9 ± 14.8	284.5 ± 15.6	154.2	2.5

Data from pages 29 and 31 of MRID 47264938.

C. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No treatment-related effects on food consumption were found.

2. **Compound intake:** Compound consumption is in Table 1.

3. **Water Consumption:** No treatment-related effect was noted for male rats. The water consumption of female rats treated with 1000 mg/kg bw/day was 10-19% lower than control females from week 2 to week 13.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related effects were found.

E. BLOOD ANALYSES:

1. **Hematology:** No treatment-related effects were found in measured WBC, RBC, or coagulation parameters.

2. **Clinical chemistry:** With the exception of the calcium concentration of treated females, no treatment-related effects were found. The plasma calcium was statistically increased 5%, but the increase was not of biological or toxicological concern.

F. URINALYSIS:

No treatment-related effects were found.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No significant treatment-related effects were noted. The absolute (1.088 g control, 1.188 g high-dose) and relative to body weight (0.414% control, 0.452% high-dose)

heart weights were increased ~10% in high-dose female rats. No histological correlates were identified. The increase was not of biological or toxicological concern.

2. **Gross pathology:** No significant treatment-related effects were noted at necropsy.

3. **Microscopic pathology:** No significant treatment-related effects were found.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded 1000 mg/kg bw/day H11 did not induce treatment-related effects in rats.

B. REVIEWER COMMENTS:

In this study, male and female rats were treated by gavage with 0 or 1000 mg/kg bw/day H11 for 90 days. No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, organ weight, or gross or microscopic pathology were found. The NOAEL for H11 for male and female Sprague Dawley rats was 1000 mg/kg bw/day.

C. STUDY DEFICIENCIES:

No deficiencies that would affect interpretation of the study results were found. Concentration, stability, and homogeneity studies of the dosing solutions were not done.

DATA EVALUATION RECORD

LAMINARIN (H11; VACCIPLANT)
OPPTS 870.3150
STUDY TYPE: SUBCHRONIC ORAL TOXICITY - DOG
MRID 47264939

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 08-025

Primary Reviewer:
H.T. Borges, Ph.D., MT(ASCP), D.A.B.T. Signature: _____
Date: _____

Secondary Reviewers:
R.A. Young, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Kimberly Slusher, M.S. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: _____ **Signature:** _____

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity (gavage) - Dog; OPPTS 870.3150 (non-rodent); OECD 409

PC CODE: 123200 **DP BARCODE:** 352311

TEST MATERIAL (PURITY): H11 (purity 90%)

SYNONYMS: Vacciplant, *Laminaria digitata* powder (Kelp powder); Laminarin; β-1-3 Glucan

CITATION: Gerard, C.A. (2001) 90-day repeated dose oral toxicity study in the dog. Centre De Recherches Biologiques. Chemin De Montifault, 18800 Baugy, France. Report No. 20000390 T/90D.TOX.DOG/H11. March 16, 2001. MRID 47264939. Unpublished.

SPONSOR: Lavoratoires GOËMAR S.A., Z.A.C. La Madeleine – BP55, 35413 Saint Malo Cedex, France

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 47264938), H11 (95% a.i., Batch No. 99S24) was administered daily to groups of four Beagle dogs/sex at concentrations of 0 or 1000 mg/kg bw/day by gavage.

No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, organ weight, or gross or microscopic pathology were found. **The NOAEL for H11 in male and female Beagle dogs was 1000 mg/kg bw/day. A LOAEL was not identified.**

This 90-day subchronic toxicity study in the dog is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day subchronic toxicity study (OPPTS 870.3150; OECD 409) in the dog.

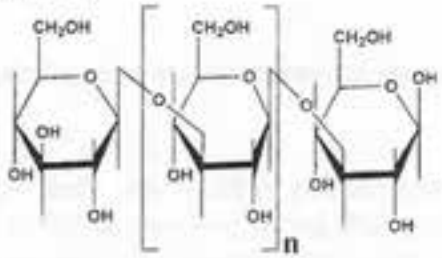
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: H11

Description: Light beige powder
Lot/batch #: 99S24
Purity: 90% a.i.
Compound stability: January 1, 2003
CAS # of TGAI: 9008-22-4
Structure:



2. Vehicle: Water

3. Test animals:

Species: Dog
Strain: Beagle
Age/weight at study initiation: ~6-9 months. Males: – 10.6: 13.05 kg; Females: 8.9 – 11.945 kg
Source: CEDES – domaine des Souches, Mezilles, 89130 Toucy, France
Housing: Individually in standard cages
Diet: UAR 125 C2 Food Product, 400 g/day
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 17-21°C
Humidity: 45-65%
Air changes: ~10/hr
Photoperiod: 12 hours light/dark
Acclimation period: 8-12 days

B. STUDY DESIGN:

1. In life dates: Start: August 2, 2000; End: November 2, 2000

2. Animal assignment: Animals were randomly assigned based on body weight to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Dose to animal (mg/kg bw/day)	# Male	# Female
Control	0	4	4
High	1000	4	4

3. Dose selection rationale: The dose was selected by the study sponsor. The test material was reported free of toxic effect, so a limit dose of 1000 mg/kg bw/day was chosen.

4. **Dose preparation and analysis:** Dose solutions were prepared daily. Samples of solutions prepared during the first week were retained and frozen for future analysis. The dosing volume was 5 mL/kg based on the most recent body weight.

Results:
Homogeneity analysis: No data on dose homogeneity was included in the study report.

Stability analysis: Doses were prepared daily, however data on dose stability were not included in the study report.

Concentration analysis: No data on the concentration of dose solutions were found in the study report.

5. **Statistics:** Incidence data were analyzed by Fisher's test. Body weights, clinical chemistry, and hematology were analyzed for each sex by two-way ANOVA for repeated measurements taking time and treatment into consideration. Continuous clinical pathology results were analyzed by one-way ANOVA. If one result from a data set was missing, an estimate based on the group mean was used. If more than one result was missing from a data set, the animal was excluded from statistical calculation. Daily clinical results, food and water consumption, ophthalmology, urinalysis and macroscopic results at necropsy were not statistically analyzed.

C. **METHODS:**

1. **Observations:**

   1a. **Cageside observations:** The dogs were examined daily approximately one hour after dosing for clinical signs of toxicity and twice daily for moribundity and mortality.

   1b. **Clinical examinations:** Detailed clinical examinations were done weekly.

   1c. **Neurological evaluations:** During the 13th week of the study, the rats were individually observed for awareness, mood, motor activity, motor in-coordination, excitation, muscle tone, body posture, and reflexes according to the method described by Irwin (1968. Comprehensive observational assessment: 1A. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 13:222-257).

2. **Body weight:** The dogs were weighed on the day of randomization, just prior to the first treatment, and weekly thereafter.

3. **Food and water consumption:** Food and water consumption were measured daily.

4. **Ophthalmoscopic examination:** Ophthalmologic examinations were done on all dogs pre-treatment and on the day of necropsy. The external ocular adnexa and the anterior segment

were examined macroscopically. If no abnormalities were present, the pupils were dilated with 0.5% tropicamide and photographs of the optic fundus and abnormalities taken.

5. **Hematology and clinical chemistry:** Blood samples were collected before treatment, once monthly during treatment, and on the day of necropsy from fasted animals into tubes containing EDTA (hematology), heparin (clinical chemistry), or citrate (coagulation studies) from all animals. The CHECKED (X) parameters were examined.

a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. **Clinical chemistry:**

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg.,*)	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine amino-transferase (also SGPT)*		
X	Aspartate amino-transferase (also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

6. **Urinalysis:** Urine samples were collected for 16 hours before treatment, once monthly during treatment, and on the day of necropsy from fasted animals. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
	Sediment (microscopic)	X	Nitrate
X	Protein*	X	Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected and examined microscopically from all animals. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall bladder*+
X	Pancreas*

X	RESPIRATORY
X	Trachea*
X	Lung*
	Nose*
	Pharynx*
	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta thoracic*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+

X	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland*
X	Vagina

X	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*

X	GLANDULAR
XX	Adrenal gland*+
X	Lacrymal gland
XX	Parathyroid*+
XX	Thyroid*+

X	OTHER
X	Bone (femur/sternum)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
+ Organ weight required for non-rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Diarrhea and/or soft stools were noted in control and treated male and female dogs; however the incidence was slightly increased in treated animals. No other treatment-related effects were noted.

2. **Mortality:** One control female was sacrificed on Day 31 of the study. This dog presented with epileptic convulsions which progressed from spaced to frequent with the dog eventually becoming unconscious. Another female dog was used to replace the animal. All other dogs survived until scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN:

No treatment-related effects were found.

TABLE 2. Average body weight and body weight gain during 90 days of treatment

Dose (mg/kg bw/day)	Body weights (kg ± SD)				Total weight gain	
	Day -2	Day 7	Day 49	Day 90	kg	% difference from control
Male						
0	12.16 ± 0.80	11.98 ± 0.76	13.23 ± 0.62	14.11 ± 0.52	2.05	
1000	12.25 ± 1.05	12.04 ± 1.06	13.19 ± 0.96	14.09 ± 1.17	1.84	-10
Female						
0	10.02 ± 1.05	10.07 ± 0.98	10.60 ± 1.58	11.78 ± 2.07	1.76	
1000	10.39 ± 1.17	10.42 ± 1.21	11.55 ± 1.27	12.47 ± 1.61	2.08	18

Data from pages 28 and 30 of MRID 47264939.

C. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No treatment-related effects were found.

2. **Compound intake:** Compound consumption is in Table 1.

3. **Water consumption:** No treatment-related effects were found.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related effects were found.

E. BLOOD ANALYSES:

1. **Hematology:** After two months of treatment, the hemoglobin, hematocrit and RBC count of treated female dogs were increased 8-11%; however, the increases were slight, the results well within the historical control range of the laboratory, and were not of biological or toxicological concern. No other treatment-related effects were noted in hematology or coagulation parameters of male and female dogs.

2. **Clinical chemistry:** No treatment-related effects were found.

F. URINALYSIS:

No treatment-related effects were found.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The absolute and relative adrenal weight of high-dose male dogs was statistically increased ~11% relative to control male dogs. In addition, the absolute and relative spleen weight of high-dose male dogs were statistically decreased ~33%. Since no microscopic correlates were found for either parameter and because the differences were slight, they were not considered of biological or toxicological concern. No other treatment-related effects were found in male or female dogs.

2. **Gross pathology:** No treatment-related effects were found.

3. **Microscopic pathology:** No treatment-related effects were found.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded 1000 mg/kg bw/day H11 did not induce treatment-related effects in dogs.

B. REVIEWER COMMENTS:

In this study, male and female Beagle dogs were treated by gavage with 0 or 1000 mg/kg bw/day H11 for 90 days. No treatment related mortality or effects on clinical signs of toxicity, neurotoxicity, hematology, clinical chemistry, body weight, body weight gain, absolute or relative organ weight, or gross and microscopic pathology were found. The H11 NOAEL for male and female Beagle dogs was 1000 mg/kg bw/day.

C. STUDY DEFICIENCIES:

No deficiencies that would affect interpretation of the study results were found. Concentration, stability, and homogeneity studies of the dosing solutions were not done.

DATA EVALUATION RECORD

LAMINARIN
OPPTS 870.300a; OECD 414
STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY- RATS
MRID No. 47264940

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
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Oak Ridge, TN 37831
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Date: _____

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

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EPA Reviewer: _____ **Date:** _____

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a ['83-3a]; OECD 414.

DP BARCODE: 352311

TEST MATERIAL (PURITY): H11 (Laminarin, 90%, a.i.; Batch No. 99S24; light beige powder)

SYNONYMS: Vacciplant; ß 1-3 glucan from *Laminaria digitate*

CITATION: Gerard, C. A. (2001) H11 (batch 99S24): Study for the effects on embryo-foetal development in the rat by the oral route. CERB, France, Laboratory Project ID No. 20000387 T, March 16, 2001.MRID 47264940. Unpublished.

SPONSOR: Laboratoires GOËMAR S.A., France

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 47264940), H11 (90% a.i.; Batch No. 99S24) was administered to 25 pregnant SPF Sprague-Dawley rats/dose by oral gavage as a solution in sterile water at dose levels of 0 or 1000 mg/kg bw/day on gestation day (GD) 6 through 17. Dams were sacrificed on GD 20 and examined grossly. The following parameters were measured: number of corpora lutea, weight of the uterus, and the number and location of the implantation sites, fetuses and resorptions (early and late). The following parameters were measured on the fetuses: number of dead and alive, fetal body weight, caudo-cranial measurements, gross evaluation and weight of the placenta, external morphological examination and sexing. One-half of the fetuses were fixed in BOUIN's fluid before Wilson's section technique for visceral examination and the other half were placed in 95% alcohol for processing for skeletal examination.

All females survived to the scheduled sacrifice and there were no clinical signs observed. Treatment did not affect body weight, body weight gain, food and water consumption or cesarean parameters measured.

The maternal NOAEL in female rats administered H11 orally from GD 6-17 was ≥ 1000 mg/kg and the LOAEL could not be determined.

There were no treatment-related effects on the number of live/dead fetuses recorded, individual fetal body weight, caudo-cranial measurement, gross evaluation and weight of placenta, and on the external, skeletal and visceral examination.

The developmental NOAEL in rats administered H11 orally was ≥ 1000 mg/kg and the LOAEL could not be determined.

The developmental toxicity study in the rat is classified as **UNACCEPTABLE/GUIDELINE (upgradable)** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat. Although only one dose was administered, this is acceptable as there were no effects and the limit dose was used. The study could be upgraded to acceptable if information as to the concentration, stability and homogeneity were added.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** H11

Description:	Light beige hygroscopic powder; pH 6.8; soluble in water
Lot/batch #:	Batch No. 99S24
Purity:	90% a.i.
Compound stability:	Not provided; report stated was responsibility of the Sponsor
CAS #:	9008-22-4
Structure:	No structure available

2. **Vehicle and/or positive control:** Sterile water was used as the vehicle in the treated animals and was administered alone to the control animals.

3. **Test animals:**

Species:	Female rats	
Strain:	SPF Sprague-Dawley	
Age/weight at study initiation:	8-11 weeks old on day of mating; body weight: 182.8 to 281.0 g	
Source:	Charles River Laboratories, France	
Housing:	Rats were housed individually in standard size cages on dust-free irradiated white wood shavings	
Diet:	SDS: RM3 (E) SQC IR, *ad libitum*	
Water:	Filtered tap water, *ad libitum*, in polycarbonate bottles	
Environmental conditions:	**Temperature:**	19-23EC
	Humidity:	45-65%
	Air changes:	10 times/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	5 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: August 1, 2000; End: August 23, 2000

2. **Mating:** Females were mated in the morning and inspected about 11:00 am for the presence of a vaginal plug. The day the vaginal plug was identified was designated as gestation day (GD) 0. Information in regard to the males used for mating was not provided.

3. **Animal assignment:** Animals were assigned randomly by body weight to dose groups as indicated in Table 1 on GD 5.

TABLE 1. Animal assignment in rats dosed with H11 by oral gavage[a]

Group	No. of pregnant females	Dose (mg/kg bwt)	Volume administered
1	25	0	5 mL/kg
2	25	1000	5 mL/kg

[a] Data from p. 14 in MRID 47264940.

4. **Dose selection rationale:** The dose levels were selected in agreement with the Sponsor as the test substance was supposed to be free of any toxic or sub-toxic effect. Therefore, a limit test was performed.

5. **Dosage preparation and analysis:** Test material-vehicle solutions were prepared daily by mixing appropriate amounts of test substance with sterile water. Storage of the test material was at room temperature. Controls were administered sterile water only. Sampling of the solutions was performed on a day drawn at random before the start of the study, on GD 6 and during the study (between GD 7-17). After preparation of the solution, one sample of ~ 5 mL was taken at room temperature before gavage and placed into a labeled and dated amber colored glass bottle and stored at – 20°C for possible subsequent analysis. No further details were provided.

Results:

Homogeneity analysis: No information was provided.
Stability analysis: No information was provided.
Concentration analysis: No information was provided.

6. **Dosage administration:** All doses were administered once daily by oral gavage, on gestation days 6 through 17, in a volume of 5 mL/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. **OBSERVATIONS:**

1. **Maternal observations and evaluations:** The animals were checked for mortality twice daily. Animals were examined for clinical signs at least once daily at the time when any possible effects would be most likely observed (approximately one hour after dosing). Body weight was recorded on the day the rats were received at the laboratory, the day of randomization, the first day of treatment and on treatment days 9, 13, 17 and 20 (day of necropsy). Food consumption data were measured before the treatment began (GD 1-5), during treatment (GD 6-17) and after the treatment period (GD 18-19) by weighing the amount of food remaining in the bowls. Water consumption was also measured by daily measurements of the amount of residual water in bottles. Dams were sacrificed on GD 20 (one day prior to parturition). Examinations at sacrifice consisted of: gross examination of all major organs, a count of the corpora lutea on the ovaries, weight of the uterus (fetuses intact), number and location of implantation sites, number and location of fetuses and number and location of resorptions (early and late).

2. **Fetal evaluations:** All fetuses from control and treated females were examined. On the day of sacrifice (day 20) or delivery on GD 17 or later, the following fetal parameters were recorded: number of live/dead fetuses recorded, individual fetal body weight, caudo-cranial measurement, gross evaluation and weight of placenta, external morphological examination and sexing. Then, half of the total number of fetuses was fixed in BOUIN's fluid prior to WILSON's section technique and the other half was fixed in 95% alcohol for skeletal examination (alizarin stain). Each fetus was placed in its own collection bottle and identified by CERB study number, mother number, fetus number, and the type of fixative it was in. Data

were recorded and reported as either fetal incidence or litter incidence (Indices used are included in Section D.2).

D. DATA ANALYSIS:

1. **Statistical analyses:** Body weight changes and water consumption were analyzed by two-way analysis of variance. If they were statistically significant, the mean of the dosed group was compared to the controls by DUNNETT's test. Food consumption results were analyzed by one-way analysis of variance at each individual time period. The number of deliveries classified as abortions or as GD 20 sacrifices was compared with that of the control group using the Chi^2 test. The distribution of the implantation sites was classified by: normal live and dead fetuses, abnormal live and dead fetuses and resorptions (early and late) in all dose groups and the control group. If possible, the treated and control groups were compared using a Chi^2 test. One-way analysis of variance was also applied to the following: mean number of copora lutea, number of implantation sites, number of live fetuses, number of dead fetuses, number of resorptions and mean percentage of reproductive indices per group and per female and the mean weight of the uterus of the females. If these were statistically significant, then DUNNETT's test was used to compare the dosed group with the control. Statistical significance was established at $p < 0.05$.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

$$\text{Pre-implantation loss (\%)} = \frac{\text{Number of corpora lutea – number of implantations}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{Number of implantations – number of live fetuses}}{\text{Number of implantations}} \times 100$$

$$\text{\% fetal incidence} = \frac{\text{Number of fetuses in group with findings}}{\text{Total number of fetuses in group}} \times 100$$

$$\text{\% litter incidence} = \frac{\text{Number of litters in group containing fetuses with findings}}{\text{Total number of litters in group}} \times 100$$

3. **Historical control data:** Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** All dams survived the study, and no clinical signs were observed.

2. **Body weight:** Body weight data are summarized in Table 2. There was no treatment-related effect on body weight during the study. The only effect on body weight gain was a transient

decrease (15%) in the treatment group females pre-treatment (GD 1-6). By GD 9, these females were similar to controls and remained similar to controls for the remainder of the study. Mean gravid uterine weight was similar between the treated and control females.

TABLE 2. Mean maternal body weight (g ∀ SD) and body weight gain (g) [a]

Interval	Control group (n = 23)	1000 mg/kg (n= 21)
Body weight		
GD 1	220.4 ± 20.9	223.9 ± 18.7
GD 6	255.4 ± 23.3	253.5 ± 23.1
GD 9	274.4 ± 24.4	275.5 ± 23.3
GD 13	295.6 ± 27.7	298.1 ± 25.4
GD 17	336.8 ± 31.3	337.2 ± 31.9
GD 20	375.3 ± 34.1	373.6 ± 35.6
Body weight gain [b]		
GD 1-6	35	29.6 (↓15%)
GD 6-9	19	22
GD 9-13	21.2	22.6
GD 13-20	79.7	75.5
GD 1-20	154.9	149.7

[a] Data obtained from Table 1, p. 22 in MRID 47264940.
[b] Body wt gain calculated by reviewer.

3. **Food and water consumption:** There were no treatment-related effects on food or water consumption.

4. **Gross pathology**: There were no treatment-related effects observed on gross pathology. Mean uterine weight in the control females was 75.43 g ± 14.20 g and in the treated females, 73.07 ± 16.08 g, indicating no significant difference.

5. **Cesarean section data:** Data are summarized in Table 3. There were no treatment-related differences observed in the 1000 mg/kg females when compared to the controls in any of the parameters measured.

TABLE 3. Cesarean section observations [a]

Observation	Control group	1000 mg/kg
# Animals assigned (mated)	25	25
# Animals pregnant	23	21
Pregnancy rate (%)	92%	84%
# Non-pregnant	2	4
Maternal wastage		
No. died	0	0
No. aborted	0	0
No. premature delivery	0	0
Total no. corpora lutea Corpora lutea/dam	400 17.4 ± 5.6	355 16.9 ± 3.6
Total no. implantations Implantations/dam	311 13.5 ± 2.6	276 13.1 ± 2.9
Total no. litters	23	21

TABLE 3. Cesarean section observations [a]		
Total no. live fetuses	290	254
Live fetuses/dam	12.6 ± 2.6	12.1 ± 2.8
Total no. dead fetuses	0	0
Total no. resorptions	21	22
Early	20	21
Late	1	1
Resorptons/dam	0.9	1.0
Early	0.9 ± 0.9	1.0 ± 1.2
Late	0.0 ± 0.2	0.0 ± 0.2
Litters with total resorptions	0	0
Mean fetal weight (g)	3.94 ± 0.27	3.95 ± 0.27
Males	not provided	not provided
Females	not provided	not provided
Sex ratio (% male) [b]	46%	53%
Mean caudo-cranial measurements (mm)	36.5 ± 1.4	36.8 ± 1.5
Mean placenta weight (g)	0.60 ± 0.07	0.60 ± 0.08
Pre-implantation loss (%)	19.0 ± 14.9	21.4 ± 17.7
Post-implantation loss (%)	6.9 ± 6.2	7.6 ± 8.4

[a] Data obtained from Tables 4-6, pages 21, 28-30 and 66-67, in MRID 47264940.

[b] The only data provided on male/female sex ratio was obtained from Table 1A, p. 91, in the study report and it was the ratio observed in the BOUIN fixed rats (total of 140 controls and 123 treated fetuses examined), and may not be a true representation of the entire fetal population.

B. DEVELOPMENTAL TOXICITY:

The number of fetuses examined for skeletal parameters was 150 and 131 in the control and treated groups, respectively. The number of fetuses examined for soft-tissue abnormalities was 140 and 123 in the control and treated groups, respectively.

1. **External examination**: The only findings on external examination were hematomas found on some of the control and treated fetuses but they were associated with the caesarean section technique, were observed in the same incidence in the control and treated fetuses and were not treatment-related.

2. **Visceral examination:** Any visceral abnormalities noted were observed at the same incidence in both the control and treated fetuses, indicating they were not treatment-related. Some of the most common findings are provided in Table 4a.

3. **Skeletal examination:** Any skeletal abnormalities noted were observed at the same incidence in both the control and treated fetuses, indicating they were not treatment-related. Some of the most common findings are provided in Table 4b.

TABLE 4a. Visceral examinations [a]		
Observation	Control group	1000 mg/kg
No. fetuses (litters) examined	140 (23)[b]	123 (21)
No. fetuses (litters) affected		
Hepatic hemorrhages	17 (10)	16 (8)
Localized internal abdomen hemorrhage	14 (8)	13 (9)
Hemorrhagic abdomen	8 (8)	11 (8)
Unilateral hyroureter	16 (8)	12 (9)
Subcutaneous hemorrhage	0 (0)	1 (1)
Subcutaneous edema	2 (2)	5 (4)

[a] Data obtained from Tables 1A and 1B on p. 91-92 in MRID 47264940.
[b] Fetal (litter) incidence

TABLE 4b. Skeletal examinations [a]		
Observation	Control group	1000 mg/kg
No. fetuses (litters) examined	150 (23)[b]	131 (21)
No. fetuses (litters) affected		
Incomplete ossification of parietals	12 (5)	10 (7)
Incomplete ossification of interparietals	32 (3)	35 (16)
Incomplete ossification of hyoid	15 (9)	13 (8)
Plaque of bone in cranial suture	0 (0)	4 (4)
Anomalous thoracic vertebral centrum(a)	29 (12)	29 (14)
6 lumbar vertebrae	150 (23)	130 (21)
13th rib rudimentary	1 (1)	0 (0)
14th rib rudimentary	8 (7)	5 (3)
1 incompletely ossified sternebrae	100 (23)	83 (20)
Small fetus with generalized reduction in ossification	0 (0)	2 (1)

[a] Data obtained from Tables 2A, 2B, 3A and 3B on p. 93-98 in MRID 47264940.
[b] Fetal (litter) incidence

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

The study author concluded that administration of H11 by oral gavage to pregnant female rats during gestation days 6-17 did not cause any maternal or developmental effects.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Treatment with 1000 mg/kg of H11 had no significant or toxicological effects on the dams. The only effect observed was a transient decrease in body weight gain occurring before treatment began.

   The maternal NOAEL in female rats administered H11 orally from GD 6-17 was ≥ 1000 mg/kg and the LOAEL could not be determined.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment with H11 at a dose of 1000 mg/kg did not result in an increased incidence of fetal death or early or late resorptions.

   b. **Altered growth:** No treatment-related effects were observed on fetal weight, ossification rates or caudo-cranial length. The only information lacking was individual fetal weight presented by gender.

   c. **Developmental variations:** The incidence of developmental variations was not significantly different in the treated rats when compared to controls.

   d. **Malformations:** There were no treatment-related effects on external, visceral or skeletal examination as the incidences of malformations were similar between the treated and control group.

The developmental NOAEL in rats administered H11 orally was ≥ 1000 mg/kg and the LOAEL could not be determined.

C. STUDY DEFICIENCIES:

Complete information in regard to the number of male and female fetuses and the mean body weight for each sex were not provided in the study report. Gender ratios for the BOUIN-fixed rats were provided and indicated a normal ratio of males/females between the control and treated rats but this may not be truly representative of the entire fetal population. This information does not appear to affect the integrity of the study but should be added if available.

No data were provided on the homogeneity, concentration or stability of the solutions administered. Although samples were obtained, they did not appear to have been analyzed. This lack of data is a major deficiency and needs to be added if available from the Sponsor/test laboratory.

DATA EVALUATION RECORD

LAMINARIAN
OPPTS 870.3700b
STUDY TYPE: PRENATAL DEVELOPMENTAL STUDY (RABBITS)
MRID 47264941

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task #: 08-025

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Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

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EPA Reviewer: _____ **Signature:** _____

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b ['83-3b]; OECD 414.

PC CODE: Not provided **DP BARCODE:** 352311

TEST MATERIAL (PURITY): H 11 (Laminarin) (90% a.i.)

SYNONYMS: Vacciplant

CITATION: Gerard, C.A. (2001) Study for the effects on embryo-foetal development in the rabbit by the oral route. CERB, Chemin de Montifault, 18800 BAUGY, France. Laboratory project ID no. 20000388 T, October 5, 2001. MRID 47264941. Unpublished.

SPONSOR: Laboratories GOËMAR S.A., Z.A.C. La Madeleine-BP55, 35413 Saint Malo Cedex, France

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 4726941) Laminarin (H 11; 90% a.i., batch # 99S24) was administered to 19 New Zealand White rabbits/dose by gavage at dose levels of 0 or 1000 mg/kg bw/day from days 6 through 15 of gestation. Does were sacrificed on day 29 of gestation, examined grossly, and the uterus, complete with ovaries, was removed and the uterine horns were examined. The following data were collected: count of corpora lutea on ovaries; weight of uterus before extraction of fetuses; number and uterine location of implantation sites; number and uterine location of fetuses; and number and uterine location of resorptions (early and late). The fetuses were examined macroscopically and the following data were recorded: number of live or dead fetuses; individual fetal weight (live and dead fetuses); caudo-cranial measurement of live and dead fetuses; gross evaluation and weight of placenta of all fetuses; external morphological examination of fetuses; soft tissue examination of all fetuses after sacrifice by subcutaneous injection of sodium pentobarbital; and sexing of all fetuses at external examination. Following examination of soft tissues, all fetuses were eviscerated. The heads were removed for approximately half of the fetuses in each litter and were fixed in Bouin's fluid for subsequent examination following freehand serial sectioning. The torsos and the remaining intact fetuses were skinned and fixed in 90% or 95% alcohol and processed for skeletal examination using the alizarin method.

All females survived to study termination. No treatment-related absolute body weight differences were observed. Mean body weight gain during the treatment period (GD 6 – GD 19) was decreased (78% lower than control group) at 1000 mg/kg bw/day. During the treatment period,

food consumption of treated females was significantly lower than control females (15% lower than control group). Water consumption was not affected by treatment. No treatment-related effects on macroscopic postmortem findings or Caesarean section data were observed.

The maternal toxicity LOAEL for Laminarin (H 11) in rabbits is 1000 mg/kg bw/day, based on decreased body weight gain during the treatment period. The maternal NOAEL is not established.

No treatment-related effects on fetal length and weight or on external fetal examinations were observed. An increase in the number of fetuses with supernumerary ribs at the thoraco-lumbar border and the number of fetuses with supernumerary ribs with an additional (8^{th}) lumbar vertebra was observed in the treated group. Comparison of the treated group with historical control values from the testing laboratory in the same strain of rabbit during the same time period showed similar values. Therefore, the skeletal findings are not considered treatment-related.

The developmental toxicity LOAEL for Laminarin (H 11) in rabbits is not established. The developmental NOAEL is 1000 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified **Unacceptable (not upgradable)/Guideline** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in the rabbit. An insufficient number of animals was used.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** H11 (Laminarin)

Description:	Light beige powder
Lot/batch #:	99S24
Purity:	90% a.i.
Compound stability:	Expiration date: January 1, 2003
CAS #of TGAI:	9008-22-4
Structure:	Not available

2. **Vehicle and/or positive control:** Sterile water

3. **Test animals:**

Species:	Rabbit
Strain:	Albino SPF (Specific Pathogen Free) New-Zealand White
Age/weight at study initiation:	4 months on day of mating/approx. 3.5 kg on first day of treatment
Source:	Charles River Laboratories, Chatillon Sur Chalaronne, France
Housing:	"individually in standard size cages"
Diet:	SDS:STANRAB (P) SQC IR *ad libitum*
Water:	Tap water *ad libitum*

Environmental conditions:	**Temperature:**	17-21EC
	Humidity:	45-65%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: November 13, 2000; End: December 15. 2000

2. **Mating:** The animals were mated at the animal provider and were delivered to the testing laboratory on gestation day (GD) 1. The day of mating was designated as GD 0.

3. **Animal Assignment:** Animals were randomized into the control and treated groups on the basis of body weight, as indicated in Table 1. The study was conducted in five parts with the start of the experimental phase ranging over a five-day period.

TABLE 1: Animal Assignment

Dose (mg/kg bw/day)	0	1000
Number of Females	19	19
Volume administered	5 mL/kg	5 mL/kg

4. **Dose selection rationale:** The testing was conducted at the limit dose of 1000 mg/kg bw/day.

5. **Dosage preparation and analysis:** The test material-vehicle mixture was prepared daily by mixing appropriate amounts of test substance with sterile water and stored at room temperature. Sampling was performed on GD 6 of each part of the study and ten days after the start of the fifth part (date drawn at random before the start of the study). The 5 mL samples were collected and stored at -20°C for possible subsequent analysis.

   Results:

   Homogeneity analysis: No information was provided.
   Stability analysis: No information was provided.
   Concentration analysis: No information was provided.

6. **Dosage administration:** All doses were administered once daily by oral gavage, on GD 6 through GD 19, in a volume of 5 mL/kg of body weight/day. Dosing was based on the body weight on the most recent body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs twice daily. Body weight data were recorded on GD 1 (day of arrival at testing laboratory), GD 5 (the day of randomization), GD 6 (first day of treatment) and on GDs 9, 12, 15, 19, 21, 24, 27 and 29. Food and water consumption were measured before, during and after the treatment period by daily weighing the amount of food remaining in bowls and by daily measurement of the amount of residual water in bottles. Dams were sacrificed on day

29 of gestation (approximately 2 days prior to parturition) by subtotal exsanguination following sodium pentobarbital anesthesia. The uterus, complete with ovaries, was removed and the uterine horns were examined. Main organs (liver, spleen, kidneys, stomach, intestines, gonads/reproductive tract, lungs and heart) were examined macroscopically. The following data were collected: count of corpora lutea on ovaries; weight of uterus before extraction of fetuses; number and uterine location of implantation sites; number and uterine location of fetuses; and number and uterine location of resorptions (early and late).

2. **Fetal evaluations:** At the scheduled sacrifice of dams (GD 29), the fetuses were examined macroscopically and the following data were recorded: number of live or dead fetuses; individual fetal weight (live and dead fetuses); caudo-cranial measurement of live and dead fetuses; gross evaluation and weight of placenta of all fetuses; external morphological examination of fetuses; soft tissue examination of all fetuses after sacrifice by subcutaneous injection of sodium pentobarbital; and sexing of all fetuses at external examination.

   Following examination of soft tissues, all fetuses were eviscerated. The heads were removed for approximately half of the fetuses in each litter and were fixed in Bouin's fluid for subsequent examination following freehand serial sectioning. The torsos and the remaining intact fetuses were skinned and fixed in 90% or 95% alcohol and processed for skeletal examination using the alizarin method. The fetal examinations (soft tissue examination of heads and skeletal examinations) were conducted by Tesh Consultants International, Suffolk, UK.

D. DATA ANALYSIS:

1. **Statistical analyses:** Body weight changes were analyzed by two-way analysis of variance for repeated measurements in time taking the time and treatment into consideration. Food consumption was analyzed period by period by one-way analysis of variance (treatment factor). These results were analyzed period by period, given that the duration of each of these periods was not the same and therefore not comparable. Water consumption was analyzed by two-way analysis of variance for repeated measurements in time taking the time and treatment factors into consideration.

   A one-way analysis of variance (treatment factor) was applied to the following: mean weight per litter and per group of live fetuses; mean caudo-cranial measurement per litter and per group; mean weight of the placenta per litter and per group; mean number of corpora lutea; mean number of implantation sites; mean number of live and dead fetuses, mean number of resorptions; mean percentage of reproductive indices per group and per female; and mean weight of the uterus.

   Results of daily clinical findings and of macroscopic examination of fetuses and/or organs removed were not analyzed statistically.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

   $$\text{Pre-implantation loss (\%)} = \frac{\text{Number of corpora lutea} - \text{number of implantations}}{} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{Number of implantations} - \text{number of live fetuses}}{\text{Number of implantations}} \times 100$$

Number of corpora lutea

3. **Historical control data:** Limited historical control data were provided to allow comparison with concurrent controls. Rib and lumbar vertebrae values from one control study were included.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** No deaths or clinical signs of toxicity were observed, except for one female in the treated group, no. 20001576, that had dark urine on GDs 17 and 18.

2. **Body weight:** Body weight data are summarized in Table 2. No treatment-related absolute body weight differences were observed. However, one treated female (No. 20001576) had a bodyweight loss of about 24% from GD 1 to GD 15. At the terminal necropsy, this female was pregnant but showed a total litter loss. Mean body weight gain during the treatment period (GD 6 – GD 19) was decreased (78% lower than control group) in the treated group. If data from animal no. 20001576 is excluded from the mean value, the decreased body weight gain in the treated group is even more severe (89% lower than control group). Body weight gain rebounded after the treatment period and was increased in the treated group as compared to the control group.

TABLE 2: Mean (∀SD) maternal body weight and body weight gain (kg) [a]			
Gestation Day	Dose in mg/kg bw/day (# of Dams)		
	Control (16)	Treated (13)	Treated (12)[b]
GD 1	3.47 ± 0.18	3.48 ± 0.24	3.50 ± 0.24
GD 6	3.48 ± 0.20	3.46 ± 0.33	3.52 ± 0.27
GD 19	3.66 ± 0.23	3.50 ± 0.39	3.54 ± 0.36
GD 29	3.76 ± 0.21	3.74 ± 0.43	3.79 ± 0.41
Pretreatment (GD 1-6)[c]	0.01	-0.02	0.02
Treatment (GD 6-19)[c]	0.18	0.04 (↓78)[d]	0.02 (↓89)
Post-treatment (GD 15-29)[c]	0.1	0.27	0.24

[a] Data obtained from page 22, MRID 47564941.
[b] Exclusion of female no. 20001576 from the mean results.
[c] Calculated by the reviewer; no statistical analysis was performed.
[d] Percentage difference from control value calculated by the reviewer.

3. **Food consumption:** Food consumption data are summarized in Table 3. During the treatment period, food consumption of treated females was significantly lower than control females. The food consumption was almost nil from GD 1 to GD 14 for female no. 20001576. Even if data from this female were not included in the mean food consumption value, intake during treatment was still decreased (10% lower than control group). Water consumption was not affected by treatment.

TABLE 3: Mean (∀SD) maternal food consumption (g) [a]			
Duration	Dose in mg/kg bw/day (# of Dams)		
	Control (16)	Treated (13)	Treated (12)[b]
Before treatment (GD 1 – GD 5)	556 ± 161	532 ± 206	574 ± 144
During treatment (GD 6 – GD 19)	1883 ± 318	1601* ± 604 (↓15)[c]	1688 ± 539 (↓10)
After treatment (GD 20 – GD 28)	875 ± 106	1017 ± 256	995 ± 255

[a] Data obtained from page 25, MRID 47264941.
[b] Exclusion of female no. 20001576 from the mean results.
[c] Percentage difference from control value calculated by the reviewer.
* Significantly different from control value, $p \leq 0.05$.

4. **Gross pathology:** No macroscopic findings were reported.

5. **Cesarean section data:** Data are summarized in Table 4. The number of implantation sites per dam was significantly increased in the treated group whether data from female 20001576 were included or not. The number of live fetuses per dam was significantly increased if data from female 20001576 were excluded from the mean. This animal had a total litter loss. No other treatment-related effects were observed.

TABLE 4: Cesarean section observations [a]			
Observation	Dose (mg/kg bw/day)		
	Control	Treated	Treated[b]
No. Animals pregnant	16	13	12
Maternal wastage			
No. died	0	0	0
No. Died pregnant	0	0	0
No. Died nonpregnant	0	0	0
No. Aborted	0	0	0
No. Premature delivery	0	0	0
Total No. corpora lutea Corpora lutea/Dam	182 11.37 ± 2.73	157 12.08 ± 2.75	152 12.67 ± 1.83
Weight of placenta (g)	5.34 ± 0.58	5.19 ± 0.77[b]	-
Uterine weight (g)	464.1 ± 111.3	507.8 ± 190.4	549.1 ± 123.7
Total No. implantations (Implantations/Dam)	152 9.50 ± 2.66	149 11.46* ± 2.40	139 11.58* ± 2.47
Total No. litters	16	13	12
Total No. live fetuses (Live fetuses/Dam)	139 8.69 ± 2.36	130 10.00 ± 3.85	130 10.83* ± 2.52
Total No. resorptions			
Early	6	6	6
Late	7	3	3
Resorptions/Dam			
Early	0.37 ± 0.72	0.46 ± 0.52	0.50 ± 0.52
Late	0.44 ± 1.03	0.23 ± 0.44	0.25 ± 0.45
Litters with total resorptions	0	1	0
Mean fetal weight (g)	36.75 ± 4.39	36.15 ± 5.25	-
Caudo-cranial measurement	88.6 ± 3.3	87.8 ± 3.8	-
Preimplantation loss (%)	16.30 ± 15.73	9.10 ± 12.49	9.86 ± 12.72
Postimplantation loss (%)	7.36 ± 10.70	13.91 ± 26.53	6.73 ± 6.15

[a] Data obtained from pages 29-31 and 76-77, MRID 47264941.
[b] Excludes female no. 20001576 from the mean results.
* Statistically different ($p < 0.05$) from the control.
- = not reported.

B. DEVELOPMENTAL TOXICITY:

1. **External examination:** Exencephaly was reported in one fetus of a control female. Forelimb flexure was observed in one fetus each from three treated females. This finding is commonly seen in rabbit fetuses and no skeletal abnormalities were reported in the affected limbs.

2. **Visceral examination:** No results from the visceral examinations were provided.

3. **Soft tissue examination of heads**: A statistically non-significant increase in cystic dilatation of the brain (dorsal brain/medulla, oblongata/lateral cerebellum) was observed in the treated group considering both the number of fetuses and the number of litters affected (Table 5).

Table 5: Incidence (%) of cystic dilatation of brain[a]		
Dose level (mg/kg bw/day)	0	1000
Number of fetal heads examined	67	65
Cystic dilatation	3 (4.5)	6 (9.2)
Number of litters examined	16	12
Cystic dilatation	3 (18.8)	5 (41.7)

[a] Data obtained from pages 96-97, MRID 47264941.

4. **Skeletal examination:** There was a slight increase in the proportion of fetuses with supernumerary ribs at the thoraco-lumbar border (rib count 13/13) (61.5% in the treated group compared with 42.4% in the control group). There was a decrease in the proportion of fetuses with 12/12 ribs (24.6% in the treated group compared to 40.3 in the control group). A number of fetuses with supernumerary ribs had an additional (8^{th}) lumbar vertebra (15.4% in the treated group compared with 7.2% in the control group). When the treated group was compared with the control values performed at the testing laboratory in the same strain of rabbit during the same time period, the distribution of rib numbers were essentially similar in both groups, while the proportion of fetuses with an additional lumbar vertebrae was lower in the treated group. The data are included in Table 6.

TABLE 6: Comparison of rib and lumbar vertebrae values from present and recent CERB study[a]			
% Fetuses Affected	Study no. 20000388T		Study no. 20000153T
	Control	Treated	Control
Ribs:			
12/12	40.3	24.6	25.8
12/13	17.3	13.8	13.2
13/13	42.4	61.5	61.1
Lumbar vertebrae:			
6	0.7	-	-
7	90.6	83.1	75.3
7/8	1.4	1.5	2.6
8	7.2	15.4	22.1

[a] Data obtained from page 94, MRID 47264941.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS= CONCLUSIONS:** The study author concluded that the test material administered to female rabbits at the dose of 1000 mg/kg bw/day was well tolerated. No maternal toxicity and no treatment-related fetal abnormalities were reported. The NOAEL was 1000 mg/kg bw/day.

B. **REVIEWER COMMENTS:**

Pregnant rabbits were treated at either 0 or 1000 mg/kg bw/day (limit dose) from GD 6 to GD 19. All females survived to study termination and no treatment-related clinical signs of toxicity were observed. No treatment-related body weight changes were observed; however, mean body weight gain during the treatment period was decreased in the treated group. During the treatment period, food consumption of treated females was significantly lower than that of control females. Water consumption was not affected by treatment. No treatment-

related effects on macroscopic postmortem findings or Caesarean section data were observed.

The maternal toxicity LOAEL for Laminarin (H 11) in rabbits is 1000 mg/kg bw/day, based on decreased body weight gain during the treatment period. The maternal NOAEL is not established.

No treatment-related effects on fetal length and weight or on external fetal examinations were observed. An increase in cystic dilatation of the brain (dorsal brain/medulla, oblongata/lateral cerebellum) was observed in the treated group considering both the number of fetuses and the number of litters affected. This finding is not considered treatment-related since there was no statistically significant increase and no other soft tissue findings in the head. There was an increase in the number of fetuses with supernumerary ribs at the thoraco-lumbar border and a decrease in the number of fetuses with 12/12 ribs. A number of fetuses with supernumerary ribs had an additional (8^{th}) lumbar vertebra. When the treated group was compared with the control values performed at the testing laboratory in the same strain of rabbit during the same time period, the distribution of rib numbers were essentially similar in both groups, while the proportion of fetuses with an additional lumbar vertebrae was lower in the treated group. Therefore, the skeletal findings are not considered treatment-related.

The developmental toxicity LOAEL for Laminarin (H 11) in rabbits is not established. The developmental NOAEL is 1000 mg/kg bw/day.

C. STUDY DEFICIENCIES:

1. Homogeneity, stability and concentration analyses were not conducted.
2. Sex ratios were not reported.
3. Female no. 20001576 should not have been included in the study since there was evidence of its ill health (anorexia and bodyweight loss) before initiation of dosing.
4. The prenatal development toxicity study guideline (870.3700b) specifies that each test and control group should contain a sufficient number of animals to yield approximately 20 animals with implantation sites. In the present study, data from 16 and 13 females in the treated and control groups, respectively, were analyzed.
5. Findings from visceral examination of the fetuses were not reported.

DATA EVALUATION RECORD

LAMINARIN (PRODUCT: H11)

STUDY TYPE: BACTERIAL REVERSE MUTATION TEST; OPPTS 870.5100 [§84-2]

MRID 47264942

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Secondary Reviewer: **Date:** _____

DATA EVALUATION RECORD

STUDY TYPE: Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/*Escherichia coli* mammalian activation gene mutation assay; OPPTS 870.5100 ['84-2]; OECD 471.

EPA Reg. No.: 83941-R
DECISION:

Product Name: Vacciplant
DP BARCODE: 352311

TEST MATERIAL (PURITY): H11 (Laminarin) (≥90% by HPLC)

SYNONYMS: Laminaran, Vacciplant

CITATION: Marzin, D. (2000) Mutagenicity Test on Bacteria (*Salmonella typhimurium* his$^-$ and *Escherichia coli* trp$^-$) Using B.N. Ames's Technique with H11. Institut Pasteur de Lille, Genetic Toxicology Laboratory, 1, rue du Professeur Calmette – BP.245, F-59019 Lille Cedex, France. Report No. IPL-R 991011/H11/ GOËMAR Laboratory, May 3, 2000. MRID 47264942. Unpublished.

SPONSOR: GOËMAR, La Madeleine, Avenue du Général Patton, 35418 Saint-Malo, France.

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 47264942), *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strains WP2 (pKM101) and WP2 *uvrA* (pKM101) were exposed to 0, 50, 150, 500, 1500, or 5000 µg/plate H11 (Laminarin; purity ≥ 90%, batch no. 99521) dissolved in distilled water in two experiments. Experiment 1 used a standard plate incorporation procedure, without and with activation. In Experiment 2, a standard plate incorporation method was used without activation and a pre-incubation method (60 minutes at 37°C) was used with activation. Triplicate platings were used in both experiments.

No toxicity or precipitation of the test article were noted at any dose level, without or with activation, with any bacterial strain. Statistically significant increases in the number of revertants occurred in Experiment 1 in both *E. coli* strains at 5000 µg/plate with activation (1.3 (WP2) and 1.4 (WP2 *uvrA*) times the control value), and in Experiment 2 in *E. coli* WP2 uvrA at 150, 1500, and 5000 µg/plate (1.4, 1.4, and 1.5 times the control value, respectively). However, in each case the criterion for a positive mutagenic effect was not met, *i.e.*, the mutation frequency was not at least two times the value for the solvent control, and there was no clear dose-response. These increases were thus not considered biologically significant. Concurrent solvent and positive controls gave appropriate values and were in line with historical solvent and positive controls. The investigator concluded that H11, tested at dose levels of 50, 150, 500, 1500, and 5000 µg/plate was non-mutagenic in all of the *S. typhimurium* and *E. coli* strains tested, both in the absence and in the presence of S9 mix. **There was no evidence of induced mutant colonies over background**. This is an **Acceptable/Guideline** study.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

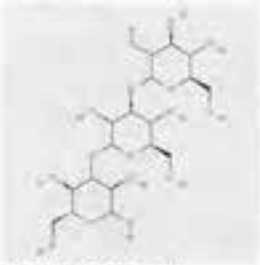
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: H11 (Laminarin)

Description: White/beige powder
Lot/Batch #: 99521
Purity: ≥90%
CAS # of TGAI: 9008-22-4
Structure:



Structure found at:
http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=439306&loc=ec_rcs

Solvent Used: Distilled water

2. Control materials:

Negative: None
Solvent (final conc'n): Distilled water / 100 µL per plate
Positive:

Nonactivation:

Both Experiment 1 and 2:
Sodium azide (SA), 1 µg/plate: TA100, TA1535
2-Nitrofluorene (2NF), 2 µg/plate: TA98
9-Aminoacridine (9AA), 50 µg/plate: TA1537
Mitomycin C (MMC), 0.125 µg/plate: *E. coli* WP2 (pKM101)
Potasium dichromate (KDC), 15 µg/plate: *E. coli* WP2 *uvrA* (pKM101)

Activation:

Experiment 1: 2-Anthramine (2AA), 2 µg/plate: TA98, TA100, TA1535, TA1537.
Benzo[a]pyrene (BAP), 5 µg/plate: *E. coli* WP2 (pKM101), *E. coli* WP2 *uvrA* (pKM101).

Experiment 2: 2-Anthramine (2AA), 1 µg/plate: TA98, TA100, TA1535, TA1537.
Benzo[a]pyrene (BAP), 2.5 µg/plate: *E. coli* WP2 (pKM101), *E. coli* WP2 *uvrA* (pKM101).

Comment: The positive control chemicals used without activation were dissolved in distilled water, while the positive control chemicals used with activation were dissolved in DMSO.

3. Activation: S9 derived from

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Noninduced		Phenobarbitol and 5,6 -Benzoflavone		Mouse		Lung
			None		Hamster		Other *(name)*
			Other *(name)*		Other *(name)*		

The S9 fraction was obtained from Sprague Dawley OFA male rats, 7-8 weeks old (180 – 200 g). Five days before sacrifice, animals were induced with a single i.p. injection of Aroclor 1250 at a dose of 500 mg/kg in a solution of corn oil at 200 mg/mL.

Description of S9-mix composition per 1 mL:

S9 fraction	0.1 mL
$MgCl_2$, 0.4 M	0.01 mL
KCl, 1.65 M	0.01 mL
Glucose-6-phosphate, 1 M	0.005 mL
NADP, 0.1 M	0.04 mL
Phosphate buffer, 0.2 M (pH 7.4)	0.5 mL
H_2O	0.335 mL

The protein concentration of the S9 fraction was 24 mg/mL (Appendix 2, p 38, MRID 47264942).

4. Test organisms: *S. typhimurium* strains *(TA#) and E. coli strains*

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538	X	*E. coli* WP2 (pKM101)	X	*E. coli* WP2 *uvr*A (pKM101)
Properly maintained?						X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?						X	Yes		No

5. Test compound concentrations used: (Triplicate plating):

Nonactivated conditions: 0, 50, 150, 500, 1500, and 5000 µg/plate in all strains
Activated conditions: 0, 50, 150, 500, 1500, and 5000 µg/plate in all strains

B. TEST PERFORMANCE:

1. Type of *Salmonella* assay:

X	Standard plate test (Experiment 1, without and with activation; Experiment 2, without activation)
X	Pre-incubation (60 minutes) (Experiment 2)
	"Prival" modification *(i.e. azo-reduction method)*
	Spot test
	Other

2. Protocol:

A preliminary cytotoxicity test was carried out in all tester strains, without and with activation, using the test article dissolved in sterile distilled water to produce doses of 0, 50, 150, 500, 1500, and 5000 µg/plate. Only one plate was tested per experimental condition and positive controls were not used. Plates were incubated for *ca.* 48-72 hours at 37°C, and the revertants were counted. Cytotoxicity was checked by examination of the background lawn and any precipitation was noted.

Mutagenicity tests:

Experiment 1

Without activation (plate incorporation method): For all strains, 0.1 mL of the test article (dissolved in distilled water at the appropriate concentration) and 0.1 mL of bacterial suspension from a culture agitated overnight at 37°C were successively added to 2 mL of top agar in a tube. For the *Salmonella* tester strains the top agar contained 10% of 0.5 mM biotin/histidine solution and for the *E. coli* strains, the top agar contained 5% Oxoid No. 2 nutrient broth and 0.5 mM tryptophan solution. The top agar was maintained at 45 °C. The contents of each tube were mixed then spread on a Petri plate containing 20 mL of minimum agar. Three plates were used per treatment. The plates were incubated at 37°C for *ca.* 48 hours, and then revertant colonies were scored for each plate. At the same time, solvent controls (0.1 mL solvent/plate) were prepared in the same way, but 6 plates were used per treatment condition. Appropriate positive reference controls (3 plates per treatment condition) were also performed. To assay for sterility of the media, 3 Petri plates containing 20 mL of minimal agar received 2 mL of top agar alone and were incubated under the same conditions. Sterility was verified if no colonies were observed after 48 hours at 37°C.

With activation (plate incorporation method): The method was the same as that described above without activation, except that immediately before spreading the top agar on the plates, 0.5 mL of S9-mix was added to the soft agar.

Experiment 2

Without activation (plate incorporation method): The same method was used as in Experiment 1 and the same doses were tested.

With activation (pre-incubation method): Based on the negative results found in the first experiment with activation, the second experiment was done using a pre-incubation method. The method was similar to the plate incorporation method, with the following changes: 100 µL of the test article dissolved in distilled water at an appropriate concentration was added to a pre-incubation tube followed by 500 µL of S9-mix, and then 100 µL of the bacterial tester strain. This mixture was incubated with stirring at 37 °C for 60 minutes prior to adding the soft agar and spreading on the Petri plates.

3. Statistical analysis:

Statistical analysis, when applied, was done using Dunnett's method, allowing the comparison of several treatment means to a solvent control.

4. Evaluation criteria:

The test was considered valid if: 1) the test article was shown to be sterile; 2) the mean frequency of spontaneous revertants for the solvent controls for each strain was within the range of the historical control values; and 3) the mean frequency of revertants for each strain, induced by the positive control chemicals, both without and with activation, was greater than the corresponding historical values.

The criteria for a positive mutagenic effect were as follows: For strains TA 1535 and TA 1537 the test article must show a dose-related increase in mutations for at least three concentrations and the highest mutation frequency must be at least three times the value for

the solvent control. For strains TA 98, TA 100, WP2 (pKM101) and WP2 uvrA (pKM101) the test article must show a dose-related increase in mutations for at least three concentrations and the highest mutation frequency must be at least two times the value for the solvent control. If the test article causes a positive response during a single assay and that result cannot be reproduced in at least 2 independent assays, the initial positive result may be considered as not significant.

II. RESULTS:

A. CYTOTOXICITY ASSAY:

The results of the preliminary cytotoxicity tests showed that the test article caused no cytotoxicity, based on no reduction in bacterial lawn growth and no reduction in revertant frequency for any tester strain, either without or with activation, at any dose tested up to a limit dose of 5000 µg/plate (Table 3, page 22 of MRID 47264942). Additionally, no precipitation of the test article was noted in any treated culture, without or with activation, up to the maximum dose of 5000 µg/plate. Based on these results, 5000 µg/plate was retained as the maximum dose tested in the mutagenicity studies, without and with activation, in all strains.

B. MUTAGENICITY ASSAY:

Experiment 1 used only the plate incorporation method. The limit dose of 5000 µg/plate, with activation, induced a statistically significant increase in the number of revertants in both *E. coli* strains (Table 1). However, in each of these two cases, the criterion for a positive mutagenic effect was not met, *i.e.*, a mutation frequency that was at least two times the value for the solvent control. The actual ratios were only 1.3 (WP2) and 1.4 (WP2 *uvrA*). In addition, there was no dose-related increase in the mutation frequency in either case. No other mutagenic effects were noted at any other doses, with any strain, either without or with activation.

Experiment 2, without activation, was a repeat of the first experiment and again used the plate incorporation method. However, with activation, the pre-incubation method was used. A summary of the results from experiment 2 are shown in Table 2. There were statistically significant increases in the number of revertants in the *E. coli* strain WP2 uvrA, with activation, at doses of 150, 1500, and 5000 µg/plate. The test article:control colony count ratios were 1.4, 1.4, and 1.5, respectively. However, the criterion for a positive mutagenic effect was not met, *i.e.*, the mutation frequencies were all less than twice that for the solvent control, and there was no clear dose-response. These increases were not considered biologically significant. No other significant increases in number of revertants were noted with any other strain, at any dose, either without or with activation. All the solvent and positive control values in both experiments were appropriate. The concurrent solvent controls were all within the range of the historical control data (Table 1, pages 19-20 of MRID 47264942).

TABLE 1. Experiment 1 (plate incorporation method). Summary of mutations induced in four tester strains of Salmonella typhimurium and two strains of Escherichia coli, without and with activation following exposure to the test article (H11).

Treatment	Number of revertant colonies per plate (mean ± standard error)					
	TA98	**TA100**	**TA1535**	TA1537	WP2 (pKM101)	WP2uvrA (pKM101)
Without S9-mix						
Solvent control (distilled water)	15 ± 3.7	83 ± 12.1	14 ± 2.3	3 ± 0.8	62 ± 7.4	179 ± 24.2
Test dose (µg/plate)						
50	21 ± 3	88 ± 3.6	7 ± 3.5	4 ± 2.1	65 ± 8.5	185 ± 23.1
150	17 ± 5.7	78 ± 7.1	12 ± 3.5	2 ± 1.2	76 ± 9	187 ± 18.5
500	17 ± 4	89 ± 10.3	7 ± 3.6	3 ± 2.1	76 ± 2.1	206 ± 20.7
1500	17 ± 1	89 ± 15.4	13 ± 1	3 ± 1.7	53± 40.6	198 ± 18.4
5000	22 ± 4.6	94 ± 6.4	13 ± 2.5	3 ± 1.5	73 ± 12.1	174 ± 10.1
Positive control (µg/plate):	2NF (2)	SA (1)	SA (1)	9AA (50)	MMC (0.125)	KDC (15)
Average mutants/plate:	599 ± 52.7**	656 ± 70.5**	370 ± 39.8 **	567 ± 86.9**	211 ± 29.4**	980 ± 42.7**
Carrier for positive control (water):	13 ± 1	105.7 ± 5.5	14 ± 0.6	3 ± 0.6	58 ± 5.5	168 ± 21.5
With S9-mix						
Solvent control (distilled water)	24 ± 5.2	87 ± 10.5	19 ± 6.5	5 ± 2.7	67 ± 4.1	161 ± 18.6
Test dose (µg/plate)						
50	21 ± 4.2	88 ± 4.4	12 ± 4.2	3 ± 2.1	75 ± 4.7	156 ± 13.5
150	17 ± 2.6	95 ± 8.1	9 ± 2.6	4 ± 1.5	75 ± 9	168 ± 12.2
500	20 ± 0.6	63 ± 2	11 ± 6.7	4 ± 1.7	80 ± 8	189 ± 15.4
1500	17 ± 3.1	62 ± 10	12 ± 5.5	6 ± 2.1	89 ± 7.5	184 ± 8.7
5000	22 ± 7	91 ± 11	12 ± 4.5	3 ± 2	90 ± 12.2**	229 ± 18.5**
Positive control (µg/plate):	2AA (2)	2AA (2)	2AA (2)	2AA (2)	BAP (5)	BAP (5)
Average mutants/plate:	1560 ± 168.9**	1675 ± 92.5**	560 ± 13.3**	262 ± 34.2**	196 ± 65.6**	707 ± 80.3**
Carrier for positive control (DMSO):	16 ± 2.5	71 ± 1.7	14 ± 2.3	5 ± 1	69± 6.2	208 ± 14.5

Data summarized from Tables 4-9, pages 23–28 of MRID 47264942.
All plating was in triplicate, except for the solvent controls which used 6 plates for each tester strain.
2NF = 2-Nitrofluorene
SA = Sodium azide
9AA = 9-Aminoacridine
MMC = Mitomycin C
KDC = Potassium dichromate
2AA = 2-Anthramine
BAP = Benzo[a]pyrene
** $p<0.01$

TABLE 2. Experiment 2. Summary of mutations induced in four tester strains of *Salmonella typhimurium* and two strains of *Escherichia coli*, without and with activation following exposure to the test article (H11). The plate incorporation method was used without activation, and the pre-incubation method was used with activation.

Treatment	Number of revertant colonies per plate (mean ± standard error)					
	TA98	TA100	TA1535	TA1537	WP2 (pKM101)	WP2uvrA (pKM101)
Without S9-mix						
Solvent control (distilled water)	18 ± 3.9	72 ± 15.9	11 ± 3.2	5 ± 2.7	54 ± 6.9	181 ± 26.6
Test dose (µg/plate)						
50	18 ± 7.4	85 ± 3.6	13 ± 2.6	6 ± 2.5	66 ± 5.7	191 ± 10.1
150	13 ± 1.2	92 ± 14	10 ± 4.4	4 ± 1.5	60 ± 5.6	183 ± 12.9
500	18 ± 5.1	90 ± 16.8	10 ± 3.1	5 ± 2	62 ± 13	188 ± 21.7
1500	18 ± 0.6	81 ± 5	12 ± 2.6	2 ± 1	60± 4.7	212 ± 17.7
5000	24 ± 2	105 ± 9.1	20 ± 3	5 ± 1.5	60± 4.7	226 ± 8.5
Positive control (µg/plate):	2NF (2)	SA (1)	SA (1)	9AA (50)	MMC (0.125)	KDC (15)
Average mutants/plate:	531 ± 15**	763 ± 94.8**	395 ± 17.2 **	393 ± 209**	377 ± 30.9**	1256 ± 273.7**
Carrier for positive control (water):	15 ± 3.6	68 ± 8.5	11 ± 4.6	4 ± 2.5	56 ± 4.6	168 ± 34.6
With S9-mix						
Solvent control (distilled water)	12 ± 3.8	65 ± 8.9	5 ± 1.8	3 ± 0.6	56 ± 5.2	154 ± 22.3
Test dose (µg/plate)						
50	17 ± 4.4	64 ± 2.3	7 ± 1.5	3 ± 0.6	51 ± 8.5	187 ± 5.1
150	17 ± 2.9	66 ± 14.1	3 ± 0.6	2 ± 1	61 ± 5.5	214 ± 9**
500	13 ± 1.2	64 ± 7	3 ± 2.3	2 ± 0.6	63 ± 11.5	185 ± 14.3
1500	14 ± 4.2	64 ± 3.8	6 ± 0.6	1 ± 0.6	60 ± 6	211 ± 11.7**
5000	15 ± 4.2	72 ± 6.4	6 ± 1	3 ± 1.5	66 ± 6.5	230 ± 4.7**
Positive control (µg/plate):	2AA (1)	2AA (1)	2AA (1)	2AA (1)	BAP (2.5)	BAP (2.5)
Average mutants/plate:	982 ± 139.6**	1109 ± 349.7**	80 ± 8**	106 ± 16.1**	147 ± 20.6**	804 ± 41.9**
Carrier for positive control (DMSO):	14 ± 0.6	60 ± 2.9	3 ± 0.6	9 ± 3.2	38± 8.4	156 ± 18

Data summarized from Tables 10-15, pages 29–34 of MRID 47264942.
All plating was in triplicate, except for the solvent controls which used 6 plates for each tester strain.
2NF = 2-Nitrofluorene
SA = Sodium azide
9AA = 9-Aminoacridine
MMC = Mitomycin C
KDC = Potassium dichromate
2AA = 2-Anthramine
BAP = Benzo[a]pyrene
** $p<0.01$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that the test article H11 did not exhibit mutagenic activity in *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and in *E. coli* strains (WP2 and WP2 *uvrA*) up to a limit dose of 5000 µg/plate, either in the absence or in the presence of an S9 mix.

B. REVIEWER COMMENTS:

The reviewer agrees with the investigator's conclusions. Occasionally, statistically significant increases in the number of revertants in the *E. coli* strains were observed, with activation, but these were all less than twice the solvent control values and are not considered biologically significant. All the solvent and positive control values in both experiments were appropriate and historical solvent and positive control data were presented that were in agreement with the concurrent controls. This is an **Acceptable/Guideline** study.

C. STUDY DEFICIENCIES:

No deficiencies were noted.

DATA EVALUATION RECORD

LAMINARIN

STUDY TYPE: *IN VITRO* MAMMALIAN CELL GENE MUTATION TEST IN L5178Y/TK+/- MOUSE LYMPHOMA CELLS; OPPTS 870.5300
MRID 47264943

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Work Assignment #08-025

Primary Reviewer:
Gary A. Sega, Ph.D.

Signature: _____
Date: _____

Secondary Reviewers:
S. Milanez, Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
K.G. Slusher, M.S.

Signature: _____
Date: _____

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Secondary Reviewer: **Date:** _____

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation Assay in L5178Y/TK+/- Mouse Lymphoma Cells; OPPTS 870.5300 ['84-2]; OECD 476.

EPA Reg. No.: 83941-R
DECISION:

Product Name: VacciPlant
DP BARCODE: 352311

TEST MATERIAL (PURITY): Laminarin (92.9% purity)

SYNONYMS: Laminaran, VacciPlant

CITATION: Haddouk, H. (2002) *In vitro* mammalian cell gene mutation test in L5178Y/TK+/- mouse lymphoma cells. Centre International de Toxicologie, B.P. 563, 27005 Evreux, France. CIT Study No. 22626 MLY, February 27, 2002. MRID 47264943. Unpublished.

SPONSOR: GOËMAR, La Madeleine, Avenue du Général Patton, 35400 Saint-Malo, France.

EXECUTIVE SUMMARY:

In a mammalian cell gene mutation assay (MRID 47264943), L5178Y/TK+/- mouse lymphoma cells cultured *in vitro* were exposed for 3 hours to Laminarin (92.9% purity, batch number 99S21) dissolved in culture medium at concentrations of 0, 312.5, 625, 1250, 2500, and 5000 µg/mL, without and with S9 activation, and for 24 hours without activation using the same doses. The S9 fraction came from rat livers induced with Aroclor 1254.

Laminarin was tested up to a limit dose of 5000 µg/mL and was soluble and non-toxic at all doses tested, without or with activation. There was no indication of a mutagenic response under any test condition. The relative mutation frequencies of the treated cells ranged from 0.5 -1.8 x the corresponding vehicle control and never reached a 2-fold increase, which was the criterion for a mutagenic response. Colony size distributions showed appropriate ratios. Solvent and positive controls gave appropriate responses. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for OPPTS 870.5300, OECD 476, *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Laminarin

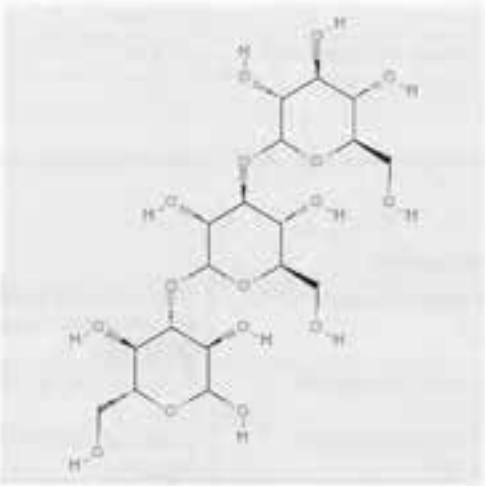
Description: Beige powder

Lot/Batch #: 99S21

Purity: 92.9%

CAS # of TGAI: 9008-22-4

Structure:



Structure found at:
http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=439306&loc=ec_rcs

Solvent Used: Culture medium

2. Control materials:

Negative control: None

Solvent control (final concentration): Culture medium (1% (v/v))

Positive controls: Nonactivation: Methyl methanesulfonate (MMS)
25 µg/mL for 3-hour treatment; 5 µg/mL for 24-hour treatment
Activation: Cyclophosphamide (CPA): 3 µg/mL

3. Activation: S9 derived from

X	Induced	X	Aroclor 1254 500 mg/kg, i.p. injection	X	Rat	X	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 mix composition: The S9 fraction was purchased from Moltox and stored in sterile tubes at -80°C until used. The S9 mix was prepared at +4°C immediately before use and maintained at this temperature until added to culture medium. The final concentration of S9 fraction in the S9 mix was 40% (v/v). Batch Nos. 1192 and 1298 had protein concentrations of 38.4 and 46.2 mg/mL, respectively.

Final amount per 5 mL of S9-mix:

S9 fraction	2 mL
NADP (25 mg/mL)	1 mL
Glucose-6-phosphate (180 mg/mL)	1 mL
KCl (150 mM)	1 mL

4. Test cells:

X	Mouse lymphoma L5178Y cells, clone 3.7.2C		V79 cells (Chinese hamster lung fibroblasts)		
	Chinese hamster ovary (CHO) cells—CHO-K_1 cells		list any others		
	Media: Described below*				No
	Properly maintained?	X	Yes		No
	Periodically checked for Mycoplasma contamination?	X	Yes		No
	Periodically checked for karyotype stability?		Yes	X	Not reported

*RPMI medium containing 10% horse serum, L-glutamine (2mM), penicillin (100 U/mL), streptomycin (100 µg/mL) and sodium pyruvate (200 µg/mL).

5. Locus examined: Thymidine kinase (TK)

			Thymidine kinase (TK)		Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		Bromodeoxyuridine (BrdU)		8-Azaguanine (8-AG)		Ouabain
			Fluorodeoxyuridine (FdU)		6-Thioguanine (6-TG) at 10 µg/mL		
		X	Trifluorothymidine (TFT) 4µg/mL				

6. Test compound concentrations used:

Nonactivated conditions:

Preliminary cytotoxicity:
3-hour treatment: 0, 10, 100, 500, 1000, 2500, and 5000 µg/mL

Main assay:
Exp. 1. 3-hour treatment: 0, 312.5, 625, 1250, 2500, and 5000 µg/mL
Exp. 2. 24-hour treatment: 0, 312.5, 625, 1250, 2500, and 5000 µg/mL

Activated conditions:

Preliminary cytotoxicity:
3-hour treatment: 0, 10, 100, 500, 1000, 2500, and 5000 µg/mL

Main assay:
Exp. 1. 3-hour treatment: 0, 312.5, 625, 1250, 2500, and 5000 µg/mL
Exp. 2. 3-hour treatment: 0, 312.5, 625, 1250, 2500, and 5000 µg/mL

B. TEST PERFORMANCE:

1. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 3 hours (Exp. 1) or 24 hours (Exp. 2) (nonactivated) and for 3 hours (Exp. 1 and 2) (activated).

b. After washing, cells were cultured for 2 days (expression period) before cell selection.

c. After expression, 2000 cells/well (one 96-well plate/culture; 2 plates/dose level) were cultured for 11-12 days in selection medium to determine numbers of mutants and 1.6 cells/well (one 96-well plate/culture; 2 plates/dose level) were cultured for at least 7 days without selective agent to determine cloning efficiency. Two 96-well plates/culture were used for the vehicle controls. Both small and large colonies were scored on mutant plates. Small colonies were <25% of the well diameter; large colonies were >25% of the well diameter. When both small and large colonies were present in the same well, two mutant colonies were counted (one small and one large).

2. Statistical methods:

The parameters that were reported were suspension growth (SG), relative suspension growth (RSG), relative total growth (RTG), relative survival (RS), cloning efficiency after treatment (CE_0), cloning efficiency at the end of the expression period (CE_2), and the relative mutation frequency (MF) per 10^6 clonable cells. 96-well plates were used in this study, so data from cytotoxicity plates (empty wells) was used to calculate CE_0 and CE_2 based on the zero term of the Poisson distribution:

CE_0 = -ln(empty wells/total wells)/no. of cells/well (*ca.* 1.6)
CE_2 was calculated in a similar way

MF was determined from the following equations:

CE_{mutant} = -ln(empty wells/total wells)/no. of cells/well (*ca.* 2000)

$MF = CE_{mutant} \times 10^6/CE_2$

Suspension growth (SG), relative suspension growth (RSG), and relative total growth (RTG) were calculated as:

SG = daily growth on day 1 x daily growth on day 2
RSG = (SG treated /SG vehicle control) x 100
$RTG = RSG \times RCE_2$

Relative survival (RS) was calculated as:

RS = [treated survival value/ vehicle control survival value] x 100

Instead of using statistical tests, arbitrary rules based on comparisons with the concurrent solvent control mutation frequency were applied to determine if results were positive, negative, or equivocal. Those rules are described in the following section on evaluation criteria. The reviewer considers this non-statistical approach to be appropriate.

3. Evaluation criteria:

The study was considered valid if the following criteria were met: 1) the cloning efficiency of the vehicle controls should be between 0.6 and 1.4 for CE_0 and between 0.7 and 1.3 for CE_2; 2) the mutation frequency of the vehicle controls should be within the range of 60-250 x 10^{-6}; 3) the mutation frequency of the positive controls should be more than two-fold higher than

the vehicle controls and should be consistent with historical data. The following arbitrary rules were applied to determine the mutagenicity of the test article. A reproducible, two-fold (or more) increase in the mutation frequency compared to the vehicle control at any dose and/or evidence of a dose-relationship were considered as a positive result. Reference to historical data, or other considerations of biological relevance might be taken into account in the evaluation of the results.

II. REPORTED RESULTS:

There was no analytical testing of the concentrations of the dosing solutions. However, the batch that was used was examined analytically before the study by the sponsor and was approved for use during the test period. All doses of the test article were adjusted to take into account its 92.9% purity.

A. PRELIMINARY CYTOTOXICITY ASSAY:

L5178Y/TK+/- mouse lymphoma cells were exposed for 3 hours to the vehicle alone and to six concentrations of the test article ranging from 10 to 5000 µg/mL in the absence and presence of S9-mix. No noteworthy cytotoxicity was noted after treatment at any dose, either without or with S9 activation (Table 1, page 16 of MRID 47264943). The cloning efficiencies of all treated cultures immediately after treatment (CE_0), as well as the relative survival (RS) of the treated cultures, were considered equivalent to those of the control cultures. In the culture medium, the dose level of 5000 µg/mL showed no precipitation, either without or with activation. At the maximum dose level, the pH was *ca.* 7.1, the same as the vehicle control and the osmolality was 299 mOsm/kg H_2O vs. 296 for the vehicle control. Based on these observations, the doses for the mutagenicity tests were set at: 0, 312.5, 625, 1250, 2500, and 5000 µg/mL.

B. MUTAGENICITY ASSAY:

Cloning efficiencies of treated cultures indicated no noteworthy toxicity in experiments 1 or 2 at any dose level, up to the limit dose of 5000 µg/mL, without or with activation (Tables 1-4). The test article also did not induce any significant increase in mutation frequency relative to the concurrent control at any dose level, without or with activation, in either experiment 1 or 2. In experiment 2 without activation (24-hour treatment), the number of mutants was increased slightly (239 vs. 131 x 10^{-6} in controls, 1.8x) but was within the historical vehicle control mutation frequency (72-242 x 10^{-6}). In the same assay, the positive control MMC mutation rate was slightly below the historical control range (401 vs. 426-1298 x 10^{-6}). These deviations were not considered biologically significant.

The proportions of small and large revertant colonies in both experiments were within the expected ranges. The mutation frequencies of the vehicle and positive controls were appropriate and were in agreement with historical control data (Appendix, pages 32-33 of MRID 47264943).

TABLE 1. Experiment 1 summary of mutagenicity results after exposure to Laminarin for 3 hours without S9 activation.

	Cytotoxicity: CE_0			Viable cells: CE_2			Mutation frequency: MF				
Dose [a] µg/mL	Empty wells	CE_0	RCE_0 %	Empty wells	CE_2	RCE_2	Empty wells	LC	SC	MF x 10^{-6}	R
0	12	1.2	100	8	1.2	100	71	19	6	110	1.0
	12			14			74	13	9		
	15			13			73	16	7		
	14			17			74	11	11		
312.5	18	1.2	97	5	1.8	148	77	13	6	54	0.5
	10			5			80	11	5		
625	29	0.9	72	12	1.1	87	74	12	11	93	0.8
	17			22			83	8	5		
1250	23	0.9	73	3	1.6	124	75	11	10	77	0.7
	22			13			76	13	8		
2500	12	1.2	101	7	1.5	118	72	17	7	77	0.7
	14			11			81	9	6		
5000	28	0.9	77	16	1.2	93	73	17	6	89	0.8
	14			14			83	11	2		
MMS 25 µg/mL	14	0.9	74	13	0.9	69	46	26	24	394	3.6
	30			35			51	25	20		

Data summarized from MRID 47264943, Table 2, page 16.
[a] Dose based on active material
LC: large colonies
SC: small colonies
R: ratio between mutation frequency of treated cells and mutation frequency of control cells

TABLE 2. Experiment 1 summary of mutagenicity results after exposure to Laminarin for 3 hours with S9 activation.

	Cytotoxicity: CE_0			Viable cells: CE_2			Mutation frequency: MF				
Dose [a] µg/mL	Empty wells	CE_0	RCE_0 %	Empty wells	CE_2	RCE_2	Empty wells	LC	SC	MF x 10^{-6}	R
0	27	0.7	100	20	0.9	100	78	13	5	145	1.0
	39			23			73	15	8		
	37			22			78	14	4		
	30			25			66	20	11		
312.5	23	0.7	101	14	1.3	141	78	11	7	116	0.8
	43			11			65	21	10		
625	28	0.7	108	16	1.1	121	70	18	9	134	0.9
	33			17			73	15	8		
1250	33	0.8	116	9	1.2	128	61	24	11	158	1.1
	23			21			72	16	8		
2500	45	0.4	62	21	1.0	108	74	15	7	164	1.1
	54			20			66	20	10		
5000	40	0.5	77	32	0.9	100	61	26	10	211	1.4
	45			13			70	14	12		
CPA 3 µg/mL	61	0.3	44	39	0.6	64	24	24	55	1144	7.9
	60			37			27	15	56		

Data summarized from MRID 47264943, Table 8, page 22.
[a] Dose based on active material
LC: large colonies
SC: small colonies
R: ratio between mutation frequency of treated cells and mutation frequency of control cells

TABLE 3. Experiment 2 summary of mutagenicity results after exposure to Laminarin for 24 hours without S9 activation.

	Cytotoxicity: CE_0			Viable cells: CE_2			Mutation frequency: MF				
Dose [a] µg/mL	Empty wells	CE_0	RCE_0 %	Empty wells	CE_2	RCE_2	Empty wells	LC	SC	MF x 10^{-6}	R
0	20	0.8	100	33	0.7	100	82	6	8	131	1.0
	29			38			83	10	3		
	30			34			80	14	2		
	29			29			78	10	8		
312.5	36	0.6	82	32	0.7	104	80	9	8	133	1.0
	32			---			---	---	---		
625	37	0.6	70	26	1.2	186	79	10	7	79	0.6
	42			1			79	11	6		
1250	39	0.6	81	40	0.6	89	86	6	4	145	1.1
	30			35			76	12	8		
2500	24	0.9	109	41	0.5	70	84	8	4	165	1.3
	24			51			81	7	8		
5000	35	0.7	89	46	0.4	62	86	3	7	239	1.8
	27			54			72	10	15		
MMS 5 µg/mL	38	0.6	72	34	0.6	91	65	13	23	401	3.1
	39			40			54	19	24		

Data summarized from MRID 47264943, Table 5, page 19.
[a] Dose based on active material
LC: large colonies
SC: small colonies
R: ratio between mutation frequency of treated cells and mutation frequency of control cells
---: cultures were not analyzed because of contamination

TABLE 4. Experiment 2 summary of mutagenicity results after exposure to Laminarin for 3 hours with S9 activation.

	Cytotoxicity: CE_0			Viable cells: CE_2			Mutation frequency: MF				
Dose [a] µg/mL	Empty wells	CE_0	RCE_0 %	Empty wells	CE_2	RCE_2	Empty wells	LC	SC	MF x 10^{-6}	R
0	33	0.8	100	34	0.7	100	83	11	2	115	1.0
	35			28			80	12	4		
	17			28			86	9	1		
	26			36			78	12	6		
312.5	31	0.6	80	37	0.7	101	81	14	1	142	1.2
	40			25			76	16	4		
625	34	0.7	89	22	0.9	132	77	15	4	89	0.8
	30			22			86	9	1		
1250	30	0.8	102	27	0.7	94	77	10	9	153	1.3
	24			40			80	12	4		
2500	27	0.7	96	25	0.7	103	88	6	2	93	0.8
	31			36			80	11	5		
5000	12	0.8	108	33	0.6	86	78	15	3	122	1.1
	38			41			88	7	1		
CPA 3 µg/mL	47	0.4	53	38	0.5	71	46	18	32	755	6.5
	53			49			45	24	28		

Data summarized from MRID 47264943, Table 5, page 19.
[a] Dose based on active material
LC: large colonies
SC: small colonies

R: ratio between mutation frequency of treated cells and mutation frequency of control cells

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that under the experimental conditions employed, Laminarin did not show any mutagenic activity in the mouse lymphoma assay.

B. REVIEWER COMMENTS:

The reviewer agrees with the conclusion of the study author. Solvent and positive controls gave appropriate responses and agreed well with the laboratory's historical controls. Laminarin was soluble and non-toxic at all doses tested, without or with activation, up to a limit dose of 5000 µg/mL. There was no indication of a mutagenic response under any test condition. The study followed proper experimental protocols and met the criteria for a valid test and a negative result. **The study is classified as Acceptable/Guideline.**

C. STUDY DEFICIENCIES:

The sex of the rats used for the S9 preparation was not given. This was a minor deficiency and did not affect the results of the study.

DATA EVALUATION RECORD

LAMINARIN

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS – BONE MARROW MICRONUCLEUS ASSAY; OPPTS 870.5395 [§84-2]
MRID .47264944

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Work Assignment #08-025

Primary Reviewer:
Gary A. Sega, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
S. Milanez, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
K.G. Slusher, M.S Signature: _____
Date: _____

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

Secondary Reviewer: **Date:**_____

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics – Bone Marrow Micronucleus assay in mouse; OPPTS 870.5395 [' 84-2]; OECD 474.

EPA Reg. No.: 83941-R
DECISION:

Product Name: VacciPlant
DP BARCODE: 352311

TEST MATERIAL (PURITY): Laminarin (94% purity)

SYNONYMS: Laminaran, Vacciplant

CITATION: Haddouk, H. (2001) Bone marrow micronucleus test by oral route in mice. Centre International de Toxicologie, B.P. 563, 27005 Evreux, France. CIT Study No. 21149 MAS, March 15, 2001. MRID 47264944. Unpublished.

SPONSOR: GOÊMAR, La Madeleine, Avenue du Général Patton, 35400 Saint-Malo, France.

EXECUTIVE SUMMARY:

In a mouse bone marrow micronucleus assay (MRID 47264944), Swiss Ico: OF1 (IOPS Caw) (5/sex/dose) were administered two daily doses of Laminarin (purity 94%, batch no. 99S10) in distilled water by gavage (oral intubation) at doses of 0, 500, 1000, or 2000 mg/kg bw. Polychromatic erythrocytes (PCEs) were examined for micronuclei in 5 animals/sex/dose. Bone marrow cells were harvested 24 hours after the final treatment.

No toxicity was noted in either sex at any dose up to the limit dose of 2000 mg/kg bw. PCEs were similarly examined in the vehicle control and in the positive control, cyclophosphamide. The vehicle and positive control treatments were also made by oral intubation. Laminarin was tested at adequate doses, up to the limit dose for the assay. The positive control induced the appropriate response. There were no statistically significant changes in the PCE/NCE ratios for any treatment group. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose in either sex.** It was concluded that the test article was negative in this *in vivo* study.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Laminarin

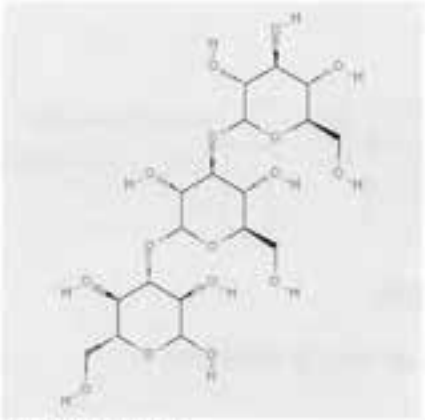
Description: Beige powder

Lot/Batch #: 99S10

Purity: 94%

CAS # of TGAI: 9008-22-4

Structure:



Structure found at:
http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=439306&loc=ec_rcs

Solvent Used: Distilled water

2. Control materials:

Negative control (if not vehicle) :		Final volume:	Route:
Vehicle:	Distilled water	Final volume: 10 mL/kg bw (2 treatments, separated by 24 h)	Route: oral intubation
Positive control :	Cyclophosphamide (CPA)	Final dose(s): 50 mg/kg bw (single dose)	Route: oral intubation

3. Test animals:

Species: Mouse

Strain: Swiss Ico: OF1 (IOPS Caw)

Age/weight at study initiation: Approximately 6 weeks of age / weights not reported

Source: Iffa Crédo, l'Arbresle, France

No. animals used per dose per harvest time	5	Males	5	Females
Properly Maintained?	x	Yes		No

Comment: Three additional animals of each sex were treated at the highest dose; they were to be used as replacements in case some of the others died from the treatment. Since no mortality occurred, bone marrow smears were not prepared from these supplementary animals.

4. Test compound administration:

	Dose levels (mg/kg bw)	Final volume	Route
Rangefinder	Limit dose of 2000 mg/kg bw, administered twice at 24-hour interval.	10 mL/kg bw	Oral intubation
Main study	0, 500, 1000, 2000	10 mL/kg bw	Oral intubation

Comment: All doses were established on the basis of the 94% purity reported for the test article.

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:	[ ]	once	[x]	twice (24 hrs apart)	[ ]	Other			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[ ]	48 hr	[ ] 72 hr
Other:									

b. Negative and/or vehicle control:

Distilled water; same schedule as for test chemical.

c. Positive control:

Dosing:	[x]	once	[ ]	twice (24 hrs apart)	[ ]	Other			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[ ]	48 hr	[ ] 72 hr
Other:									

d. Comment:

The study was performed in accordance with Study Protocol No. 21149 MAS and subsequent amendments, except that the temperature in the animal room was sometimes outside the temperature range specified in the protocol (23 to 25°C). This deviation was not considered to have any effect on the validity of the study.

2. Tissues and cells examined:

Were erythrocytes from bone marrow examined?:	Yes
No. of polychromatic erythrocytes (PCEs) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Number found in 1000 total erythrocytes
Other	

3. Details of slide preparation: After the mice were killed by carbon dioxide asphyxiation, bone marrow from both femurs of each animal was eluted using fetal calf serum. After centrifugation, the supernatant was removed and the sedimented cells were suspended by shaking. A drop of this cell suspension was spread on a slide. The slides were then air-dried, stained with Giemsa, and coded for scoring.

4. Evaluation criteria: For each animal, the number of micronucleated polychromatic erythrocytes (MNPCE) was counted in 2000 polychromatic erythrocytes. The polychromatic

(PCE) and normochromatic (NCE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PCE + NCE). The scoring of the slides was performed blind, at Microptic Cytogenetic Services, 2 Langland Close Mumbles, Swansea SA3 4LY, U.K. For a result to be considered positive the following criteria had to be met: There had to be a statistically significant increase in the frequency of MNPCEs in a treated group of animals when compared to the vehicle control group. Reference to historical control data, or other considerations of biological relevance were also taken into account in the evaluation of data.

5. **Statistical methods:** If there was no significant, within-group heterogeneity (using the heterogeneity chi-square test value), the frequency of MNPCEs for each treated group was compared with those in the concurrent vehicle control group using a 2 x 2 contingency table to calculate a χ^2 value. When there was significant, within-group heterogeneity, the treated group was compared with the control group using a Mann-Whitney, non-parametric analysis. The Student t-test was used for the PCE/NCE ratio comparison. Probability values ≤ 0.05 were flagged.

II. REPORTED RESULTS:

A. PRELIMINARY TOXICITY ASSAY:

Three male and three female mice were exposed to a limit dose of 2000 mg/kg/day on two successive days. Since no observable toxic effects were noted, 2000 mg/kg/day was chosen for the top dose in the cytogenetic test, along with doses of 1000 and 500 mg/kg/day.

B. MICRONUCLEUS ASSAY:

No clinical signs and no mortality were observed in the animals of either sex given 500, 1000, or 2000 mg/kg/day for two successive days. Results of this assay are summarized in Table 1. No biologically or statistically significant differences were found between test article-treated and vehicle control mice of either sex for the frequencies of micronucleated PCEs. Vehicle and positive control values for all parameters were appropriate, and the vehicle control and positive control frequencies of micronucleated PCEs and of PCE/NCE ratios were well within the ranges of the laboratory's historical controls. The dose levels and the route of exposure were also appropriate.

TABLE 1. Summary of micronucleus assay with Laminarin at 24 hours after treatment			
Treatment	**Number of PCEs scored per mouse for micronuclei**	**MNPCEs per 1000 PCEs (Mean ± SD)[a]**	**PCE/NCE ratio (Mean ± SD)**
MALES (5/DOSE)			
Vehicle control (distilled water)	2000	0.8 ± 0.8	0.9 ± 0.3
Laminarin (mg/kg bw)			
500	2000	1.1 ± 0.9	0.7 ± 0.2
1000	2000	1.6 ± 1.6	0.7 ± 0.1
2000	2000	1.2 ± 0.7	0.9 ± 0.1
Cyclophosphamide (50 mg/kg bw)	2000	31.5 ± 9.6***	1.0 ± 0.1
FEMALES (5/DOSE)			
Vehicle control (corn oil)	2000	0.8 ± 0.7	0.8 ± 0.2
Laminarin (mg/kg bw)			
500	2000	1.0 ± 0.5	0.9 ± 0.1
1000	2000	0.9 ± 0.7	0.9 ± 0.1
2000	2000	0.8 ± 0.6	1.1 ± 0.2
Cyclophosphamide (50 mg/kg bw)	2000	30.9 ± 8.7***	1.0 ± 0.2

Data obtained from MRID 46977412, Tables 8 and 10 on pages 31 and 33, respectively
PCEs = polychromatic (immature) erythrocytes
NCEs = normochromatic (mature) erythrocytes
MNPCEs = micronucleated PCEs
***Statistically significantly higher than vehicle control, $p < 0.001$
[a] MNPCEs were evaluated on 2000 PCEs but expressed on the basis of 1000 PCEs.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that, under the conditions of the study, Laminarin did not induce any damage to the chromosomes or the mitotic apparatus of mouse bone marrow cells after two oral administrations, with a 24-hour interval, at dose levels of 500, 1000, and 2000 mg/kg/day.

B. REVIEWER COMMENTS:

The reviewer agrees with the investigator's conclusions. The test article was tested up to the limit dose for the assay, and there was no suggestion that it induced micronucleated PCEs.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

C. STUDY DEFICIENCIES:

None.

DATA EVALUATION RECORD

LAMINARIN
(VACCIPLANT)

STUDY TYPE: Waiver Request for Immunotoxicity (OPPTS 870.7800)

MRID 47264945

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Immunotoxicity (OPPTS 870.7800)
MRID NO:	47264945
DECISION NO:	385527
DP BARCODE:	DP352311
TEST MATERIAL:	Vacciplant (a.i., 3.51% laminarin)
PROJECT STUDY NO:	Not provided
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Not applicable
TITLE OF REPORT:	Toxicology Waiver Request - Immunotoxicity
AUTHOR:	Smith, F.T.
STUDY COMPLETED:	October 10, 2007
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not required to meet the requirements of 40 CFR Part 160.
CONCLUSION:	The information provided is sufficient to justify a waiver for immunotoxicity testing of laminarin. If a waiver of immunotoxicity is being requested for the end use product Vacciplant, the inert ingredients in the product will also need to be addressed.

Test Material

Vacciplant (a.i., 3.51% laminarin)

Product Description

Vacciplant is an end use product to stimulate the natural defense reactions of crop plants. The active ingredient is 3.51% laminarin.

Waiver Request

The registrant is requesting a waiver for Immunotoxicity (OPPTS 870.7800).

Registrant's Justification

Laminarin is a naturally-occurring β-glucan that is extracted from the brown seaweed *Laminaria digitata*. Glucans are polysaccharides of D-glucose monomers linked by glycosidic bonds. β-Glucans are abundant in the bran of barley and oats, and to a much lesser degree, in rye and wheat. They are useful in human nutrition as texturing agents and as soluble fiber supplements. *L. digitata* is used for sea-vegetable production in Ireland and France, and is an important food item in Japan.

Laminarin is not structurally related to any known immunotoxic chemical. Numerous studies confirm that it has virtually no acute, subacute, or subchronic toxicity, and no mutagenic effects to animals (Table 1). In the subchronic and teratology studies, there were no effects on the immune system (e.g., lymph nodes, thymus, spleen), and no effects were seen in the dermal sensitization study.

TABLE 1. Mammalian toxicity of laminarin	
Study	**Results**
Acute oral (870.1100)	Rats (5/sex) received 2000 mg/kg laminarin (91% purity) at a volume of 10 mL/kg in water and were monitored for 14 days. No mortality occurred and no adverse clinical signs were noted. There was no effect on body weight, and there were no visible lesions at gross necropsy. The LD_{50} was >2000 mg/kg.
Acute subcutaneous No guideline	Rats (5/sex) received subcutaneous administration of 1000 mg/kg laminarin (91% purity) in a volume of 5 mL/kg in water and were monitored for 14 days. No mortality occurred and no adverse clinical signs were noted. There was no effect on body weight, and no visible lesions at gross necropsy. The acute subcutaneous toxicity was >1000 mg/kg.
Acute dermal (870.1200)	5000 mg/kg laminarin (91% purity) was applied in a volume of 8 mL/kg to the skin of 5 rats/sex and the animals were monitored for 14 days. No mortalities occurred, and no adverse clinical signs were noted. There was no effect on body weight, and no visible lesions at gross necropsy. The LD_{50} was >5000 mg/kg.
Acute inhalation (870.1300)	Rats (5/sex) were exposed for 4 hours to an aerosol of laminarin (91% purity) as a 10% solution in distilled water. The maximum attainable concentration was 1.02 mg/L. The animals were observed for 14 days. No mortality occurred, and no adverse clinical signs were noted. There was no effect on body weight, and no visible lesions at gross necropsy. The LC_{50} was >1.02 mg/L.
Primary eye irritation (870.2400)	Three rabbits received 0.1g of laminarin (91% purity) in the conjunctival sac of the left eye. The mean indices for chemosis, iritis, and corneal effects at 24, 48 and 72 hrs were all zero. The mean index for redness was 0 in one rabbit and 0.33 in the other two rabbits. Laminarin was non-irritating.
Primary dermal irritation (870.2500)	Three rabbits had 0.5 g of laminarin (91% purity) moistened with 0.5 mL of water applied to the shaved flank under a semiocclusive dressing for 4 hours. The mean index for edema for all rabbits was zero at 24, 48, and 72 hrs. The mean index for erythema was zero for two rabbits, and 1.00 for the third rabbit. Laminarin was non-irritating.
Dermal sensitization (870.2600)	In a Magnusson and Kligman maximization test, laminarin was not a sensitizer to guinea pigs.
4-Week oral toxicity (870.3050)	Rats (5/sex) received a daily oral gavage of laminarin (97.6% purity) at a dose of 1000 mg/kg for 28 consecutive days. There were no toxicologically significant findings for mortality, observations, neurotoxicity assessment, body weight, food consumption, hematology, clinical chemistry, organ weight, or macroscopic or microscopic observations. The NOEL was 1000 mg/kg/day.
90-Day oral toxicity (870.3100)	Laminarin (94.9% purity) was administered orally to rats at the limit dose of 1000 mg/kg/day for 90 consecutive days. There were no deaths, and no treatment-related changes in clinical observations, body weight, food and water consumption, organ weight, hematology, clinical chemistry, urinalysis, histopathology, ophthalmology, or functional tests. The NOAEL was 1000 mg/kg/day.
90-Day oral toxicity (870.3150)	Laminarin (94.9% purity) was administered orally to dogs (4/sex) at the limit dose of 1000 mg/kg/day for 90 consecutive days. There were no treatment-related deaths, and no treatment-related clinical (except for a slight increase of diarrhea and/or soft stools in the treated group) or macroscopic changes. There were no changes in functional tests or ophthalmology. There were no statistical differences in body weight, food and water consumption, organ weight, hematology, clinical chemistry, or urinalysis. No treatment-related histopathological changes were seen. The NOAEL was 1000 mg/kg/day.
Mutagenicity (870.5100)	Laminarin (97.6% purity) was tested with and without S9 activation against *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strains WP2 (pKM101) and WP2uvrA (pKM101) using a maximum concentration of 5000 µg/plate. In two independent assays, no biologically significant increase in the number of revertants was noted in the strains tested. It is noted that with metabolic activation a slight, but statistically significant, increase in the number of revertants was seen at the 5000 µg/plate dose in the first assay in the *E. coli* strains, and at doses of 150, 1500, and 5000 µg/plate in the second assay in the WP2uvrA (pKM101) strain only. This effect was not biologically significant, not dose-related, and was not attributed to significant mutagenic activity.
In vivo bone marrow	Mice (5/sex) received two oral treatments of laminarin (94% purity) at 500, 1000, or 2000

micronucleus test (870.5395)	mg/kg/day at a 24-hour interval. The animals were killed 24 hrs after the last treatment and bone marrow smears were prepared. The mean number of micronucleated polychromic erythrocytes and the polychromatic erythrocyte/normochromic erythrocyte ratio of treated mice were equivalent to those of the controls.
In vivo mammalian cell gene mutation (870.5300)	In two independent experiments, laminarin was tested with and without S9 activation at concentrations of 312.5, 625, 1250, 2500, and 5000 µg/mL in L5178Y TK+/- mouse lymphoma cells. Laminarin did not induce any noteworthy increases in the mutation frequency, with or without S9.
Teratogenicity (870.3700)	Laminarin was administered orally to 21 pregnant SPF rats at 1000 mg/kg/day (number of days not reported) as a solution of 5 mL/kg of sterile water. There were no treatment-related deaths, clinical changes, or macroscopic changes. Body weight, food and water consumption, and uterus weight were similar to controls. There were no significant reproductive effects or fetal abnormalities. The NOAEL was 1000 mg/kg/day.
Teratogenicity (870.3700)	Laminarin as administered orally to 13 pregnant albino SPF New-Zealand White rabbits at 1000 mg/kg/day (number of days not reported) as a solution of 5 mL/kg of sterile water. There were no treatment-related deaths, clinical changes, or macroscopic changes. Body weight, food and water consumption, and uterus weight were similar to controls. There were not significant treatment-related reproductive effects or fetal abnormalities. The NOAEL was 1000 mg/kg/day.

Based on the results of these studies, the registrant requests that the requirement for an immunotoxicity study be waived.

Reviewer's Comments

The information provided is sufficient to justify a waiver for immunotoxicity testing of laminarin. The information was apparently submitted to support registration of Vacciplant. If that is the case, information concerning the inert ingredients in the product will need to be submitted.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)

MRID 47264947

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:	
STUDY TYPE:	Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)
MRID NO:	47464947
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin
STUDY NO:	10041220
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Institut fur Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany
TITLE OF REPORT:	Acute Toxicity of Laminarin to *Daphnia magna* in a 48-Hour Semi-Static Immobilization Test.
AUTHOR:	Hert, J.
STUDY COMPLETED:	March 30, 2001
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with 40 CFR Part 160.
STUDY SUMMARY:	In a semi-static acute toxicity test, groups of *Daphnia magna* were exposed to nominal concentrations of 0, 10, 30, or 100 mg/L laminarin in reconstituted water for 48 hours. The test solutions were renewed after 24 hours. No daphnid mortality or immobility was seen in any of the test groups after 24 or 48 hours. The measured test material concentration in fresh 100 mg/L solution ranged from 84 to 90% of nominal, and in 24-hour-old solution ranged from 81 to 97% of nominal. In this study, the 48-hr NOEC and EC_0 were each ≥100 mg/L, and the LOEC and EC_{50} were >100 mg/L.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (94% purity), Batch No. 99S10, a beige solid with an expiration date of January 1, 2003. A certificate of analysis was provided on p. 46 of MRID 47264947. After receipt at the testing facility, the test material was stored in the original container at room temperature in darkness.

Test Methods

A 48-hour semi-static acute toxicity test was conducted to determine the acute toxicity of the test material to *Daphnia magna*. The daphnids were bred at the testing facility under temperature, lighting and water conditions similar to those used for the test. The line was originally supplied in 1997 by the Umweltbundesamt, Institut fur Wasser-, Boden-, und Lufthygiene, Berlin. The test organisms were not first brood progeny. The daphnids were acclimated to the test conditions for six hours prior to test start.

The test water was reconstituted deionized water with a hardness of 250 mg/L as $CaCO_3$. Constituents of the reconstituted water are given on p. 13 of MRID 47264947. The test material concentrations used in the test were 0, 10, 30, and 100 mg/L. The test solutions were prepared by dissolving 200 mg of the test material in 2 L of the test water to provide a nominal concentration of 100 mg/L. This stock solution was diluted with the appropriate volume of test water to provide the 10 and 30 mg/L test solution concentrations.

The test containers were 100 mL glass beakers containing 50 mL of the appropriate test solution. At test start 10 daphnids were added to each test container. Each of the test material concentrations and the control (test water only) was replicated twice. The test solutions were renewed after 24 hours. The pH, dissolved oxygen concentration, and temperature were determined for each test solution at test start and end. Additionally, samples of the test solutions were collected at test start and incubated for 24 hours under the test conditions (but without daphnids) prior to HPLC analysis to determine the actual test material concentration in the solutions.

The test containers were placed in a controlled environment room set to maintain a temperature of 21°C with a photoperiod of 16 hours light:8 hours darkness. The light intensity was 480 lux.

The test organisms were monitored for immobility and mortality at 24 and 48 hours. Daphnids not able to swim within 15 seconds after gentle agitation of the test container were considered immobile. The NOEC and EC_0 were determined from the raw data.

Results Summary

No daphnid mortality or immobility was seen in any of the test groups after 24 or 48 hours. During the test the pH ranged from 7.8 to 7.9, and the dissolved oxygen concentration was ≥8.4 mg/L (>60% saturation). The water temperature was 21°C.

Analysis of the 100 mg/L test solution samples at test start showed that the actual test material concentration ranged from 84 to 90% of nominal. After 24 hours of incubation, the actual test material concentration ranged from 81 to 97% of nominal. The analytical method is described in Appendix III of MRID 47264947. The 10 and 30 mg/L solutions were not analyzed since there were no adverse effects to any daphnids during the test.

Study Author's Conclusions

The study author concluded that the 48-hr NOEC and EC_0 were each ≥100 mg/L, and the LOEC and EC_{50} were >100 mg/L.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)

MRID 47264948

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
MRID NO:	47464948
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin
STUDY NO:	00-907005-021
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	SEPC, ZAC de Milieux, 42160 Andrezieux Boutheon, France
TITLE OF REPORT:	Acute Toxicity in Freshwater Fish (96h) *Oncorhynchus mykiss* [*sic*]
AUTHOR:	Licata-Messana, L.
STUDY COMPLETED:	January 22, 2001
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with GLP standards (1999/11/CE – Decret n 98-1312).
STUDY SUMMARY:	In a semi-static acute toxicity test, groups of *Danio rerio* were exposed to a nominal concentration of 0 or 100 mg/L laminarin in tap water for 96 hours. The test solutions were renewed after 24, 48, and 72 hours. No mortality or adverse clinical signs were seen in any of the test groups. The geometric mean measured test material concentration in test material solutions was determined to be 111.4 mg/L. In this study, the 96-hr LC_{50} for laminarin in *Danio rerio* was >100 mg/L.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (98% purity), Code H11, Batch No. S 210300, a beige powder with an expiration date of January 1, 2003. A certificate of analysis was provided on p. 15 of MRID 47264948. After receipt at the testing facility, the test material was stored at room temperature in darkness.

Test Methods

A 96-hour semi-static acute toxicity test was conducted to determine the acute toxicity of the test material to the freshwater fish *Danio rerio*. The fish were supplied by HB Developpment elevage (Batch No. D000216) and were acclimated for 12 days prior to the test. The holding water was not described, except that the pH ranged from 7.75 to 8.19 and the temperature from 21.0 to 22.0°C. The fish food was not identified, but feeding was stopped about about 29 hours before test start. Length of the test fish ranged from 3.2 to 3.8 cm (measured at the end of the test), with a mean of 3.5 cm (from 10 randomly selected). The biological loading rate was 1.05 g/L. No mortality occurred in the 7 days preceding the test.

Prior to the main study, a preliminary test was conducted (no test details were provided). Since microbial growth was observed and no mortality was seen in the preliminary test, the main test used a semi-static system with a single concentration of 100 mg/L.

The dilution water was tap water with a measured hardness of 90 to 100 mg $CaCO_3$. No other water quality parameters were provided.

Fresh test solutions were prepared at test start and at 24, 48, and 72 hours. The solutions were prepared by weighing 400 mg of test material into 4 L of tap water to produce a nominal concentration of 100 mg/L. The pH, dissolved oxygen concentration, and temperature of the control and test material solutions were determined at 0 (new solutions), 24, (old and new solutions), 48 (old and new solutions), 72 (old and new solutions), and 96 hours (old solutions). Additionally, a sample was collected from each fresh control and test material solution prepared at 0, 24, and 96 hours. The samples were frozen and transmitted to the analytical testing facility (Defitraces, ZA "Les Andres", 150, rue Pre-Magne, F-69126 Brindas) to determine the test material concentrations.

The test containers were not described. Each test group contained 10 fish. The photoperiod during the test was 12 hours light:12 hours darkness.

Results Summary

The measured concentration of the test material in the 100 mg/L solution was 155 mg/L in the 0 hour samples. The measured concentrations in the 24 hour and 96 hour samples were below 100 mg/L, and were estimated to be 80 and 55 mg/L, respectively. These values had to be estimated since the limit of quantitation (LOQ) for laminarin is 100 mg/L. The geometrical mean for the test material concentrations was calculated as 111.4 mg/L. Since the variation of the test concentration levels was <20%, all concentrations are expressed as nominal. All samples of the control solutions were below the LOQ for laminarin.

The solution temperature ranged from 20.3 to 22.0°C, and pH ranged from 7.20 to 8.13. The dissolved oxygen concentration in the solutions ranged from 96 to 107% of saturation.

No mortality occurred in the control or test material group fish, and clinical observations during the test were unremarkable.

The study states that a reference control of potassium dichromate (Carlo erba, Batch 810292281) was used to validate the test, producing an LC_{50} of 221 mg/L.

Study Author's Conclusions

The study author concluded that the 96-hr LC_{50} for laminarin in *Danio rerio* was >100 mg/L.

Reviewer's Conclusion

The loading rate (1.05 g) slightly exceeded the OPPTS 850.1075 recommended rate of 0.8 g. The fasting period prior to the test was 29 hours rather than the recommended 48 hours. Tap water (presumably dechlorinated) was used as the dilution water, but the recommended daily chlorine analysis was not performed. The reviewer does not believe that these variations invalidate the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)

MRID 47264948

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
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Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
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Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
MRID NO:	47464949
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin
STUDY NO:	00-907005-021
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	SEPC, ZAC de Milieux, 42160 Andrezieux Boutheon, France
TITLE OF REPORT:	Acute Toxicity in Freshwater Fish (96h) *Danio rerio* [*sic*] *mykiss*
AUTHOR:	Licata-Messana, L.
STUDY COMPLETED:	January 22, 2001
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with GLP standards (1999/11/CE – Decret n 98-1312).
STUDY SUMMARY:	In a semi-static acute toxicity test, groups of *Oncorhynchus mykiss* were exposed to a nominal concentration of 0 or 100 mg/L laminarin in tap water for 96 hours. The test solutions were renewed at 24, 48, and 72 hours. No mortality or adverse clinical signs were seen in any of the test groups. The geometric mean measured test material concentration in the test material solutions was determined to be 81.2 mg/L. In this study, the 96-hr LC_{50} for laminarin in *Oncorhynchus mykiss* was >100 mg/L.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (90% purity), Code H11, Batch No. S 01/2000, a beige powder with an expiration date of January 1, 2003. A certificate of analysis was provided on p. 15 of MRID 47264949. After receipt at the testing facility, the test material was stored at room temperature in darkness.

Test Methods

A 96-hour semi-static acute toxicity test was conducted to determine the acute toxicity of the test material to the freshwater fish *Oncorhynchus mykiss*. The fish were supplied by S.A. Charles Murgat (Batch No. OM 000921) and were acclimated for 12 days prior to the test. The holding water was not described, but the pH ranged from 7.43 to 7.81 and the temperature from 11.9 to 14.2°C. The fish food was not identified, but feeding was stopped about 24 hours before test start. Length of the test fish ranged from 6.8 to 9.5 cm (measured at the end of the test), with a mean of 8.5 cm (from 7 randomly selected). The biological loading rate was 1.3 g/L. The batch mortality was 1.9% in the 7 days preceding the test.

Prior to the main study, a static 96-hr preliminary test was conducted using test material concentrations of 0, 1, 5, 10, 50, and 100 mg/L. Since microbial growth was observed and no mortality was seen in the preliminary test, the main test used a semi-static system and a single concentration of 100 mg/L.

The dilution water was tap water with a measured hardness of 90 to 105 mg $CaCO_3$. No other water quality parameters were provided.

Fresh test solutions were prepared at test start and at 24, 28, and 72 hours. The solutions were prepared by weighing 3000 mg (the study incorrectly gives 3000 g) of test material into 30 L of tap water to produce a nominal concentration of 100 mg/L. The pH, dissolved oxygen concentration, and temperature of the control and test material solutions were determined at 0 (new solutions), 24, (old and new solutions), 48 (old and new solutions), 72 (old and new solutions), and 96 hours (old solutions).

Additionally, a sample was collected from each fresh control and test material solution prepared at 0, 24, and 96 hours. The samples were frozen and transmitted to the analytical testing facility (Defitraces, ZA "Les Andres", 150, rue Pre-Magne, F-69126 Brindas) to determine the test material concentrations.

The test containers were not described. Each test group contained 7 fish. The photoperiod during the test was 12 hours light:12 hours darkness.

Results Summary

The measured concentration of the test material in the 100 mg/L solution was 110 mg/L in the 24-hour sample. The measured concentrations in the 0 hour and 96 hour samples were below 100 mg/L, and were estimated to be 60 and 55 mg/L, respectively. These values had to be estimated since the limit of quantitation (LOQ) for laminarin is 100 mg/L. The geometrical mean for the test material concentrations was calculated as 81.2 mg/L. Since the variation of the test concentration

levels was <20%, all concentrations are expressed as nominal. All samples of the control solutions were below the LOQ for laminarin.

The solution temperature ranged from 15.4 to 16.3°C, and pH ranged from 7.41 to 8.00. The dissolved oxygen concentration in the solutions ranged from 81 to 104% of saturation.

No mortality occurred in the control or test material group fish, and clinical observations during the test were unremarkable.

The study states that a reference control of potassium dichromate (Merck, Batch K26852864 947) was used to validate the test, producing an LC_{50} of 572.3 mg/L.

Study Author's Conclusions

The study author concluded that the 96-hr LC_{50} for laminarin in *Oncorhynchus mykiss* was >100 mg/L.

Reviewer's Conclusion

The loading rate (1.3 g) slightly exceeded the OPPTS 850.1075 recommended rate of 0.8 g. The fasting period prior to the test was 24 hours rather than the recommended 48 hours. Tap water (presumably dechlorinated) was used as the dilution water, but the recommended daily chlorine analysis was not performed. The reviewer does not believe that these variations invalidate the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Avian Acute Oral Toxicity (OPPTS 850.2100)

MRID 47264950

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Avian Acute Oral Toxicity (OPPTS 850.2100)
MRID NO:	47464950
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin (85% purity)
STUDY NO:	GOM/001/022173
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England
TITLE OF REPORT:	Laminarin. Acute Oral Toxicity (LD_{50}) to the Bobwhite Quail
AUTHOR:	Rodgers, M.H.
STUDY COMPLETED:	April 23, 2002
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with 40 CFR Part 160
STUDY SUMMARY:	In an acute oral toxicity study, 14-month-old northern bobwhite (*Colinus virginianus*) were administered a single nominal oral dose of 0, 500, 1000, or 2000 mg/kg body weight laminarin (purity, 85%) and observed for 14 days. There were no mortalities or adverse clinical signs, and all birds appeared healthy during the test. Body weight and food consumption were similar among all groups, and macroscopic examination revealed no abnormalities in any birds. The acute oral LD_{50} was >2000 mg/kg, the highest dose tested. The NOEL was 2000 mg/kg.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (85% purity), Batch No. S012000, a light brown powder with an expiration date of January 1, 2003. A certificate of analysis is provided on p. 21 of MRID 47264950. After receipt at the testing facility the test material was stored at room temperature in the dark.

Test Methods

A 14-day study was conducted to evaluate the acute toxicity of the test material when administered as a single oral dose to northern bobwhite (*Colinus virginianus*). Apparently healthy northern bobwhite were obtained from Monkfield Nutrition (Cambridgeshire), where they had been fed commercial pheasant pre-breeder pellets and had received no medication. All birds were from the same hatch and phenotypically indistinguishable from wild birds. The birds were acclimated to the test facility for 15 days prior to dosing, and at the time of treatment were approximately 14 months old.

Throughout acclimation and testing, the birds were fed standard HRC layer diet pellets (Special Diet Services, Witham, Essex). Diet composition is given in Appendix 4 of MRID 47264950. The diet was not analyzed for contaminants, but nominally contained no added antibiotics or other non-nutritional feed additives other than the binding agent. Food was offered *ad libitum*, with the exception of the 19-hour period prior to dosing. Potable water suitable for human consumption was available *ad libitum*.

The birds were assigned to treatment groups on the basis of body weight, to ensure all groups had similar mean body weights and body weight distributions. Each group consisted of five males and five females. The groups were then randomly assigned to the treatments.

Birds were housed by sex in groups of two or three/cage. The cages were constructed of plastic-coated steel wire mesh and measured approximately 0.31 x 0.39 x 0.24 m. Each cage contained an automatic drinker and food hopper. The cages were housed in a room with mean daily maximum and minimum temperatures of 20°C and 18°C, respectively. Mean daily relative humidity was 40%. Ventilation was provided by fans, and the photoperiod was 10 hrs light:14 hrs darkness.

Prior to the study, a rangefinding study was conducted using one male and one female from the testing facility stock. Housing, environmental conditions, food, and dose preparation and procedures were similar to those used in the main study. The treatments were 200 and 2000 mg/kg laminarin. There were no moralities and no clinical signs of toxicity. Based on the rangefinding results, dose levels of 0, 500, 1000, and 2000 mg/kg laminarin were selected for the main study. The dose levels were for the test substance as supplied, and were not adjusted for purity.

The birds received a single dose of the appropriate treatment by oral intubation, using a disposable syringe and a Ch 10 Nelaton plastic catheter. Prior to formulating the dose solutions, the test material was ground with a mortar and pestle. Methylcellulose (1%) was used as the control and as the vehicle for the test material. The birds were dosed at a rate of 10 mL/kg body weight. Care was taken to ensure each bird had ingested the entire dose before being returned to its cage.

The birds were observed four times on the day of dosing and twice daily thereafter. Observations were for mortality, health, and clinical signs. Individual body weight was recorded on test days -15,

-7, 0 (immediately prior to dosing), 7, and 14. Group mean food consumption was determined for days -15 to -8, -7 to -1, 1 to 7, and 8 to 14.

At study end, the birds were sacrificed, and all control birds and all birds in the 2000 mg/kg group were examined macroscopically. Tissues examined included the digestive tract, liver, kidneys, heart, spleen, muscle, and subcutaneous fat.

Results Summary

No mortalities occurred in any group. There were no treatment-related clinical signs, and all birds remained in good health for the duration of the study. Body weight and food consumption were similar among all groups (Tables 1 and 2). Macroscopic examination revealed no abnormalities in any birds.

TABLE 1. Group mean body weights and body weight changes (g)

Treatment	Sex	Day of study								
		Body weight					Body weight change			
		-15	-7	0	7	14	-15 to -7	-7 to 0	0 to 7	7 to 14
Control	M	189	189	186	188	188	0	-3	+2	0
	F	187	187	184	186	186	0	-3	+2	0
Laminarin (500 mg/kg)	M	189	188	182	185	186	-1	-6	+3	+1
	F	186	184	178	181	182	-2	-6	+3	+1
Laminarin (1000 mg/kg)	M	188	183	181	180	181	-5	-2	-1	+1
	F	186	188	184	185	185	+2	-4	+1	0
Laminarin (2000 mg/kg)	M	187	183	183	188	190	-4	0	+5	+2
	F	186	183	182	182	182	-3	-1	0	0

Data from p. 16, MRID 47264950

TABLE 2. Group mean food consumption (g/bird/day)

Treatment	Sex	Day of study			
		-15 to -8	-7 to -1	1 to 7	8 to 14
Control	M	13	14	14	13
	F	12	13	13	13
Laminarin (500 mg/kg)	M	12	12	14	13
	F	10	12	13	13
Laminarin (1000 mg/kg)	M	11	13	13	14
	F	12	12	13	12
Laminarin (2000 mg/kg)	M	12	14	15	14
	F	12	13	13	13

Data from p. 17, MRID 47264950

Study Author's Conclusions

The study author concluded that under the conditions of this study, the acute oral LD_{50} value for laminarin in bobwhite was >2000 mg/kg, the highest dose tested. The NOEL was 2000 mg/kg.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Avian Dietary Toxicity (OPPTS 850.2200)

MRID 47264951

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Avian Dietary Toxicity (OPPTS 850.2200)
MRID NO:	47464951
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin (85% purity)
STUDY NO:	GOM/002/014410
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England
TITLE OF REPORT:	Laminarin. Dietary Toxicity (LD_{50}) to the Bobwhite Quail
AUTHOR:	Rodgers, M.H.
STUDY COMPLETED:	April 23, 2002
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with 40 CFR Part 160.
STUDY SUMMARY:	In a dietary toxicity study, bobwhite (*Colinus virginianus*) chicks were provided diet containing 0, 156, 313, 625, 1250, 2500, or 5000 ppm laminarin (purity, 85%) for 5 days, then provided untreated diet for an additional three days. There were no treatment-related effects on mortality, body weight, or feed consumption, and post-mortem macroscopic examination was unremarkable. The dietary LD_{50} was >5000 ppm, and the NOEL was 5000 ppm.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (85% purity), Batch No. S012000, a light brown powder with an expiration date of January 1, 2003. A certificate of analysis is provided on p. 19 of MRID 47264951. After receipt at the testing facility the test material was stored at room temperature in the dark.

Test Methods

The study was conducted to evaluate the dietary toxicity of the test material to the northern bobwhite (*Colinus virginianus*). Apparently healthy northern bobwhite were obtained from Monkfield Nutrition (Roysten, Herts.), where they had been fed pheasant breeder diet and had received no medication. The birds were one day old on arrival at the test facility and were phenotypically indistinguishable from wild birds. Due to the size and age of the birds, no determination of sex was attempted. The birds were acclimated to the test facility for 7 days.

Upon arrival, birds were provided the basal diet (HLS chick meal, Special Diet Services, Witham, Essex). Diet composition is given in Appendix 4 of MRID 47264951. The diet was known to contain no antibiotics or other non-nutritional feed additives. The birds were offered the diet until the test diets were introduced (control birds continued to receive the basal diet during the test). Diet and domestic quality potable water were available *ad libitum* at all times.

The birds were assigned to treatment groups on the basis of body weight, to ensure all groups had similar mean body weights and body weight distributions. Each group consisted of 10 birds. The groups were then randomly assigned to the treatments.

Birds were housed by group in printboard boxes (approximately 80 x 60 x 50 cm) fitted with wire mesh lids. Each box contained a low level feeder covered with wire mesh to minimize spillage, and a drinker. Wood shavings (RS Biotech, Finedon, Northamptonshire) were provided as bedding material. An infrared heat source was suspended over each pen. The pens were housed in a room with mean daily maximum and minimum temperatures of 28°C and 26°C, respectively. Mean daily relative humidity was 54%. Ventilation was provided by fans, and the photoperiod was 14 hrs light:10 hrs darkness.

The treatments consisted of basal diet containing 0 (two groups), 156, 313, 625, 1250, 2500, or 5000 ppm laminarin. The dose levels were for the test material as supplied, with no correction for purity. To prepare the treated diet, a pre-mix was prepared by mixing the appropriate amount of the test material with untreated basal diet. The pre-mix was blended in a T10 Turbula mixer for a minimum of 5 minutes, and the individual treatment diets were prepared by diluting the pre-mix with the basal diet. The test diets were then mixed for a minimum of 5 minutes. The test diets were prepared one day prior to the test and were stored at ambient temperature for the duration of the test. Analysis for stability, homogeneity, and concentration of the test material in the diet was not conducted, due to previous problems in other matrices (uspecified). The birds were fed the appropriate test diet *ad libitum* for 5 days, then fed the untreated basal diet for three additional days.

The birds were observed for mortality, health, and clinical signs at least 5 times/day on test days 1-5, and at least twice/day on days 6-8. Group mean bodyweights were determined on days -3, 0 (immediately prior to introduction of the test diets), 5, and 8. Group mean food consumption was

determined for days -3 to -1, 1 to 5 (daily), and 6 to 8, by weighing the hopper contents at the beginning and end of each time period.

At test end, the birds were sacrificed and all birds in the 5000 ppm group and one of the control groups were examined macroscopically. Tissues examined included the digestive tract, liver, kidneys, heart, spleen, muscle, and subcutaneous fat. Premature decedents also received a macroscopic examination.

Results Summary

One control group bird and one 2500 ppm group bird were found dead on day 5. Both birds had appeared subdued prior to death. The mortality of the 2500 ppm bird was not considered to be treatment-related, since there was no mortality or adverse clinical signs in the 5000 ppm group. The remaining birds were in good health throughout the study, and the excreta of all groups appeared normal. Body weight gain and group mean food consumption were variable, with no treatment related effects (Tables 1 and 2). No abnormalities were found in any bird, including the premature decedents, at macroscopic examination.

TABLE 1. Group mean body weights and body weight changes (g)

Treatment	Day of study						
	Body weight				Body weight change		
	-3	0	5	8	-3 to 0	0 to 5	5 to 8
Control 1	10.2	13.8	21.0	24.3	3.6	7.2	3.3
Control 2	10.1	13.3	20.0*	23.2	3.2	6.7	3.2
Laminarin (156 ppm)	10.2	13.6	18.9	22.5	3.4	5.3	3.6
Laminarin (313 ppm)	10.0	13.3	20.1	23.7	3.3	6.8	3.6
Laminarin (625 ppm)	10.1	13.5	19.9	23.6	3.4	6.4	3.7
Laminarin (1250 ppm)	10.0	13.0	19.1	22.5	3.0	6.1	3.4
Laminarin (2500 ppm)	10.1	13.1	18.3*	21.7*	3.0	5.2	3.4
Laminarin (5000 ppm)	10.1	13.6	19.4	22.4	3.5	5.8	3.0

Data from p. 17, MRID 47264951
*Only 9 birds weighed

TABLE 2. Group mean food consumption (g/bird/day)								
Treatment	Day of study							
	-3 to 1	1	2	3	4	5	1 to 5	6 to 8
Control 1	3.8	5.4	4.3	4.3	4.6	4.3	4.6	6.1
Control 2	3.5	6.5	3.8	3.8	3.5	4.2	4.4	5.6
Laminarin (156 ppm)	3.4	5.6	3.3	3.3	4.2	3.9	4.2	6.0
Laminarin (313 ppm)	3.2	4.6	4.1	4.1	4.3	4.6	4.3	5.8
Laminarin (625 ppm)	3.6	6.3	4.7	4.7	4.4	4.6	4.9	5.0
Laminarin (1250 ppm)	3.3	4.5	2.7	2.7	3.6	3.1	3.5	3.7
Laminarin (2500 ppm)	2.9	4.5	2.9	2.9	3.2	3.3	3.3	4.4
Laminarin (5000 ppm)	3.1	4.8	3.3	3.3	3.5	3.5	3.8	4.7

Data from p. 18, MRID 47264951

Study Author's Conclusions

The study author concluded that there were no treatment-related mortalities, and that the dietary LD_{50} value for laminarin to the bobwhite was >5000 ppm. The NOEL was 5000 mg/kg.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Honey Bee Acute Contact Toxicity (OPPTS 850.3020)

MRID 47264952

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Honey Bee Acute Contact Toxicity (OPPTS 850.3020)
MRID NO:	47464952
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin (91% purity)
STUDY NO:	20001342/01-BLEU
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Arbeitsgemeinschaft, GAB Biotechnologie GmbH & IFU Umveltanalytik GmbH, Eutinger Str. 24, D-75223 Niefern-Oschelbronn, Germany
TITLE OF REPORT:	Assessment of Side Effects of Laminarin to the Honey Bee, *Apis mellifera* L. in the Laboratory
AUTHOR:	Kling, A.
STUDY COMPLETED:	November 13, 2000
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with OECD GLP guidelines.
STUDY SUMMARY:	Laboratory limit tests were conducted to determine the acute oral and acute contact toxicity of laminarin (91% purity) to the honey bee (*Apis mellifera*). Both tests used a nominal dose of 100.00 µg laminarin/bee. In the oral toxicity test, groups of caged bees were provided the test material in a 50% w/v sucrose solution for six hours, then monitored for mortality after 2, 4, 24, and 48 hours. The test also included an untreated control group (sucrose solution only) and a reference control group (dimethoate). After 48 hours, there was no difference in mortality of the untreated control and test material groups. Response of the reference control bees was appropriate. In the contact

	toxicity test, bees were anesthetized with carbon dioxide and received an individual application of tap water (untreated control), laminarin, or dimethoate to the ventral thorax. After 48 hours, the uncorrected mean mortality was 10.0% for the untreated control group and 4.0% for the test material group. Response of the reference control bees was appropriate. In this test, the 48-hr oral toxicity LD_{50} for laminarin was >118.64 µg/bee, and the 48-hr contact toxicity LD_{50} was >100.00 µg/bee.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (91% purity), Batch No. S012000, a white hygroscopic powder with an expiration date of January 1, 2003. A certificate of analysis was provided on p. 32 of MRID 47264952. After receipt at the testing facility, the test material was stored at ambient temperature under dry conditions.

The reference control was Perfekthion (400 g/L dimethoate), with an expiration date of October 31, 2000.

Test Methods

Laboratory limit tests were conducted to determine the acute oral and acute contact toxicity of the test material to the honey bee. The bees were approximately 22 to 32 days old and were derived from a healthy colony that descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany. The colonies were randomly inspected periodically according to the rules of beekeeping, and were examined for a reportable bee epidemic by a bee specialist. The test bees were collected randomly from the outer combs of the colony.

In both tests, the bees were kept in 10 x 5.5 x 8.5 cm steel cages with perforated board bottoms to supply ventilation. The front side of the cages contained a transparent pane for observation. The cages were lined with filter paper. Each cage contained 10 bees and each treatment was replicated five times.

Both tests used a nominal dose of 100.00 µg laminarin/bee. The test material solutions were slightly over-formulated to ensure a dose in excess of nominal. For the oral toxicity test, the test material was dissolved in tap water to provide a stock solution. The appropriate amount of stock solution was mixed with the appropriate amount of 50% w/v aqueous sucrose solution such that the target dose was found in 20 µL of test solution. The reference control bees received a nominal dose of 0.17 µg dimethoate/bee. Untreated control bees received sucrose solution only. The bees were starved for one hour and 52 minutes prior to the test. The test solution (250 µL) was offered for approximately 6 hours. After the test solution was withdrawn, the bees were supplied with 50% sucrose solution *ad libitum*.

For the contact toxicity test, the test material was dissolved in tap water to produce the required concentration. The bees were anesthetized with carbon dioxide and treated individually by topical

application of 2 µL of test solution to the ventral side of the thorax using a microapplicator. The reference control bees received a dose of 0.30 µg dimethoate/bee, and the untreated control bees received tap water only. After application, the bees were returned to their cages and fed 50% sucrose solution *ad libitum*.

During both tests, the bees were kept in darkness. They were observed under red light for abnormal behavior, and the number of dead bees in the individual cages was recorded after 2, h, 24, and 48 hours. The average mortality in each treatment was calculated after correcting for control mortality using the Schneider-Orelli formula. During the test the temperature ranged from 24.0 to 25.0°C and the relative humidity from 62 to 80%.

Results Summary

Results of the oral toxicity test are summarized in Table 1. The uncorrected mean 48-hr mortality was 2% for both the untreated control and test material groups, and there was no difference in the behavior of bees between the two groups. Response of the reference control bees was appropriate.

TABLE 1. Mean mortality of honey bees exposed orally to laminarin

Treatment	Actual intake (µg/bee)	Mortality (%)		Corrected mortality (%)[a]	
		24 hrs	48 hr	24 hrs	48 hrs
Untreated control (50% w/v sucrose solution)	--	2.0	2.0	--	--
Laminarin 100.00 µg/bee	118.64	0.0	2.0	0.0	0.0
Reference control Perfekthion 0.17 µg dimethoate/bee	0.18	76.0	92.0	75.5	91.8

Data from p. 12, MRID 47264952

[a]Corrected for control mortality using Schneider-Orelli formula

Results of the contact toxicity test are summarized in Table 2. The uncorrected mean 48-hr mortality was 10.0% for the untreated control group and 4.0% for the test material group. There was no difference in the behavior of bees between the two groups. Response of the reference control bees was appropriate.

TABLE 2. Mean mortality of honey bees receiving laminarin applied to the thorax

Treatment	Mortality (%)		Corrected mortality (%)[a]	
	24 hrs	48 hr	24 hrs	48 hrs
Untreated control (Tap water)	10.0	10.0	--	--
Laminarin 100.00 µg/bee	0.0	4.0	0.0	0.0
Reference control Perfekthion 0.17 µg dimethoate/bee	94.0	94.0	93.3	93.3

Data from p. 13, MRID 47264952

[a]Corrected for control mortality using Schneider-Orelli formula

Study Author's Conclusions

The study author concluded that the 48-hr oral toxicity LD_{50} for laminarin was >118.64 µg/bee, and the 48-hr contact toxicity LD_{50} was >100.00 µg/bee.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Algal Toxicity, Tiers I and II (OPPTS 850.5400)

MRID 47264953

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Algal Toxicity, Tiers I and II (OPPTS 850.5400)
MRID NO:	47464953
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	H11
STUDY NO:	990714
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	Istituto di Ricerche Biomediche, "A. Marxer" RBM S.p.a., Via Ribes, 1, 10010 Colleretto Giacosa (TO), Italy
TITLE OF REPORT:	H11. Algal Growth Inhibition Study
AUTHOR:	Gnemi, P.
STUDY COMPLETED:	May 17, 2000
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with OECD GLP guidelines.
STUDY SUMMARY:	A 72-hour laboratory study was conducted to determine the effects of H11 (100 mg/L, nominal) on the growth of the unicellular freshwater green alga *Selenastrum capricornutum*. An untreated control was also included in the test. At test end, cell growth and density were similar in the test material and control group. The 72-hour EbC_{50} and ErC_{50} for H11 were >100 mg/L, and the NOECb and NOECr were >100 mg/L. The mean measured concentrations of H11 in the test solutions at 0 and 72 hours were 101.64% and 97.75% of nominal, respectively.
CLASSIFICATION:	**Acceptable**, provided that confirmation that H11 is laminarin is submitted.

Test Material

H11, Batch No. 99521, a pale yellow powder supplied by the study sponsor. No expiration date or certificate of analysis were provided.

Test Methods

A laboratory study was conducted to determine the effects of the test material on the growth of the unicellular freshwater green alga *Selenastrum capricornutum*. The test algae were cultured from a line purchased from CCAP Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, England. The algae were cultured in a chamber at 23 ± 2°C with continuous uniform illumination of about 6000 lux in the spectral range 400-700 nm. The culture medium was double-strength EPA algal assay medium (water hardness 40 mg $CaCO_3$).

The test vessels were 100 mL silylated glass flasks. The test solution was prepared by adding 20 mg of the test material to 200 mL of algal growing medium to obtain a final nominal concentration of 100 mg/L, and adding 50 mL of the solution to the test vessels. The control vessels received untreated algal medium only. The control group consisted of six replicates, while the test material group consisted of three replicates.

The algal inoculum was taken from a preculture that had been incubated for about three days under the test conditions. The initial cell concentration was approximately 10^4 cells/mL. During the test, the algae were kept in suspension by placing the test vessels on an orbital shaker at about 100 rpm. The cell concentration was determined after 24, 48, and 72 hours using a microscope with a counting chamber. At 0 and 72 hours, the pH of the test and control solutions was measured with a pH meter. Samples of the test material solutions were taken at 0 and 72 hours and analyzed for H11 content using HPLC. A description of the method is provided in Appendix 1 of MRID 47264953.

The EbC_{50}, ErC_{50}, and their statistical limits were calculated using the modified Probit method of the Flemish Institute for Technological Research. The NOEC was determined using ANOVA. The methods for calculating the area under the growth curve, the growth rate, and the percentage inhibition of growth rate are provided on p. 12 of MRID 47264953.

Results Summary

At test start, the mean measured concentration of the test material in the test solution was 101.64 mg/L, 101.64% of nominal. At 72 hours the mean measured concentration was 97.75 mg/L, 97.75% of nominal.

Results for growth inhibition are summarized in Table 1. The mean cell density and growth were similar for the test material and control groups at 72 hours.

TABLE 1. Growth of *Selenastrum capricornutum* exposed to H11 for 72 hours				
	0 hours	24 hours	48 hours	72 hours
Control				
Cell density (10^4 cells/mL)	0.594	4.95	51.69	227.47
pH	7.8	--	--	8.8
Mean area under growth curve	--	52.27	717.72	4053.42
Mean specific growth rate	--	0.088	0.093	0.083
H11 (100 mg/L)				
Cell density (10^4 cells/mL)	0.594	3.39	45.57	208.33
pH	7.79	--	--	8.79
Mean area under growth curve	--	33.55	606.86	3639.48
Percent inhibition	--	35.81	15.45	10.21
Mean specific growth rate	--	0.073	0.090	0.081
Percent mean specific growth rate inhibition	--	17.85	2.82	1.48

Data from pp. 23-24, MRID 47264953

Study Author's Conclusions

The study author concluded that the EbC_{50}, ErC_{50}, NOECb, and NOECr for the test material at 24, 48, and 72 hours were each >100 mg/L.

Reviewer's Conclusion

Based on the information provided, the reviewer agrees with the study author's conclusions. However, it should be confirmed that the H11 test material is actually laminarin.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Ready Biodegradability (OPPTS 835.3110)

MRID 47264954

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Ready Biodegradability (OPPTS 835.3110)
MRID NO:	47464954
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Laminarin
STUDY NO:	00-907005-024
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	SEPC, ZAC de Milieux, 42160 Andrezieux Boutheon, France
TITLE OF REPORT:	Ready Biodegradability Modified Strum [*sic*] Test
AUTHOR:	Licata-Messana, L.
STUDY COMPLETED:	October 25, 2000
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with GLP standards 1999/11/CE – Decret n 98-1312.
STUDY SUMMARY:	A modified Sturm test was conducted to determine the ready biodegradability of laminarin. The inoculum source was activated sewage sludge. The inoculum was incubated in mineral nutrient medium for 28 days, during which CO_2 production was measured. The test also included an untreated control (inoculum only), a reference control (sodium acetate trihydrate), and a toxicity control (test material + reference material + inoculum). Biodegradation of the test material was 76% after 28 days. Biodegradation in the reference material and toxicity controls was 71% and 65%, respectively, after 14 days. Laminarin was concluded to be readily biodegradable under the test conditions.
CLASSIFICATION:	**Acceptable**

Test Material

Laminarin (90% purity), Batch No. S 01/2000, a beige powder with an expiration date of January 1, 2003. A certificate of analysis was provided on p. 14 of MRID 47264954. After receipt at the testing facility, the test material was stored in at room temperature in darkness.

The reference control was sodium acetate trihydrate (Merck), Batch No. TA331667829.

Test Methods

A modified Sturm test was conducted to determine the ready biodegradability of laminarin. The inoculum source was activated sewage sludge from the Le Porchon sewage treatment plant (La Fouillouse, Loire, France). The sludge was aerated for 4 hours and 15 minutes, stirred for 2 minutes, and allowed to stand for 1 hour prior to centrifugation. The inoculum (300 mL of supernatant) was incubated at 35°C, and contained 2,240,000 to 2,680,000 microorganisms/L.

Composition of the mineral nutrient medium is given in Table 1. The solutions were prepared in demineralized water, and the medium was aerated for 22.5 hours prior to the test start.

TABLE 1. Composition of mineral nutrient medium

	Stock solution (g/L)	Mineral medium (mg/L)
Nutrient solution A		
KH_2PO_4	8.50	85.00
K_2HPO_4	21.75	217.50
$Na_2HPO_4 \cdot 2H_2O$	33.40	334.00
NH_4Cl	0.50	5.00
Nutrient solution B		
$CaCl_2$	27.50	27.50
Nutrient solution C		
$MgSO_4 \cdot 7H_2O$	22.50	22.50
Nutrient solution D		
$FeCl_3 \cdot 6H_2O$	0.25	0.25

Data from p. 20, MRID 47269454

The TOC in the test material was 0.40015 mg/mg (15 mg/L). Based on a concentration of 37.49 mg/L of test material in the nutrient medium, the theoretical CO_2 production for 3 L was calculated to be 164.99 mg. The TOC in the reference item was 0.1765 mg/mg (12.355 mg/L). Based on a concentration of 70 mg/L of the reference material in the medium, the theoretical CO_2 production for 3 L was calculated to be 135.87 mg.

The test containers were glass flasks. Two control flasks (inoculum only, 2 test material flasks, 1 reference material flask, and 1 toxicity control flask (undefined, presumably test material + reference material + inoculum) were used in the test. Each flask contained 3 L of the appropriate test solution, which was aerated with CO_2-free air at a rate of 1 to 2 bubbles/second. The flasks were incubated (it was not stated if in darkness or in diffused light) at 20.3 to 28.6°C for 28 days. The CO_2 produced was trapped in $Ba(OH)_2 \cdot 8H_2O$. Calculations for determining CO_2 production and biodegradation are provided on p. 22 of MRID 47264954.

Results Summary

Results of pH monitoring are provided in Table 2.

TABLE 2. pH values for solutions in 28-day biodegradability test					
Time	Control 1	Control 2	Test material 1	Test material 2	Reference material
At test start	7.21	7.24	7.31	7.36	7.40
At test end (day 28)	7.41	7.51	7.46	7.49	7.84

Data from p. 10, MRID 47264954

CO_2 production by the control flasks is given in Table 3.

TABLE 3. CO2 production by controls in 28 day biodegradability test						
Day	Flask 1		Flask 2		Mean volume of HCl (0.05M) (mL)	Mean CO_2 produced (mg)
	Volume of HCl (0.05) (mL)	CO_2 produced (mg)	Volume of HCl (0.05) (mL)	CO_2 produced (mg)		
2	45.8	4.62	45.8	4.62	45.8	4.62
5	45.0	5.50	45.6	4.84	45.3	5.17
7	43.2	7.48	46.8	3.52	45.0	5.50
9	44.4	6.16	46.4	3.96	45.4	5.06
12	43.2	7.48	46.1	4.29	44.7	5.83
14	44.8	5.72	48.3	1.87	46.6	3.74
19	47.8	2.42	47.4	2.86	47.6	2.64
23*	32.7	19.03	47.7	2.53	40.2	10.78
28*	31.5	20.35	45.6	4.82	38.6	12.54
Total	--	78.76	--	33.33	--	55.88
29*	39.2	11.88	48.2	1.98	43.7	6.93

Data from p. 10, MRID 47264954

*Due to slight bubbling problems, the calculations in Table 4 using data from these days were for the individual flasks, not the mean of the two flasks, e.g., the results for test material flask 1 were corrected for the results of control flask one only.

Biodegradation results for the test material are given in Table 4. Biodegradation was 76% after 28 days, and was 65% at the end of the 10-day window. Biodegradation of the reference control was 71% after 14 days, and biodegradation of the toxicity control was 65% after 14 days.

TABLE 4. Biodegradability of laminarin over 28 days					
Day	CO_2 produced (mg)*				Biodegradation (%)
	Flask 1	Flask 2	Mean	Cumulative values	
2	8.7	9.5	9.1	9.1	6
5	40.6	39.4	40.0	49.1	30
7	28.1	26.2	27.2	76.3	46
9	16.1	15.7	15.9	92.2	56
12	12.8	13.1	13.0	105.2	64
14	7.3	8.0	7.7	112.9	68
19	3.6	7.8	5.7	118.6	72
23	3.3	7.7	5.5	124.1	75
28	0.7	2.8	1.8	125.9	76
Cumulative values in each flask	121.2	130.2	--	--	--
Biodegradation (%)	73	79	--	--	--

Data from p. 11, MRID 47246954
*Corrected for production by control

Study Author's Conclusions

The study author concluded that laminarin is readily biodegradable under the test conditions.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Nontarget Insect Testing (OPPTS 880.4350)

MRID 47264979

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

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Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
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Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Nontarget Insect Testing (OPPTS 880.4350)
MRID NO:	47464979
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Phyliq (37 g/L laminarin)
STUDY NO:	01APGOL25
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	PROMO-VERT S.A., Z. I. du Hant Ossau, Rue d'Aste Beon, B.P. 27, F-64121 Serres Castet
TITLE OF REPORT:	Phyliq. The Effects of Phyliq (37 g/L Laminarin) on *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) on Artificial Substrate in Laboratory: LR_{50} Estimation and Reproduction Assessment
AUTHOR:	Tessier, C.
STUDY COMPLETED:	March 15, 2001
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with OECD GLP guidelines.
STUDY SUMMARY:	In a laboratory study, male and female adult parasitic wasps (*Aphidius rhopalosiphi*) were exposed for 48 hours to Phyliq (37 g/L laminarin) sprayed on glass plates at rates equivalent to 0, (untreated control) 0.1, 0.3, 1.0, 3.0, or 10.0 L/ha. A reference control group was exposed to 0.85 mL/ha Callidim 40 (400 g/L dimethoate). There was no statistically significant difference in the 24-hour or 48-hour mortality between the test material and untreated control groups. The response of the reference control group was appropriate. Surviving females in the 3.0 and 10.0 L/ha test material groups and the untreated control group were transferred to separate test chambers containing aphids on barley seedlings for approximately 30 hours. The wasps

were then removed, and the number of parasitized aphid mummies in each chamber was determined 14 days later. The number of mummies/female wasp was significantly lower in the 10.0 L/ha test material group, but not in the 3.0 L/ha group, compared to the untreated control group.

CLASSIFICATION: **Acceptable**

Test Material

Phyliq, Batch No. L001204, a dark orange cloudy liquid containing 40 g/L laminarin, with an expiration date of January 12, 2002. A certificate of analysis giving the laminarin concentration as 40 g/L is provided on p. 18 of MRID 47264979. The test material was stored in darkness, above 0°C.

The reference control was Callidim 40 (400 g/L dimethoate), Batch No. 8000633, with an expiration date of December 20, 2002.

Test Methods

A laboratory study was conducted to determine the effects of the test material on mortality and fecundity of the parasitic wasp *Aphidius rhopalosiphi*. The test organisms were obtained as parasitized aphid mummies from PK Nutzlingszuchten in Germany and maintained in a controlled environment cabinet at temperatures of 17 to 23°C and 70% relative humidity. Emerged adults were kept in a controlled environment cabinet set at 20°C and 70% relative humidity and were fed a honey solution. Adults less than 48 hours old were used in the test. Aphids (*Rhopalosiphum padi*) obtained from the same source as the wasps were used as hosts for the fecundity portion of the study. Prior to the study, the aphids were maintained on barley plants at the test facility.

The test chambers for the mortality assessment were composed of two 11 x 11 cm glass plates separated by a 9 x 9 x 1.5 cm tall metal frame. The frame contained holes covered with synthetic 0.3 mm mesh to provide ventilation.

Prior to assembly of the test chambers, the appropriate treatments were applied to the glass plates (Table 1). Each treatment was replicated three times. Application was by a Potter tower compressed air sprayer previously calibrated for the amount delivered on glass and the evenness of the spray pattern. The average output was 200.7 L/ha after calibration. The untreated control plates were treated first, followed by the test material plates (beginning with the lowest rate), and then the reference control plates. A stock solution of the test material (10 L/ha) was made by adding 10.53 g to 189.43 g of deionized water. The lower treatment rates were made by successive dilutions of the stock solution. The sprayer was rinsed three times with distilled water between the different application rates of the test material. It was decontaminated with 6% Ammoniac solution and rinsed three times with distilled water after the last application of the test material and before application of the reference control.

TABLE 1. Study treatments	
Treatment	**Rate**
Untreated control (deionized water)	--
Phyliq (37 g/L laminarin)	0.1, 0.3, 1.0, 3.0, 10.0 L/ha
Callidim 40 (400 g/L dimethoate)	0.85 mL/ha

Data from p. 5, MRID 47264980

The treated plates were allowed to dry for approximately one hour in a ventilated area before the test. Five male and five female wasps were then placed in each test chamber and cotton wool soaked in honey solution was placed in the introduction hole. The chambers were placed in a ventilated controlled environment cabinet at 20°C, 70% relative humidity, and a photoperiod of 16 hrs light:8 hrs darkness. Mortality was assessed after 24 and 48 hours.

After the 48 hour mortality assessment, surviving females in the 3.0 and 10.0 L/ha test material groups and the untreated control group were transferred to fecundity chambers (one female/chamber). The chambers consisted of a 20 cm tall x 11 cm diameter plastic cylinder placed over a pot of barley and closed with a 0.3 mm mesh lid. The chambers were ventilated by an air pump moving air out of the cylinder. Each pot of barley contained 20 to 40 seedlings about 10 cm tall and infested with approximately 100 aphids. The females were removed after spending approximately 30 hours in the chambers. The number of parasitized aphid mummies in each chamber was determined 14 days later.

Mean percent mortality and mean percent corrected mortality using Abbott's formula were calculated for each treatment. The results were analyzed using one-way ANOVA with a 5% significance level. Fecundity results were analyzed using a t-test with 5% significance level.

Results Summary

Mortality results are summarized in Table 2. There was no statistically significant difference in the 24 or 48 hour mortality among the test material and untreated control groups. The NOEC was 10 L/ha. The response of the reference control group was appropriate.

TABLE 2. Mortality of *Aphidius rhopalosiphi* exposed to Phyliq for 48 hours				
Treatment	**Mean mortality (%)**			
	24 hour		**48 hour**	
	Uncorrected	**Corrected[a]**	**Uncorrected**	**Corrected[a]**
Untreated control (deionized water)	0.0	--	0.0	--
Phyliq				
0.1 L/ha	0.0	0.0	0.0	0.0
0.3 L/ha	3.3	3.3	6.7	6.7
1.0 L/ha	0.0	0.0	0.0	0.0
3.0 L/ha	0.0	0.0	0.0	0.0
10.0 L/ha	0.0	0.0	3.3	3.3
Reference control				
Callidim 40 (400 g/L dimethoate)	100.0	100.0	100.0	100.0

[a]Corrected using Abbott's formula

Data from p. 14, MRID 47264979

Fecundity results are summarized in Table 3. There was no statistically significant difference in the number of mummies/female between the 3.0 L/ha test material group and the untreated control. The number of mummies/female in the 10.0 L/ha test material group was significantly lower than in the untreated control group.

TABLE 3. Mean fecundity of *A. rhopalosiphi* females exposed to Phyliq

Treatment	Mean number of mummies/female/24 hours
Untreated control (deionized water)	8.5
Phyliq	
3.0 L/ha	6.3
10.0 L/ha	4.6*

*Significantly different from untreated control ($p \leq 0.05$)
Data from p. 15, MRID 47264979

Study Author's Conclusions

The study author concluded that exposure of *A. rhopalosiphi* to the test material at rates ≤10.0 L/ha did not have a statistically significant effect on mortality, and exposure to 3.0 L/ha did not have a significant effect on the fecundity of *A. rhopalosiphi* females.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions. Exposure to 10.0 L/ha of the test material did significantly lower the fecundity of *A. rhopalosiphi* females compared to the untreated control. However, the product label for Vacciplant recommends an application rate of 9.7 to 14.4 oz/A, which the reviewer calculates to be equivalent to 0.7 to 1.05 L/ha, well below the 10.0 L/ha rate at which fecundity was affected.

DATA EVALUATION RECORD

LAMINARIN
(Vacciplant)

STUDY TYPE: Nontarget Insect Testing (OPPTS 880.4350)

MRID 47264980

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-025

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M. A.

Signature: _____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Nontarget Insect Testing (OPPTS 880.4350)
MRID NO:	47464980
DP BARCODE:	DP352311
DECISION NO:	385527
SUBMISSION NO:	Not provided
TEST MATERIAL:	Phyliq (37 g/L laminarin)
STUDY NO:	01TYGOL24
SPONSOR:	Laboratoires Goemar SA, Z.A.C. La Madeleine, Avenue General Patton, 35400 Saint-Malo, France
TESTING FACILITY:	PROMO-VERT S.A., Z. I. du Hant Ossau, Rue d'Aste Beon, B.P. 27, F-64121 Serres Castet
TITLE OF REPORT:	Phyliq. The Effects of Phyliq (37 g/L Laminarin) on *Typhlodromus pyri* (Acari, Phytoseiidae) on Artificial Substrate in Laboratory: LR_{50} Estimation and Reproduction Assessment
AUTHOR:	Tessier, C.
STUDY COMPLETED:	March 15, 2001
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was conducted in compliance with OECD GLP guidelines.
STUDY SUMMARY:	In a laboratory study, predatory mite (*Typhlodromus pyri*) protonymphs were exposed for 14 days to Phyliq (37 g/L laminarin) sprayed on glass plates at rates equivalent to 0, (untreated control) 0.1, 0.3, 1.0, 3.0, or 10.0 L/ha. A reference control group was exposed to 0.5 L/ha Danitol (100 g/L fenpropathrin). After 7 days, mortality in the 1.0 and 10.0 L/ha groups, but not the 3.0 L/ha group, was significantly increased compared to the untreated control. The response of the reference control group was appropriate. After 14 days, there was no statistically significant difference between the test material groups and

the untreated control for the mean number of eggs produced/female. The NOEC mortality was designated as 3.0 L/ha. The LR_{50} was calculated as 5.0 L/ha.

CLASSIFICATION: **Acceptable**

Test Material

Phyliq, Batch No. L001204, a dark orange cloudy liquid containing 37 g/L laminarin, with an expiration date of January 12, 2002. A certificate of analysis giving the laminarin concentration as 40 g/L is provided on p. 22 of MRID 47264980. The test material was stored in darkness, above 0°C.

The reference control was Danitol (a.i., 100 g/L fenpropathrin), Batch No. B0000002DAN, with an expiration date of January 2, 2002.

Test Methods

A laboratory study was conducted to determine the effects of the test material on mortality and fecundity of the predatory mite *Typhlodromus pyri*. The test organisms were obtained as eggs from the laboratory culture of PK Nutzlingszuchten in Germany. Four days prior to test start, the eggs were placed in a controlled environment cabinet at 25°C and 70% relative humidity, with a photoperiod of 16 hrs light:8 hrs darkness. After hatching, the protonymphs were fed a 50:50 mixture of apple and walnut pollens.

The test chambers consisted of two 7.5 x 5.0 x 0.2 cm glass plates held side by side with glass bars. A narrow gap was left between the two plates to allow access to water. A sticky barrier was applied around the edge of the chamber to prevent the mites from escaping, and a plastic shelter was provided to allow for egg laying. After treatment, the test chambers were placed on a tissue-covered sponge, which was placed in a 13.5 x 15.0 x 6.0 cm box filled with mineral water.

Before placement on the sponge, the glass plates received the appropriate treatments (Table 1). Each treatment was replicated three times. Application was by a Potter tower compressed air sprayer previously calibrated for the amount delivered on glass and the evenness of the spray pattern. The average output was 203.9 L/ha after calibration. The untreated control plates were treated first, followed by the test material plates (beginning with the lowest rate), and then the reference control plates. A stock solution of the test material (10 L/ha) was made by adding 10.53 g to 189.43 g of deionized water. The lower treatment rates were made by successive dilutions of the stock solution. The sprayer was rinsed three times with distilled water between the different application rates of the test material. It was decontaminated with 6% Ammoniac solution and rinsed three times with distilled water after the last application of the test material and before application of the reference control.

TABLE 1. Study treatments	
Treatment	**Rate**
Untreated control (deionized water)	--
Phyliq (37 g/L laminarin)	0.1, 0.3, 1.0, 3.0, 10.0 L/ha
Danitol (100 g/L fenpropathrin)	0.5 L/ha

Data from p. 5, MRID 47264979

The treated plates were allowed to dry for approximately one hour in a ventilated area before being placed in the prepared boxes. A 50:50 mixture of apple and walnut pollens was placed in each test chamber, and 20 *T. pyri* protonymphs were introduced. The boxes were then covered with a mesh lid and placed in a controlled environment cabinet at 25°C and 70% relative humidity, with a photoperiod of 16 hrs light:8 hrs darkness. The pollen was renewed approximately every two days.

Mortality assessments were made at 1, 3, and 7 days after treatment, and the number of eggs produced was assessed on days 7, 10, 12, and 14.

Mean percent mortality and mean percent corrected mortality using Abbott's formula were calculated for each treatment. The results were analyzed using one-way ANOVA with a 5% significance level. Fecundity results were analyzed using a t-test with 5% significance level.

Results Summary

Mortality results are summarized in Table 2. After seven days, the uncorrected mortality ranged from 26.7 to 88.3% in the test material treatments; corrected mortality ranged from 13.7 to 86.2%. Mortality in the 1.0 and 10.0 L/ha groups was significantly increased compared to the untreated control. The LR_{50} (residue level producing 50% mortality) was calculated as 5.0 L/ha ($1.66 < LR_{50} < 14.93$).

TABLE 2. Mortality of *Typhlodromus pyri* exposed to Phyliq for 7 days						
Treatment	**Mean mortality (%) at day after application**					
	1 day		**3 days**		**7 days**	
	Uncorrected	**Corrected[a]**	**Uncorrected**	**Corrected[a]**	**Uncorrected**	**Corrected[a]**
Untreated control	6.7	--	11.7	--	15.0	--
Phyliq						
0.1 L/ha	6.7	0.0	18.3	7.5	28.3	15.7
0.3 L/ha	5.0	-1.8	23.3	13.2	26.7	13.7
1.0 L/ha	13.3	7.1	28.3	18.8	40.0**	29.4
3.0 L/ha	18.3	12.5	33.3	24.5	38.3	27.5
10.0 L/ha	48.3	44.6	86.7	84.9	88.3**	86.2
Reference control	100.0*	100.0*				

*Significantly different from the untreated control and test material groups ($p \leq 0.05$)
** Significantly different from the untreated control ($p \leq 0.05$)
[a]Corrected using Abbott's formula
Data from p. 13, MRID 47264980

Fecundity results are summarized in Table 3. There was no significant difference between the treated and untreated groups for the mean number of eggs produced/female at any time point.

TABLE 3. Fecundity of *Typhlodromus pyri* females exposed to Phyliq				
Treatment	**Mean number of eggs/female at days after treatment**			
	10 days	**12 days**	**14 days**	**Total**
Untreated control	2.1	2.7	2.3	7.0
Phyliq				
0.1 L/ha	1.6	2.3	2.3	6.1
0.3 L/ha	2.6	2.8	2.3	7.7
1.0 L/ha	2.8	2.2	2.3	7.3
3.0 L/ha	2.9	2.0	2.1	7.0
10.0 L/ha	1.0	3.1	2.4	6.4

Data from p. 15, MRID 47264980

Study Author's Conclusions

The study author concluded that the test material did not have any significant effect on the mortality of *T. pyri* when applied at rates ≤3.0 L/ha, stating that the statistical significance of the increased mortality at 1.0 L/ha was slight. The NOEC was designated as 3.0 L/ha . The study author also concluded that the fecundity of *T. pyri* was not significantly affected by the test material applied at a rate ≤ 10.0 L/ha.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusions, since the statistically significant increase in mortality at 1.0 L/ha was not seen at 3.0 L/ha. The product label for Vacciplant recommends an application rate of 9.7 to 14.4 oz/A, which the reviewer calculates to be equivalent to 0.7 to 1.05 L/ha, well below the maximum rate of 10 L/ha used in this test.

SAFETY DATA SHEET

072002M7

I - IDENTIFICATION OF THE PRODUCT AND OF THE COMPANY

NAME OF THE PRODUCT	***Laminarin***
Use	Agriculture R&D product
SUPPLIER Manufacturer	**Laboratoires GOËMAR** Parc Technopolitain Atalante St Malo - CS 41908 St Jouan des Guérets 35400 SAINT MALO - FRANCE Phone : +33.(0)2.99.19.19.19 Fax. : +33.(0)1.41.30.99.63 fds@goemar.com
EMERGENCY TELEPHONE	ORFILA +33.(0)1.45.42.59.59.(24/24H) ANTI-POISON CENTER (Rennes) +33.(0)2.99.59.22.22.(24/24H)

II - HAZARDS IDENTIFICATION

MAIN HAZARDS	Not classified as dangerous.
Adverse physico-chemical effects	
Adverse human health effects	
Adverse environmental effects	
Additional information	Do not release the product into draining system or in the environment.

III - COMPOSITION / INFORMATION ON INGREDIENTS

PREPARATION - chemical nature

	denomination / CAS n° / EC n°	%w/w	classification
Components involved in the hazards	none		
Components presenting a hazard	none		

Additional information

IV - FIRST AID MEASURES

IN CASE OF :

Eye contact	Flush immediately with plenty of water for 15 mn. In case of persistent irritation, ask the advice of an ophthalmologist .
Skin contact	Take off dirty clothes. Rinse skin with plenty of water.
Ingestion	Wash out mouth with water. Do not give to drink. Do not induce vomiting. If necessary seek medical advice, and show this SDS, or for lack, the label.
Inhalation	Remove from the contaminated area and bring to fresh air.
Collective emergency means	Eyes shower. Safety shower.

V - FIRE FIGHTING MEASURES

Suitable extinguishing media	All extinguishing agents usable (water, foam, powders, CO_2, …)
Specific hazards	
Precautions	Evacuate the place. Fight fire in early stage ONLY IF SAFE to do so. Provide for holding contaminated water.
Protective equipment	Professional fire-fighters only : use a self-contained breathing apparatus.

VI - ACCIDENTAL RELEASE MEASURES

Personal precautions	Avoid contact with eyes and skin. Use Personnal Protective Equipments proposed hereafter.
Environmental precautions	Limit and impound discharge. Prevent entry into drains, waters or soil. Avert authorities.
Methods for cleaning up	Do not water down. Collect with a neutral absorbent material and sweep it . Do not reintroduce into original container ; treat as waste.

VII - HANDLING AND STORAGE

HANDLING	For personal protective equipments, see point 8 for details.
Precautions for use	Avoid accidental dispersion and spatter.
STORAGE	
Technical measures	Recommended limit height of stacking : 2 pallets
Conditions of storage	Store out of frost (-10°C) and if possible in a cool place. Store away from children, domestic animals, food, drink and animal feedingstuffs.
Packaging	Keep the product into original container labelled and closed.

VIII - EXPOSURE CONTROLS / PERSONAL PROTECTION

Technical measures	No specific recommendations.
Exposure limit values	
Monitoring procedures	
PPE : Personal	The product doesn't require specific measures.
Protection Equipments	
Respiratory protection	Mask recommended while spraying the mixture.
Hand protection	Protective gloves, such as latex, PVC, rubber.
Eye protection	Safety goggles.
Skin and body protection	Protective clothing, such as overall.
Hygiene measures	Wash hands with water after handling the product. Do not eat or drink during the handling.
Environmental protection	

IX - PHYSICAL AND CHEMICAL PROPERTIES

Appearance : physical state, colour	Liquid brown
Odour	odourless
pH	4,07-4,57
Characteristic temperatures :	
Solidification	no data
Boiling point	no data
Flash point	no data
Auto-ignition temperature	Not applicable (no flammable component)
Oxidising properties	Not applicable (no oxidising component)
Explosive properties	Not applicable (no explosive component)
Relative density (water = 1)	1,029-1,044
Solubility	Not applicable
Viscosity	no data
Vapour density	no data
Evaporation rate	no data

X - STABILITY AND REACTIVITY

Stability	The product is stable in normal conditions of use.
Hazardous reactions	
Conditions to avoid	No hazardous reaction known in normal conditions of use.
Materials to avoid	none
Hazardous decomposition products	

XI - TOXICOLOGICAL INFORMATION

Acute toxicity / Local effects	**Data for pure substance** (solution = 50g/L of pure substance) acute oral tox. (rat) : LD 50 > 2000 mg / Kg acute sub-cutaneous tox. (rat) : LD 50 > 5000 mg / Kg acute inhalation tox. (rat) : LC 50 > 1,02 mg / L / 4h
Eye contact	Non-irritant for the eye of the rabbit. Nevertheless the solution can cause slight irritations (pH slightly acid)
Skin contact	Non-irritant for the skin of the rabbit.Nevertheless the solution can cause slight irritations (pH slightly acid)
Sensitisation	No sensitising capacity in the Guinea pig (Test of maximization Magnusson and Kligman)
Toxicity by repeated administration	4 weeks oral tox. (rat) :Ineffective dose NOAEL > 1000 mg / Kg / day acute subchronic oral tox. (rat) 90days: Ineffective dose NOAEL > 1000 mg/kg/day acute subchronic oral tox. (dog) 90 days: Ineffective dose NOAEL > 1000 mg/kg/day
Specific effects	Mutagenicity Ames's test : no mutagenic activity in the 4 *salmonella typhimurium* and the 2 *Escherichia coli* stains tested. Test of the micropit (mouse): no damage in chromosomes or in device mitotique cells of marrow after 2 administrations at 12 pm of interval, at 500, 1000, or 2000mg/kg/day In vitro essay of gene mutation on cells of mammal (mouse): no mutagenic activity The studies of the effects on the embryo-foetal development to the rat and to the rabbit led both to NOAEL>1000 mg/kg/day
Others informations:	The essays were realized on various lots of substance of purity included between 89 % and 99 %

XII - ECOLOGICAL INFORMATION

Ecotoxicity	LC 0 and LC 50 fish of warm water - Zebra fish (*Brachydanio rerio*) > 100 mg/L/96h LC 0 and LC 50 fish of cold water - Rainbow trout *(Oncorhynchus mykiss)* > 100 mg/L/96h LC0 and LC50 daphnies >100mg/L/48h EbC50 seaweed (24, 48 and 72h) > 100 mg / L ErC50 seaweed (24, 48 and 72h) > 100 mg / L NOECb seaweed (24, 48, and 72 h) > 100 mg / L NOECr seaweed (24, 48 and 72 h) > 100 mg / L Bees : oral LD50 (48h) > 118,64 µg/bee, contact LD50 (48h) > 100 µg/bee Bird: oral LD50 >2000 mg/kg, diet > 5000ppm

XIII - DISPOSAL CONSIDERATIONS

Residues waste	Do not empty into drains.
Destruction / disposal	Dispose of in authorised waste collection plant.
Contaminated packaging	
Decontamination / cleaning	Rinse with water and pour into the spraying tank.
Destruction / disposal	Dispose of rinsed plastic packagings and cardboard in an authorised plant (incineration or recycling).

XIV - TRANSPORT INFORMATION

International regulations	Not concerned.
Road (ADR)	
Rail (RID)	
Sea (IMDG)	
Air (OACI/IATA)	

XV - REGULATORY INFORMATION

CE LABELLING	
Symbol of danger	None
Special risks (R sentences)	None
Safety advice (S sentences)	S2 : keep out of the reach of children. S20/21 : when using do not eat, drink or smoke.
Other information	None.

XVI - OTHERS INFORMATIONS

BIBLIOGRAPHICAL REFERENCES	written following ISO Norm 11014-1
For communal regulation	Directive1907/2006/EC. Directive 1999/45/EC modified. Directive 67/548/EEC modified.
For transport	ADR 2011 IMDG 2011

symbol points out a modification with regard to the previous sheet.

N .B. : This sheet completes the technical leaflet but doesn't replace it. The information written here above are based on our relative knowledge of the concerned product, at the time of writting this safety data sheet. They are honestly written. A list of the main regulation, legislation and administrative texts can be joined to this sheet as indication. The use of the product for other purposes than those indicated on the label may present a risk.

Material Safety Data Sheet R&D - last update : 03/08/12*1