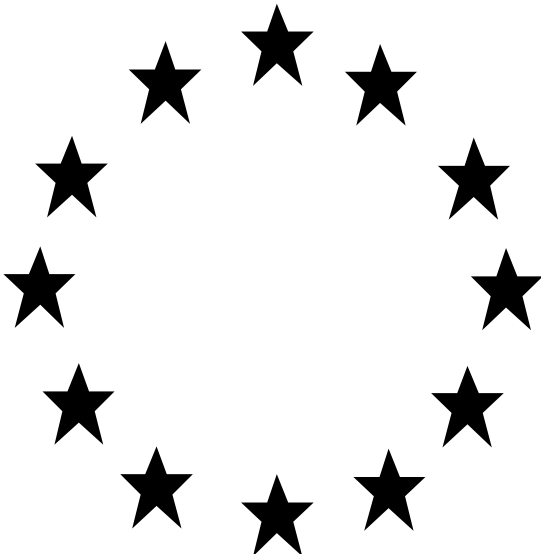

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State France for the new active substance

HEPTAMALOXYLOGLUCAN

as referred to in Article 8(1) of Council Directive 91/414/EEC

June 2009

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European Commission

Program for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC

HEPTAMALOXYLOGLUCAN

VOLUME 1 - CORRIGENDUM 1

Rapporteur Member States Summary, Evaluation And Assessment Of The Data And Information

Rapporteur Member State: ***France***

January 2009

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
251, rue de Vaugirard
75732 PARIS CEDEX 15
France

2.5. FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.5.1 DEFINITION OF THE RESIDUES RELEVANT IN THE ENVIRONMENT

Heptamaloxyloglucan is considered to be the only relevant compound expected in soil, groundwater, surface water, sediment and air.

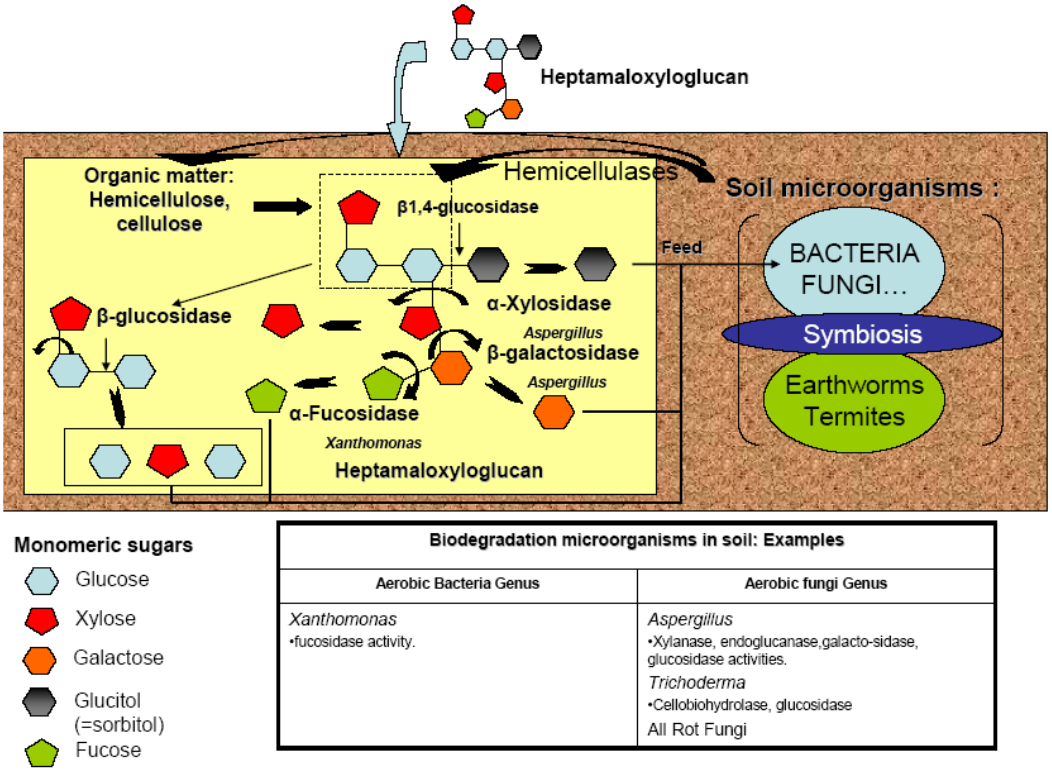
2.5.2 FATE AND BEHAVIOUR IN SOIL

Considering that heptamaloxyloglucan could be an intermediate compound of natural organic matter decomposition process which could undergo degradation by endogenous soil microorganisms naturally occurring in soil, no specific study on the rate and route of degradation was deemed necessary.
The literature data confirm that xyloglucans belong to the Hemicellulose family, one of the principal components of plant cell wall, as well as xylans, mixed glucans (Warren, 1996). The monosaccharide units of xyloglucan are the same as heptamaloxyloglucan (De Vries & Visser, 2001), (Wershaw, 2004). These monomers (or hemicellulosic sugars) are part of natural organic matter found in soils (Karroum et al., 2004).
As microorganisms that degrade cellulose also degrade hemicellulose (and thus xyloglucans), cellulases and hemicellulases are considered as components of systems for the enzymatic cleavage/hydrolysis of plant cell walls. (Warren, 1996). Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose. An effective hydrolysis of cellulose also requires β-glucosidases (Pérez & Muñoz-Dorado, 2002). The hydrolysis of hemicelluloses occurs by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of polymers or oligosaccharides leading to the release of various mono- or disaccharides (Aro et al., 2004), (Wershaw, 2004). In this complex system of biodegradation, heptamaloxyloglucan could be considered as an intermediate compound which will be further degraded into monosaccharide.
During chemical and biochemical processes involved in the degradation of natural organic matter (humification), cellulotic microorganisms transform organic matter into smaller molecules (Tuomela et al., 2000). The structural polysaccharides (cellulose and hemicelluloses) of ligno-cellulosic material of plants undergo a degradation depending on the depth (Karroum et al., 2004). Hemicellulose recycling from plant biomass is indispensable for the carbon cycle (Pérez & Muñoz-Dorado, 2002). Heptamaloxyloglucan as an intermediate of biodegradation should be fully part of the carbon cycle.
Different classes of enzymes are involved in plant cell wall polysaccharide degradation. They are produced by several saprophytic[1] organisms such as bacteria, archea and fungi (for example *Aspergilli*, *Cellulomonas fimi*, a mesophilic aerobic soil bacterium and *Thermonospora fusca*, a thermophilic actinomycete common in compost) (De Vries & Visser., 2001); (Warren, 1996). Plant cell wall hydrolysis requires in particular enzymes hydrolyzing β–1,4 and β–1,3 glycosidic bonds; β–1,4 xylosidic bonds and β–1,4 mannosidic bonds. Four different enzymes are required to degrade the common hemicellulose O-acetyl-4-O-methylglucuronxylan: endo-1-4-β-xylanase, acetyl esterase, α-glucuronidase, and β-xylosidase. (Wershaw, 2004). Aspergilli produce different classes of these accessory enzymes that act on plant cell wall polysaccharides : for example, α-D-Xylosidases, α-galactosidases and β-galactosidases

[1] organisms that survive by decomposing dead or decaying organic matter

Talking about the glucitol residue, it is well known that soil microorganisms thoroughly catabolize this sugar. The pathway of glucitol (sorbitol) metabolism described in *Pseudomonas, Areobacter, Cellvibiro, Rhizobium* and *Stenotrophomonas* genuses oxydize glucitol as a carbon source for organism growth use. (Brechtel, 2002); (Kelker, 1971).

Taking into account of all information given by the cited literature, a pathway of biodegradation was proposed (see figure thereafter). The representation of heptamaloxyloglucan molecule and monomeric sugars are schematic. Real spatial formula illustration could be found under B.1.1.7. To facilitate the review of the scheme, the notifier used the same symbols for the glucidic monomer units of heptamaloxyloglucan and its expected degradation products (monomeric sugars). Therefore in heptamaloxyloglucan representation, D-glucopyranosyl, D-glucitol, D-xylopyranosyl, D-galactopyranosyl and L-fucopyranosyl are represented by the glucose, glucitol, xylose, galactose and fucose monomeric sugar symbols, respectively.



Biodegradation microorganisms in soil: Examples	
Aerobic Bacteria Genus	**Aerobic fungi Genus**
Xanthomonas •fucosidase activity.	*Aspergillus* •Xylanase, endoglucanase,galacto-sidase, glucosidase activities. *Trichoderma* •Cellobiohydrolase, glucosidase All Rot Fungi

Figure 1: fate and behavior of Heptamaloxyloglucan in soil.

No studies regarding adsorption, desorption and mobility of the active substance in soil were deemed necessary as heptamaloxyloglucan which is used at very low application rate may undergo degradation by soil micro-organisms and presents no toxicological or ecotoxicological concerns. Moreover the 0.1 µg/L limit for ground water is not expected to be reached for this kind of molecule.

2.5.3 FATE AND BEHAVIOUR IN WATER

Heptamaloxyloglucan is hydrolytically stable. It is expected to be stable under photo-irradiation.
Heptamaloxyloglucan is readily biodegradable (biodegradation reached 78% at the end of the 28-day modified Sturm test).

No water sediment test was deemed necessary as heptamaloxyloglucan is not acutely toxic to aquatic organisms. Moreover, it can be produced from xyloglucan by enzymatic degradation naturally occurring in plant and could therefore be considered as taking part of vegetal debris naturally brought to open water bodies by plant decays, which is expected to be degraded in natural aquatic systems.

Heptamaloxyloglucan is neither a fungicide nor a bactericide and should enter water bodys at levels of the nanograms per liters. Consequently, no data on the impact of heptamaloxyloglucan on water treatment procedures is deemed necessary.

2.5.4 FATE AND BEHAVIOUR IN AIR

The calculated vapour pressure of heptamaloxyloglucan is $1.1*10^{-11}$ Pa and therefore, heptamaloxyloglucan is not expected to volatilize from plant and soil surface in the air compartment. No study on fate and behaviour of heptamaloxyloglucan was performed.

2.6 EFFECTS ON NON-TARGET SPECIES

2.6.1 EFFECTS ON BIRDS

No toxicity data are available on effects of heptamaloxyloglucan on birds. However literature data show that heptamaloxyloglucan which belongs to the carbohydrates family may undergo fermentation in gastrointestinal tract of birds allowing the production of short-chain fatty acids by endogenous bacteria and convertion of nitrogenous compounds into ammonia and microbial protein and synthetize B vitamins (Stevens & Hume, 1998). Heptamaloxyloglucan which belongs to the carbohydrates family may undergo the same metabolisation in gastrointestinal tract of birds.
Estimation of exposure for birds has been done according to the guidance given in the 4145/SANCO document for an application on early stage of vine. Level of exposure were compared to mammalian toxicity data for acute and short-term risk assessment (see table below). Long-term exposure is judged not relevant as log K_{ow} is equal to –15.96.

Exposed group	Time-scale	Diet	Endpoint	ETE (mg/kg/day)	Theoretical TER
insectivorous bird	acute	small insects	LD_{50} > 5000 mg a.s/kg/day (acute oral rat)	0.024	> 208333
	short-term	small insects	LD_{50} > 1000 mg a.s./kg/day (28-day oral toxicity rat)	0.013	> 76923

The theoretical TER between the mammalian toxicity endpoints and the estimated exposure of insectivorous birds to heptamaloxylogucan are very high compared to the trigger value of 10 for acute and short-term indicating an acceptable risk. Therefore no acute and short-term risk on birds is expected following applications of PEL101GV on vine.

The log K_{ow} of heptamaloxyloglucan is low (–15.96). Therefore it is not expected that heptamaloxyloglucan accumulates along the food chains. Consequently no risk for secondary poisoning is anticipated.

Therefore, no risk on birds is expected following applications of PEL101GV on vine.

2.6.2 EFFECTS ON AQUATIC SPECIES

Toxicity data for aquatic organisms

Heptamaloxyloglucan was not acutely toxic to fish, daphnia or algae with L(E)C_{50} > 150 mg a.s./L.

Substance	Species	Exposure	LC_{50} or EC_{50}	NOEC
Heptamalo-xyloglucan	*O. mykiss*	Acute, 96h	>150 mg/L	150 mg/L
	D. magna	Acute, 48h	>150 mg/L	150 mg/L
	S. subspicatata	Chronic, 72 h	>150 mg/L	150 mg/L

Chronic toxicity tests on aquatic species are not required, since heptamaloxyloglucan is expected to be quickly degraded and is not acutely toxic to aquatic organisms.

As the log K_{OW} of heptamaloxyloglucan is low (–15.96), no bioconcentration of the active substance or its degradation products is expected.

Toxicity/exposure ratios for aquatic organisms

The assessment of surface water contamination by heptamaloxyloglucan assuming no degradation of the active substance between applications lead to an initial PEC after the fourth and last applications of PEL101GV of 0.0143 µg a.s./L. The corresponding TER meet Directive 91/414/EC triggers:

Test species	Endpoint	Result (µg as/L)	Initial PEC µg as/L	TER
O. mykiss	LC_{50}, 96h	>150000	0.6025	> $20*10^4$
D. magna	EC_{50}, 48h	>150000	0.6025	> $20*10^4$
S. subspicatata	E_bC_{50}, 72 h	>150000	0.6025	> $20*10^4$

Risk assessment conducted under the worst case assumption with 1 application of 1.76 g a.s./ha (equivalent to no degradation of heptamaloxyloglucan after 4 applications) show that no risk on aquatic organisms are expected following applications of PEL101GV.

2.6.3 EFFECTS ON TERRESTRIAL VERTEBRATES OTHER THAN BIRDS

Toxicity data for mammals

Studies performed on mammals to investigate the acute and short-term toxicity of EL101GV (technical heptamaloxyloglucan) show the low toxicity of the active substance.

Test species	Test system	Results
Rat	Acute oral toxicity	LD_{50} > 5000 mg EL101GV/kg b.w
Rat	28-day oral toxicity	NOEL = 1000 mg EL101GV/kg b.w./d

Toxicity/exposure ratios for mammals

Risk assessment for mammals done according to the guidance given in the 4145/SANCO document for an application on early stage of vine show that the acute risk of heptamaloxyloglucan for vertebrates other than birds is acceptable. The log K_{ow} of heptamaloxyloglucan is low (–15.96). Therefore it is not expected that heptamaloxyloglucan accumulates along the food chains. Consequently no risk for secondary poisoning is anticipated.

Exposed group	Time-scale	Endpoint	Diet	ETE (mg/kg/day)	TER
Small herbivorous mammal	acute	LD_{50} > 5000 mg a.s/kg	Short grass	# 0.11	> 45455

2.6.4 EFFECTS ON BEES AND OTHER ARTHROPOD SPECIES

Bees

Heptamaloxyloglucan was not acutely toxic to bees (oral and contact LD_{50} > 100 µg a.s./bee). Literature data show that some sugars could be poisonous to bees or cause reduction in bee longevity (Winston, 1987). Some such as mannose can not be used by bees and are present in glycopeptide form in royal jelly to avoid a poisonous effect (Haydak, 1970). One of the possible degradation product of heptamaloxyloglucan, i.e. galactose, was found to have lethal effects compared to the control at a dose level of 8%, 2.16 mg/bee/day, in a 16-days dietary test (Barker, 1977). A LD_{50} of galactose comprised between 1620 µg/bee/day and 2160 µg/bee/day could be derived from this publication.

Hazard quotient following application of 0.44 g heptamaloxyloglucan/ha, as well as the hazard quotient for galactose (assuming the same application rate as heptamaloxyloglucan), was well below 50 (*i.e.* 0.44/100=0.0044 and 0.44/1620=0.00027, respectively). The risk to bees following application of PEL101GV on vine is considered to be acceptable.

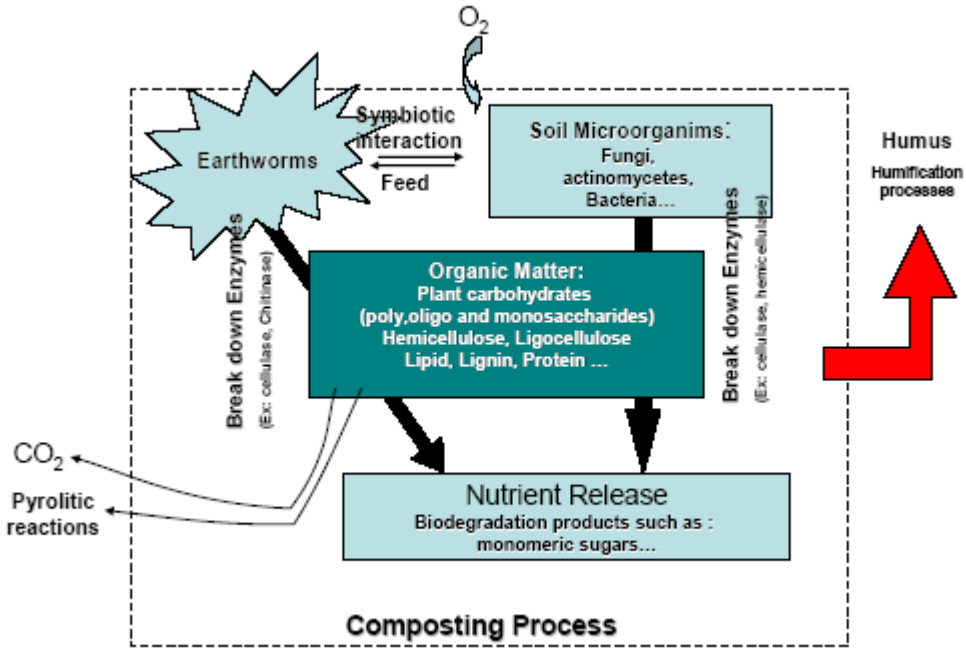
Other non-target arthropods
Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples and composed of 7 hexose residues (glucopyranosyl, fucopyranosyl, xylopyranosyl and galactopyranosyl). All these hexose are natural components of the apple and of other dicotyledonous plants, where they are major constituents of cellulose and hemicellulose molecules, which are the principal components of cell walls. As such, heptamaloxyloglucan takes part of usual food on arthropods.
Heptamaloxyloglucan is not toxic to honey bees (oral and contact LD_{50} > 100 µg/bee).
For these reasons, no test on non-target arthropods is deemed necessary.

2.6.5 EFFECTS ON EARTHWORMS AND OTHER SOIL MACRO-ORGANISMS

No data was provided on toxicity of heptamaloxyloglucan or formulated product PEL101GV to earthworms and other soil macro-organisms.

Literature data show that earthworms use organic matter as a source of nutrition but depend upon protozoa, rotifers, nematodes, bacteria, fungi and other micro-organisms, for their nutrients. Soil ingested microorganisms and earthworms have developed symbiotic/mutualist relationship to digest soil organic matter (Lattaud and al., 1997, II A 8.4/4). Though earthworms possess glycolytic enzyme activity (cellulase, hemicellulase, amylase…), they need microflora to complete enzymatic equipment that they lack. In this general situation, ingested microflora is able to degrade organic matter as simple elements that will be absorb again trough the earthworm gut walls (Zhang and al. 1993, II A 8.4/5, Lattaud and al. 1997, II A 8.4/4, Garvin and al. 2000, II A 8.4/6).

Therefore, it is assumed that heptamaloxyloglucan is in the same way, degraded by ingested soil microorganism as glucidic monomers inside the different parts of the earthworm gut. The following relationship scheme describing earthworm/microorganisms interactions in the composting process (humification) was proposed.



Initial PEC_{soil} of heptamaloxyloglucan calculated based on worst-case (unrealistic) assumptions of no degradation and no crop interception has been estimated to be equal to

0.00235 mg a.s./kg after 4 applications of PEL101GV to grapevines at growth stage BBCH 7 to BBCH 16.

It was demonstrated that micro-organisms ingested by earthworms together with soil are able to degrade oligosaccharides into monomeric sugars. Glucose, xylose, fucose, galactose and glucitol, the monomeric sugars expected to result from degradation of heptamaloxyloglucan, are naturally occuring into organic matter of soil (see also B.8.1 on fate and behaviour in soil). The very low amounts of residues due to the use of heptamaloxyloglucan (with no consideration of degradation, initial PEC_{soil} are comprised between 0.59 and 2.35 µg a.s./kg after 1 and 4 applications, respectively) is not expected to change the qualitative composition of the organic matter which reaches the soil or to cause damage on soil macro-organisms. Therefore no testing on earthworm is needed.

2.6.6 EFFECTS ON SOIL MICRO-ORGANISMS

Heptamaloxyloglucan is a possible degradation product of plant cell walls as it can be produced from xyloglucan by enzymatic degradation naturally occurring in plant or in soil by micro-organisms. Plant decay is a natural substrate for soil micro-organisms growth.
The initial PEC_{soil} of heptamaloxyloglucan were calculated after the 4^{th} application of PEL101GV (see B.8.3 for detail of calculations), and found to be 0.00235 mg/kg dry soil. Such low concentrations are not susceptible to change the qualitative composition of the organic matter which reaches the soil. Therefore heptamaloxyloglucan is not expected to have any adverse effects on the function of soil micro-organisms ecosystems.

2.6.7 EFFECTS ON OTHER NON-TARGET ORGANISMS (FLORA AND FAUNA)

Heptamaloxyloglucan (EL101GV) had no adverse effects on the vegetative vigour of wheat, mustard and red cover at 0.2, 2.0 and 20.0 g a.s./ha application rate.
A negligible risk is expected since there is no adverse affects observed following application of 20 g heptamaloxyloglucan/ha, i.e. 45-fold greater than the initial application rate of heptamaloxyloglucan.

2.6.8 EFFECTS ON BIOLOGICAL METHODS OF SEWAGE TREATMENT

Heptamaloxyloglucan is a xyloglucan molecule and made of 7 glucidic monomer units, which are all natural components of cell walls of the apple (from which it is extracted) and of other dicotyledonous plants. Moreover it could be produced by degradation of xyloglucan by enzymes naturally occurring in plant or soil micro-organisms (see B.8.1.2.3 and B.8.1.2.4). As such it is a classical part of sewage. Therefore it is not expected to have any detrimental effect on biological methods for sewage treatment. No study is therefore deemed necessary.

3.1 BACKGROUND OF THE PROPOSED DECISION

Add this paragraph

"Concerning identity, active substance from actual plant production is acceptable. Concerning physical and chemical properties all studies required by Directive 91/414/EEC are available and were conducted according to Guideline requirements and GLP regulations.

Analytical methodology is available for the determination of the active substance and the impurities in Heptamaloxyloglucan as manufactured and in the formulated product. The methods are fully validated for determination of active substance."

EUROPEAN COMMISSION

Program for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC

HEPTAMALOXYLOGLUCAN

VOLUME 3, ANNEX B.2 ADDENDUM 1

Rapporteur Member States Summary, Evaluation And Assessment Of The Data And Information

Rapporteur Member State: ***France***
January 2009

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
251, rue de Vaugirard
75732 PARIS CEDEX 15
France

B.2.1. PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

B.2.2.7.3. SHELF LIFE FOLLOWING STORAGE AT AMBIENT TEMPERATURE

In January 2009, a shelf life study was provided by the notifier.

<table>
<tr><th>Test or Study Annex point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Results</th><th>Comments</th><th>GLP</th><th>Reference</th></tr>
<tr><td>Shelf life following storage at ambient temperature
B.2.2.7.3
(IIIA, 2.7.3)</td><td>According to GIFAP Monograph No. 17</td><td>Batch AND0205</td><td>The preparation was packed in amber glass flasks and stored at ambient temperature (20°C) during 2 years.
Results:
<table>
<tr><th></th><th>T0</th><th>T 2 years</th></tr>
<tr><td>Content %</td><td>85.5</td><td>85.3</td></tr>
<tr><td>pH at 1%</td><td>6.23 after 1 min
7.02 after 10 min</td><td>60.4 after 1 min
7.09 after 10 min</td></tr>
<tr><td>Wettability without swirling</td><td>immediate</td><td>immediate</td></tr>
<tr><td>Degree of dissolution at 1.2% w/v</td><td>No residue after 5 min and for 18h</td><td>0.1% w/w of residue after 5 min and 0% after 18 h</td></tr>
<tr><td>Wet sieving 45 µm sieve</td><td>All the preparation pass through the sieve</td><td>Most of the preparation pass through the sieve. A negligible amount of preparation (not quantifiable) were observed on sieve</td></tr>
</table></td><td>Acceptable.
The preparation is stable for 2 years at 20°c</td><td>Y</td><td>Ferron N, Ricau H, 2009</td></tr>
</table>

REFERENCE RELIED ON

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIIA, 2.7.1	Ferron N, Ricau H	2009	Chemical stability for 2 years at 20 +/- 2 °C and physico-chemical tests after the storage procedure on the preparation PEL101GV	N	Elicityl

EUROPEAN COMMISSION

Program for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC

HEPTAMALOXYLOGLUCAN

VOLUME 3, ANNEX B.2
CORRIGENDUM 1

Rapporteur Member States Summary, Evaluation And Assessment Of The Data And Information

Rapporteur Member State: ***France***
January 2009

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
251, rue de Vaugirard
75732 PARIS CEDEX 15
France

B.2.1. PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

B.2.1.1./3C MELTING, FREEZING OR SOLIDIFICATION POINT

In the column results, delete “this sticky paste has a little tendency to blow up”

B.2.1.4. APPEARANCE : PHYSICAL STATE, COLOUR AND ODOUR

In the column GLP, read :“No”

Instead of :”No, the quality assurance report was not signed. Data required”

B.2.2.1.1. APPEARANCE : PHYSICAL STATE, COLOUR AND ODOUR

In the column GLP, read :“No”

Instead of :”No, the quality assurance report was not signed. Data required”

B.2.2.4.2. PH

In the column findings, read “The pH measured is 6.2 at 21°C after 1 min and 7.02 at 21°C after 10 min in water D”
Instead of “The pH measured is 6.2 at 21°C after 1 min and 7 at 21°C after 10 min in water D”

B.2.2.7.1. ACCELERATED STORAGE STABILITY

In the column GLP, read :“No”

Instead of :”No, the quality assurance report was not signed. Data required”

In the column findings, add “the packaging is a amber glass flask.”

B.2.1.10. STABILITY IN AIR, PHOTOCHEMICAL DEGRADATION, IDENTITY OF BREAKDOWN PRODUCTS

In the column Results, add “Hydroxyl-ion concentration is 1.5 10^6 OH/cm^{3}”

EUROPEAN COMMISSION

Program for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC

HEPTAMALOXYLOGLUCAN

VOLUME 3, ANNEX B.8
CORRIGENDUM 1

Rapporteur Member States Summary, Evaluation And Assessment Of The Data And Information

Rapporteur Member State: ***France***
January 2009

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
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B.8. ENVIRONMENTAL FATE AND BEHAVIOUR

Xyloglucan is the principal hemicellulosic component of primary cell walls of dicotyledonous and non-graminaceous monocotyledonous plants. Xyloglucan plays a physiological key role in maintaining cell wall integrity by cross-linking individual cellulose microfibrils in the primary cell wall. Specific oligosaccharides such as heptamaloxyloglucan can be produced naturally from xyloglucan by partial hydrolysis with cellulase (β-1,4-D-glucanase) and various other enzymes which are present in plants and soil micro-organisms. It has been demonstrated that these specific oligosaccharides accumulate extracellularly in plants and act at very low levels as signaling molecules that participate in cell-cell and wall-nucleus communication (Fry et al., 1993[2]; Buchanan et al., 2000[3]).

Technical Heptamaloxyloglucan (coded EL101GV) is prepared from dry apple pomace by enzymatic hydrolysis and deacetylation/reduction after fractioning and purification. Samples are then purified and conditioned by lyophilisation.

The active substance heptamaloxyloglucan (MW = 1078 g/mol, CAS number [870721-81-6], minimum purity of 78%) is a xyloglucan-derived oligosaccharide made of 7 glycosidic monomer units (polymerisation degree = 7). There are β-1,4 linkages on the main chain between the two D-glucopyranosyl units and terminal D-glucitol, and α-1,2, β-1,2 and α-1,6 linkages between the various monomer units present in side chains. The latter side chain-monomers are D-xylopyranosyl (α-1,6-linked to D-glucopyranosyl), D-galactopyranosyl (β-1,2-linked to D-xylopyranosyl) and L-fucopyranosyl (α-1,2-linked to D-galactopyranosyl).

Heptamaloxyloglucan acts as a stimulator of plant defence natural mechanisms (“elicitor”) which must preserve the chemical structure and conformation of the xyloglucan heptamer XFG in order to increase the cold resistance of the grapevine. As heptamaloxyloglucan occurs in plants and soil at very low levels, the manufacturing process mimics natural phenomenons in order to accelerate the rate of natural biochemical processes and thus increase heptamaloxyloglucan yields.

The representative formulation (PEL101GV) is equivalent to the technical active substance EL101GV PEL101GV is a lyophilisat water-soluble formulation used as an anti-freezing in grapevine. The proposed use for heptamaloxyloglucan is post-emergence applications of 0.44 g a.s./ha (4 applications maximum with a minimal interval between applications of 4 days) on grapevine at BBCH 7 to 16 (early spring).

B.8.1. ROUTE AND RATE OF DEGRADATION IN SOIL (ANNEX IIA 7.1.1; ANNEX IIIA 9.1.1)

Notifier mentions that xyloglucans are degraded by enzymatic cleavage in soil. Saprotrophic organisms[4] such as bacteria, archea and fungi could produce the required enzymes. As heptamaloxyloglucan could be produced under natural

[2] Fry. S.C., Aldington S., Hetherington P.R., Aitken J., 1993. “Oligosaccharides as Signals and Substrates in the Plant Cell Wall“.Plant Physiol., Vol. 103 (1993), pp. 1-5.
[3] Buchanan B.B. et al, 2000. “Chapter 2: The Cell Wall. Biochemistry and molecular Biology of Plants”. B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89.
[4] Organisms that survive by decomposing dead or decaying organic matter

conditions by enzymatic degradation of xyloglucans (component of plant cell wall) by soil micro-organisms (see B.8.1.2.3 and B.8.1.2.4), it could occurred naturally in soil organic matter. Therefore the notifier has not conducted specific studies on rate and route of degradation in soil.

B.8.1.1. ROUTE OF DEGRADATION (ANNEX IIA 7.1.1.1)

No studies have been performed to test the degradation of heptamaloxyloglucan as several enzymes and micro-organisms present in soil can degrade xyloglucans under aerobic and anaerobic conditions. Moreover photodegradation is not relevant for xyloglucans.

B.8.1.1.1. Aerobic degradation of heptamaloxyloglucan (Annex IIA 7.1.1.1.1)

Notifier explains that there is mainly three classes of enzymes involved in the degradation of xyloglucans:

- hydrolytic enzymes, including cellulases, glucanases and glucosidases, which convert these polymers into simple sugars for fungal metabolism (Warren, 1996; De Vries & Visser, 2001; see B.8.1.2.3, references II A 7.1/2 and II A 7.1/7);
- oxidative enzymes;
- oxidoreductase enzymes.

Finally, the sugar residues take part in the micro-organism metabolism and are finally incorporated into micro-organism cellular structures or mineralized to CO_2 (Wershaw, 2004; II A 7.1/6).

B.8.1.1.2. Supplementary studies (Annex IIA 7.1.1.1.2)

B.8.1.1.2.1 Anaerobic degradation of heptamaloxyloglucan (Annex IIA 7.1.1.1.2a)

The notifier argue that xyloglucans may be degraded by fermentation under anaerobic conditions (Warren, 1996; see B.8.1.2.3, Reference II A 7.1/2) leading to production of short-chain fatty acids, as final product. These molecules are involved in the micro-organism metabolism and are finally incorporated into micro-organism cellular structures or mineralized to CO_2 (Wershaw, 2004; II A 7.1/6).

It may be noticed that anaerobic conditions may be considered as not relevant regarding the timing of application, i.e. early spring to prevent damage from frost.

B.8.1.1.2.2 Soil photolysis (Annex IIA 7.1.1.1.2b)

As the notifier did not provide any information on that aspect, it cannot be conclude that heptamaloxyloglucan is not sensitive to photodegradation in soil. However, photolysis is not expected to be the major dissipation process in the case of early spring application of heptamaloxyloglucan. Even if some photolysis would happen, it is emphasised that the degradation products will not lead to concentration of concern. In case heptamaloxyloglucan is stable under soil photolysis it may anyway be degraded into monomeric sugars by soil micro-organisms.

B.8.1.2. RATE OF DEGRADATION (ANNEX IIA 7.1.1.2)

The release of xyloglucans from the cell wall is due to leaves and plant debris fractionation and to enzymatic cleavage of short-chain oligosaccharides. According to the notifier, the rate of degradation of xyloglucans is directly correlated to the soil biomass (which produces the various enzymes necessary to the degradation) and to the soil temperature, which controls the activity of the enzymes. In addition, short chain soluble oligosaccharide, like heptamaloxyloglucan are more easily degraded than long-chain insoluble molecules, so the degradation rate of the active substance in soil is likely to be quite rapid. Furthermore heptamaloxyloglucan is readily biodegradable as shown in L'haridon J. (2006) under point B.8.4.3.1.

B.8.1.2.1 Laboratory studies (Annex IIA 7.1.1.2.1)

No study has been performed since the active substance, which could be produced under natural conditions by enzymatic degradation of xyloglucans by soil micro-organisms, is shown to be readily biodegradable and is intended to be used at very low rates (max 0.5 g a.s./ha).

B.8.1.2.2 Field studies (Annex IIA 7.1.1.2.2)

No study is required.

B.8.1.2.3 Additional informations

Notifier provides literature data to show that heptamaloxyloglucan as other oligosaccharides belonging to carbohydrates family could be degraded under natural conditions in soil.
The literature data was judged acceptable when it was possible to derive useful informations (even general or qualitative ones) on how xyloglucan, carbohydrates or oligosaccharides could be degraded, assimilated or used by the different organisms and when the complete scientific article is provided. A summary of each publication has been done by the RMS in regard to these conditions and in the purpose of hazard identification and assessment of risk. A general conclusion on the rate and route of degradation is also provided at the end of the current point of B.8.

- **Summaries of literature data from RMS focusing on xyloglucan informations:**

Reference number:	II A 7.1/1
Report:	Ishii T., 1997 Structure and functions of feruloylated polysaccharides Plant Science 127 (1997) 111-127 Published
GLP compliance	No
Acceptability	**No, no reliable informations regarding degradation in soil. The information related to xyloglucans as part of plant cell wall component. Moreover it was already addressed in B.9.1 and B.9.6.4 (Buchanan B.B. et al, 2000).**

This article is a review of knowledge on feruloylated polysaccharides. Small amounts of ester-linked hydroxycinnamic acid derivatives contained in cell wall polysaccharides (e.g. ferulic acids) can be coupled oxidatively to form the acid dimers. Formation of such dimers in a growing plant cell wall caused the cross-linkage of cell wall polysaccharides leading to an increase of wall rigidity. Feruloyl oligosaccharides, which are derived from feruloyl polysaccharides, inhibit cell elongation growth induced by auxin or gibberellins. Feruloyl polysaccharides are

shown to be critical entities in directing wall cross-linking and in limiting biodegradability by microorganisms.
In this article, the major components of plant cell walls were summarised. In growing plant cell, cellulose, hemicellulose (*i.e.* polysaccharides hydrogen-binding to cellulose such as xylans and xyloglucans...) and pectins are of equal abundance in dicotyledons (*ca.* 30%) whereas monocots possess much less pectin (5% *vs*. 30% cellulose and 65% hemicellulose). Other components such as lignin or phenolic acids are present in less amounts. At maturity, wall composition changes, pectins are present in very few amounts in both dicots and grasses. In grasses, hemicellulose became more present than cellulose (40-50% *vs.* 25-35%) whereas the opposite is observed in dicots (25% hemicellulose *vs*. 45-50% cellulose).

Reference number: II A 7.1/2
Report: Warren R.A.J., 1996
Microbial hydrolysis of polysaccharides
Annu. Rev. Microbiol. 1996. 50:183-212
Published
GLP compliance No
Acceptability **Yes**

This publication refers to the systems of enzymes produced by microorganisms for the hydrolysis of polysaccharides to metabolizable products.

The naturally occurring substrates are insoluble and microorganisms utilizing them must use extracellular enzymes, free or associated with the cell surface, to convert the polysaccharides to soluble products that are transportable into the cells.

Microorganisms efficiently degrade starch, chitin and polysaccharides in plant cell walls. The diversity of enzymes involved in cellulose hydrolysis must be a consequence of the different sugars and linkages present in plant cell walls. Among polysaccharides, cellulose is more tightly associated with plant cell wall than mannans or xylans. Plant cell walls comprise also minor components such as xyloglucans, galactomannans, pectins, and some glucans.

The microbial degradation of polysaccharides entails diverse glycoside hydrolases with different specificities and modes of action. The enzyme systems involved are complex as many of the individual enzymes are modular proteins comprising one or more catalytic domains linked to ancillary domains that often include one or more substrate-binding domains, and as the systems comprise from a few to 20 or more enzymes, all of which hydrolyze a particular substrate. Systems for the hydrolysis of plant cell walls usually contain more components than systems for the hydrolysis of starch and chitin because the cell walls contain several polysaccharides. As organisms that degrade hemicellulose usually degrade hemicelluloses also, cellulases and hemicellulases are considered as components of systems for hydrolysis of plant cell walls. The bacteria *Cellulomonas fimi* and *Thermonospora fusca* (a mesophilic aerobic soil bacterium and a thermophilic actinomycete common in compost, respectively) for example have similar plant cell wall hydrolysing systems.
Microorganisms degrading polysaccharides produce multi-component enzyme systems of varying degrees of complexity (depending on the substrate). These systems have a number of characteristics in common. Plant cell wall hydrolysis requires enzymes hydrolyzing β–1,4 and β–1,3 glycosidic bonds; β–1,4 xylosidic bonds and β–1,4 mannosidic bonds; and perhaps others. Two basic types of systems are involved in the hydrolysis of plant cell walls: complexed systems like the cellulosome and non-associated systems like those in aerobic microorganisms. Both types of systems contain multiple endoglucanases and multiple xylanases. Cell wall hydrolyzing systems appear to be more complex than those hydrolyzing starch and chitin.
The hydrolysis of plant cell walls is a relatively slow process. This is compensating by the high efficiency of the enzymes involved in hydrolysis of glycosidic bonds.

Reference number:	II A 7.1/3
Report:	Karroum M. et al., 2004 Importance et devenir des biopolymères (lignines et polysaccharides) dans les sols d'une chronoséquence de hêtraies (*Fagus sylvatica*) en forêt de Fougères (France) / Importance and fate of biopolymers (lignins and polysaccharides) in soils of *Fagus sylvatica* stands of various ages in Fougères forest (Britany - France) Ann. For. Sci 61 (2004) 211-233 Published
GLP compliance	No
Acceptability	**Yes. The publication was in french but notifier provides translation in english of main part of this publication related to the polysaccharides degradation in soil. This study provides interesting informations on humification and fate of polysaccharides under field conditions (forest).**

The humification process rests on the transformation of initial components of microbial or phyto-inherited organic matter comprising mainly lignin, polysaccharides (pectins, hemicelluloses and cellulose) and polypeptids. The aim of this study was to precise the route of transformation of lignins and polysaccharides, major biopolymers reaching the soil via plant falls. In soil, these polymers were transformed and degraded according to different rates due to the diversity of humus type and depending on the stability of the associations formed with other components such as polyphenols, oligopeptids and amino acids. The inventory and quantification of polysaccharides by gaz chromatography was based on an acid hydrolysis technic in 2 steps, which allow distinguishing the structural entities (cellulose and hemicelluloses) that are destroyed and the microbial exo-polysaccharides produced by the microflora.

In this study, four beech stands of various ages were selected in 1997 to study the evolution of lignins and structural polysaccharides (cellulose, hemicelluloses) in the soil cover. The 10-year-old station has a mull type humus. In the 27-year-old stand there is a mull-moder mosaic whereas in the 87 and 145-year-old stands humus is a moder. Twenty-one soil profiles were sampled by separating the different humus layers, *i.e.,* OL and OF, present in mull and moder, and the OH layer, only present in the moder. Organo-mineral A11 and A12 horizons were also sampled with some A13 horizons in the mull stations.
Lignins are abruptly degraded in mull where they represent 52‰ of the total organic carbon (TOC) in OL and only 12‰ in A1 horizons. Similarly, polysaccharides undergo degradation from 236‰ (OL) to 105‰ (A1) of TOC. The fast decrease in the concentration of these components over a small depth interval is indicative of a strong biological activity. The lignin alteration is evidenced by the decrease of the ratio of syringic compounds over vanillic compounds that reveal methoxyl group losses and the increase of vanillic acid over vanillic aldehyde which indicates oxidative depolymerization of lignins.
Whatever is the age of the plot, the OL, OF and OH organic layer present a high level of total sugar with a prevalence of hemicellulosic sugars. The structural

polysaccharides (cellulose and hemicelluloses) of ligno-cellulosic material of plants undergo a degradation depending on the depth. However, at the same time, there is a neosynthesis of a new microbial polysaccharides phase dominating by glucose, mannose and galactose in *Fagus sylvatica* stands. The major monosaccharides are glucose, xylose, arabinose, and galactose whereas mannose and rhamnose are of less importance, fucose and ribose are not very present. All these polysaccharides which are unstable and soluble in the surface layer disappear mainly in the organo-mineral major horizons.
In old stands (87, 145-y.) where humus was of an uniform moder type, the decrease of the xylose/mannose ratio in the OF and OH layers reveals the production of microbial sugars at the expense of the phyto-inherited polysaccharides, like cellulose and hemicelluloses which decrease, whereas lignins are strongly degraded. The decrease of the structural polysaccharides continues in the underlying A1 horizon, similar to the evolution observed in the transition from mull to moder although at a slower degradation rate.

Reference number:	II A 7.1/4
Report:	Martens D.A. & Loeffelmann K.L., 2002 Improved accounting of carbohydrates carbon from plants and soil Soil Biology and Biochemistry 34 (2002) 1393-1399 Published
GLP compliance	No
Acceptability	**No, study related to optimisation of an analytical method**

This study investigated the efficiency of glucose recovery from purified cellulose by different extraction methods. Optimised conditions were employed to evaluate the carbohydrates values (hemicellulose and cellulose plus uronic acids) from different plant biomass and soils.

Reference number: II A 7.1/5
Report: Aro N. et al., 2005
Transcriptional regulation of plant cell wall degradation by filamentous fungi
FEMS Microbiology Reviews 29 (2005) 719-739
Published
GLP compliance No
Acceptability **Yes, a part of the article is related to the degradation of polysaccharides by some soil micro-organisms.**

This review summarises current knowledge on transcriptional regulation of genes coding for enzymes involved in the breakdown of plant cell wall biopolymers. It provides general information on plant cell wall composition and hydrolysis.
Plant cell wall consists mainly of the large biopolymers cellulose, hemicellulose, lignin and pectin. These biopolymers are degraded by many microorganisms, in particular filamentous fungi (such as the brown-rot fungi *Trichoderma reesei*, *Aspergillus niger* and *Penicillium* and the ligninolytic white-rot fungus *Phanerochaete*), with the aid of extracellular enzymes acting synergistically. Filamentous fungi have a key role in degradation of the most abundant biopolymers found in the environment, cellulose and hemicelluloses, and therefore are essential for the maintenance of the global carbon cycle. The production of plant cell wall degrading enzymes, cellulases, hemicellulases, ligninases and pectinases, is regulated mainly at the transcriptional level in filamentous fungi.
Hemicelluloses are the second most abundant polysaccharide in the environment, and have a heterogeneous composition of various sugar units. Their hydrolysis occur by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and ancillary enzymes cleaving the side chains of the polymers or oligosaccharides leading to the release of various mono- and disaccharides depending on the hemicellulose type. From the *Aspergillus* species nearly 20 genes encoding endoxylanases and β-xylosidases have been cloned. Xylanase genes have been cloned from a number of other fungi as well, e.g. *Penicillium*, *A. bisporus*, and the rice blast fungus *Magnaporthe grisea*. Numerous other genes encoding hemicellulose backbone and side chain cleaving enzymes have been characterized, most from the species of *Aspergillus*. Hemicelluloses are chemically highly variable and their hydrolysis leads to the formation of a variety of pentose and hexose sugars and acids.

Reference number: II A 7.1/6
Report: Wershaw R., 2004
Evaluation of conceptual models of natural organic matter (humus) from a consideration of the chemical and biochemical processess of humification
Scientific investigations report 2004-5121, US Geological Survey, Reston (Virginia)
Published
GLP compliance No

Acceptability	**Yes, a part of this report is related to the enzymatic degradation of carbohydrates such as cellulose and hemicellulose.**

In this report the degradation pathways of the components of plant tissue and the interactions of the resulting degradation products in soil and natural water were discussed in order to develop a compartmental model of natural organic matter.

Natural organic matter is depicted as being composed of molecular aggregates (supramolecular aggregates) of plant degradation products held together by non-covalent bonds. The most commonly used name of natural organic matter fractions are: humus, humic substances, humic acid, fulvic acid, and humin.

Biodegradation of primary molecules (also called primary metabolites) is carried out both by the organisms that originally produced the metabolites and by exogenous organisms. For example, carbohydrates and proteins that are produced in plant leaves are degraded during senescence by endogenous enzyme systems to monomeric species that can be stored for use in the following growing season. In addition, heterotrophic, exogenous microorganisms are dependent on primary metabolites produced by plants in their metabolic processes. The enzyme systems that catalyze these endogenous and exogenous reactions are often very similar. The biodegradation of primary metabolite polymers generally involves hydrolytic depolymerization reactions that ultimately produce monomeric species. As these reactions proceed, the average size of the molecules continually decreases until only monomers are left. Primary metabolites also undergo oxidative degradation during cell respiration to produce energy (catabolism). Organic acids produced during catabolism ultimately are utilized in one of the cell respiration cycles such as the citric acid cycle. Size reduction also occurs during oxidative degradation.

The hemicellulose components are mainly composed of β-1-4-linked pentoses and hexoses; however, some β-1-3-gycosidic bonds may also be present. Some branching of the chains is present in most of the hemicellulose components. The monomeric units of hemicelluloses are: D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methylglucuronic acid, D-galacturonic acid, and D-glucuronic acid.
Cellulose is degraded aerobically by eubacteria and fungi to carbon dioxide and water, and is degraded anaerobically by protozoa and slime molds to methane and water. These organisms secrete a variety of enzymes that attack cellulose in different ways.
The hydrolytic degradation of hemicelluloses by hemicellulases produces monomeric saccharides and acetic acid. Enzymes specific for the different saccharides and bonds in a given type of hemicellulose are required for the degradation of the particular hemicellulose. Four different enzymes are required to degrade the common hemicellulose O-acetyl-4-O-methylglucuronxylan: endo-1-4-β-xylanase, acetyl esterase, α-glucuronidase, and β-xylosidase.

The review shows that a compartmental model of natural organic matter would be more representative of natural organic matter in soils and sediments than other type of models (for example, molecular-agregate model). In this model natural organic matter is divided into the following compartments: (1) partially degraded plant tissue,

(2) biomass from microorganisms, (3) organic coatings on mineral grains, (4) pyrolytic carbon, (5) organic precipitates, and (6) dissolved organic matter in water.

Reference number:	II A 7.1/7
Report:	De Vries R. & Visser J., 2001 Aspergillus enzymes involved in degradation of plant cell walll polysaccharides Microbiology and Molecular Biology Reviews, Vol. 65, No. 4, p. 497-522 Published
GLP compliance	No
Acceptability	**Yes, a part of this review is related to the enzymatic degradation of plant cell wall polysachharides by enzymes produced *Aspergilli*., naturally occuring in hay and compost.**

This review summarizes current knowledge on the different classes of enzymes involved in plant cell wall polysaccharide degradation produced by *Aspergilli,* the genes encoding these enzymes, and the regulation of these genes. It also provides some general information on xyloglucans role and enzymes involved in their degradation.

Plant cell wall polysaccharides are the most abundant organic compounds found in the environment. They make up 90% of the plant cell wall and can be divided into three groups: cellulose, hemicellulose, and pectin. Cellulose represents the major constituent of cell wall polysaccharides and consists of a linear polymer of β-1,4-linked D-glucose residues. The cellulose polymers are present as ordered structures (fibers), and their main function is to ensure the rigidity of the plant cell wall. Hemicelluloses are more heterogeneous polysaccharides and are the second most abundant organic structure in the plant cell wall.
Xyloglucans are present in the cell walls of dicotyledonae and some monocotylodonae (e.g., onion). Xyloglucans consist of a β-1,4- linked D-glucose backbone substituted by D-xylose. L-Arabinose and D-galactose residues can be attached to the xylose residues, and L-fucose has been detected attached to galactose residues in xyloglucan. Xyloglucans interact with cellulose microfibrils by the formation of hydrogen bonds, thus contributing to the structural integrity of the cellulose network.
Four classes of enzymes are involved in the biodegradation of cellulose : endoglucanases hydrolyze cellulose to glucooligosaccharides; cellobiohydrolases release cellobiose from crystalline cellulose; β-Glucosidases degrade the oligosaccharides to glucose and exoglucanases release glucose from cellulose and glucooligosaccharides. All four classes of enzymes have been identified in *Aspergilli*. Endoglucanases and β-glucosidases are also able to degrade the backbone of xyloglucan. Some accessory enzymes act on the substituents or the side chains of the plant cell wall component structures. Some of these enzymes act on linkages between a main-chain residue and a substituent, whereas other enzymes cleave internal or terminal linkages of side chains.
Aspergilli produce different classes of these accessory enzymes that act on plant cell wall polysaccharides. For example, α-D-Xylosidases can release α-linked xylose

residues from xyloglucan. The removal of D-galactose residues from plant cell wall polysaccharides requires the action of α-galactosidases and β-galactosidases.

Reference number:	II A 7.1/8
Report:	Jeffries T.W., 1994 Biodegradation of lignin and hemicelluloses Biochemistry of Microbial Degradation, C. Ratledge (ed.), p 233–277 Published
GLP compliance	No
Acceptability	**No, review focusing on enzyme characteristics and not on degradation process**

The present review is related to molecular characteristic of enzymes involved in degradation of lignin and hemicelluloses. In this publication hemicellulose refers to a group of homo- and heteropolymers consisting largely of anhydro-□-(1□ 4}D-xylopyranose, mannopyranose, glucopyranose, and galactopyranose main chains with a number of substituents. Hemicelluloses are generally found in association with cellulose in the secondary walls of plants, but they are also present in the primary walls.

Reference number:	II A 7.1/9
Report:	Tuomela M. et al., 2000 Biodegradation of lignin in a compost environment: a review Bioressource Technology 72 (2000) 169-183 Published
GLP compliance	No
Acceptability	**Yes, this literature survey provides qualitative informations on degradation of lignin in compost by micro-organisms that naturally occurred in soil or plant litter.**

This literature survey reviewed knowledge on biodegradability and compostability of lignocellulosic materials and briefly the microbial activity during composting. The main emphasis is made on thermophilic fungi because of their occurrence in compost and other fungi such as white-rot fungi as it is the most important group of lignin biodegraders in the environment.

Composting is nowadays a general treatment method for municipal solid waste. Organic material is converted to carbon dioxide, humus and heat by compost microorganisms (such as *Bacillus*, *Aspergillus*, *Pseudomonas*...). It is assumed that humus is formed mainly from lignin. Thus, lignin is not totally mineralized during composting. The elevated temperatures found during the thermophilic phase are essential for rapid degradation of lignocellulose. After the easily degradable carbon sources have been consumed, more resistant compounds such as cellulose, hemicellulose and lignin are degraded and partly transformed into humus. Humus is

the end product of the humification process, in which compounds of natural origin are partially transformed into relatively inert humic substances. Complex organic compounds like lignin are mainly degraded by thermophilic microfungi and actinomycetes.
In the environment, lignocellulose accounts for the major part of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle (Fig. 1).

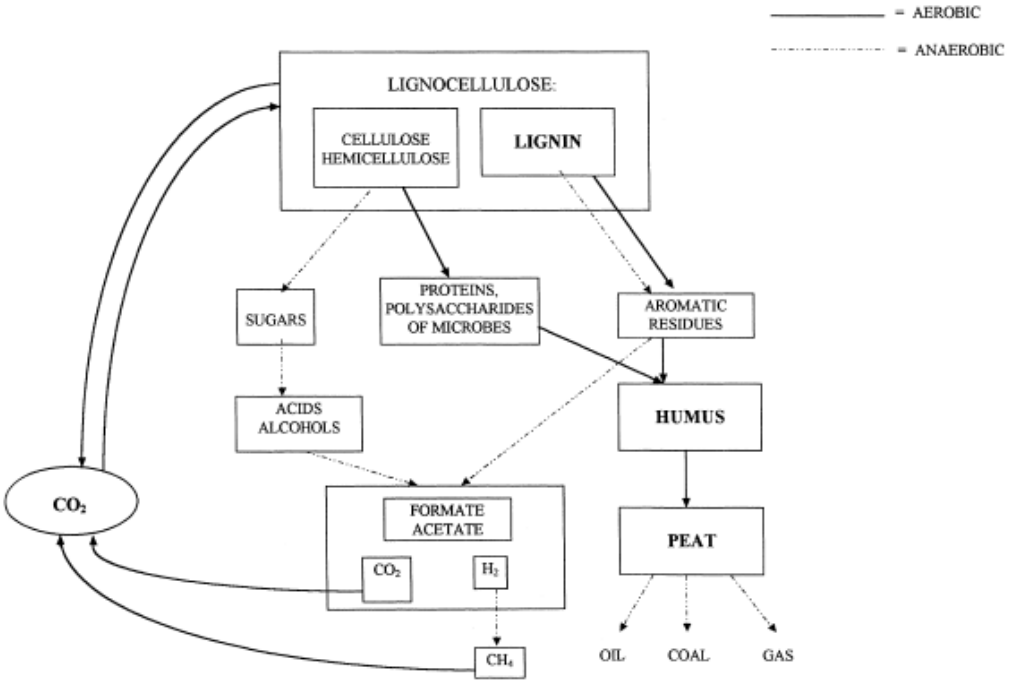


Fig. 1. Global carbon cycle (Brown, 1985; Colberg, 1988).

In mushroom compost, thermophilic fungi are responsible for the degradation of lignocellulose, which is a prerequisite for the growth of the edible fungus. However, most of them are known to be able to degrade wood or other lignocellulose, cellulose or hemicelluloses. The ability of fungi to hydrolyse hemicelluloses is probably more common than cellulose hydrolyzation.

Reference number:	II A 7.1/10
Report:	Pérez J. & Muñoz-Dorado J., 2002 Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview Int Microbiol (2002) 5: 53-63 Published
GLP compliance	No
Acceptability	**Yes**

The aim of this publication is to provide an overview of the degradation of cellulose, hemicellulose, and lignin and the enzymatic systems involved, with first a brief description of the structure of the cell wall and its components.

In the environment, lignocellulose derives from wood, grass, agricultural residues, forestry wastes and municipal solid wastes. It is the major component of biomass. It

consists of three types of polymers: cellulose, hemicellulose, and lignin. These polymers are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages. A great variety of fungi and bacteria can fragment these macromolecules by using a battery of hydrolytic or oxidative enzymes.

The interactions between cellulotic and non-cellulotic microorganism populations (synergistic relationship) lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions. Microorganisms capable of degrading cellulose produce a battery of enzymes with different specificities, working together. Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose. Traditionally, they are divided into two classes referred to as endoglucanases and cellobiohydrolases. Endoglucanases (endo-1,4-β-glucanases) can hydrolyze internal bonds (preferably in cellulose amorphous regions) releasing new terminal ends. Cellobiohydrolases (exo-1,4-β-glucanases) act on the existing or endoglucanase-generated chain ends. An effective hydrolysis of cellulose also requires β-glucosidases, which break down cellobiose releasing two glucose molecules. Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms or other microbes living in the environment where cellulose is being degraded.

Hemicelluloses are biodegraded to monomeric sugars and acetic acid. Hemicellulases are frequently classified according to their action on distinct substrates. Xylan is the main carbohydrate found in hemicellulose. Its complete degradation requires the cooperative action of a variety of hydrolytic enzymes. In addition, hemicellulose biodegradation needs accessory enzymes such as xylan esterases, ferulic and p-coumaric esterases, α-l-arabinofuranosidases, and α-4-O-methyl glucuronosidases acting synergistically to efficiently hydrolyze wood xylans and mannans.
In the case of O-acetyl-4-O-methylglucuronxylan, one of the most common hemicelluloses, four different enzymes are required for degradation: endo-1,4-β-xylanase (endoxylanase), acetyl esterase, α-glucuronidase and β-xylosidase.

In conclusion, the recycling of cellulose, lignocellulose and lignin, major sources of plant biomass in the environment, is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms (such as *Trichoderma*, *Pseudomanas*, *Streptomyces*, *Thermonospora* [...] naturally occurring in soil) which produce a battery of enzymes that work synergically.

Reference number:	II A 7.1/11
Report:	Brechtel E. et al., 2002 L-Glucitol catabolism in *Stenotrophomonas maltophilia* Ac Applied and environmental microbiology, Vol. 68, No. 2, p582-587 Published
GLP compliance	No
Acceptability	**Yes, this study provide information on possible degradation of glucitol (D-glucitol is one of the glucidic monomer units of heptamaloxyloglucan)**

degradation by *Stenotrophomonas maltophilia* which could be found in soil

The purpose of this study is to characterize a new pathway through which *Stenotrophomonas maltophilia* is capable of utilizing rare and unnatural carbohydrate substrates.

Stenotrophomonas maltophilia Ac (previously named *Pseudomonas* sp. strain Ac, or also *Xanthomanas maltophilia*) was isolated from soil using a mineral medium and the unnatural polyol L-glucitol as the selective carbon source. This bacterium is known to convert the unnatural polyol L-glucitol to D-sorbose during growth on the former as the sole source of carbon and energy. All enzymes operating in a pathway that channels L-glucitol via D-sorbose into compounds of the intermediary metabolism were demonstrated, and for some prominent reactions the products of conversion were identified. D-Sorbose was converted by C-3 epimerization to D-tagatose, which, in turn, was isomerized to D-galactose. D-Galactose was the initial substrate of the De Ley-Doudoroff pathway, involving reactions of NAD-dependent oxidation of D-galactose to D-galactonate, its dehydration to 2-keto-3-deoxy-D-galactonate, and its phosphorylation to 2-keto-3-deoxy-D-galactonate 6-phosphate. Finally, aldol cleavage yielded pyruvate and D-glycerate 3-phosphate as the central metabolic intermediates.

Reference number:	II A 7.1/12
Report:	Kelker N.E. & Anderson R.L., 1971 Sorbitol metabolism in *Aerobacter aerogenes* Journal of Bacteriology, Vol. 105, No. 1, p 160-164 Published
GLP compliance	No
Acceptability	**Yes, this study provide information on possible degradation of glucitol (D-glucitol is one of the glucidic monomer units of heptamaloxyloglucan) degradation by *Aerobacter aerogenes* which could be found in soil**

In this study, sorbitol (D-glucitol) metabolism in *Aerobacter aerogenes* PRL-R3 is shown to proceed via the pathway: sorbitol → sorbitol 6-phosphate → D-fructose 6-phosphate.
Sorbitol phosphorylation is mediated by a phosphoenolpyruvate (PEP): sorbitol 6-phosphotransferase system, and sorbitol 6-phosphate oxidation by a pyridinenucleotide- linked dehydrogenase. Mutants deficient in sorbitol 6-phosphate dehydrogenase or a component (enzyme I) of the phosphotransferase system did not grow on sorbitol, whereas revertants, which had regained these enzymatic activities, grew normally.
The pathway of sorbitol metabolism in *A. aerogenes* PRL-R3 thus is distinct from the pathway(s) occurring in *Acetobacter suboxydans*, *Pseudomonas fluorescens*, *Celivibrio polyoltrophicus*, *Bacillus subtilis*, and *Rhizobium meliloti*, in which sorbitol is oxidized to D-fructose or L-sorbose.

B.8.1.2.4. Conclusion on route and rate of degradation

Considering that heptamaloxyloglucan could be an intermediate compound of natural organic matter decomposition process, which could undergo degradation by endogenous soil microorganisms naturally occurring in soil, no specific study on the rate and route of degradation was deemed necessary.

The literature data confirm that xyloglucans belong to the Hemicellulose family, one of the principal components of plant cell wall, as well as xylans, mixed glucans (Warren, 1996). The monosaccharide units of xyloglucan are the same as in heptamaloxyloglucan (De Vries & Visser, 2001), (Wershaw, 2004). These monomers (or hemicellulosic sugars) are part of natural organic matter found in soils (Karroum et al., 2004).

As microorganisms that degrade cellulose also degrade hemicellulose (and thus xyloglucans), cellulases and hemicellulases are considered as components of systems for the enzymatic cleavage/hydrolysis of plant cell walls. (Warren, 1996). Cellulases hydrolyze the β-1,4-glycosidic linkages of cellulose. An effective hydrolysis of cellulose also requires β-glucosidases (Pérez & Muñoz-Dorado, 2002). The hydrolysis of hemicelluloses occurs by the concerted action of endo-enzymes cleaving internally the main chain, exo-enzymes liberating monomeric sugars and

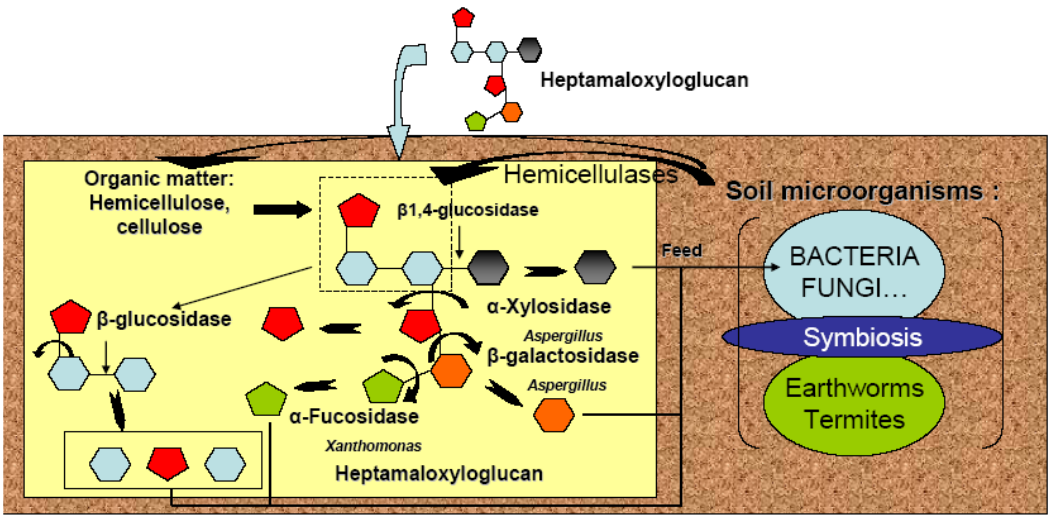
ancillary enzymes cleaving the side chains of polymers or oligosaccharides leading to the release of various mono- or disaccharides (Aro et al., 2004; Wershaw, 2004). In this complex system of biodegradation, heptamaloxyloglucan could be considered as an intermediate compound which will be further degraded into monosaccharide. During chemical and biochemical processes involved in the degradation of natural organic matter (humification), cellulotic microorganisms transform organic matter into smaller molecules (Tuomela et al., 2000). The structural polysaccharides (cellulose and hemicelluloses) of ligno-cellulosic material of plants undergo a degradation depending on the depth (Karroum et al., 2004). Hemicellulose recycling from plant biomass is indispensable for the carbon cycle (Perez, 2002). Heptamaloxyloglucan as an intermediate of biodegradation should be fully part of the carbon cycle.

Different classes of enzymes are involved in plant cell wall polysaccharide degradation. They are produced by several saprophytic[5] organisms such as bacteria, archea and fungi (for example *Aspergilli*, *Cellulomonas fimi*, a mesophilic aerobic soil bacterium and *Thermonospora fusca*, a thermophilic actinomycete common in compost) (De Vries & Visser, 2001;Warren, 1996). Plant cell wall hydrolysis requires in particular enzymes hydrolyzing β–1,4 and β–1,3 glycosidic bonds; β–1,4 xylosidic bonds and β–1,4 mannosidic bonds. Four different enzymes are required to degrade the common hemicellulose O-acetyl-4-O-methylglucuronxylan: endo-1-4-β-xylanase, acetyl esterase, α-glucuronidase, and β-xylosidase. (Wershaw, 2004). Aspergilli produce different classes of accessory enzymes that act on plant cell wall polysaccharides : for example, α-D-Xylosidases, α-galactosidases and β-galactosidases

Talking about the glucitol residue, it is well known that soil microorganisms thoroughly catabolize this sugar. The pathway of glucitol (sorbitol) metabolism described in *Pseudomonas, Areobacter, Cellvibiro, Rhizobium* and *Stenotrophomonas* genuses oxydize glucitol as a carbon source for organism growth use (Kelker, 1971; Brechtel, 2002).

Taking into account of all information given by the cited literature, the notifier proposed a pathway of biodegradation in figure 1. The representation of heptamaloxyloglucan molecule and monomeric sugars are schematic. Real spatial formula illustration of heptamaloxyloglucan could be found under B.1.1.7. To facilitate the review of the scheme, the notifier used the same symbols for the glucidic monomer units of heptamaloxyloglucan and its expected degradation products (monomeric sugars). Therefore in heptamaloxyloglucan représentation, D-glucopyranosyl, D-glucitol, D-xylopyranosyl, D-galactopyranosyl and L-fucopyranosyl are represented by the glucose, glucitol, xylose, galactose and fucose monomeric sugar symbols, respectively.

[5] organisms that survive by decomposing dead or decaying organic matter



Monomeric sugars

- Glucose
- Xylose
- Galactose
- Glucitol (=sorbitol)
- Fucose

Biodegradation microorganisms in soil: Examples

Aerobic Bacteria Genus	Aerobic fungi Genus
Xanthomonas •fucosidase activity.	*Aspergillus* •Xylanase, endoglucanase,galacto-sidase, glucosidase activities. *Trichoderma* •Cellobiohydrolase, glucosidase All Rot Fungi

Figure 1: fate and behavior of Heptamaloxyloglucan in soil.

- **Comments of RMS regarding route and rate of degradation:**

Notifier provides general informations on the way oligosaccharides such as xyloglucan and its expected degradation product heptamaloxyloglucan could be degraded in soil by enzymatic action of the micro-organisms. These informations are useful to understand how heptamaloxyloglucan could be degraded in soils. Moreover the assimilation and degradation of xyloglucan like molecule by soil macro-organisms such as earthworms is facilitated by soil micro-organisms that they ingest together with soil (see B.9.7). Therefore the fate and behaviour of heptamaloxyloglucan in soil and possible assimilation and degradation by soil micro- and macro-organisms are well described.

B.8.2. ADSORPTION, DESORPTION AND MOBILITY IN SOIL (ANNEX IIA 7.1.2 AND 7.1.3, ANNEX IIIA 9.1.2)

B.8.2.1. ADSORPTION AND DESORPTION OF THE ACTIVE SUBSTANCE (ANNEX IIA 7.1.2)

Calculation of a Koc is not relevant as heptamaloxyloglucan is part of the organic matter of the soil.

B.8.2.2. ADSORPTION AND DESORPTION OF RELEVANT METABOLITES (ANNEX IIA 7.1.2)

This point is set as not relevant by the notifier. This is judged acceptable as no relevant metabolites are expected to occur following applications of heptamaloxyloglucan on vine.

B.8.2.3. COLUMN LEACHING STUDIES (ANNEX IIA 7.1.3)

No study, not required.

B.8.2.4. AGED RESIDUE COLUMN LEACHING (ANNEX IIA 7.1.3.2)

No study, not required.

B.8.2.5. LYSIMETER AND FIELD LEACHING STUDIES (ANNEX IIA 7.1.3.3)

No study, not required as the 0.1 µg/L trigger for groundwater is not expected to be reached for this compound (see B.8.6.1).

B.8.2.6. CONCLUSION ON ADSORPTION, DESORPTION AND MOBILITY

No studies regarding adsorption, desorption and mobility of the active substance in soil were deemed necessary as heptamaloxyloglucan which is used at very low application rate may undergo degradation by soil micro-organisms and presents no toxicological or ecotoxicological concerns. Moreover the 0.1 µg/L limit for ground water is not expected to be reached for this kind of molecule.

B.8.3. PREDICTIVE ENVIRONMENTAL CONCENTRATIONS IN SOIL (ANNEX IIIA 9.1.3)

PEL101GV (874 g/kg heptamaloxyloglucan) is intended to be applied on grapevines at a maximum rate of 0.5 g/ha, *i.e.* 0.44 g a.s./ha, by spraying from BBCH 7 to BBCH 16 (early spring). A maximum of 4 applications at 4-day intervals are expected.

Notifier calculated the PEC_{soil} without consideration of crop interception factor.

After a single application, the PEC_{soil} was calculated according to the following equation:

$$PEC_{s,ini} = \frac{A \cdot (1 - f_{int})}{100 \cdot DEPTH_{soil} \cdot bd_{soil}} \qquad (1)$$

where A = application rate of heptamaloxyloglucan (0.44 g a.s./ha)
f_{int} = fraction intercepted by plant cover (= 0)
$DEPTH_{soil}$ = depth of soil layer (= 5 cm)
bd_{soil} = bulk soil density (= 1.5 g/cm^3)

For repeated applications, no degradation between applications was considered.

The estimated initial PEC_{soil} values (mg/kg) for single and multiple applications of PEL101GV on grape vines are listed in Table B.8.3/1.

Table B.8.3/1: PECsoil values (mg as/kg soil) following 1 to 4 applications of PEL101GV at the maximum rate of 0.5 g./ha to grapevines at BBCH 7 to BBCH 16
Assumptions: 0% of crop interception, no degradation between applications

Number of applications	1	2	3	4
PECsoil ini (mg a.s./kg)	0.000587	0.00117	0.00176	0.00235

Initial PEC_{soil} of heptamaloxyloglucan, calculated based on assumptions of no degradation and no crop interception, will be 2.35 µg a.s./kg after 4 applications of PEL101GV to grapevines at growth stage BBCH 7 to BBCH 16.

B.8.4. FATE AND BEHAVIOUR IN WATER (ANNEX IIA 7.2.1, ANNEX IIIA 9.2.1, 9.2.3)

B.8.4.1. HYDROLYSIS

Reference number:	II A 7.2.1.1/1 and II A 7.2.1.1/2 (addendum)
Report:	Ricau H. 2005 Abiotic degradation on the technical heptamaloxyloglucan, pH dependent hydrolysis (Test C7) Defitraces unpublished report N°05-905012-003, 2006-01-13
Dates of works:	January 13, 2006
GLP compliance	Yes
Guidelines	Directive 92 / 69 / EEC paragraph C7 of July 31st, 1992
Deviations	pH values of 5, 7 and 9 were used in the test instead of 4, 7 and 9 No temperature recording is available although temperature of water-bath was followed during the study to be at 50±0.5°C. These minor deviations are not expected to modify study conclusions.
Acceptability	**Yes**

Methods:
Hydrolysis of heptamaloxyloglucan was investigated in buffer solutions with pH adjusted to 5, 7 and 9.
For each pH, test solutions of 12.4 g technical heptamaloxyloglucan/L (batch AND0205, purity 88%) was prepared by adding 310 mg of technical heptamaloxyloglucan into 25 mL of buffer (pH 5, 7 and 9).
At test initiation, and after 2.4 hours and 5 days, 2 mL of the test solution was diluted 25 times with water before analysis, except for pH 5 and 7 after 5 days where dilution was 20 times. Test was conducted with duplicate flasks for each of the three pH.
Heptamaloxyloglucan was analysed by HPLC and detected by pulsed amperometric electrochemical technique using the method described in study report 05-905012-007).

Results:
The pH values at test initiation and after 5 days at 20°C were 4.96, 7.01, 8.89 and 5.02, 6.98, 8.72, respectively. The mean temperature was 50.0±0.5°C.
Results are summarized in the following table:

Table B.8.4.1/1: Concentration of heptamaloxyloglucan and percentage of hydrolysis depending on pH

	Concentration (mean value,g/L)			% of hydrolysis		
Time	0	2.4 h	5 d	0 (1)	2.4 h	5 d
pH 5	11.54	11.77	11.77	0 [6.9]	ns [5.1]	ns [5.1]
pH 7	11.66	11.56	12.18	0 [6.0]	ns [6.8]	ns [1.8]
pH 9	11.46	11.67	12.37	0 [7.6]	ns [5.9]	ns [0.2]

(1) notifier assumed that at t = 0 the hydrolysis was 0%
ns: not significant
Values under brackets are the percent of hydrolysis recalculated by RMS based on initial mean concentration of 12.4 g heptamaloxyloglucan/L

No significant change in heptamaloxylglucan concentration was observed during the study.

Conclusion:
No significant hydrolysis of heptamaloxyloglucan was observed at pH 5, 7 and 9 after 5 days.
EL101GV is hydrolytically stable.

B.8.4.2. PHOTODEGRADATION IN WATER

Heptamaloxyloglucan is photochemically stable as it has no peak absorption with molecular absorption coefficient higher than 10 l/mol/cm at wavelength >290nm (see B.2.1.9).

B.8.4.3. BIOLOGICAL DEGRADATION (ANNEX IIA 7.2.1.3)

B.8.4.3.1. Ready biodegradability

Reference number: II A 7.2.1.3.1/1
Report: Havet S. 2006
An assessment of the ready biodegradability
CERMAV – CNRS unpublished report EL101GV-160106-04
Dates of works: January 16, 2006
GLP compliance No
Guidelines based on principles of Directive 92 / 69 / EEC paragraph C4 of July 31st, 1992
Deviations No reference chemical to check the test procedure. No detailed informations on test conditions: pH, incubation conditions (dark/light). No information regarding water checked for DOC beforehand and if just one batch of water was used
Acceptability **No**

Methods:
The biodegradability of heptamaloxyloglucan was tested by specific chemical analysis of the active substance and intermediate substance formed (glucose, galactose, xylose and fucose). The method is based on EEC Directive 92/69/EEC paragraph C4.
Test material was technical EL101GV (batch ALD0104, purity of 88%).
Microorganisms were obtained from Grenoble plant works. A solution of activated sludge at 1.4 g/L (3 determinations after oven drying for 48 hours) was stirred during 2 minutes, then let to settle for 30 min; the supernatant was then used as a floating inoculum for the assays, at the rate of 10 mL/L.
The study was conducted on four controls in parallel, including one with no microbial inoculum and N_3Na as bacterial growth inhibitor (abiotic degradation), and one with medium plus microbial inoculum only (blank), as summarized in Table B.8.4.3.1/1.

Table B.8.4.3.1/1: Heptamolaloxyloglucan ready biodegradability test design

Methods		EL101GV 100 g/L	Culture medium	Inoculum	N_3Na 22 g/L	Water MiliQ
A	Biological breakdown	0.5 mL	50 mL	0.5 mL	-	0.5 mL
B	Inoculum control sample	-	-	0.5 mL	-	51 mL
C	Abiotic degradation	0.5 mL	50 mL	-	0.5 mL	0.5 mL
D	Adsorption control sample	0.5 mL	50 mL	0.5 mL	0.5 mL	-
E	Development control sample		50 mL	0.5 mL	-	1 mL

EL101GV was added to obtain the final concentration of 1 mg/mL. The bottles were incubated at *ca* 25°C for 63.65 hours.
The bacterial growth was quantified by measuring the absorbance at 550 nm.
The EL101GV degradation was quantified by measuring the concentration of EL101GV and of residues from product degradation (i.e. hexoses constituents of heptamaloxyloglucan : glucose, galactose, xylose and fucose). Measurement of heptamaloxyloglucan was done by High Performance Anion Exchange (HPAE) with pulsed amperometric detection (PAD). Monosaccharides were analysed following the Dionex analytical procedure (Technical note 40) and determined by HPAE-PAD.

The bacterial growth was calculated by:

$$DO(550\ \ nm) = DO(550\ \ nm)_A - \sum_{B \le X \le E} DO(550\ \ nm)_X$$

$$= DO(550\ \ nm)_A - DO(550\ \ nm)_B + DO(550\ \ nm)_C + DO(550\ \ nm)_D + DO(550\ \ nm)_E$$

The biodegradation of heptamaloxyloglucan was calculated by:

$$(\%\text{Biodegradation})_t = (\%\text{Degradation A})_t - \sum_{X=B,C,D,E} (\%\text{Degradation X})_t$$

$$(\%\text{Degradation X})_t = 100 \times \frac{[X]_0 - [X]_t}{[X]_0}$$

$$\Leftrightarrow (\%\text{Biodegradation})_t = 100 \times \left[\frac{[A]_0 - [A]_t}{[A]_0} - \left(\frac{[B]_0 - [B]_t}{[B]_0} + \frac{[C]_0 - [C]_t}{[C]_0} + \frac{[D]_0 - [D]_t}{[D]_0} + \frac{[E]_0 - [E]_t}{[E]_0} \right) \right]$$

with $[A]_t$, $[B]_t$, $[C]_t$, $[D]_t$ and $[E]_t$ being the respective concentrations at time t of heptamaloxyloglucan in the test solutions A, B, C, D and E (see table B.8.4.3.1/1).

Results:
The results of the bacterial growth and biodegradation are summarized in Table B.8.4.3.1/2.

Table B.8.4.3.1/2: Results of bacterial growth and biodegradation of EL101GV

Date	Hour	Time (h)	Absorbance (550 nm)	Biodegradation (%)
26/07/2004	17:30	0	0	0%
27/07/2004	09:10	15.33	0.0114	0%
27/07/2004	13:30	19.66	0.0153	1%
27/07/2004	18:30	24.66	0.0488	0%
28/07/2004	09:10	38.99	0.8276	82%
28/07/2004	14:30	44.32	0.7940	102%
28/07/2004	18:00	47.82	0.7643	99%
29/07/2004	10:10	63.65	0.7155	98%

The results are illustrated in Figure B.8.4.3.1/1.

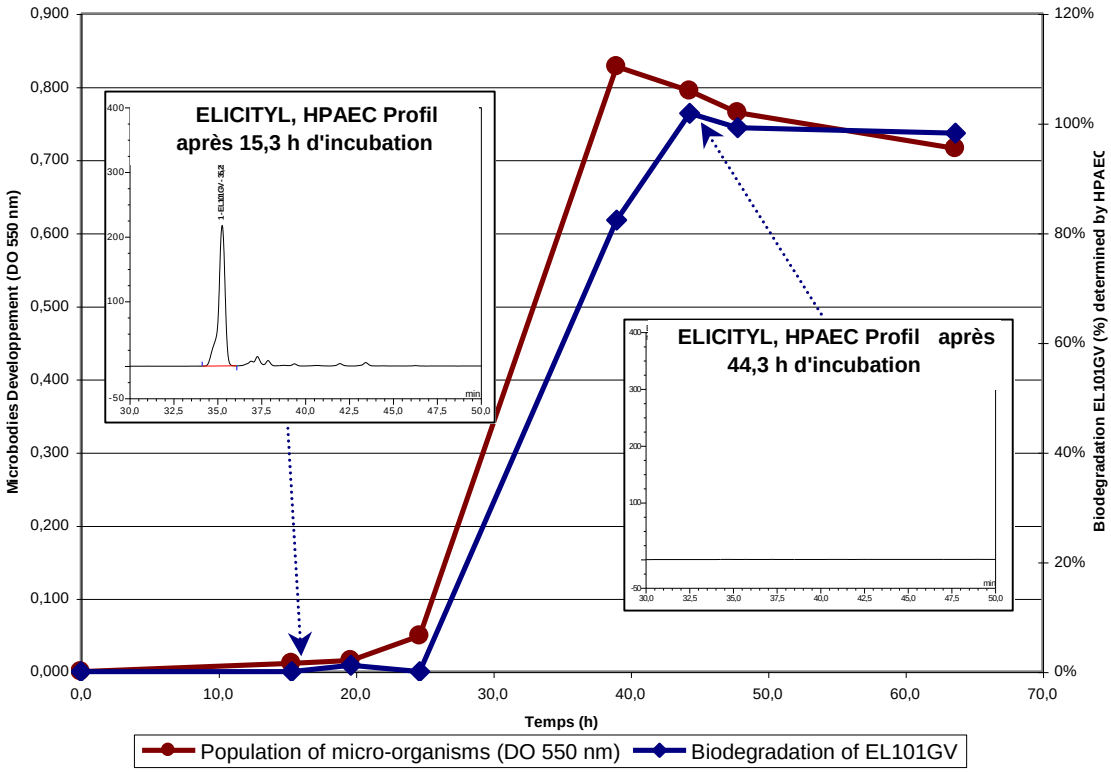


Figure B.8.4.3.1/1: Bacterial growth and biodegradation of EL101GV

The biodegradation of heptamaloxyloglucan was completed within about 10 hours and paralleled the growth of micro-organisms.
The residues of degradation (monosaccharides) did not accumulate in the culture medium but were used by the micro-organisms.

Conclusion:

When incubated with micro-organisms from water plant activated sludge, EL101GV at the concentration of 1 mg/mL was biodegraded within 10 hours. The biodegradation is paralleled to the growth of micro-organisms. No monosaccharide accumulated within the culture medium.

- **Comments of RMS:**

The protocol of this study is based on principles of Directive 92/69/EEC paragraph C4 and allow measurement of micro-organisms growth and follow-up of heptamaloxyloglucan degradation. However the aim of the C4 method is to demonstrate the total mineralisation of the substance either by direct measure of CO_2 or by measuring the oxygen consumption. The study was not acceptable for ready biodegradability for which a valid study was provided (see L'haridon, 2006).

Reference number:	II A 7.2.1.3.1/1 (study plan), II A 7.2.1.3.1/3 (summary) and II A 7.2.1.3.1/3 (final report)
Report:	L'Haridon, J. 2006 EL101GV: Determination of the ready biodegradability CO_2 evolution test (Final report) CIT unpublished report number 31237ECS
Dates of works:	March 2-31, 2006
GLP compliance	Yes
Guidelines	Directive 92/69/EEC paragraph C.4-C of July 31st, 1992 - Directive 93/21/EEC (27th April 1993) - OECD guideline No. 301B (17th July 1992)
Deviations	pH of the mineral medium was not controlled (and adjusted at 7.4 ± 0.2) before the start of the test, but since the test medium was prepared according to test guideline, the study was considered to be valid.
Acceptability	**Yes**

Methods:

Technical heptamaloxyloglucan (EL101GV, batch ANN0304, purity 78.2%w/w) was dissolved in reconstituted water (OECD mineral medium) prepared from deionized water with conductivity < 10 µS/cm.

Five flasks were used to determine the quantity of carbon dioxide evolved by the degradation of the test item:

- two flasks containing the inoculum (inoculum blanks),
- two flasks containing the test item (at 10.0 mg/L of total organic carbon (TOC)) and inoculum (test solutions),
- one flask containing the reference item (sodium acetate at 10.0 mg/L of TOC) and inoculum (procedure control).

The inoculum consisted of sewage sludge sampled from the aeration tank of a sewage treatment plant and then aerated for 6 days. Inoculum concentration was 20.0 mg/L (dry weight) in all test vessels.

CO_2 scrubbed air was bubbled through the flasks for the 28-day test period.

Results:

Environmental parameters were recorded as: pH: 7.48 to 7.74, room temperature: 20°C to 23°C.

The reference item degraded normally under the test conditions.

The percentage of degradation is reported in the following table:

Table B.8.4.3.1/3: Degradation of heptamaloxyloglucan (% of initial concentration)

Day	Test item			Reference item
	flask 1	flask 2	average	
1	0.00	0.00	0.00	5.30
4	29.02	22.23	25.63	37.02
6	52.70	44.91	48.81	57.50
8	68.29	64.39	66.34	68.19
11	77.33	72.43	74.88	72.63
14	80.48	76.28	78.38	74.58
18	82.18	78.38	80.28	75.38
22	82.18	77.78	79.98	75.88
25	81.18	78.18	79.68	75.78
28	79.33	76.33	77.83	76.03

Conclusion:
The biodegradation of heptamaloxyloglucan reached 76% at the end of the 10-day window (the 10 days immediately following reaching of 10% biodegradation) and 78% at the end of the test.
Under the experimental conditions, the test item heptamaloxyloglucan was therefore readily biodegradable in the 28-day modified Sturm test.

- **Comments of RMS:**

As the test item has a minimum purity of only 78.2% it could be questionable whether readily biodegradability observed in this 28-day modified Sturm test was totally provided by heptamaloxyloglucan. However as the impurities have a structure close to Heptamaloxyloglucan structure, results of the study is judged acceptable, i.e. EL101GV is set as a redily biodegradable compound.

B.8.4.3.2. Degradation in water sediment systems (Annex IIA, 7.2.1.3.2)

Heptamaloxyloglucan can be produced from xyloglucan by enzymatic degradation naturally occurring in plant and could therefore be considered as taking part of vegetal debris naturally brought to open water bodies by plant decays, which is expected to be rapidly degraded by the micro-organisms present in water and sediments. The ready biodegradability study (L'Haridon, J., 2006) demonstrated a rapid biodegradation through bacterial metabolism. Additionally heptamaloxyloglucan is not toxic to aquatic organisms ($L(E)C_{50}$ > 150 mg a.s./L, see B.9.2). Therefore, no water sediment test is deemed necessary.

B.8.4.4. DEGRADATION IN SATURED ZONE (ANNEX IIA 7.2.1.4)

The test was not considered necessary.

B.8.4.5. CONCLUSION ON FATE AND BEHAVIOUR IN WATER

Heptamaloxyloglucan is hydrolytically stable. It is expected to be stable under photo-irradiation.
Heptamaloxyloglucan is readily biodegradable (biodegradation reached 78% at the end of the 28-day modified Sturm test). No metabolites are expected.
No water sediment test was deemed necessary as heptamaloxyloglucan can be produced from xyloglucan by enzymatic degradation naturally occurring in plant and could therefore be considered as taking part of vegetal debris naturally brought to open water bodies by plant decays, which is expected to be degraded in natural aquatic systems. Moreover heptamaloxyloglucan was not acutely toxic to aquatic organisms (see B.9.2).

B.8.5. IMPACT ON WATER TREATMENT PROCEDURES (ANNEX IIIA 9.2.2)

Heptamaloxyloglucan is neither a fungicide nor a bactericide and should enter water bodies at levels of the nanograms per liters. Consequently, no data on the impact of heptamaloxyloglucan on water treatment procedures is deemed necessary.

B.8.6. PREDICTED ENVIRONMENTAL CONCENTRATIONS IN GROUND WATER AND IN SURFACE WATER (PECGW, PECSW) (ANNEX IIIA 9.2.1 AND 9.2.3)

B.8.6.1. PREDICTED ENVIRONMENTAL CONCENTRATIONS IN GROUNDWATER

The notifier did not provide calculation of predicted environmental concentration in groundwater according to FOCUS as neither the DT_{50} nor the K_{OC} are precisely known or can be estimated.

The estimation of initial maximum PEC_{soil} indicates concentration of 0.00235 mg a.s./ka soil after 4 applications. Moreover, short-chain soluble oligosaccharides such as heptamaloxyloglucan are readily accessible to enzymatic degradation on the soil and in its superficial layers. Therefore it is not expected that heptamaloxyloglucan reach groundwater at levels > 0.1 μg/L following applications of PEL101GV to vines in the Europe.

In the first version of the DAR, the RMS proposed estimations of PEC_{GW} based on conservative worst-case parameters, as indicative information. But, as no ADI and MRL were determined for heptamaloxyloglucan, the limit value of 0.1 μg/L is not applicable in this case.

B.8.6.2. PREDICTED ENVIRONMENTAL CONCENTRATIONS IN SURFACE WATER

A small stagnant water body of 30 cm depth, a sediment depth of 5 cm and a ratio between size of field and water body of 10:1 were considered for calculations of predicted environmental concentrations *via* spray drift entry.

The PECsw and PECsed for heptamaloxyloglucan were calculated using FOCUS Step 1 for 1 application of 1.76 g/ha on vines (early applications). Conservative worst-case parameters are used by RMS for DT_{50} (1000 days), K_{oc} (0 L/kg for PECsw and 10000 for PECsed) and solubility of 5.10^5 mg/L. The results of PECsw and PECsed are reported in Table B.8.6.2/2.

Table B.8.6.2/2 : PECsw and PECsed for heptamaloxyloglucan – FOCUS Step 1

FOCUS STEP 1 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
	0h	0.6025		4.093	
	24h	0.6021	0.6023	4.2006	4.1468
	2d	0.6017	0.6021	4.1977	4.173
	4d	0.6008	0.6017	4.1919	4.1839
	7d	0.5996	0.601	4.1831	4.1854
	14d	0.5967	0.5996	4.1629	4.1792
	21d	0.5938	0.5981	4.1428	4.1704
	28d	0.5909	0.5967	4.1227	4.161
	42d	0.5852	0.5938	4.0829	4.1416

B.8.6.3. PREDICTED ENVIRONMENTAL CONCENTRATIONS IN SEDIMENT

Considering that all heptamaloxyloglucan present in water after the last application (0.0143 µg/L) will be transferred to the sediment layer, the initial PEC_{SED} is calculated to be 0.107 µg/kg after 4 applications of PEL101GV in a lentic water body of 30 cm depth with a layer of 5 cm of sediment having a density of 0.8 (40 kg/m^2).

B.8.7. FATE AND BEHAVIOUR IN AIR (ANNEX IIA 7.2.2; ANNEX IIIA 9.3)

The calculated vapour pressure of heptamaloxyloglucan is $1.1*10^{-11}$ Pa and therefore, heptamaloxyloglucan is not expected to volatilize from plant and soil

surface in the air compartment. No study on fate and behaviour of heptamaloxyloglucan was performed.

Due to the very low vapor pressure and application rate (0.44 g heptamaloxyloglucan/ha), the predicted environmental concentrations in air are expected to be very low.

B.8.8. DEFINITION OF THE RESIDUE (ANNEX IIA 7.3)

Heptamaloxyloglucan could be produced by degradation of xyloglucan by enzymes naturally occurring in plant or soil micro-organisms (see B.8.1.2.3 and B.8.1.2.4). As such it could occur naturally in soil organic matter. According to the B.6 and B.9 points of the DAR, there is no toxicologically or eco-toxicologically relevant residue. For the purpose of risk assessments the residue in soil, groundwater, surface water, sediment and air will be defined as heptamaloxyloglucan.

B.8.9. MONITORING DATA (ANNEX IIA 7.4)

No data submitted, or deemed necessary by the RMS (see also B.8.6.1).

B.8.10. REFERENCES RELIED ON

Annex II Data and Information

Author(s)	Annex point/ reference number	Year	Title **Source** (where different from company) **Company name, Report No., Date, GLP status** (where relevant)**, published or not**	Data protect. claimed	Owner
Warren R.A.J.	II A 7.1/2	1996	Microbial hydrolysis of polysaccharides Annu. Rev. Microbiol. 1996. 50:183-212 Not GLP, published	No	-
Karroum M. et al.	II A 7.1/3	2004	Importance et devenir des biopolymères (lignines et polysaccharides) dans les sols d'une chronoséquence de hêtraies (*Fagus sylvatica*) en forêt de Fougères (France) / Importance and fate of biopolymers (lignins and polysaccharides) in soils of *Fagus sylvatica* stands of various ages in Fougères forest (Britany - France) Ann. For. Sci 61 (2004) 211-233 Not GLP, published	No	-
Aro N. et al.	II A 7.1/5	2005	Transcriptional regulation of plant cell wall degradation by filamentous fungi FEMS Microbiology Reviews 29 (2005) 719-739 Not GLP, published	No	-
Wershaw R.	II A 7.1/6	2004	Evaluation of conceptual models of natural organic matter (humus) from a consideration of the chemical and biochemical processess of humification Scientific investigations report 2004-5121, US Geological Survey, Reston (Virginia) Not GLP, published	No	-

Author(s)	**Annex point/ reference number**	**Year**	**Title** **Source** (where different from company) **Company name, Report No., Date,** **GLP status** (where relevant)**, published or not**	**Data protect. claimed**	**Owner**
De Vries R. & Visser J.	II A 7.1/7	2001	Aspergillus enzymes involved in degradation of plant cell walll polysaccharides Microbiology and Molecular Biology Reviews, Vol. 65, No. 4, p. 497-522 Not GLP, published	No	-
Tuomela M. et al.	II A 7.1/9	2000	Biodegradation of lignin in a compost environement: a review Bioressource Technology 72 (2000) 169-183 Not GLP, published	No	-
Pérez J. & Muñoz-Dorado J.	II A 7.1/10	2002	Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview Int Microbiol (2002) 5: 53-63 Not GLP, published	No	-
Brechtel E. et al.	II A 7.1/11	2002	L-Glucitol catabolism in *Stenotrophomonas maltophilia* Ac Applied and environmental microbiology, Vol. 68, No. 2, p582-587 Not GLP, published	No	-
Kelker N.E. & Anderson R.L.	II A 7.1/12	1971	Sorbitol metabolism in *Aerobacter aerogenes* Journal of Bacteriology, Vol. 105, No. 1, p 160-164 Not GLP, published	No	-
Ricau H	II A 7.2.1.1/1 and II A 7.2.1.1/2 (addendum)	2005	Abiotic degradation on the technical heptamaloxyloglucan, pH dependent hydrolysis (Test C7) Defitraces report N°05-905012-003, 2006-01-13 Elicityl GLP, unpublished	Yes	Elicityl

Author(s)	**Annex point/ reference number**	**Year**	**Title** **Source** (where different from company) **Company name, Report No., Date,** **GLP status** (where relevant)**,** **published or not**	**Data protect. claimed**	**Owner**
L'Haridon J.	II A 7.2.1.3.2/1 (Study plan) II A 7.2.1.3.2/2 (Summary) II A 7.2.1.3.2/3 (Final report)	2006	EL101GV: Determination of the ready biodegradability CO_2 evolution test CIT, Evreux, France, 31237ECS, 2006-07-21 Elicityl Yes, unpublished	Yes	Elicityl

Annex III Data and Information

None

EUROPEAN COMMISSION

Program for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC

HEPTAMALOXYLOGLUCAN

VOLUME 3, ANNEX B.9
CORRIGENDUM 1

Rapporteur Member States Summary, Evaluation And Assessment Of The Data And Information

Rapporteur Member State: ***France***
January 2009

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
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B.9. ECOTOXICOLOGY

Xyloglucan is the principal hemicellulosic component of primary cell walls of dicotyledonous and non-graminaceous monocotyledonous plants. Xyloglucan plays a physiological key role in maintaining cell wall integrity by cross-linking individual cellulose microfibrils in the primary cell wall. Specific oligosaccharides such as heptamaloxyloglucan can be produced naturally from xyloglucan by partial hydrolysis with cellulase (β-1,4-D-glucanase) and various other enzymes which are present in plants and soil micro-organisms. It has been demonstrated that these specific oligosaccharides accumulate extracellularly in plants and act at very low levels as signaling molecules that participate in cell-cell and wall-nucleus communication (Fry et al., 1993[6]; Buchanan et al., 2000[7]).

Technical Heptamaloxyloglucan (coded EL101GV) is prepared from dry apple pomace by enzymatic hydrolysis and deacetylation/reduction after fractioning and purification. Samples are then purified and conditioned by lyophilisation.

The active substance heptamaloxyloglucan (MW = 1078 g/mol, CAS number [870721-81-6], minimum purity of 78%) is a xyloglucan-derived oligosaccharide made of 7 glycosidic monomer units (polymerisation degree = 7). There are β-1,4 linkages on the main chain between the two D-glucopyranosyl units and terminal D-glucitol, and α-1,2, β-1,2 and α-1,6 linkages between the various monomer units present in side chains. The latter side chain-monomers are D-xylopyranosyl (α-1,6-linked to D-glucopyranosyl), D-galactopyranosyl (β-1,2-linked to D-xylopyranosyl) and L-fucopyranosyl (α-1,2-linked to D-galactopyranosyl).

Heptamaloxyloglucan acts as a stimulator of plant defence natural mechanisms (“elicitor”) which must preserve the chemical structure and conformation of the xyloglucan heptamer XFG in order to increase the cold resistance of the grapevine. As heptamaloxyloglucan occurs in plants and soil at very low levels, the manufacturing process mimics natural phenomenons in order to accelerate the rate of natural biochemical processes and thus increase heptamaloxyloglucan yields.

The representative formulation (PEL101GV) is equivalent to the technical active substance EL101GV PEL101GV is a lyophilisat water-soluble formulation used as an anti-freezing in grapevine. The proposed use for heptamaloxyloglucan is post-emergence applications of 0.44 g a.s./ha (4 applications maximum with a minimal interval between applications of 4 days) on grapevine at BBCH 7 to 16 (early spring).

Regarding literature data submitted by the notifier, RMS judged them acceptable when it is possible to derive useful informations on how xyloglucan, carbohydrates or oligosaccharides could be degraded and assimilated or used by the different organisms and when the complete scientific article is provided. A summary of each publication has been done by the RMS in regard to these conditions and in the purpose of hazard identification and assessment of risk.

[6] Fry. S.C., Aldington S., Hetherington P.R., Aitken J., 1993. “Oligosaccharides as Signals and Substrates in the Plant Cell Wall“.Plant Physiol., Vol. 103 (1993), pp. 1-5.
[7] Buchanan B.B. et al, 2000. “Chapter 2: The Cell Wall. Biochemistry and molecular Biology of Plants”. B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89.

B.9.1. EFFECTS ON BIRDS (ANNEX IIA 8.1; ANNEX IIIA 10.1)

No studies have been conducted to determine the toxicity of heptamaloxyloglucan on birds.

Based on literature data, the notifier showed that xyloglucans, among which heptamaloxyloglucan, are natural constituents of cell wall of dicotyledons, in which they account for *ca.* 10% of the whole constituents (Buchanan et al, 2000,II 8.1/1). Herbivorous animals such as birds ingest hemicelluloses (typical name of xyloglucan), which are fermented by the bacteria of the hindgut and caecum and transformed in short-chain fatty acids. Fatty acids are absorbed and provide a substantial amount of the maintenance energy required by these animals (Stevens C.E., and Hume I.D., 1998, II A 8.1/2). As hemicelluloses are relatively soluble and therefore more easily fermented than the large polymers of cellulose, they account for the major part of digestible fibers in these animals.
Regarding insectivorous birds, they may ingest heptamaloxyloglucan via consumption of insects feeding on plants or plant debris.
Heptamaloxyloglucan is intended to be used at very low dose rate (0.0005 to 0.44 g/ha), the level of expected residues on plants and insects is estimated to be such low that no significant change in the diet of birds eating leaves, grass or insects is expected after application of PEL101GV on vine (see Table B.9.1.7.3-1)

- **Summary of literature data from RMS focusing on xyloglucan informations:**

Reference number:	II A 8.1/1, II A 8.3.1/1, II A 8.4/1, II A 8.5/1
Report:	Buchanan B.B. et al, 2000 Chapter 2: The Cell Wall Biochemistry and molecular Biology of Plants B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89 Published
GLP compliance	No
Acceptability	**Yes, provide justification on the natural occurrence of xyloglucan in plant cell wall.**

This document provides informations on plant cell wall constituents, architecture and biosynthesis.
The plant wall structure is a highly organised matrix of many different molecules: polysaccharides, protein and aromatic substances, acting as fibers or cross-linked matrix. The carbohydrates structures offer an important possibility of linkage at multiple positions and therefore a great functional flexibility.
Xyloglucans are one of the 2 major cross-linking glycans (also named “hemicelluloses”) are polysaccharides that can coat cellulose microfibrils, span distance between microfibrils and link them to form a network. Links between xyloglucan and cellulose are made by hydrogen bonds.
Polymers of the plant cell wall, such as xyloglucans, are synthetised in the Golgi apparatus. They can be modified by esterification, acetylation or arabinosylation for solubility during transport in Golgi-derived apparatus. In order to cross-link into the cell wall, they are later deesterifyed, deacetylated or dearabinosylated by extracellular enzymes.
Xyloglucan molecules are part of the cell wall constituents of the majority of dicotyledonous plants and of the noncommelinoid monocots, in which they could be

present in same amount as cellulose (*i.e*. 15 to 30% of dry mass of primary cell walls).

Reference number:	II A 8.1/2
Report:	Stevens C.E., Hume I.D. 1998 Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients Physiol.Rev., 1998 Apr, 78(2), pp 393-427 Published
GLP compliance	No
Acceptability	**Yes**

This publication presents an overview of the gastrointestinal tract among the vertebrates and the contribution of endogenous bacteria for the production and conservation of nutrients.
Dietary cellulose, hemicellulose and pectin are major substrates in the hindgut of herbivores. Most of herbivorous animals have a great gut capacity and digesta retention time that allow additionnal fermentation of structural carbohydrates of plant cell walls. Bacteria which colonize the gastrointestinal tract of all vertebrates (particularly hindgut and caecum or colon for herbivorous) produce short-chain fatty acids by fermentation of carbohydrates and convert nitrogenous compounds into ammonia and microbial protein and synthetize B vitamins.
The size of gastrointestinal tract organs tends to vary with the diet and also degree of capacity of flying. Thus granivorous and herbivorous birds have generally a larger crop and more vascular gizzard. Most of them belong to galliforms who tend to fly only short distance.
The microbial fermentation takes place in the caecum or colon for most of herbivorous animals. The limited gut capacity and high rate of metabolism of small herbivorous birds and mammals are compensated by specific adaptation of retention time in caecum (i.e. selective retention of fluid and small particles in caecum and more rapid excretion of larger digesta particles).

- **Comments from RMS:**

The literature data provided some qualitative informations which confirmed that xyloglucans are part of the plant cell wall and gave an idea of how heptamaloxyloglucan as other carbohydrates could be assimilated by birds and mammals. However these data may not be enough focused on xyloglucans or oligosaccharides themselves and there is no informations on the quantity of such molecules in a bird usual diet.

B.9.1.1. Acute oral toxicity (Annex IIA 8.1.1; Annex IIIA 10.1.1)

No acute studies on toxicity of heptamaloxyloglucan and the formulation PEL101GV have been performed since heptamaloxyloglucan is part of bird diet.

B.9.1.2. Short term dietary toxicity (Annex IIA 8.1.2)

No short-term dietary toxicity study has been conducted.

B.9.1.3. Subchronic and reproductive toxicity of heptamaloxyloglucan to birds (Annex IIA 8.1.3)

No subchronic and reproduction toxicity study has been provided.

B.9.1.4. Supervised cage or field trials (Annex IIIA 10.1.2)

Since neither toxicity nor long-term exposure are expected, supervised cage or field trials are not deemed necessary.

B.9.1.5. ACCEPTANCE OF BAIT, GRANULES OR TREATED SEEDS BY BIRDS (ANNEX IIIA 10.1.3)

Not applicable because the test material is not supposed to be applied to baits, granules or seeds.

B.9.1.6. EFFECTS OF SECONDARY POISONING (ANNEX IIIA 10.1.4)

The log K_{ow} of heptamaloxyloglucan is low (-15.96). Therefore no accumulation is expected along the food chains and there is no risk for secondary poisoning.

B.9.1.7. RISK ASSESSMENT FOR BIRDS

B.9.1.7.1. Toxicity endpoints for birds

As no study was performed with birds, no endpoint could be set.

B.9.1.7.2. Exposure scenario for calculations of ETE for birds

Birds may be exposed to PEL101GV by the consumption of contaminated food picked from the treated area. Guidance to estimate the exposure of birds and mammals to plant protection products (PPP) is provided in SANCO document 4145/2000. Beside others, standard exposure scenarios for the application of PPP in orchards/vine/hops are described in the guidance document. This scenario describes the situations in vine most suitably and therefore is considered in the present risk assessment. After an application of PEL101GV in early spring, insectivorous birds feeding small insects (wren, tit) may be at risk. The presented risk assessment focus on this group. Standard values for body weight and food intake rate were used.

B.9.1.7.3. Calculation of ETE and TER for birds

Risk assessment for birds according to the guidance given in the 4145/SANCO document was based on one single application, default DT_{50} values and the following data:

- grapevine: a maximum application rate of 124 mg product/mL, i.e. 0.44 g a.s./ha, applied up to 4 times with a minimum interval of 4 days

The calculated ETE for acute, short-term and long-term exposure are presented in Table B.9.1.7.3-1.

Table B.9.1.7.3-1: First tier acute, short and long term risk assessment for insectivorous birds (wren, tit), 4 applications of 124 mg/mL of PEL101GV, i.e. 0.44 g a.s./ha in vine

Time scale	**Crop scenario**	**Indicator species**	**FIR / bw**	**Food category**	**RUD**	**MAF**	**Factor (t.w.a.)**	**Use rate (kg a.s./ha)**	**ETE (mg/kg bw)**
acute	Orchard/vine/hops	insectivorous bird	1.04	small insects	52	-	-	0.00044	0.024
short term	Orchard/vine/hops	insectivorous bird	1.04	small insects	29	-	-	0.00044	0.013
long term	Orchard/vine/hops	insectivorous bird	1.04	small insects	29	-	-	0.00044	0.013

B.9.1.7.4. Conclusion on acute, short- term risks for birds

No TERs have been calculated by the notifier since no toxicity data are available. However, as heptamaloxyloglucan is a possible degradation product of xyloglucans entering in the composition of animal diet for which calculated ETE is extremely low no risk is expected following an application of heptamaloxyglucan as used according to the intended GAP proposed for PEL101GV.

- **Proposition of RMS:**

In an attempt to quantify the risk, it is proposed to assess the margin of safety existing between mammalian toxicity data and the estimated theoretical exposure of birds in vine following applications of heptamaloxyloglucan in early spring.

Table 9.7.7.4-1: Margin of safety for birds exposed to heptamaloxyloglucan					
		Results of mammalian toxicity studies	**ETE of insectivorous birds in vineyard**	**Theoretical TER**	**Margin of safety between toxicity to mammals and toxicity to birds**
Acute		Acute oral toxicity Rat LD_{50} > 5000 mg a.s./ kg bw/d * (Freulon I., 2004, B.6.2.1)	0.024 mg/kg bw/d	208333	20833
Short term		28-day oral toxicity Rat LD_{50} > 1000 mg a.s./kg bw/d**	0.013 mg/kg bw/d	76923	7692
* no observed effect at 2000 mg/kg/d, highest dose tested ** NOEL = 1000 mg a.s./kg bw/d, highest tested dose					

The theoretical TER between the mammalian toxicity endpoints and the estimated exposure of insectivorous birds to heptamaloxylogucan are very high compared to the trigger value of 10 for acute and short-term indicating an acceptable risk. Therefore no acute and short-term risk on birds is expected following applications of PEL101GV on vine. No further studies are therefore necessary.

B.9.1.7.5. Conclusion on long-term risks for birds

As there is no toxicological concern from acute and short-term exposure and as bioaccumulation along food chains is expected to be low (log K_{ow} of –15.96), no long-term risk is expected following applications of heptamaloxyloglucan on vine.

B.9.1.8. Conclusion on effects of heptamaloxyloglucan on birds

No toxicity data are available on effects of heptamaloxyloglucan on birds. However literature data show that heptamaloxyloglucan which belongs to the carbohydrates family may undergo fermentation in gastrointestinal tract of birds allowing the production of short-chain fatty acids by endogenous bacteria.
Estimation of exposure for birds has been done according to the guidance given in the 4145/SANCO document for an application on early stage of vine. Comparisons of level of exposure to mammalian toxicity data show that the acute and short-term risk is acceptable. Long-term exposure is judged not relevant. No risk on birds is expected following applications of PEL101GV on vine.

B.9.2. Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

Acute toxicity studies have been performed with the active substance heptamaloxyloglucan on rainbow trout, daphnia and one algae species. Short-term and long-term studies were not conducted as heptamaloxyloglucan is not acutely toxic for the species tested. Additionally no test has been performed with the formulation PEL101GV as only heptamaloxyloglucan enters in the composition of PEL101GV.

B.9.2.1. ACUTE TOXICITY TO FISH (ANNEX IIA 8.2.1; ANNEX IIIA 10.2.1)

B.9.2.1.1. Acute toxicity of heptamaloxyloglucan (EL101GV) to fish (Onccorhynchus mykiss, semi-static)

Reference number:	II A 8.2/1
Report:	L'Haridon J. 2006a EL101GV: Acute toxicity in the Rainbow trout under semi-static conditions CIT, unpublished report No 30711 EAP
Dates of works:	October 31-November 4, 2005
GLP compliance	Yes
Guidelines	Directive 92/69/EEC C.1 (1992), OECD N° 203 (1992), OCDE Series on testing and assessment, Guidance document on aquatic testing of difficult substances and mixtures (2000)
Deviations	None
Acceptability	**Yes**

Methods:
One group of 7 rainbow trouts (body length 40-46 mm, mean body weight 1.06 g) were exposed for 96 hours under semi-static conditions (renewal every 24 hours) to nominal technical EL101GV (batch No ANN0304, purity 78.2%) concentration of 150 mg/L at 13-17°C. There was one control group without any treatment. A photoperiod of 16/8 hour was applied during the test.
The test substance was dissolved in test water directly.
Observations for mortalities and clinical signs were performed at test initiation, after 2 hours and then daily.
Chemical analysis of heptamaloxyloglucan and water characterisation in control and treatment group (temperature, pH, oxygen, water hardness) were realised at the beginning of the test, before and after each renewal of solution and at the end of test.

Results:
Temperature was recorded between 14.4 and 16.1°C, dissolved oxygen concentrations were comprised between 6.8 and 9.6 mg/L, pH between 7.76 and 8.46 and water hardness between 145 and 158 mg/L of $CaCO_3$.
Concentrations of heptamaloxyloglucan (EL101GV) during the test were ranged from 157 mg/L (105%) and 165 mg/L (110%). Toxicity results (mortalities and clinical signs) were therefore expressed as nominal concentration.
There were no mortalities or clinical signs at nominal concentration of 150 mg/L.

Conclusion:
The rainbow trout 96h semi-static LC_{50} appears to be higher than 150 mg heptamaloxyloglucan/L. The NOEC was determined as 150 mg a.s./L, the highest tested concentration. This is equivalent to 117 mg/L pure heptamaloxyloglucan.

B.9.2.1.2. Acute toxicity of PEL101GV to fish (Annex IIIA 10.2.1)

PEL101GV contains only heptamaloxyloglucan (EL101GV) without any additional formulant. Additionally the test performed with the active substance was realised with a purity of 78.2% which is very closed to the minimum purity of heptamaloxyloglucan, i.e. 78%, and which considered therefore maximum account of impurities.
For these reasons, no test on PEL101GV toxicity to fish is deemed necessary.

B.9.2.1.3. Acute toxicity of metabolites, degradation or reaction products to fish (Annex IIA 8.2.1)

No toxicologically relevant metabolites are expected to occur as heptamaloxyloglucan is readily biodegradable in water and no relevant metabolites are expected to occur (B.8.4). Test on metabolite is not relevant.

B.9.2.2. CHRONIC TOXICITY TO FISH (ANNEX IIA 8.2.2)

B.9.2.2.1. Chronic toxicity of heptamaloxyloglucan to fish (Annex IIA 8.2.2.1, 8.2.2.2, 8.2.2.3)

In environment, heptamaloxylglucan is degraded in smaller oligosaccharides, then to monomers and finally to CO_2. It is soluble in water and is expected to be quickly degraded (see section B.8).

Additionally, the application rate is very low and PEC surface water after 4 applications of PEL101GV will 0.0143 µg a.s./L. This value is negligible in comparison to the quantity of xyloglucan that may reach surface water following plant decay. Moreover the acute NOEC to rainbow trout has been determined to be 150 mg a.s./L (equivalent to 117 mg pure heptamaloxyloglucan/L), demonstrating that no adverse effects are expected from chronic exposure to minimal amounts of heptamaloxyloglucan that may occur in surface water following applications of PEL101GV to vines.

As there was no evidence of acute toxicity and PEC_{sw} at ng/L level are expected, the margin of safety is judged sufficient and no chronic tests are deemed necessary.

B.9.2.2.2. Chronic toxicity of PEL101GV (Annex III A 10.2)

As PEL101GV contains only heptamaloxyloglucan (EL101GV) without any additional formulant, assumptions made for the active substance are relevant for the formulated product and no tests are required.

B.9.2.2.3. Bioconcentration in fish (Annex IIA 8.2.3)

The notifier considered that unchanged Heptamaloxyloglucan will not absorbed from the gastro-intestinal tract due to molecular weight greater than 1000 g/mol, but microbiologically degraded into natural hexoses and/or short chain fatty acids, which do not bioaccumulate. Therefore, no bioconcentration of the active substance or its degradation products is expected.
Furthermore, as outlined in the EU-Directive 91/414/EEC and the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4), a log K_{OW} >3 should be used as a general trigger for a fish bioconcentration study. As the log K_{OW} of heptamaloxyloglucan is low (-15.96), no study is required.

B.9.2.3. ACUTE TOXICITY TO AQUATIC INVERTEBRATES (ANNEX IIA 8.2.4; ANNEX IIIA 10.2.1)

B.9.2.3.1. Acute toxicity of heptamaloxyloglucan to daphnids (D. magna; static test)

Reference number:	II A 8.2.4/1
Report:	L'Haridon J. 2006b EL101GV: Acute toxicity in *Daphnia magna* under static conditions CIT, unpublished report No 30710EAD
Dates of works:	November 2-4, 2005
GLP compliance	Yes
Guidelines	Directive 92/69/EEC C.2 (1992), OECD N° 202 (2004), OCDE Series on testing and assessment, Guidance document on aquatic testing of difficult substances and mixtures (2000)
Deviations	None
Acceptability	**Yes**

Methods:
Four replicats of 5 *Daphnia magna* (less than 24 hours old) were exposed for 48 hours under static conditions to nominal technical EL101GV (batch No ANN0304, purity 78.2%) concentration of 150 mg/L at 18-22°C. There was one control group of 20 daphnids (4 replicats of 5 daphnids) without any treatment. A photoperiod of 16/8 hour was applied during the test.
The test substance was dissolved in test water directly.
Observations for immobilisation of daphnids were performed at 0, 24 and 48 hours.
One sample was taken in each replicate and pooled per group (control/test solution) for chemical analysis of the active substance and water characterisation in control and treatment groups (temperature, pH, oxygen, water hardness) at 0 and 48 hours. Samples were also taken at 24 hours but were not analysed.

Results:
During the test, temperature was recorded between 19.6 and 20.6°C, dissolved oxygen concentrations were comprised between 8.4 and 8.7 mg/L (94-98%), pH between 8.08 and 8.55 and water hardness of 303 mg/L of $CaCO_3$.
Concentration of heptamaloxyloglucan (EL101GV) at test initiation was 144 mg/L (96%) and 126 mg/L (84%) at test termination. Therefore toxicity results (immobilisation) are based on nominal concentration.
There were no mortalities or clinical signs at nominal concentration of 150 mg/L.

Conclusion:
Daphnia magna 48h static EC_{50} appears to be higher than 150 mg heptamaloxyloglucan/L. The NOEC was determined as 150 mg a.s./L, the highest tested concentration. This is equivalent to 117 mg/L pure heptamaloxyloglucan.

B.9.2.3.2 Acute toxicity of PEL101GV to Water Flea (Daphnia magna Straus, static test)

This point is addressed by the test on the active substance as PEL101GV contains only heptamaloxyloglucan (EL101GV) without any additional formulant. Additionally the test performed with the active substance was realised with a purity of 78.2% which is very closed to the minimum purity of heptamaloxyloglucan, i.e. 78%, and which considered therefore maximum account of impurities.
For these reasons, no test on PEL101GV toxicity to *Daphnia magna* is deemed necessary.

B.9.2.3.3. Acute toxicity of metabolites to the Water Flea

No toxicologically relevant metabolites are expected to occur as heptamaloxyloglucan is readily biodegradable in water and no relevant metabolites are expected to occur (B.8.4). Test on metabolite is not relevant.

B.9.2.3.4. Acute toxicity for at least one representative species of Aquatic insects, Aquatic crustaceans (species unrelated to Daphnia) and Aquatic gastropod molluscs (Annex II A 8.2.4)

None of these studies are required as heptamaloxyloglucan is not applied directly on water surfaces.

B.9.2.4. CHRONIC TOXICITY TO AQUATIC INVERTEBRATES (ANNEX IIA 8.2.5)

B.9.2.4.1. Chronic toxicity of heptamaloxyloglucan to daphnids (D. magna; static test)

A chronic toxicity test on *Daphnia magna* is not required, since heptamaloxyloglucan is expected to be quickly degraded (see section B.8.4) and is not acutely toxic to *Daphnia magna*.

B.9.2.4.2.Chronic toxicity of PEL101GV to daphnids

As PEL101GV contains only heptamaloxyloglucan (EL101GV) without any additional formulant, there is no need to conduct test on the formulation.

B.9.2.4.3. Chronic toxicity for at least one representative species of Aquatic insects, Aquatic crustaceans (species unrelated to Daphnia) and Aquatic gastropod molluscs (Annex II A 8.2.5)

None of these studies are required as heptamaloxyloglucan is not applied directly on water surfaces.

B.9.2.5. EFFECTS ON ALGAL GROWTH (ANNEX IIA 8.2.6; ANNEX IIIA 10.2.1)

B.9.2.5.1. Effects of heptamaloxyloglucan on growth of the green alga (Scenedesmus subspicatus; static test)

Reference number:	II A 8.2.6/1
Report:	L'Haridon J. 2006c EL101GV: Algal growth inhibition test CIT, unpublished report No 30709EAD
Dates of works:	October 31-November 3, 2005
GLP compliance	Yes
Guidelines	Directive 92/69/EEC C.3 (1992), OECD N° 201 (1984), OCDE Series on testing and assessment, Guidance document on aquatic testing of difficult substances and mixtures (2000)
Deviations	pH varies from more than 1.5 units during the test. This deviation is not considered to impact results of the study
Acceptability	**Yes**

Methods:
One group of six replicates of *Scenedesmus subsipacatus* (cell density 10^4 cells/mL at test initiation) and one without alga was exposed for 72 hours under static conditions to nominal technical EL101GV (batch No ANN0304, purity 78.2%) concentration of 150 mg/L at 21-25°C. There was one control group with six replicates containing alga without any treatment. Continuous illumination was applied during the test.
The test substance was dissolved in test water directly.
The number of cells was determined at 0, 24, 48 and 72 hours.
One sample was taken in each replicate containing alga and pooled per group (control/test solution) at 0, 24, 48 and 72 hours. Chemical analysis of the active substance and water characterisation in control and treatment groups (temperature, pH) were recorded at 0 and 72 hours. One sample was taken in replicate without alga of the 150 mg/L group in order to determine the influence of adsorption at the surface of algae cells and/or bioaccumulation on the possible decrease in test item concentration throughout the test.

Results:
Temperature was recorded between 23.5 and 24.0°C, pH from 7.74 to 9.89 in control and 7.41 to 10.15 in treatment group.
Concentrations of heptamaloxyloglucan (EL101GV) during the test were equal to 155 mg/L (103%) at 0h and 158 mg/L (105%) at 72h in replicate without algae, and at 165 mg/L (110%) in replicate with algae. Toxicity results were therefore expressed as nominal concentrations.
The Table B.9.2.5.1-1 summarised the main results obtained during the study.

Table B.9.2.5.1-1: Cell densities, growth rate and biomass at each concentration

Group	**Min-max cell densities (x 10^4/mL)**			**Specific growth rate (% inhibition)**			**Biomass 0-72h cell x mL^{-1} x h [area under growth curve] (% inhibition)**
	24h	48h	72h	24h	48h	72h	
0	7.4-9.1	50.0-65.5	261.0-297.0	0.0878*	0.0848*	0.078*	4940.0
150 mg/L	7.0-10.0	54.0-72.0	272.0-373.0	0.0880* (-0.23%**)	0.0862* (-1.65%)	0.080* (-2.6%)	5598.4 (-13.3%)
* approximation done by RMS ** recalculated by RMS							

Heptamaloxyloglucan has no inhibition effects on growth rate or biomass.

Conclusion:
The alga (*Scenedesmus subsipacatus*) 72h static EC_{50} appears to be higher than 150 mg/L. This is equivalent to 117 mg/L pure heptamaloxyloglucan.

B.9.2.5.2. Effects of heptamaloxyloglucan on a second algae species (diatom or blue-green algae)

Not required as heptamaloxyloglucan does not show toxicity nor on green alga neither on non target plant at concentration well above the expected exposure resulted from the use of heptamaloxyloglucan.

B.9.2.5.3. Effects of PEL101GV on alga

This point is addressed by the test performed with heptamaloxyloglucan which is the only component of PEL101GV.

B.9.2.5.4. Effects of metabolites on growth of alga

No toxicologically relevant metabolites are expected to occur as heptamaloxyloglucan is readily biodegradable in water and no relevant metabolites are expected to occur (B.8.4). Test on metabolite is not relevant.

B.9.2.6. EFFECTS ON SEDIMENT DWELLING ORGANISMS (ANNEX IIA 8.2.7)

As outlined in the EU-Directive 91/414/EEC and the Aquatic Guidance Document, test on sediment dwelling organisms such as *Chironomus sp*. is required:

- for insect growth regulator,
- if 48h-$EC_{50(insect)}$ < 1/10 48h-EC_{50} (*Daphnia*) or if TER (insect, acute) < 100
- if more than 10% applied radioactivity in sediment and NOEC (*Daphnia*) < 0.1 mg/L

None of these criteria is met. Moreover, heptamaloxyloglucan may enter to surface water and reach sediment layer via plant decay in amounts far above the initial PEC_{SED} calculated to be 4.093 µg/kg after 1 application of 1.76 g a.s./ha.
For these reasons no tests are deemed necessary.

B.9.2.7. EFFECTS ON AQUATIC PLANTS (ANNEX IIA 8.2.8)

Heptamaloxyloglucan is a plant elicitor and as such could be considered as a plant growth regulator. Nevertheless, no tests on aquatic plants are deemed necessary as no phytotoxicity on terrestrial plants or on alga have been observed and exposure is very low.

B.9.2.8. MICROCOSM OR MESOCOSM STUDY (ANNEX IIIA 10.2.2)

Study not needed.

B.9.2.9. RESIDUE DATA IN FISH (ANNEX IIIA 10.2.3)

There is no indication for a bioaccumulation potential of heptamaloxyloglucan due to low log $K_{ow.}$

B.9.2.10. RISK ASSESSMENT FOR AQUATIC ORGANISMS

B.9.2.10.1. Toxicity endpoints for aquatic organisms

The toxicity data are summarized in the Table B.9.2.10.1-1.

Table B.9.2.10.1-1: Heptamaloxyloglucan toxicity data for aquatic organisms

Substance	**Species**	**Exposure**	**LC_{50} or EC_{50}**	**NOEC**
Heptamalo-xyloglucan	*O. mykiss*	Acute, 96h	>150 mg/L *	150 mg/L *
	D. magna	Acute, 48h	>150 mg/L *	150 mg/L *
	S. subspicatata	Chronic, 72 h	>150 mg/L *	150 mg/L *

* equivalent to 117 mg pure heptamaloxyloglucan/L

B.9.2.10.2. Exposure scenario for calculations of TER for aquatic species

For prediction of the concentrations of PEL101GV and heptamaloxyloglucan in surface waters, the following considerations were taken into account:
- according to the GAP, PEL101GV is recommended to be applied at a maximum of 1.76 g a.s./ha on vines (worst case than 4 applications of 0.44 g a.s./ha). Results of PECsw and sed are reported in Table B.9.2.10.2-1.

Table B.9.2.10.2-1 : PECsw and PECsed for heptamaloxyloglucan – FOCUS Step 1

FOCUS STEP 1 Scenario	Day after overall maximum	PEC_{SW} (µg/L)		PEC_{SED} (µg/kg)	
		Actual	TWA	Actual	TWA
	0h	0.6025		4.093	
	24h	0.6021	0.6023	4.2006	4.1468
	2d	0.6017	0.6021	4.1977	4.173
	4d	0.6008	0.6017	4.1919	4.1839
	7d	0.5996	0.601	4.1831	4.1854
	14d	0.5967	0.5996	4.1629	4.1792
	21d	0.5938	0.5981	4.1428	4.1704
	28d	0.5909	0.5967	4.1227	4.161
	42d	0.5852	0.5938	4.0829	4.1416

B.9.2.10.3. Risk assessment for the parent compound, heptamaloxyloglucan

The TER values for heptamaloxyloglucan is reported in Table B.9.2.10.4-1:

Table B.9.2.10.4-1 Toxicity-exposure ratio (TER) for heptamaloxyloglucan

Test species	Endpoint	Result (µg as/L)	Initial PEC µg as/L	TER
O. mykiss	LC_{50}, 96h	>150000	0.6025	$> 20*10^4$
D. magna	EC_{50}, 48h	>150000	0.6025	$> 20*10^4$
S. subspicatata	E_bC_{50}, 72 h	>150000	0.6025	$> 20*10^4$

All acute TERs meet the requirements of Directive 91/414/EEC. There is no potential acute risk for heptamaloxyloglucan to fish, aquatic invertebrates or algae.

No long-term risk is expected following application of PEL101GV.

B.9.2.11. CONCLUSION ON EFFECTS OF HEPTAMALOXYLOGLUCAN ON AQUATIC ORGANISMS

Heptamaloxyloglucan was not acutely toxic to fish, daphnia or algae with no effects at the highest test concentration (150 mg a.s./L).
Risk assessment conducted under the worst case assumption with 1 application of 1.76 g a.s./ha (equivalent to no degradation of heptamaloxyloglucan after 4 applications) show that no risk on aquatic organisms are expected following applications of PEL101GV.

B.9.3. EFFECTS ON TERRESTRIAL VERTEBRATES OTHER THAN BIRDS (ANNEX IIIA 10.3)

According to current specifications, the minimal accepted purity of heptamaloxyloglucan is 78%. The toxicology studies on mammals have been performed with heptamaloxyloglucan batches of 96.24% purity. Since impurities have a structure similar to the one of heptamaloxyloglucan, *i.e*. sugar molecules with greater molecular weight, RMS believes that assessment of the active substance will cover the impurities and accepted studies with higher purity than the one of specification.

B.9.3.1. TOXICITY STUDIES

Studies have been performed on mammals to investigate the acute and short-term toxicity of EL101GV (technical heptamaloxyloglucan). They are reported in the relevant section of the present document (B.6.) and summarised in Table B 9.3.1-1.

Table 9.3.1-1: Toxicity of heptamaloxyloglucan to mammals

Test species	Test system	Results	References
Rat	Acute oral toxicity	LD_{50} > 5000 mg EL101GV/kg b.w.*	Freulon I., 2004 (B.6.2.1)
Rat	28-day oral toxicity	NOEL = 1000 mg EL101GV/kg b.w./d **	Barbier E., 2006 (B.6.3.1)

* equivalent to 4810 mg Heptamaloxyloglucan /kg b.w (based on the purity of 96.2%)
** equivalent to 886 mg Heptamaloxyloglucan /kg b.w (based on the purity of 88.6%), highest tested dose

B.9.3.2. ACCEPTANCE OF BAIT, GRANULES OR TREATED SEEDS

Not relevant, since heptamaloxyloglucan is not used in these scenarios.

B.9.3.3. EFFECTS OF SECONDARY POISONING

The log K_{ow} of heptamaloxyloglucan is low (-15.96). Therefore it is not expected that heptamaloxyloglucan accumulates along the food chains. Consequently no risk for secondary poisoning is anticipated.

B.9.3.4. SUPERVISED CAGE OR FIELD TRIALS OR OTHER APPROPRIATE STUDIES

No such studies are required as TER are all above the trigger values.

B.9.3.5. RISK ASSESSMENT FOR VERTEBRATES OTHER THAN BIRDS

B.9.3.5.1. Toxicity endpoints for mammals

Toxicity values reported in Table B.9.3.1/1 are used for the purpose of risk assessment.

B.9.3.5.2. Exposure scenario for calculations of ETE for mammals

Mammals may be exposed to PEL101GV by the consumption of contaminated food picked from the treated area. Guidance to estimate the exposure of birds and mammals to plant protection products (PPP) is provided in SANCO document 4145/2000. Beside others, standard exposure scenarios for the application of PPP in orchards/vine/hops are described in the guidance document. This scenario describes the situations in vine most suitably and therefore is considered in the present risk assessment. After an application of PEL101GV in early spring, small herbivorous mammals (vole) feeding short grass may be at risk. The presented risk assessment focus on this group. Standard values for body weight and food intake rate were used. Additionally, an interception factor of 40%, usually used for insecticides and fungicides, was taken into account in the following assessment, as PEL101GV will be applied by foliar application.

Table B.9.3.5.2-1: Exposure assessment for heptamaloxyloglucan in vine (Tier 1) [1]

Crop stage	Indicator species	FIR (fresh) / body weight	Food type	RUD [2] [mg a.s./kg]	PT	PD		f_{twa}	MAF [3]	Use Rate [kg a.s./ha]	ETE [mg a.s./kg]
Acute											
Early	Small herbiv. mammal	1.39	Short grass	85	1	1		--	2.00 *	0.00044	0.1040
									2.18 **		0.1133
Long-term											
Early	Small herbiv. mammal	1.39	Short grass	46	1	1		0.53	2.77	0.00044	0.0413

1) Sanco 4145/2000, rev. 6, 25.09.02 [1]
2) A deposition factor of 0.6 for application of insecticides/fungicides in vine at early stage (according to Sanco 4145/2000) is included within RUD value
3) * Acute MAF has been extrapolated by notifier based on Sanco 4145/2000, rev. 6, 25.09.02 and set at 2.00.
** According to PSD ETE model acute MAF for short grass following 4 applications of PEL101GV with 4 days interval will be 2.18 with a default DT_{50} of 10 days.

B.9.3.5.3. Calculation of ETE and TER for mammals

B.9.3.5.3.1. Acute risk assessment

TER will be greater than 45455 based on an acute toxicity of greater than 5000 mg EL101GV/kg b.w and an ETE around 0.11 mg/kg/d.

B.9.3.5.3.2. Long-term-risk assessment

As there was no toxicological concerns from acute and short-term exposure and as bioaccumulation along food chains was expected (log K_{ow} of –15.96), no long-term risk is expected following applications of heptamaloxyloglucan on vine.

B.9.3.5.4. Conclusion on risks for mammals

Acute and long-term risk to mammals exposed to heptamaloxyloglucan in vine meet requirements of Directive 91/414/EC.

B.9.3.6. CONCLUSION ON EFFECTS OF HEPTAMALOXYLOGLUCAN ON VERTEBRATES OTHER THAN BIRDS

Studies performed on mammals to investigate the acute and short-term toxicity of EL101GV (technical heptamaloxyloglucan) show the low toxicity of the active substance.
Risk assessment for mammals done according to the guidance given in the 4145/SANCO document for an application on early stage of vine show that the risk of heptamaloxyloglucan for vertebrates other than birds is acceptable.

B.9.4. EFFECTS ON BEES (ANNEX IIA 8.3.1; ANNEX IIIA 10.4)

B.9.4.1. ACUTE TOXICITY (ANNEX IIA 8.3.1.1; ANNEX IIIA 10.4.1)

B.9.4.1.1. Acute contact and oral toxicity on honey bees (A. mellifera; laboratory studies)

B.9.4.1.1.1 Acute toxicity of heptamaloxyloglucan

Reference number:	II A 8.3.1.1/1
Report:	Servajean E. 2006a Laboratory determination of the contact and oral acute toxicity of a formulation to honey bees (*Apis mellifera*) (OECD 213 and OECD 214, September 1998) Phytosafe unpublished report number 05-27-064-ES
Dates of works:	June 15 – July 2, 2006
GLP compliance	Yes
Guidelines	OECD 213 and OECD 214
Deviations	None
Acceptability	**Yes**

Methods:
Honeybees (*Apis mellifera,* unknown age) were subjected to contact and oral limit tests with technical heptamaloxyloglucan (EL101GV, batch AND0205, purity: 87.1%). For each test, 3 replicates were exposed to nominal concentration of 100 µg/bee. A control group made of 3 replicates was added.
Dimethoate was used as toxic reference. Bees were exposed to concentrations between 0.10 and 0.30 µg/bee (contact exposure) or 0.35 µg/bee (oral exposure), with 3 replicates per concentrations.
For each test and treatment, 25 bees were isolated. Males and moribund females were discarded. The exact number of bees treated per replicate is specified in the tables of results.
For oral toxicity testing, treatment solution was prepared by diluting 99.9 mg of technical heptamaloxyloglucan in 10 mL of a 500 g/L sucrose solution in water.
Mortality was recorded in each replicate after 4, 24 and 48 hours of exposure. The amount of sucrose ingested will be recorded after 24 and 48 hours. Bee behaviour was also inspected regularly.

Results:
In the oral test, the ingestion of technical heptamaloxyloglucan treated syrup had no adverse effect on the post-treatment feeding dynamic (percentage to the control: 113 % for the 0-24h period; 143 % for the 24-48h period). Post-treatment feeding dynamic was affected for bees exposed to 0.10 µg dimethoate/bee (percentage to the control: 60 % for the 0-24h period; 34 % for the 24-48h period).

The results of mortality are presented in the following table.

Table B.9.4.1.1.1-1: Oral toxicity of technical heptamaloxyloglucan to bees

Test Group (µg/bee)	Replicate	Number of bee treated	Number of dead			% mortality		
			4h	24h	48h	4h	24h	48h
Control	1	18	0	3	3	0.0 %	16.7 %	16.7 %
	2	19	0	0	0	0.0 %	0.0 %	0.0 %
	3	16	0	0	0	0.0 %	0.0 %	0.0 %
	mean					**0.0 %**	**5.6 %**	**5.6 %**
EL101GV (100)	1	19	0	0	1	0.0 %	0.0 %	5.3 %
	2	19	0	1	2	0.0 %	5.3 %	10.5 %
	3	19	0	0	0	0.0 %	0.0 %	0.0 %
	mean					**0.0 %**	**1.8 %**	**5.3 %**

Heptamaloxyloglucan had no oral adverse effects on bees at the limit dose of 100 µg/bee.
The reference substance (dimethoate) reached 50%, 89.5% and 100% mortality after 48 hours exposure at 0.10, 0.15 and 0.30 µg a.s./bee, respectively.

In the contact test, the ingestion of technical heptamaloxyloglucan treated syrup had no adverse effect on the post-treatment feeding dynamic (percentage to the control: 87 % for the 0-24h period; 84 % for the 24-48h period). Post-treatment feeding dynamic was affected for bees exposed to 0.10-0.16 µg dimethoate/bee (percentage to the control: 74-85 % for the 0-24h period; 26-35 % for the 24-48h period).
The results of mortality are presented in the following table.

Table B.9.4.1.1.1-2: Contact toxicity of technical heptamaloxyloglucan to bees

Test Group (µg/bee)	**Replicate**	**Number of bee treated**	**Number of dead**			**% mortality**		
			4h	**24h**	**48h**	**4h**	**24h**	**48h**
Control	1	20	0	1	2	0.0 %	5.0 %	10.0 %
	2	20	0	1	1	0.0 %	5.0 %	5.0 %
	3	20	0	0	0	0.0 %	0.0 %	0.0 %
	mean					**0.0 %**	**3.3 %**	**5.0 %**
EL101GV (100)	1	20	0	1	1	0.0 %	5.0 %	5.0 %
	2	20	0	0	0	0.0 %	0.0 %	0.0 %
	3	20	0	1	1	0.0 %	5.0 %	5.0 %
	mean					**0.0 %**	**3.3 %**	**3.3 %**

Heptamaloxyloglucan had no contact adverse effects on bees at the limit dose of 100 µg/bee.
The reference substance (dimethoate) reached 25%, 85% and 96.7% mortality after 48 hours exposure at 0.10, 0.16 and 0.25 µg a.s./bee, respectively.

Conclusion:

In both oral and contact toxicity test, the 48h LD_{50} was > 100 µg a.s./bee.

B.9.4.1.1.2 Additionnal information on acute toxicity of carbohydrates to bees

RMS has summarised literature data submitted and proposed its conclusion in regard to risk assessment under paragraph B.9.4.6.4.

Reference number:	II A 8.3.1/2
Report:	Winston M.L. 1987 The biology of the honey bee Harvard University Press, 1987, 281 pp Published
GLP compliance	No
Acceptability	**Yes**

Honeybee castes (workers, queens or drones) satisfy their food requirements via different nutritional need and feeding mechanisms but with same starting materials for both brood and adult bees.
In the diet of bees, carbohydrates are provided in sugar form via nectar whereas protein, lipids, vitamins and mineral are supplied by pollen. The main sugars found in nectar are sucrose, glucose and fructose. Some sugars such as mannose, galactose and rhamnose could be toxic to bees or cause reduction in bee longevity. Enzymes from the hypopharyngeal glands, specifically diastase, invertase and glucose oxidase could break down sugars into simple forms easily digestible by bees.
Pollen wall contains hard and undigestible components that rapidly reach the midgut. They do not seem to be degraded and digestion of usable nutrients takes place either through the pollen germination spores or through the pollen wall.

Reference number:	II A 8.3.1/3
Report:	Haydak M.H. 1970 Honey bee nutrition Annu. Rev. Entomol., Vol. 15, pp. 143-156, 1970 Published
GLP compliance	No
Acceptability	**Yes**

For most insects carbohydrates serve as a convenient source of energy. Nectar and honey contributes mostly mono and oligo saccharides to the food of bees.
“Sweet” sugars, such as glucose, fructose, saccharose (sucrose), maltose, trehalose, melezitose, and “unsweet” sugars, such as arabinose, xylose, galactose, cellobiose, raffinose, mannitol and sorbitol can be used by bees. Whereas others such as rhamnose, fucose, mannose, sorbose, lactose melibiose, dulcitol, erythritol or inositol can not be used. Mannose which is poisonous to honeybees will be present in glycopeptide form in royal jelly to avoid detrimental effects.

Reference number:	II A 8.3.1/4, II A 8.3.1.2/1
Report:	Barker R.J. 1977 Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees J. Nutr., Vol. 107, pp. 1859-1862, 1977 Published

GLP compliance No
Acceptability **Yes**

Some carbohydrates of pollen could be toxic to bees which may used nectar for dilution of galactosides or pectins to non-toxic levels. The aim of this study is to exposed honey bees to some carbohydrates found in pollen and see the importance of dilution to safe levels by sucrose.

Methods:
Groups of 60 bees were held at 30°C and under a photoperiod of 8 hours light/16 hours dark. Water and treated syrups were supplied by separate feeders. Treated groups consisted of dilution of carbohydrates (D-galactose, lactose, raffinose, stachyose, glucuronic acid, galacturonic acid, polygalacturonic acid) and pectin in 50% sucrose syrup. Concentration of carbohydrates and pectin depend of their solubility. Tested concentration were therefore: 2, 4, 6, 8, 10% D-galactose; 2, 4, 6, 8, 10% lactose; 2, 4, 6, 8, 10% raffinose; 2 and 8% stachyose; 2, 4 and 8% glucuronic acid; 2, 4 and 8% galacturonic acid; 2 and 4% polygalacturonic acid and 4% pectin. Control consisted on 50% sucrose syrup.
After 8 days, dead bees were counted and removed; feeders were weighed, cleaned and replenished. At 16 days, live and bees were counted. Each test was replicated 3 times.

Results:
During days 0 to 8, daily syrup consumption was 28 ± 10 mg/bee/day. Taking into account this food intake, concentrations of 2, 4, 6, 8 and 10% carbohydrates correspond to 0.56, 1.12, 1.68, 2.24 and 2.8 mg/bee/day, respectively.
During days 9 to 16, daily syrup consumption was 27 ± 10 mg/bee/day. Taking into account this food intake, concentrations of 2, 4, 6, 8 and 10% carbohydrates correspond to 0.54, 1.08, 1.62, 2.16 and 2.7 mg/bee/day, respectively.
Sucrose consumption was found to be in excess of 4 mg/bee/day. Taking into account this food intake, concentrations of 50% sucrose correspond to more than 2 mg/bee/day.

The main results are summarized in the table below.

Group (tested concentration)		**% dead in 8-day test**	**% dead in 16-day test**
Sucrose control	(50%)	1 (f)	6 (f)
D-galactose	(2%)	0 (f)	2 (f)
	(4%)	5 (f)	24 (d,e,f)
	(6%)	2 (f)	19 (d,e,f)
	(8%)	34 (d,e,f)	**82 (a,b,c)**
	(10%)	**56 (b,c,d)**	**93 (a,b,c)**
Lactose	(2%)	0 (f)	**57 (a,b,c,d)**
	(4%)	**15 (f)**	**81 (a,b,c)**
	(6%)	**42 (d,e)**	**96 (a,b)**
	(8%)	**50 (c,d,e)**	**100 (a)**
	(10%)	**76 (b,c)**	**100 (a)**
Raffinose	(2%)	1 (f)	3 (f)
	(4%)	5 (f)	12 (e,f)
	(6%)	5 (f)	34 (d,e,f)

Group (tested concentration)		% dead in 8-day test	% dead in 16-day test
	(8%)	27 (e,f)	**58 (a,b,c,d)**
	(10%)	**54 (c,d,e)**	**81 (a,b,c)**
Satchyose	(2%)	2 (f)	**80 (a,b,c)**
	(8%)	**40 (d,e)**	**99 (a)**
Glucuronic acid	(2%)	4 (f)	16 (e,f)
	(4%)	6 (f)	**46 (c,d,e)**
	(8%)	11 (f)	**96 (a,b)**
Galacturonic acid	(2%)	6 (f)	**54 (b,c,d)**
	(4%)	19 (f)	**78 (a,b,c)**
	(8%)	18 (f)	**98 (a)**
Polygalacturonic acid	(2%)	4 (f)	**45 (c,d,e)**
	(4%)	12 (f)	**87 (a,b,c)**
Pectin	(4%)	**82 (b)**	**100 (a)**
Values reported are means of 3 replicates. Means with a common letter are not significantly different at P< 0.05 (SNK Multiple Range Test)			

In the 16-day test, mortality in groups with 6% raffinose or galactose (*i.e*. 1.62 mg/bee/day) or with 2% glucuronic acid (*i.e*. 0.54 mg/bee/day) did not differ from mortality recorded in syrup control.
Significant differences from control were recorded in the 8-day test from 6% lactose (*i.e.* 1.68 mg/bee/day), 8% stacchyose (*i.e*. 2.24 mg/bee/day), 10% raffinose (*i.e*. 2.8 mg/bee/day), 10% galactose (*i.e*. 2.8 mg/bee/day) and 4% pectin (*i.e*. 1.12 mg/bee/day).

In the 16-day test toxic effects compared to control were observed from 2% lactose (*i.e*. 0.54 mg/bee/day), 2% stacchyose (*i.e.* 0.54 mg/bee/day), 2% galacturonic acid (*i.e.* 0.54 mg/bee/day), 2% polygalacturonic acid (*i.e*. 0.54 mg/bee/day), 4% glucuronic acid (*i.e* 1.08 mg/bee/day), 8% raffinose (*i.e*. 2.16 mg/bee/day) and 8% galactose(*i.e*. 2.16 mg/bee/day).

Conclusion:
Some carbohydrates found in pollen could be toxic for bee when their concentrations exceed certain levels. These toxins need to be diluted or degraded to a safe level before bees can feed on the pollen. For the authors, production and storage of honey by bees seems to be a mechanism to dilute toxins.

- **Overall conclusion of literature data by RMS:**

In the diet of bees, carbohydrates serve as a convenient source of energy. Nectar and honey contributes mostly as mono and oligo saccharides to the food of bees. Some sugars could be poisonous to bees or causing reduction of longevity (e.g. mannose, galactose, rhamnose) (see II A 8.3.1/3).
The following sugar monomers of heptamaloxyloglucan could de used by bees : glucose, xylose, galactose and sorbitol, whereas others such as fucose can not. Galactose was the heptamaloxyloglucan monomer identified by the literature as poisonous to bees and therefore a risk assessment is proposed by the RMS in B.9.4.6.

B.9.4.1.2. Acute toxicity of PEL101GV to bees

This point is addressed by the active substance which is the only component of PEL101GV.

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

No study was performed.

B.9.4.3. Residue test (Annex IIIA 10.4.2)

No study was performed.

B.9.4.4. Cage tests (Annex IIIA 10.4.3)

No study was performed.

B.9.4.5. Field or tunnel tests (Annex IIIA 10.4.4 and 10.4.5)

No study was performed.

B.9.4.6. RISK ASSESSMENT FOR BEES

B.9.4.6.1. Toxicity endpoints for bees

The oral and contact LD_{50} of heptamaloxyloglucan is greater than 100 µg a.s./bee. One of the possible degradation product of heptamaloxyloglucan, i.e. galactose, was found to have lethal effects compared to the control at a dose level of 8%, 2.16 mg/bee/day, in a 16-days dietary test (Barker, 1977).

B.9.4.6.2. Exposure scenario for calculations of HQ for bees

According to GAP, PEL101GV is applied up to 4 times at a maximal rate of 0.44 g a.s./ha in vine. This was used for a first-tier, in-field exposure assessment.

B.9.4.6.3. Calculation of HQ for bees

To assess the risk to honey bees following the use PEL101GV, the hazard quotient (HQ) which is the ratio of the application rate (in g/ha) and the acute toxicity (LD_{50} in µg/bee), was determined based on the results from available studies to be: **HQ: 0.44/100 = 0.0044**.
NOEL of galactose was found at 6% level, i.e. 1620 µg/bee/day. The LD_{50} is greater than 1620 µg/bee/day and lower than 2160 µg/bee/day. This lead to a hazard quotient of: **HQ: 0.44/1620 = 0.00027** when considering that all heptamaloxyloglucan applied will be degraded in galactose units.

B.9.4.6.4. Conclusion on risks for bees

Hazard quotients are all well below 50. Moreover exposure of bees in vine at BBCH 7 to 16 is negligible. Therefore, the risk to bees is considered to be acceptable following application of PEL101GV on vine.

B.9.4.7. CONCLUSION ON THE EFFECTS OF HEPTAMALOXYLOGLUCAN ON BEES

Heptamaloxyloglucan was not acutely toxic to bees (oral and contact LD_{50} > 100 µg a.s./bee).
Literature data show that some sugars could be poisonous to bees or cause reduction in bee longevity (Winston, 1987). Some such as mannose can not be used by bees and are present in glycopeptide form in royal jelly to avoid a poisonous effect (Haydak, 1970). One of the possible degradation product of heptamaloxyloglucan, i.e. galactose, was found to have lethal effects compared to the control at a dose level of 8%, 2.16 mg/bee/day, in a 16-days dietary test (Barker, 1977). A LD_{50} of galactose comprised between 1620 µg/bee/day and 2160 µg/bee/day could be derived from this publication.
Hazard quotient following application of 0.44 g heptamaloxyloglucan/ha, as well as the hazard quotient for galactose (assuming the same application rate as heptamaloxyloglucan), was well below 50 (*i.e.* 0.44/100=0.0044 and

0.44/1620=0.00027, respectively). The risk to bees following application of PEL101GV on vine is considered to be acceptable.

B.9.5. EFFECTS ON OTHER ARTHROPOD SPECIES (ANNEX IIA 8.3.2; ANNEX IIIA 10.5)

Assessment of toxicity to non-target arthropods is always required where exposure is possible.

Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples and composed of 7 hexose residues (glucopyranosyl, fucopyranosyl, xylopyranosyl and galactopyranosyl). All these hexose are natural components of the apple and of other dicotyledonous plants, where they are major constituents of cellulose and hemicellulose molecules, which are the principal components of cell walls. As such, heptamaloxyloglucan takes part of usual food on arthropods.
Heptamaloxyloglucan is not toxic to honey bees (oral and contact LD_{50} > 100 µg/bee).
For these reasons, no test on non-target arthropods is deemed necessary.

B.9.6. EFFECTS ON EARTHWORMS (ANNEX IIA 8.4; ANNEX IIIA 10.6.1)

B.9.6.1. ACUTE TOXICITY TO EARTHWORMS (ANNEX IIA 8.4.1; ANNEX IIIA 10.6.1.1)

B.9.6.1.1. Acute toxicity of heptamaloxyloglucan

No study has been conducted since heptamaloxyloglucan is a xyloglucan molecule extracted from apples. Furthermore heptamaloxyloglucan can be produced from xyloglucan by enzymatic degradation naturally occurring in plant. As such it could enter in the diet of earthworms via fall of leaves.

B.9.6.1.2. Acute toxicity of PEL101GV

This point is addressed by the active substance as PEL101GV contains only heptamaloxyloglucan.

B.9.6.2. EFFECTS ON REPRODUCTION (ANNEX IIA 8.4.2; ANNEX IIIA 10.6.1.2)

No study needed.

B.9.6.3. FIELD STUDIES (ANNEX IIIA 10.6.1.3)

No study needed.

B.9.6.4. ADDITIONNAL INFORMATIONS (ANNEX IIIA 10.6.1.3)

Reference number:	II A 8.1/1, II A 8.3.1/1, II A 8.4/1, II A 8.5/1
Report:	Buchanan B.B. et al, 2000 Chapter 2: The Cell Wall Biochemistry and molecular Biology of Plants B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89 Published
GLP compliance	No
Acceptability	**Yes, provide justification on the natural occurrence of xyloglucan in plant cell wall.**

For summary see B.9.1.

Reference number:	II A 8.4/2
Report:	Edwards C. A., Fletcher E. 1988 Interaction between earthworms and microorganism in organic-matter breakdown Agriculture, Ecosystems & Environnement, Vol. 24 (1-3), pp. 235-247, 1988 Published
GLP compliance	No
Acceptability	**No, publication on importance of presence of micro-organisms for earthworm nutrition**

The aim of this publication is to review current knowledge of microbial-earthworm interactions and attempt to generalize their importance in the maintenance of soil structure and fertility and their role in dynamic soil processes.

The digestive system of earthworms consists of a pharynx, oesophagus and gizzard (“reception zone”) followed by an anterior intestine that secretes enzymes and a posterior intestine that absorbs nutrients. During progress through this digestive system there is a dramatic increase in numbers of micro-organisms of up to 1000 times. There is experimental evidence that micro-organisms provide food for

earthworms. Bacteria are of minor importance in the diet, algae are of moderate importance; protozoa and fungi are major sources of nutrients. Worms, produced under sterile conditions, could live on individual cultures of certain bacteria, fungi and protozoa, but grew best on various mixtures of organisms.
Symbiotic interactions between earthworms and micro-organisms break down and fragment organic matter progressively, finally incorporating it into water-stable aggregates. The mineral nutrients in earthworm casts and lining earthworm burrows are in a form readily available to plants. There is evidence that interactions between earthworms and micro-organisms not only provide these available nutrients, but stimulate plant growth indirectly in other ways.

Reference number:	II A 8.4/3
Report:	Edwards C. A., Bohlen P.J. 1996 Biology and Ecology of earthworms, 3rd edition Chapman & Hall, 1996, 426 pp Published
GLP compliance	No
Acceptability	**No, review focusing on biology and ecology, not enough information on oligosaccharides**

Earthworms use organic matter as a source of nutrition and depend upon microorganisms such as protozoa, rotifers, nematodes, bacteria, fungi (…) for supply of nutrients. Even lumbric species, such as *L. terrestris* that feed directly on leaves, depend upon the microorganisms growing upon them for nutrition.
Earthworms break down organic matter and recycle nutrients containing in it. They remove partially decomposed plant litter and crop residues from the soil surface, ingest it, fragment it and transport it to the subsurface layers. In fact organic matter ingested by earthworms passed through the gut and is broken down in earthworm casts into much finer particles, so that a greater organic matter surface area is exposed to microbial decomposition. Earthworms can increase the rates of mineralisation of organic matter and convert organic forms of nutrients into inorganic forms that can be taken up by plants.

Reference number:	II A 8.4/4
Report:	Lattaud C. et al., 1997 Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (megascoloecidae) Soil Biol. Biochem., Vol 29 (3/4), pp 335-339, 1997 Published
GLP compliance	No
Acceptability	**Yes, in complement with the literature data provided in B.8.1 regarding the possibility of micro-organisms to reduce xyloglucan polymer into monomeric sugars**

Endogeic geophagous earthworms from tropical areas seem to digest soil organic matter through a mutualist earthworm microflora-digestion system and the intestinal mucus produced by earthworms was supposed to play a central role in the process of digestion. A large range of glucosidic substrates characteristic of plant material was used to reveal the activities of digestive enzymes in the gut (wall and contents) of *Polypheretima elongata*. This worm consumes some plant substrates tested and is able mainly to degrade root and fungal substrates. It corroborates that tropical endogeic earthworms feed on litter debris and soils poor in organic matter. These glucosidic activities were higher than those found previously in *Pontoscolex corethrum*.
The gut of *P. elongata*, like that of *P. corethrurus*, shows a certain specific activity on cellulose and hemicellulose: it shows that these earthworms are likely to use most of the vegetal components in the soil for their nutrition. Moreover, in *P. elongata*, the presence of cellulolytic and mannanase activities in not only the gut, but also in tissues and in their culture medium, allows to infer that these enzymes are secreted

by the earthworm itself without the micro-organisms of the ingested soil. In nature, few organisms possess these enzymes in order to degrade cellulose and mannan which are the main plant constituents and they make use, like *P. corethrurus* and mannanase activities, or of symbiotic bacteria in order to degrade the insoluble substrates.
The *in vitro* tissue culture of gut wall allowed us to infer that *P. elongata* can synthesize by itself all its extra and intracellular enzymes, contrary to *P. corethrurus* which requires the microflora of the soil ingested in order to hydrolyse some substrates such as cellulose and mannan.

Reference number:	II A 8.4/5
Report:	Zhang B.G. and al., 1993 Activity and origin of digestive enzymes in gut of tropical earthworm *Pontoscolex corethrurus* Eur. J. Soil Biol., Vol 29 (1), pp 7-11, 1993 Published
GLP compliance	No
Acceptability	**Yes, in complement with the literature data provided in B.8.1 regarding the possibility of micro-organisms to reduce xyloglucan polymer into monomeric sugars**

The aim of this study was to identify enzymatic activities in the gut of the tropical earthworm *Pontoscoloex corethrurus* and to determine whether the enzymes were produced by the worm itself or by micro-organisms contained in the ingested soil.

Activities of glucidic digestive enzymes in the gut (content plus walls) of a tropical endogeic earthworm, *Pontoscolex corethrurus*, have been assayed. In order to determine the origin of the enzymes found in the gut, the wall tissues were cultured *in vitro*, and enzymatic activities were measured both in the cultured tissues and in the culture medium.
The earthworm possesses a weak but quite complete enzyme system, especially for cellulase, hemicellulases, cellobiase, and β-D-glucopyranosidase. In the gut, the enzymes were capable of degrading the following substrates: heteroside (N-acetylglucosamine), oligosaccharides (maltose, laminaribose) and polysaccharides (starch, laminaran, pullulan, microcrystalline cellulose, carboxymethylcellulose, mannan, glucomannan and caroub galactomannan, lichenin). The strongest enzymatic activities were located in the foregut and midgut. The weak enzymatic activities of the earthworm are coherent with its feeding habitats; being endogeic earthworm, *Pontoscolex corethrurus* feeds on soils poor in organic matter and litter debris.
Among the main enzymes found in the gut, cellulase and mannanase were neither detected in the cultured tissues nor in the culture medium, which indicates that these two enzymes were produced by micro-organisms ingested with the soil. The oligosaccharidase and heterosidase activities were higher in the cultured tissues than in the medium, which was not the case for the polysaccharidases.

Reference number:	II A 8.4/6
Report:	Garvin M.H. and al., 2000 Activity of glycolytic enzymes in the gut of *Hormogaster elisae* (Oligochaeta, Hormogastridae) Soil Biology & Biochemistry, Vol. 32, pp 929-934, 2000 Published
GLP compliance	No
Acceptability	**Yes, in complement with the literature data provided in B.8.1 regarding the possibility of micro-organisms to reduce xyloglucan polymer into monomeric sugars**

The aim of the study was to identify glycolytic activities in the gut of *Hormogaster elisae* and to determine whether these enzymes were produced by the worm itself or by the micro-organisms ingested with the soil.

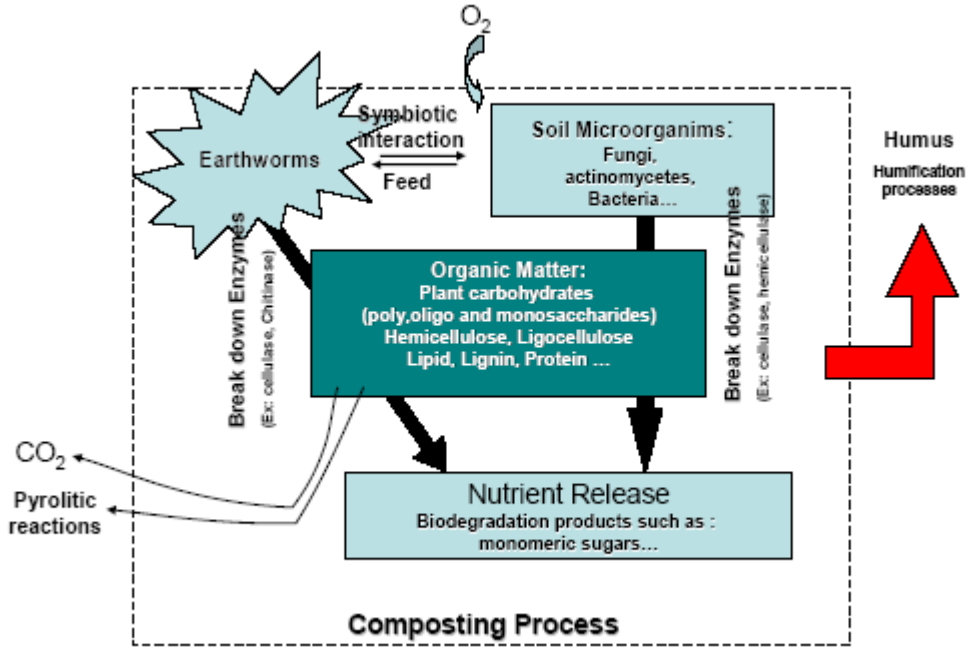
Most endogeic earthworms have weak enzymatic complement and they usually establish mutualistic relationships with soil microflora to digest some organic compounds. Therefore, the intestinal wall tissues were cultured in vitro to assess the origin of the glycolytic enzymes found in the gut and enzymatic activities were measured in both cultured tissues and culture media. *H. elisae* had a wide but not very strong enzyme complement, since all substrates were degraded but most of them at a low rate. All the polysaccharides were degraded in the gut except cellulose and mannose. This species cannot produce cellulase and mannanase, so for the digestion of these substrates it probably uses the digestive enzymatic capabilities of the ingested microflora. The especially weak activity on cellulose, hemicelluloses, cellobiose and most heterosides is in accordance with the ecological requirements of *H. elisae*, since it is an endogeic oligohumic species that feeds on soil low in organic matter.

B.9.6.5. CONCLUSION ON EFFECTS ON EARTHWORMS

As discussed under B.8.1.2.3 and B.8.1.2.4, Heptamaloxyloglucan, as all oligosaccharide polymers, may undergo hydrolysis by several enzymes, which are found in a large number of plants, bacteria and yeast, and release hexose monomers. It may also be fermented by bacteria and transformed into short-chain fatty acids.

Based on the above literature data, the notifier shows that earthworms use organic matter as a source of nutrition but depend upon protozoa, rotifers, nematodes, bacteria, fungi and other micro-organisms, for their nutrients. Soil ingested microorganisms and earthworms have developed symbiotic/mutualist relationship to digest soil organic matter (Lattaud and al., 1997, II A 8.4/4). Though earthworms possess glycolytic enzyme activity (cellulase, hemicellulase, amylase…), they need microflora to complete enzymatic equipment that they lack. In this general situation, ingested microflora is able to degrade organic matter as simple elements that will be absorb again trough the earthworm gut walls (Zhang and al. 1993, II A 8.4/5, Lattaud and al. 1997, II A 8.4/4, Garvin and al. 2000, II A 8.4/6).

Therefore, the notifier assumes that heptamaloxyloglucan is in the same way, degraded by ingested soil microorganism as glucidic monomers inside the different parts of the earthworm gut. The following relationship scheme describing earthworm/microorganisms interactions in the composting process (humification) is proposed.



As a conclusion, Heptamaloxyloglucan could enter in earthworm diet via enzymatic degradation of xyloglucans of plant cell walls by several micro-organisms and no toxicity is expected towards these organisms.

B.9.6.6. RISK ASSESSMENT FOR EARTHWORMS

Initial PEC_{soil} of heptamaloxyloglucan calculated based on worst-case (unrealistic) assumptions of no degradation and no crop interception has been estimated to be equal to 0.00235 mg a.s./kg after 4 applications of PEL101GV to grapevines at growth stage BBCH 7 to BBCH 16.

The notifier shows that micro-organisms ingested by earthworms together with soil are able to degrade oligosaccharides into monomeric sugars. Glucose, xylose, fucose, galactose and glucitol, the monomeric sugars expected to result from degradation of heptamaloxyloglucan, are naturally occuring into organic matter of soil (see also B.8.1 on fate and behaviour in soil). The very low amounts of residues due to the use of heptamaloxyloglucan (with no consideration of degradation, initial PEC_{soil} are comprised between 0.59 and 2.35 µg a.s./kg after 1 and 4 applications, respectively) is not expected to change the qualitative composition of the organic matter which reaches the soil or to cause damage on soil macro-organisms. Therefore the RMS agrees with the notifier that no testing on earthworm is needed.

B.9.7. EFFECTS ON OTHER SOIL NON-TARGET MACRO-ORGANISMS (ANNEX IIA 8.3.2; ANNEX IIIA 10.6.2)

As for earthworms, the low concentrations of the natural cell wall component heptamaloxyloglucan (maximum PEC_{soil} after 4 applications of 0.00235 mg a.s./kg, without consideration of degradation between applications) is not expected to be toxic to these organisms and to induce any risk to the population.

B.9.8. EFFECTS ON SOIL NON-TARGET MICRO-ORGANISMS (ANNEX IIA 8.5; ANNEX IIIA 10.7)

Heptamaloxyloglucan is a possible degradation product of plant cell walls as it can be produced from xyloglucan by enzymatic degradation naturally occurring in plant or in soil by micro-organsims. Plant decay is a natural substrate for soil micro-organism growth.

The initial PEC_{soil} of heptamaloxyloglucan were calculated after the 4^{th} application of PEL101GV (see B.8.3 for detail of calculations), and found to be 0.00235 mg/kg dry soil. Such low concentrations are not susceptible to change the qualitative composition of the organic matter which reaches the soil. Therefore heptamaloxyloglucan is not expected to have any adverse effects on the function of soil micro-organisms ecosystems.

No study has been conducted since heptamaloxyloglucan is a xyloglucan molecule extracted from apples. Furthermore heptamaloxyloglucan can be produced from xyloglucan by enzymatic degradation naturally occurring in plant. As such it could enter in the diet of soil non target micro-organisms via fall of leaves.

B.9.9. EFFECTS ON OTHER NON-TARGET ORGANISMS (FLORA) BELIEVED TO BE AT RISK (ANNEX IIA 8.6; ANNEX IIIA 10.8)

Notifier indicates that observations on adverse effects of heptamaloxyloglucan on other non-target organisms made during screening and developmental phase did not result in particular risks. Moreover exposure of plants to a possible degradation product of plant cell wall components (i.e. xyloglucan) by enzymes naturally occurring in plants, is not expected to have any detrimental effect on in-crop and off-crop plants, when applied according to the proposed GAP.

Nevertheless, considering that heptamaloxyloglucan is a plant growth regulator (elicitor) the notifier performed a study on side effect of heptamaloxyloglucan on the vegetative growth of terrestrial plants which is summarised below.

B.9.9.1. STUDY

Reference number:	II A 8.6/1 (interim report) and II A 8.6/2 (final report)
Report:	L'Haridon J (interim report), Servajean E. (final report) 2006 Semi-field assessment of the side-effect of a substance on the vegetative growth of terrestrial plants (non-GLP study) CIT unpublished report number 05-27-072-ES
Dates of works:	April 19 - May 5, 2006
GLP compliance	No
Guidelines	Not specified

Deviations	No
Acceptability	**Yes, despite no analytical check of applied rates nor positive control**

Methods:
Effects of EL101GV (technical heptamaloxyloglucan, batch AND0205, purity 87.1%) on vegetative vigour of 3 plant species (i.e. red cover, wheat and mustard) were followed during the study. The test was conducted in semi-field, protected from rain on a natural soil.
48 plots of 0.25m^2 were planted with 10 plants each. Treatment concentrations of 0.2, 2 and 20 g a.s./ha were applied in a randomised block with 4 plots (replicates) per concentration and per plant species (1 application at the 2-4 leaves stages). A negative control (water) was added. The plots will be individually sprayed using 10 mL of a 0.4, 4 and 40 mg/L solution, thus corresponding to a 500 L/ha application rate.
Phytotoxic effects will be observed twice per week for 2 weeks after the treatment applications. After 2 weeks the plants were collected and dried until constant weight. The number of collected plants per plot, total weight of the collected plants per plot and total dry weight of the collected plants per plots were recorded. Mean weight of the treated plots (wet weight and dry weight) was compared to that of the control plants for each concentration and each plant species. Temperature and humidity have been continuously recorded.

Results:
Temperature was ranged from 7.0 to 16.0°C during night and 22.0 to 39.5°C during the day.
No sign of phytotoxicity was recorded. There was no difference between the number of plants per plot in control and in the treatment groups. There were no statistically significant differences between control and treatment groups in term of dry and wet weight as shown in the table B.9.9.1-1.

Table B.9.9.1-1: Dry and wet biomass of wheat, mustard and red cover exposed to heptamaloxyloglucan

Treatment		Wet weight	Dry weight
		mean of 4 replicates (F-variance value)	
Wheat			
Water control		1.06	0.21
EL101GV	0.2 g/ha	1.12 (0.07)	0.22 (0.08)
EL101GV	2.0 g/ha	0.93 (0.34)	0.20 (0.23)
EL101GV	20.0 g/ha	1.26 (0.50)	0.24 (0.31)
Mustard			
Water control		2.26	0.32
EL101GV	0.2 g/ha	2.18 (0.05)	0.32 (0.00)
EL101GV	2.0 g/ha	2.47 (0.20)	0.34 (0.15)
EL101GV	20.0 g/ha	2.62 (0.43)	0.35 (0.26)
Red cover			
Water control		0.18	0.04
EL101GV	0.2 g/ha	0.17 (0.08)	0.04 (0.19)
EL101GV	2.0 g/ha	0.16 (0.83)	0.03 (0.45)
EL101GV	20.0 g/ha	0.20 (1.08)	0.04 (2.02)

F-variance value: result statistically significant (compared to the water control) at 5% confidence level if the F-variance value > 5.99

Conclusion:
Heptamaloxyloglucan (EL101GV) had no adverse effects on the vegetative vigour of wheat, mustard and red cover at 0.2, 2.0 and 20.0 g a.s./ha application rate.

B.9.9.2. RISK ASSESSMENT

A negligible risk is expected since there is no adverse affects observed following application of 20 g heptamaloxyloglucan/ha, i.e. 45-fold greater than the initial application rate of heptamaloxyloglucan.

B.9.10. EFFECTS ON BIOLOGICAL METHODS OF SEWAGE TREATMENT (ANNEX IIA 8.7)
Heptamaloxyloglucan is a xyloglucan molecule and made of 7 glucidic monomer units, which are all natural components of cell walls of the apple (from which it is extracted) and of other dicotyledonous plants. Moreover it could be produced by degradation of xyloglucan by enzymes naturally occurring in plant or soil micro-organisms (see B.8.1.2.3 and B.8.1.2.4). As such it is a classical part of sewage. Therefore it is not expected to have any detrimental effect on biological methods for sewage treatment. No study is therefore deemed necessary.

B.9.11. REFERENCES RELIED ON

Annex II and III Data and Information

Author(s)	Annex point/ reference number	Year	TITLE **Source** (where different from company) **Company name** **Report No.** **GLP status** **published or not**	Data protect. claimed	Owner
Buchanan B.B. et al	II A 8.1/1 II A 8.3.1/1 II A 8.4/1 II A 8.5/1	2000	Chapter 2: The Cell Wall Biochemistry and molecular Biology of Plants B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89 Not GLP, published	No	-
Stevens C.E., Hume I.D.	II A 8.1/2	1998	Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients Physiol.Rev., 1998 Apr, 78(2), pp 393-427 Not GLP, published	No	-
L'Haridon J	II A 8.2.1/1	2006a	EL101GV: Acute toxicity in the Rainbow trout under semi-static conditions CIT, BP 563, 27005 Evreux, France 30711 EAP GLP, unpublished	Yes	Elicityl
L'Haridon J.	II A 8.2.4/1	2006b	EL101GV: Acute toxicity in *Daphnia magna* under static conditions CIT, BP 563, 27005 Evreux, France 30710 EAD GLP, unpublished	Yes	Elicityl
L'Haridon J	II A 8.2.6/1	2006c	EL101GV: Algal growth inhibition test. CIT, BP 563, 27005 Evreux, France 30709 EAA GLP, unpublished	Yes	Elicityl
Servajean E.	II A 8.3.1.1/1	2006a	Laboratory determination of the contact and oral acute toxicity of a formulation to honey bees (*Apis mellifera*) (OECD 213 and OECD 214, september 1998) Phytosafe unpublished Report number 05-27-064-ES GLP, unpublished	Yes	Elicityl

Author(s)	**Annex point/ reference number**	**Year**	**TITLE** **Source** (where different from company) **Company name** **Report No.** **GLP status** **published or not**	**Data protect. claimed**	**Owner**
Winston M.L	II A 8.3.1/2	1987	The biology of the honey bee Harvard University Press, 1987, 281 pp Not GLP, published	No	-
Haydak M.H.	II A 8.3.1/3	1970	Honey bee nutrition Annu. Rev. Entomol., Vol. 15, pp. 143-156, 1970 Not GLP, published	No	-
Barker R.J.	II A 8.3.1/4 II A 8.3.1.2/1	1977	Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees J. Nutr., Vol. 107, pp. 1859-1862, 1977 Not GLP, published	No	-
Lattaud C. et al.	II A 8.4/4	1997	Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (megascoloecidae) Soil Biol. Biochem., Vol 29 (3/4), pp 335-339, 1997 Not GLP, published	No	-
Zhang B.G. et al.	II A 8.4/5	1993	Activity and origin of digestive enzymes in gut of tropical earthworm *Pontoscolex corethrurus* Eur. J. Soil Biol., Vol 29 (1), pp 7-11, 1993 Not GLP, published	No	-
Garvin M.H. and al.	II A 8.4/6	2000	Activity of glycolytic enzymes in the gut of *Hormogaster elisae* (Oligochaeta, Hormogastridae) Soil Biology & Biochemistry, Vol. 32, pp 929-934, 2000 Not GLP, published	No	-
L'Haridon J. (interim report) Servajean E.. (final report)	II A 8.6/1 (interim report) and II A 8.6/2 (final report)	2006	Semi-field assessment of the side-effect of a substance on the vegetative growth of terrestrial plants (non GLP study) PHYTOSAFE, 2 rue Marx Dormoy, 64000 PAU, France 05-27-072-ES Not GLP, unpublished	Yes	Elicityl

No Reference regarding the preparation PEL101GV.

European Commission

Programme for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Praper MEETING

heptamaloxyloglucan

Volume 2(Addendum 1)

May 2009

Rapporteur Member State: ***France***

Ministère de l'Agriculture et de la Pêche
Direction Générale de l'Alimentation
251, rue de Vaugirard
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Following the to praper meeting 66, this reference is added to the list of reference relied on.

METHODS OF ANALYSIS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
IIA, 1.8/02	Anonymous	1993	***Méthode Iso 8128-1 : Jus de pommes, concentrés de jus de pommes et boissons à base de jus de pommes -- Détermination de la teneur en patuline -- Partie 1: Méthode par chromatographie en phase liquide à haute performance***		

European Commission

Programme for inclusion of Active Substances in Annex I of Council Directive 91/414/EEC (Articles 5 and 6 of Council Directive 91/414/EEC)

Praper MEETING

heptamaloxyloglucan

Volume 4 Annex C (Addendum 1)

May 2009

Rapporteur Member State: ***France***
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