ANNEX B

Laminarin

B.8 Environmental fate and behaviour

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

No study has been conducted on the fate and behaviour of laminarin in soil (metabolism in soil, degradation rate, mobility in soil).

However, an extensive literature search has been performed by the notifier on

- the distribution of ∃-1,3 glucans in various plants :
Brown algae such as the *Laminaria* genus store carbohydrate as laminarans, a class of low-molecular weight ∃-1,3 glucans. The active substance is a polysaccharide of 25-30 glycosyl units. Laminaran are hydrolyzed by laminarase enzymes.
- ∃-1,3 glucans are also a major constituents of cell walls in yeast, filamentous fungi.
- Callose is a ∃-1,3 glucan which is found in a variety of situations in the tissues of higher plants : sieve tubes, young tracheids, lactifers, anthers and pollen mother cells, pollen grain and pollen tubes, root hairs, leaf and stem hairs, cystoliths, pericarp, abcission tissue, lenticels, root endodermis, pit canals and wound tissue.
- Lichenin, oat glucan and barley glucan are closely related and have a ∃-1,3 ∃-1,4 glucan structure. (review by Clarke *et al*, 1963)

- the occurrence of various types of glucanases and more specifically laminarases (also known as β-1,3-glucanases)
Various soil microorganisms (eubacteria, streptomycetes, fungi, algae, protozoa), soil macroorganisms (annelids, arthropods, molluscs) and higher plants possess β-1,3-glucanases. Laminarinase activity is the result of a multienzyme system (review by Clarke *et al*, 1963 ; review by Bull *et al*, 1966)

- the β-1,3-glucanase activity in soil
'The effect of various physical and chemical treatments on accumulated β-1,3-glucanase activity in soil was investigated in the laboratory. Enzyme activity was remarkably constant during long term storage of unamended soil irrespective of incubations conditions. The enzyme system was unaffected by flooding and NPK fertilizer, but was stimulated by pig-slurry and glucose and inhibited by ground limestone. (Lethbridge *et al*, 1980),
- the role of laminarase in the biological control of pathogenic fungi
'A number of bacteria of lysing *Fusarium oxysporum* were isolated from soil. One of these, a strain of Bacillus cereus was studied in detail. Lysis of *Pythium, Streptomyces, Agrobacterium* and *Pseudomonas* species appeared to be associated with chitinase and laminarase activity of B. cereus' (Mitchell, 1962)
- 'Soil treatment with either of 2 fungal cell-wall constituents, chitin or laminarin, resulted in decline in severity of diseases caused by several soil-borne fungal pathogens (Fusarium species). The evidence indicated that adding chitin or laminarin to soil stimulated a microbial flora capable of digesting mycelium of specific fungi' (Mitchell, 1963),

- the analytical determination of of β-1,3-glucanase activity in soil (Hayano, 1973) (Lethbridge *et al*, 1978)

The main conclusions which can be drawn from this literature survey is that :

- β-1,3- glucans are common plant polysaccharides
- β-1,3-glucanases do exist in soil
- β-1,3-glucanases are very common in bacteria, fungi, algae, higher plants, molluscs
- they are able to hydrolyze β-1,3-glucans like laminarin or callose
- The degradation of laminarin by soil micro-organisms would lead to smaller-sized oligosaccharides and monosaccharides (glucose). No other relevant metabolites degradation or reaction products is expected to appear.

- The DT50soil of laminarin cannot be determined easily. Nevertheless, soil persistence should not exceed a few weeks.

The evaluation of the risk to soil organisms will be based on initial PEC which would represent a worst case situation.

The estimations of the initial PECsoil were calculated assuming that :

- 1 application of 37 g a.s./ha/season is performed
- Equal distribution in the top 5 cm of the soil with a bulk density of 1.5 g/cm^3
- The substance is applied on cereals between full tillering and the 1 cm ear stage (BBCH 29-30) : 50% of the spray reaching the soil)

Table B.8.1-1 : PEC soil

	Cereals 37 g a.s./ha 1 application, 50% of applied dose reaching the soil
Day 0	0.025 mg a.s./kg soil

PECgw
Laminarin and its metabolites don't pose a risk to groundwater. PECgw are not required.

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2)

B.8.4.1 Hydrolysis rate of relevant metabolites, degradation and reaction products (Annex IIA 7.2.1.1)

Laminarin is hydrolytically stable in sterile water at pH 4, 7 and 9.

B.8.4.2 Direct phototransformation of relevant metabolites, degradation and reactions products in water (Annex IIA 7.2.1.2)

Laminarin is photostable.

B.8.4.3 Ready biodegradability of the active substance (Annex IIA 7.2.1.3.1)

Ready biodegradability – Modified Sturm test (Licata-Messana, 2000)
Guidelines :
OECD 301B (1992)
GLP :
yes
Material and Methods :
Test material : laminarin, purity on dry matter 88.9%
Test conditions : 37.49 mg a.s./l has been incubated in a water medium inoculated with activated sewage micro-organisms at 20.3-28.6°C, pH 7.31-7.49. The biodegradation was monitored over a 28-day period by means of CO_2 analysis, CO_2 being released and incorporated to $BaCO_3$. Control assay without any test substance and assay using sodium acetate as reference substance were run in simultaneously.

Findings :
- Biodegradation of laminarin :

46 % at day 7
56% at day 9
76 % at day 28

- The biodegradation of the reference substance was 50% at day 7, 60% at day 9, 73% at day 28.

Conclusions :
Laminarin is readily biodegradable in the conditions of the test.

B.8.4.4 Water/sediment study (Annex IIA 7.2.1.3.2)

Not required :
- The a.s. is readily biodegradable (DT_{50} a.s. | CO_2 ~ 8 days)
- The degradation of laminarin by micro-organisms would lead to smaller-sized oligosaccharides and glucose.

B.8.4.5 Degradation in the saturated zone of active substance, metabolites, degradation and reaction products (Annex IIA 7.2.1.4)

Not required

B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

The a.s. is readily biodegradable ; it would not endanger water treatment procedures.

B.8.6 Summary of behaviour in water and predicted environmental concentrations in surface water and in ground water (PECsw, PECgw) (Annex IIIA 9.21, 9.2.3)

Laminarin is hydrolytically stable in sterile water at pH 4, 7 and 9.
Laminarin is photostable.

Laminarin is readily biodegradable (56-64% biodegradation at day 9-12 ; 76% biodegradation at day 28)

The degradation of laminarin which is a polysaccharide would lead to smaller-sized oligosaccharides and glucose. No other relevant metabolites degradation or reaction products is expected to appear.

The estimations of the initial PECsw were calculated assuming that :

- 1 application of 37 g a.s./ha/season is performed
- Spray drift scenarios according to 'Guidance document 8075/VI/97, rev 8'
- The waterbody is 30 cm deep
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Table B.8.6-1 : PEC surface water

	Cereals 37 g a.s./ha 1 application, 1 m drift 2.77% of applied dose reaching the waterbody	Cereals 37 g a.s./ha 1 application, 5 m drift 0.57% of applied dose reaching the waterbody
Day 0	0.342 µg a.s./l	0.070 µg a.s./l

Chronic PEC were not required because the a.s. is applied once per season ; the a.s. is readily biodegradable (DT_{50} a.s. | CO_2 ~ 8 days)

B.8.7 Fate and behaviour in air (Annex IIA, 7.2.2; Annex IIIA 9.3)

Laminarin having a very low vapour pressure (<2.6 10^{-5} Pa at 25°C) and a very low Henry's law constant (< 1.5 10-6 $Pa.m^3.mol^{-1}$), no risk of volatilisation is to be expected in the recommended conditions of use.

B.8.9 Definition of the residue (Annex IIA 7.3)

The notifier proposes this residue definition : 'Laminarin being a polysacharide, its decomposition in the environment will give rise to oligosaccharides and to glucose. The most significant residue in soil would therfore be glucose itself, what is in fact the most accurate way to quantify laminarin after total acid hydrolysis.'

The rapporteur considers that :
- The natural background level of mono-, di- or polysaccharides in soil and water is expected to be high and variable.
- Laminarin and its mono-, di- or polysaccharides metabolites have no (eco)toxicological significance.

Therefore, the establishment of residue definition in soil, water and air is not required.

B.8.10 References relied on

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