

ANNEX B

Laminarin

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

With respect to the test substances used for the determination of the physical and chemical properties, following purity values should be taken into account :

- for the purified a.s. : 98% on dry matter (batch N° S210300)
- for the a.s. as manufactured : 89% on dry matter (batch N° S012000)

Table B.2.1-1 : Physical and chemical properties of Laminarin

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	- EEC A1 (capillary method – heater block) - GLP-compliance stated	purified a.s. : no melting point could be determined (degradation of test item during test – see B.2.1.3)	Acceptable	Licata-Messana, 2000a
B.2.1.2 Boiling point (IIA 2.1.2)		purified a.s. : boiling point is not determinable, taking into account results of melting point determination (see B.2.1.1)	Acceptable	Licata-Messana, 2000b (amended study plan)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	- EEC A1 (capillary method – heater block) - GLP-compliance stated	purified a.s. : at 216°C : test item turned brown at about 316°C : test item was retracted and black coloured	Acceptable	Licata-Messana, 2000a
B.2.1.4 Relative density (IIA 2.2)	- EEC A3 (pycnometer method) - GLP-compliance stated	purified a.s. : $D_4^{20} = 1.502 \pm 0.026$	Acceptable	Licata-Messana, 2000c
B.2.1.5 Vapour pressure (IIA 2.3.1)	- EEC A4 (vapour pressure balance) - GLP-compliance stated	purified a.s. : vapour pressure at 25°C : $< 2.6 \times 10^{-5}$ Pa (max. value estimated by imposing a shallow regression slope of –1500 on a chosen data point and extrapolating to 25°C)	Acceptable	Tremain, 2001

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.6 Volatility – Henry's law constant (IIA 2.3.2)	- Calculation - GLP-compliance not relevant	purified a.s. : <ul style="list-style-type: none"> vapour pressure at 25°C : $< 2.6 \times 10^{-5}$ Pa water solubility at 23°C : 301.5 g/L or 60.3 mol/m³ (using MW-value of 5000 g/mol) \Rightarrow H at 23-25°C : $< 4.3 \times 10^{-7}$ Pa.m ³ .mol ⁻¹	Acceptable	Ambrosi, 2002
B.2.1.7 Physical state, colour (IIA 2.4.1)		purified a.s. : white crystals a.s. as manufactured (TC) : beige powder	Acceptable	Licata-Messana, 2000a Licata-Messana, 2000d
B.2.1.8 Odour (IIA 2.4.2)		purified a.s. : odourless a.s. as manufactured (TC) : odourless	Acceptable	Tier II Licata-Messana, 2001a

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References												
B.2.1.9 Spectra of the active substance (IIA 2.5.1)	- GLP-compliance stated	<p>purified a.s. : <i>Following spectra were provided :</i> UV/VIS (water; spectra measured between 200-900 nm) ¹H-NMR (250 MHz, deuterated water) ¹³C-NMR (63 MHz, deuterated water) IR (KBr; sample scanned over range 4000 to 600 cm⁻¹) The different spectra were found to be consistent with the proposed chemical structure of Laminarin (i.e. spectra showed no evidence that contradicts proposed structure), although full individual peak assignment was not possible due to complex nature of test material. No useable mass spectra were acquired (both positive and negative ion Electrospray (ES) and Fast Atom Bombardement (FAB) mass spectrometry were attempted).</p> <p><i>UV/VIS absorption characteristics :</i></p> <table><thead><tr><th></th><th><u>λ_{max} (nm)</u></th><th><u>ε (L.mol⁻¹.cm⁻¹)</u></th></tr></thead><tbody><tr><td>acidic (pH 1.9)</td><td>264</td><td>245 to 294</td></tr><tr><td>neutral (pH 7.0)</td><td>260</td><td>242 to 290</td></tr><tr><td>alkaline (pH 11.8)</td><td>258</td><td>264 to 317</td></tr></tbody></table> <p>⇒ λ_{max} : ~ 260 nm</p> <p>at 290 nm : ε-values in the range of 121 to 169 L.mol⁻¹.cm⁻¹</p>		<u>λ_{max} (nm)</u>	<u>ε (L.mol⁻¹.cm⁻¹)</u>	acidic (pH 1.9)	264	245 to 294	neutral (pH 7.0)	260	242 to 290	alkaline (pH 11.8)	258	264 to 317	<p>Acceptable. Values of ε are quoted as a range, due to the MW range of the test material.</p> <p>As far as mass spectra are concerned, study quotes information from literature suggesting that compounds of this type are difficult to ionise and therefore difficult to analyse by MS. Nevertheless, other published articles show that it is possible to obtain useable spectra (discussed hereafter)</p>	Cuthbert, 2001
	<u>λ_{max} (nm)</u>	<u>ε (L.mol⁻¹.cm⁻¹)</u>														
acidic (pH 1.9)	264	245 to 294														
neutral (pH 7.0)	260	242 to 290														
alkaline (pH 11.8)	258	264 to 317														
	- no GLP-compliance stated (published article)	<p>Laminarans from different species of brown algae (among which <i>L. digitata</i>), commercial product or isolated (no purity stated) : MALDI-MS (both in native and methylated form) and FAB-MS (as permethylated derivatives) confirmed existence of M- and G-chains for some laminarans (among which <i>L. digitata</i>) and absence of M-chains for others.</p>	<p>Not acceptable : spectra of Laminaran from <i>L. digitata</i> are not shown in article</p>	Chizhov et al., 1998 (published article)												

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
	- no GLP-compliance stated	purified a.s : MALDI-TOF mass spectra (acquired in positive ion mode; matrix : 2,5-dihydroxybenzoic acid (DHB)) mainly show a large distribution of MNa^+ ions in which oligomers are separated by 162 Da. This corresponds to the glucose linkage, leading to a loss of 18 Da. Zooming on an oligomer shows a double distribution separated by two mass units. The first one could be dedicated to a polysaccharide only formed by glucose monomers, even in the end groups. The second one could be explained by a D-mannitol end group (MW 182 g/mol)	Acceptable, although not GLP	Delolme, 2000
	- no GLP-compliance stated (published article)	Laminarin from <i>Laminaria digitata</i> , commercial product (Sigma, no purity stated) : MALDI-TOF mass spectra (acquired in positive ion mode; matrix : DHB, resp. arabinosazone) show m/z differences between adjacent peaks to be 162. Oligomeric envelope observed in the mass spectrum obtained in arabinosazone, favouring higher m/z, is more representative of the sample composition than that observed with DHB. Presumably, the higher laser power used with DHB caused some unwanted fragmentation.	Acceptable as additional information (confirms findings by Delolme, stated above)	Chen et al., 1997 (published article)
	- no GLP-compliance stated (published article)	Laminarin from <i>Laminaria digitata</i> , commercial product (Sigma, Lot 110H3841) : ESI-mass spectra (acquired in positive ion mode) of permethylated Laminarin revealed a mean molecular size of 25 glucosyl residues and an approximately 3:1 molar ratio of M-series and G-series molecules	Acceptable as additional information	Read et al., 1996 (published article)
B.2.1.10 Spectra of the impurities (IIA 2.5.2)	- Applicant's statement	"Not applicable as none of the impurities is considered to be of toxicological, ecotoxicological or environmental significance"	Acceptable	

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.11 Solubility in water (IIA 2.6)	- EEC A6 (flask method + GC-FID of glucose derivative) - GLP-compliance stated	purified a.s. : solubility at 20°C in water (pH ≈ 6) : > 88.6 g/L glucose	Not acceptable : method not fully in accordance with EEC A6 (instead of using 5 times the expected saturation amount, less than the saturation amount was used).	Licata-Messana, 2001b
	- Adaptation of flask method (EEC A6) + HPIC with amperometric detection (global method ME-0248 (Trad)) - GLP-compliance stated	purified a.s. : solubility at 23°C in water (pH 5.5-7) : 301.5 g/L determination of water solubility in acidic or alkaline range : not relevant (a.s. is not capable of forming ions – see B.2.1.17)	Acceptable	Anding, 2002
B.2.1.12 Solubility in organic solvents (IIA 2.7)	- Method adapted from EEC A6 (flask method + GC-FID of glucose derivative) - GLP-compliance stated	purified a.s. : solubility at about 20°C in <ul style="list-style-type: none"> • n-heptane : < 10 mg/L glucose • xylene : < 10 mg/L glucose • 1,2-dichloroethane : < 10 mg/L glucose • methanol : 60 mg/L glucose • acetone : 21 mg/L glucose • ethylacetate : < 10 mg/L glucose 	Acceptable	Licata-Messana, 2001c Licata-Messana, 2001d Licata-Messana, 2001e Licata-Messana, 2001f Licata-Messana, 2001g Licata-Messana, 2001h

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.13 Partition coefficient n-octanol/water (IIA 2.8)	- EEC A8 (shaking flask method + GC-FID of glucose derivative) - GLP-compliance stated	purified a.s. : at 20°C : $\log P_{ow} = -1.6$ effect of pH : not relevant (a.s. is not acidic nor basic – see B.2.1.17)	Acceptable	Quintelas, 2001a
B.2.1.14 Hydrolysis rate at pH 4, 7 and 9 under sterile conditions in the absence of light (IIA 2.9.1)	- EEC C7 - GLP-compliance stated	purified a.s. : <i>Tests at 50°C at pH 4, 7 and 9 :</i> at all pH : hydrolytic stability (= less than 10% degradation of laminarin to glucose after 5 d) → no further tests required ⇒ no hydrolysis of laminarin into glucose under environmental conditions	Acceptable, as far as <u>hydrolysis to glucose</u> is concerned. As far as eventual hydrolysis into smaller sized oligosaccharides is concerned, a new hydrolysis study is ongoing (study protocol N° GOM/004 by Huntingdon Life Sciences).	Quintelas, 2001b Comb, 2002 (protocol)

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.15 Direct phototransformation in purified water using artificial light under sterile conditions (IIA 2.9.2)	- Justification	The molar decadic absorption coefficient (ϵ) at 290 nm ($\approx 160 \text{ L.mol}^{-1}.\text{cm}^{-1}$) slightly exceeds the trigger value of $10 \text{ L.mol}^{-1}.\text{cm}^{-1}$, and a phototransformation study could thus be justified. However, such a study would be extremely difficult to realize due to the fact that it is impossible to obtain radiolabelled material and the difficulties encountered in analysis of the required low concentrations. No good study could thus be conducted. As a worst case it is therefore considered that the a.s. in water is stable to the light and that the same photodegradation products (if any) would be produced as would appear with glucose.	Acceptable	
B.2.1.16 Quantum yield of direct phototransformation in water (IIA 2.9.3)		see B.2.1.15	see B.2.1.15	
B.2.1.17 Dissociation in water – pKa value(s) (IIA 2.9.4)	- Justification	The comparison of the UV/VIS spectra at different pH's shows that there is no dissociation in water. Therefore no dissociation constant can be determined.	Acceptable (see B.2.1.9)	
B.2.1.118 Estimated photochemical oxidative degradation (IIA 2.10)	- Justification	Laminarin being an oligosaccharide, no such estimation of the photochemical oxidative degradation can be submitted, as the calculation according to Atkinson et al. does not apply to polymers. However, it must be remembered that Laminarin has a very low vapour pressure and a very low Henry's law constant.	Acceptable	

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.19 Flammability (IIA 2.11.1)	- EEC A10 - GLP-compliance stated	a.s. as manufactured (TC) : <i>preliminary test</i> : flame propagates along 200 mm in 1 min 17 sec <i>main test (6 burning rate assays)</i> : shortest burning time : 46 sec (close to cut-off level of 45 sec!) ⇒ a.s. is not considered as highly flammable	Acceptable	Licata-Messana, 2000d
	- EEC A12 - GLP-compliance stated	a.s. as manufactured (TC) : <i>steps 1 to 4</i> : no development or ignition of gas is observed after contact with water ⇒ a.s. is not considered as hazardous	Acceptable	Licata-Messana, 2000e
B.2.1.20 Auto-flammability (IIA 2.11.2)	- EEC A16 - GLP-compliance stated	a.s. as manufactured (TC) : exothermic reaction is observed at ca 236°C → test item temperature reaches max. 363°C ⇒ no self-ignition temperature up to 420°C	Acceptable	Licata-Messana, 2000f
B.2.1.21 Flash point (IIA 2.12)			Not applicable (melting point > 40°C)	
B.2.1.22 Explosive properties (IIA 2.13)	- EEC A14 - GLP-compliance stated	a.s. as manufactured (TC) : <ul style="list-style-type: none"> • no thermal sensitivity • no mechanical sensitivity with respect to shock • no mechanical sensitivity with respect to friction ⇒ a.s. is not considered to have explosive properties	Acceptable	Licata-Messana, 2000g

Study	Guidelines/methods and GLP	Findings	Evaluation and conclusion	References
B.2.1.23 Surface tension (IIA 2.14)	- EEC A5 (ring method) - GLP-compliance stated	purified a.s. : $\sigma = 72.2 \text{ mN/m}$ at 20°C (1 g/L solution) \Rightarrow a.s. is not considered as surface active	Acceptable	Licata-Messana, 2000h
B.2.1.24 Oxidizing properties (IIA 2.15)	- EEC A17 - GLP-compliance stated	a.s. as manufactured (TC) : <i>main test</i> : <ul style="list-style-type: none"> max. burning rate of reference mixtures = 3.086 mm/s (barium nitrate/cellulose 20/80 % w/w) max. burning rate of test mixtures = 2.459 mm/s (test substance/cellulose 10/90 % w/w) \Rightarrow a.s. does not present oxidizing properties	Acceptable	Licata-Messana, 2000i

Summary and conclusions

Laminarin, an oligosaccharide with a molecular mass of ca. 5000 g/mol, is an odourless beige powder (TC). Its melting point and boiling point cannot be determined due to decomposition starting at ca. 216°C . Its low vapour pressure and Henry's law constant indicate that Laminarin is very slightly volatile. Laminarin is readily soluble in water and slightly to moderately soluble in organic solvents. Its very low octanol/water partition coefficient indicates no significant potential for bioaccumulation. Laminarin is hydrolytically stable, whatever the pH (as far as hydrolysis to glucose is concerned) and the light should have no influence on this stability. Laminarin shows no dissociation in water and is not considered as surface active. The a.s. is not highly flammable, not self heating and exhibits no explosive or oxidizing properties.

B.2.2. Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

All tests were conducted on Lot L000713 (37.8 g/L Laminarin), unless indicated otherwise.

Table B.2.2-1 : Physical, chemical and technical properties of **PHYLIQ** (Soluble concentrate : 37 g/L laminarin)

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.1 Physical state, colour (IIIA 2.1)	- GLP-compliance stated	clear brown liquid	Acceptable	Licata-Messana, 2001g
B.2.2.2 Odour (IIIA 2.1)	- GLP-compliance stated	slight odour	Acceptable	Licata-Messana, 2001g
B.2.2.3 Explosive properties (IIIA 2.2.1)	- Justification	"Not necessary : the preparation does not contain any component susceptible of exothermic reaction and is composed mainly of water (> 80 %)."	Acceptable	
B.2.2.4 Oxidizing properties (IIIA 2.2.2)	- Justification	"Not necessary : the preparation does not contain any component able to react exothermically with combustible materials and is composed mainly of water (> 80 %)."	Acceptable	
B.2.2.5 Flash point (IIIA 2.3)			Not applicable (preparation contains no flammable solvents and is composed mainly of water (> 80 %))	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.6 Flammability (IIIA 2.3)			Not applicable (liquid preparation)	
B.2.2.7 Auto-flammability (IIIA 2.3)	- Justification	"Not necessary : the preparation is a SL composed mainly of water (> 80 %)."	Acceptable	
B.2.2.8 Acidity or alkalinity and pH value (IIIA 2.4.1)	- CIPAC MT 75.1 - CIPAC MT 31 (electrometric procedure) - GLP-compliance stated	undiluted product at 21.7°C : pH = 4.01 free acidity = 0.25% w/w	Acceptable. As far as free acidity is concerned, CIPAC MT 31 was performed without dissolution in acetone, because precipitation was observed when mixing acetone with the test item. Acetone was replaced by equal amount of water.	Licata-Messana, 2001a Licata-Messana, 2001b
B.2.2.9 pH of a 1% aqueous dilution, emulsion or dispersion (IIIA 2.4.2)	- CIPAC MT 75.2 - GLP-compliance stated	1% w/v aqueous dilution at 22.7°C : pH = 4.19	Acceptable	Licata-Messana, 2001c
B.2.2.10 Kinematic viscosity (IIIA 2.5.1)			Not applicable (preparation not intended for ULV-use)	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.11 Viscosity (IIIA 2.5.2)	- OECD 114 – ISO 3219 (rotational viscometer) - GLP-compliance stated	at 20°C : η between 43.6 ± 0.5 mPa.s (shear rate 77.5 s^{-1}) and 64.3 ± 2.5 mPa.s (shear rate 15.5 s^{-1}) at 40°C : η between 10.8 ± 0.2 mPa.s (shear rate 258.2 s^{-1}) and 12.2 ± 0.5 mPa.s (shear rate 77.5 s^{-1}) \Rightarrow PHYLIQ has non-Newtonian properties	Acceptable	Licata-Messana, 2001d
B.2.2.12 Surface tension (IIIA 2.5.3)	- EEC A5 (ring method) - GLP-compliance stated	undiluted product at 20°C : $\sigma = 29.7 \pm 0.7$ mN/m \Rightarrow PHYLIQ is considered as surface active	Acceptable.	Licata-Messana, 2001e
B.2.2.13 Relative density (IIIA 2.6.1)	- EEC A3 (pycnometer method) - GLP-compliance stated	$D_4^{20} = 1.057$	Acceptable	Licata-Messana, 2001f
B.2.2.14 Bulk or tap density (IIIA 2.6.2)			Not applicable (liquid preparation)	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.15 Stability after storage for 14 days at 54°C (IIIA 2.7.1)	- CIPAC MT 46 + GC-FID of glucose derivative + CIPAC MT 75.1 + CIPAC MT 75.2 + CIPAC MT 47.1 + CIPAC MT 41 - GLP-compliance stated	after storage for 14 d at 54°C : <ul style="list-style-type: none"> • <i>chemical stability of a.s.</i> : Laminarin content : 46.7 g/L (initial : 45.3 g/L) • <i>physical stability</i> : appearance : clear brown liquid; slight odour (initial : idem) pH (undiluted) : 4.00 at 20.5°C (initial : 4.01 at 21.7°C) pH (1% w/v) : 4.01 at 21.3°C (initial : 4.19 at 22.7°C) persistent foaming (2% v/v) : 3 mL after 10 s; 0 mL after 40 s (initial : 2 mL after 10 s; 0 mL after 15 s) dilution stability (2% v/v) : no separated material was observed (initial : idem) <p>⇒ PHYLIQ is chemically and physically stable</p>	Acceptable as far as <u>physical stability</u> is concerned. <u>Chemical</u> <u>stability</u> results however are not acceptable, because the analytical method used is unsuitable to detect eventual degradation of the a.s. in the preparation. Laminarin is determined by total acid hydrolysis of the polysaccharide into glucose and subsequent quantification of glucose as its TMS-derivative by GC- FID, without taking into account the glucose content before hydrolysis. Eventual degradates are thus co- determined. Also, a.s. content does not comply with FAO-tolerances.	Licata-Messana, 2001g Licata-Messana, 2001h Licata-Messana, 2001i Licata-Messana, 2001j Licata-Messana, 2001k Licata-Messana, 2001l

Study	Guidelines and GLP	Findings			Evaluation and conclusion	References
	- CIPAC MT 46 - GLP-compliance stated	PHYLIQ, batch N° 020219 (41 g/L laminarin) :			Study is acceptable : a.s. content was now determined according to the “global method”, e.g. without preceding hydrolysis step. It should however be noted that the a.s. content apparently does not comply with FAO tolerances : determined content exceeds the declared content by more than 10%, both before and after storage. Also, after storage, the active substance content has decreased by 13.8%.	Mourgues, 2002
			before storage	after 14 d at 54°C		
		Laminarin content (g/kg) (global method ME-0250 (mod.) : HPIC with amperometric detection)	46.08	39.70		
		acidity (% w/w) (CIPAC MT 31)	0.34	0.35		
		pH (undiluted) (CIPAC MT 75.1)	3.24 at 22.6°C	3.44 at 22°C		
		pH (1% w/v) (CIPAC MT 75.2)	3.84 at 24.6°C	3.74 at 22.5°C		
		dilution stability (CIPAC MT 41 : 2% v/v in water D, 18 h at 20°C)	T ₀ : beige and cloudy solution, uniform, no phase observed T ₁₈ : idem. However, brown residues were observed at bottom of solution	T ₀ : yellowish solution, uniform, no phase observed T ₁₈ : idem. However, yellowish/beige residues were observed at bottom of solution		
		appearance	thick brown mixture, low odour	dark brown liquid phase on upper part; rest is a thick granular dark brown liquid, stark odour		

Study	Guidelines and GLP	Findings				Evaluation and conclusion	References
B.2.2.16 Stability after storage for other periods and temperatures (IIIA 2.7.1)	- Internal procedure C.Mi.01/2 : counting of total germs, yeasts and moulds after incubation of seeded agar plates - No GLP-compliance stated	PHYLIQ, batch N° 020219 (41 g/L laminarin) :				Acceptable	Burosse, 2002
		<i>Bacteriological control</i>	after 7 weeks at ambient temp.		after 7 weeks at +40°C		
			free of microbial contamination				
		<i>pH</i>	at production	after 9 weeks at ambient temp.	after 9 weeks at +40°C		
			3.41	3.39	3.42		
		<i>laminarin content</i>	at production	after 15 weeks at ambient temp.	after 15 weeks at +40°C		
41 g/L	40 g/L		40 g/L				
B.2.2.17 Minimum content after heat stability testing (IIIA 2.7.1)		see B.2.2.15 : Min. content after 14 days at 54°C = 39.70 g/kg → decrease by 13.8%.					
B.2.2.18 Effect of low temperatures on stability (IIIA 2.7.2)	- CIPAC MT 39 - GLP-compliance stated	storage at 0±1 °C : after 1 h : no separated material after 7 d : no separated material ⇒ PHYLIQ is stable at low temperature				Acceptable (temp. < -1°C for 2 days did not affect results)	Licata-Messana, 2001m
B.2.2.19 Shelf life at ambient temperature (IIIA 2.7.3)		Real time study at ambient temperature in commercial packaging material is currently under way.				We await submission of the report	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.20 Wettability (IIIA 2.8.1)			Not applicable (liquid preparation)	
B.2.2.21 Persistent foaming (IIIA 2.8.2)	- CIPAC MT 47.1 - GLP-compliance stated	dilution of preparation with CIPAC water C to a conc. of 2% v/v : after 10 s : 2 mL foam after about 15 s : foam had disappeared	Test complies with CIPAC MT 47 and is thus acceptable. However, as it is not in accordance with FAO-manual (5th ed.), a new study according to CIPAC MT 47.2, using water D at 30°C, will be provided with the application for registration at MS-level.	Licata-Messana, 2001n
B.2.2.22 Suspensibility (IIIA 2.8.3)			Not applicable (preparation is not a water dispersible product)	
B.2.2.23 Spontaneity of dispersion (IIIA 2.8.3)			Not applicable (preparation is not a water dispersible product)	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.24 Dilution stability (IIIA 2.8.4)	- CIPAC MT 41 - GLP-compliance stated	dilution of preparation with CIPAC water C to a conc. of 2% v/v : after 18 h at 20°C : no separated material was observed ⇒ dilution of PHYLIQ is considered as stable	Test complies with CIPAC MT 41 and is thus acceptable. However, as it is not in accordance with FAO-manual (5th ed.), a new study according to CIPAC MT 41, using water D and 18 h standing at 30°C will be provided with the application for registration at MS-level.	Licata-Messana, 2001o
B.2.2.25 Dry sieve test and wet sieve test (IIIA 2.8.5)			Not applicable (preparation is neither a dustable powder nor a water dispersible product)	
B.2.2.26 Size distribution of particles – Nominal size range of granules (IIIA 2.8.6.1)			Not applicable (preparation is neither a powder nor a granule)	
B.2.2.27 Dust content and particle size of dust (IIIA 2.8.6.2)			Not applicable (preparation is not a granule)	
B.2.2.28 Friability and attrition characteristics of granules (IIIA 2.8.6.3)			Not applicable (preparation is not a granule)	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.29 Emulsifiability, emulsion stability, re- emulsifiability (IIIA 2.8.7.1)			Not applicable (preparation does not form emulsions)	
B.2.2.30 Stability of dilute emulsions (IIIA 2.8.7.2)			Not applicable (preparation does not form emulsions)	
B.2.2.31 Flowability (IIIA 2.8.8.1)			Not applicable (preparation is not a granule)	
B.2.2.32 Pourability (including rinsed residue) (IIIA 2.8.8.2)			Not applicable (preparation is not a suspension)	
B.2.2.33 Dustability following accelerated storage (IIIA 2.8.8.3)			Not applicable (preparation is not a dustable powder)	

Study	Guidelines and GLP	Findings	Evaluation and conclusion	References
B.2.2.34 Physical compatibility of tank mixes (IIIA 2.9.1)	- Internal method : <i>static test</i> : mixing + observation of homogeneity, flocculation, colour and sedimentation (filtration on 150 µm) <i>dynamic test (if needed)</i> : agitating during 15 min + filtration (150 µm)	PHYLIQ was found to be <u>fully compatible</u> with (according to static test) : Alto, Alto + Sportak HF, Amistar, Bref C, Cycocel C5BASF, Cycocel CL, Medax, Moddus, Ogam, Sportak HF, Unix + Alto <u>partly compatible</u> with (according to static test) : Unix : compatible only when respecting a particular order of mixing (i.e. first PHYLIQ and then Unix)	Acceptable	Joubert, 2001a
B.2.2.35 Chemical compatibility of tank mixes (IIIA 2.9.2)	- Internal method : mixing + observation of pH variation	PHYLIQ was found to be chemically stable when mixed with following plant protection products tested : <u>fungicides</u> : Alto, Amistar, Fortress, Horizon EW, Ogam, Opus, Pyros (= Sportak HF), Unix <u>plant growth regulators</u> : Bref C, Cycocel C5 BASF, Cycocel CL 2000, Moddus, Mondium	Acceptable	Joubert, 2001b
B.2.2.36 Distribution and adhesion to seeds (IIIA 2.10)			Not applicable (preparation not intended for seed treatment)	

Summary and conclusions

PHYLIQ is a soluble concentrate (SL) to be used alone or in combination with fungicides for the protection of cereals against pathogens. It is a clear brown liquid with a slight odour and it is not to be classified as a dangerous preparation.

The technical properties of the formulation indicate that no particular problems are to be expected when it is used as recommended : it is not very acidic, its dilution is stable, and it produces no foam. The only peculiarity is that the product has to be considered as surface active.

Its physical stability observed after 14 days at 54°C suggests that storage under practical conditions should pose no problems; chemical stability on the other hand appears to be problematic after accelerated storage (a.s. content decreases by 13.8% after 14 days at 54°C); shelf life study at ambient temperature in commercial packaging is still ongoing. Justification is required for the fact that the difference between the determined a.s. content and that declared exceeds the FAO tolerance.

When used in tank mixes, no problem is expected although in some cases a particular procedure for mixing might be required (i.e. respecting a particular order of adding the tank mix partners).

B.2.3 References relied on**B.2.3.1 Physical and chemical properties of the active substance**

Annex Point / Reference number	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIA 2.1/01	LICATA-MESSANA L.	2000a	Laminarin – Melting point SEPC - Study N° 00-907005-003 GLP, unpublished	Y	GOË
IIA 2.1/02	LICATA-MESSANA L.	2000b	Laminarin – Boiling point SEPC - Study N° 00-907005-004 GLP, unpublished	Y	GOË
IIA 2.1/03 (location : IIA 2.1/01)	LICATA-MESSANA L.	2000a	Laminarin – Melting point SEPC - Study N° 00-907005-003 GLP, unpublished	Y	GOË
IIA 2.2/01	LICATA-MESSANA L.	2000c	Laminarin – Relative density SEPC - Study N° 00-907005-005 GLP, unpublished	Y	GOË
IIA 2.3/01	TREMAIN S.P.	2001	Laminarin: Determination of Vapour Pressure Safepharm Laboratories Limited Study N° SPL Project Number: 1303/005 GLP, unpublished	Y	GOË
IIA 2.3/02b	AMBROSI D.	2002	Calculation of Henry's law constant ASC - Non GLP, unpublished	Y	GOË
IIA, 2.4/01 (location : IIA 2.1/01)	LICATA-MESSANA L.	2000a	Laminarin – Melting point SEPC - Study N° 00-907005-003 GLP, unpublished	Y	GOË
IIA 2.4/02 (location : IIA 8.2/02)	LICATA-MESSANA L.	2001a	Laminarin – Acute toxicity in freshwater fish (96 hours) <i>Danio rerio</i> SEPC - Study N°00-907005-021 GLP, unpublished	Y	GOË
IIA 2.4/03 (location : IIA 2.11/01)	LICATA-MESSANA L.	2000d	Laminarin – Flammability of solids SEPC - Study N° 00-907005-016 GLP, unpublished	Y	GOË
IIA 2.5/01	CUTHBERT J.	2001	Laminarin - Determination of Spectra Safepharm Laboratories Limited Study N° 1476/001 GLP, unpublished	Y	GOË

Annex Point / Reference number	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIA 2.5/02	CHIZHOV A.O., DELL A., MORRIS H.R., REASON A.J., HASLAM S.M., McDOWELL R.A., CHIZHOV O.S., USOV A.I.	1998	Structural analysis of laminarans by MALDI and FAB mass spectrometry. Carbohydrate Research 310, 203-210 Non-GLP, published	N	-
IIA 2.5/03	DELOLME F.	2000	Analysis by MALDI-TOF mass spectrometry CNRS Report N° FC 000828 Non-GLP, unpublished	Y	GOË
IIA 2.5/04	CHEN P., BAKER A.G., NOVOTNY M.V.	1997	The use of osazones as matrices for the Matrix-Assisted Laser Desorption/Ionisation mass spectrometry of carbohydrates Analytical Biochemistry, 244, 144-151 Non-GLP, published	N	-
IIA 2.5/05 (location : IIA 1.7/03)	READ S.M., CURRIE G., BACIC A.	1996	Analysis of the structural heterogeneity of Laminarin by Electrospray-ionisation-mass spectrometry Carbohydrate Research, 281, 187-201 Non-GLP, published	N	-
IIA 2.6/01	LICATA-MESSANA L.	2001b	Laminarin – Solubility in water SEPC - Study N° 00-907005-008 GLP, unpublished	Y	GOË
IIA 2.6/02	ANDING C.	2002	Hydrosolubility of Laminarin DEFITRACES – Study N° SEP/01-069 GLP, unpublished	Y	GOË
IIA 2.7/01	LICATA-MESSANA L.	2001c	Laminarin – Solubility in n-Heptane SEPC - Study N° 00-907005-009 GLP, unpublished	Y	GOË
IIA 2.7/02	LICATA-MESSANA L.	2001d	Laminarin – Solubility in Xylene SEPC - Study N° 00-907005-010 GLP, unpublished	Y	GOË
IIA 2.7/03	LICATA-MESSANA L.	2001e	Laminarin – Solubility in 1,2 Dichloroethane SEPC - Study N° 00-907005-011 GLP, unpublished	Y	GOË
IIA 2.7/04	LICATA-MESSANA L.	2001f	Laminarin – Solubility in Methanol SEPC - Study N° 00-907005-012 GLP, unpublished	Y	GOË

Annex Point / Reference number	Author(s)	Year	Title Testing facility, Report n°, GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIA 2.7/05	LICATA-MESSANA L.	2001g	Laminarin – Solubility in Acetone SEPC -Study N° 00-907005-013 GLP, unpublished	Y	GOË
IIA 2.7/06	LICATA-MESSANA L.	2001h	Laminarin – Solubility in Ethyl acetate SEPC - Study N° 00-907005-014 GLP, unpublished	Y	GOË
IIA 2.8/01	QUINTELAS G.	2001a	Laminarin – Determination of the partition coefficient of Laminarin DEFITRACES – Study N° SEP/00-074 GLP, unpublished	Y	GOË
IIA 2.9/01	QUINTELAS G.	2001b	Laminarin –Abiotic degradation of Laminarin pH dependent hydrolysis (Test C7) DEFITRACES – Study N° SEP/00-075 GLP, unpublished	Y	GOË
IIA 2.9/02	COMB A.L .	2002	Laminarin – Abiotic degradation : hydrolysis as a function of pH Huntingdon Life Sciences – Study (protocol) N° GOM/004 GLP, unpublished	Y	GOË
IIA 2.11/01	LICATA-MESSANA L.	2000d	Laminarin – Flammability of solids SEPC - Study N° 00-907005-016 GLP, unpublished	Y	GOË
IIA 2.11/02	LICATA-MESSANA L.	2000e	Laminarin – Flammability in contact with water SEPC - Study N° 00-907005-017 GLP, unpublished	Y	GOË
IIA 2.11/03	LICATA-MESSANA L.	2000f	Laminarin – Self ignition temperature of solids SEPC - Study N° 00-907005-019 GLP, unpublished	Y	GOË
IIA 2.13/01	LICATA-MESSANA L.	2000g	Laminarin – Explosive properties Laminarin N° 00-907005-018 GLP,unpublished	Y	GOË
IIA 2.14/01	LICATA-MESSANA L.	2000h	Laminarin – Surface tension SEPC - Study N° 00-907005-007 GLP, unpublished	Y	GOË
IIA 2.15/01	LICATA-MESSANA L.	2000i	Laminarin – Oxidizing properties SEPC - Study N° 00-907005-020 GLP, unpublished	Y	GOË

B.2.3.2 Physical and chemical properties of the plant protection product

Annex Point / Reference Number	Author(s)	Year	Title Testing facility, Report No., GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIIA 2.1/01 (location : IIIA, 2.7/01)	LICATA-MESSANA L.	2001g	Phyliq – Storage stability : accelerated procedure by heating (at +54°C for 14 days) SEPC Report No. 00-907005-034 GLP, unpublished	Y	GOË
IIIA 2.4/01	LICATA-MESSANA L.	2001a	Phyliq – Free acidity or alkalinity SEPC Report No. 00-907005-027 GLP, unpublished	Y	GOË
IIIA 2.4/02	LICATA-MESSANA L.	2001b	Phyliq – Determination of pH value of the test item SEPC Report No. 00-907005-026 GLP, unpublished	Y	GOË
IIIA 2.4/03	LICATA-MESSANA L.	2001c	Phyliq – Determination of pH value of a 1% aqueous dilution SEPC Report No. 00-907005-028 GLP, unpublished	Y	GOË
IIIA 2.5/01	LICATA-MESSANA L.	2001d	Phyliq – Viscosity SEPC Report No. 00-907005-029 GLP, unpublished	Y	GOË
IIIA 2.5/02	LICATA-MESSANA L.	2001e	Phyliq – Surface tension SEPC Report No. 00-907005-031 GLP, unpublished	Y	GOË
IIIA 2.6/01	LICATA-MESSANA L.	2001f	Phyliq – Relative density SEPC Report No. 00-907005-030 GLP, unpublished	Y	GOË
IIIA 2.7/01	LICATA-MESSANA L.	2001g	Phyliq – Storage stability : accelerated procedure by heating (at +54°C for 14 days) SEPC Report No. 00-907005-034 GLP, unpublished	Y	GOË

Annex Point / Reference Number	Author(s)	Year	Title Testing facility, Report No., GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIIA 2.7/02	LICATA-MESSANA L.	2001h	Phyliq – Free acidity or alkalinity After an accelerated storage procedure at +54°C for 14 days SEPC Report No. 00-907005-036 GLP, unpublished	Y	GOË
IIIA 2.7/03	LICATA-MESSANA L.	2001i	Phyliq – Determination of pH value of the test item <i>After an accelerated storage procedure at +54°C for 14 days</i> SEPC Report No. 00-907005-035 GLP, unpublished	Y	GOË
IIIA 2.7/04	LICATA-MESSANA L.	2001j	Phyliq – Determination of pH value of a 1% aqueous dilution <i>After an accelerated storage procedure at +54°C for 14 days</i> SEPC Report No. 00-907005-037 GLP, unpublished	Y	GOË
IIIA 2.7/05	LICATA-MESSANA L.	2001k	Phyliq – Persistent foaming <i>After an accelerated storage procedure at +54°C for 14 days</i> SEPC Report No. 00-907005-038 GLP, unpublished	Y	GOË
IIIA 2.7/06	LICATA-MESSANA L.	2001l	Phyliq – Dilution stability <i>After an accelerated storage procedure at +54°C for 14 days</i> SEPC Report No. 00-907005-039 GLP, unpublished	Y	GOË
IIIA 2.7/07	LICATA-MESSANA L.	2001m	Phyliq – Low temperature stability SEPC Report No. 00-907005-040 GLP, unpublished	Y	GOË
IIIA 2.7/08	MOURGUES L.	2002	Phyliq : physico-chemical tests before and after CIPAC MT 46 test 14 days at 54°C Defitraces Report N° 02-907005-001 GLP, unpublished	Y	GOË

Annex Point / Reference Number	Author(s)	Year	Title Testing facility, Report No., GLP or GEP Status published or not	Data Protection Claimed Y/N	<u>Owner</u>
IIIA 2.7/09	BUROSSE V.	2002	Phyliq – Bacteriological control : total germs, yeasts and moulds counting after 7 weeks storage at ambient temperature and +40°C Laboratoires Goëmar S.A. Report N° VB 0204-01 non-GLP, unpublished	Y	GOË
IIIA 2.8/01	LICATA-MESSANA L.	2001n	Phyliq – Persistent foaming SEPC Report No. 00-907005-032 GLP, unpublished	Y	GOË
IIIA 2.8/02	LICATA-MESSANA L.	2001o	Phyliq – Dilution stability SEPC Report No. 00-907005-033 GLP, unpublished	Y	GOË
IIIA 2.9/01	JOUBERT J.M.	2001a	Phyliq – Physical compatibility of tank mixes Laboratoires GOËMAR S.A. Report No. JMJ010111 Non-GLP, unpublished	Y	GOË
IIIA 2.9/02	JOUBERT J.M.	2001b	Phyliq – Chemical compatibility of tank mixes Laboratoires GOËMAR S.A. Report No. VB 0111-07 Non-GLP, unpublished	Y	GOË