# *European Commission*

**Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009**

# Heptamaloxyloglucan

## Volume 3 – B.9 (PPP) – PEL101GV

Rapporteur Member State: France
Co-Rapporteur Member State: Spain

# Version History

| When | What |
|---|---|
| 2020-09 | Initial RAR |
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The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

# Table of contents

# B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

The representative formulation (PEL101GV) is equivalent to the technical active substance heptamaloxyloglucan (EL101GV). (Please refer to Volume 3 CP B.1.1 and Volume 1 point 1.4.4.1.)

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).
All these hexose and hexol residues are natural components of the apple and of other dicotyledone plants, where they are major constituents of cellulose and hemicellulose molecules, which are the principal components of cell walls.
For more details on the active substance, please refer to Vol 3 CA B.9.

PEL101GV is a lyophilisat water-soluble formulation used as an anti-freezing in grapevine. The proposed use for heptamaloxyloglucan is post-emergence applications of 0.56 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).

**Table 9.1**: **Table of critical GAP**

| Crop | Maximum number of applications | Interval between applications (days) | Application rate (mg a.s./ha/application) | Growth stage |
|---|---|---|---|---|
| Vine | 4 | 4 | 0.69 - 560 | BBCH 07-16 |

## B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

### B.9.1.1. Effects on birds

No avian toxicity test has been performed with the product PEL101GV.

### B.9.1.2. Effects on terrestrial vertebrates other than birds

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

As the product PEL101GV is identical to the technical active substance EL101GV, no additional test on mammals was necessary.

## B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

### B.9.2.1. Risk assessment for birds

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

#### *B.9.2.1.1. Acute dietary risk assessment*

Risk assessment for birds according to the guidance given in the EFSA Journal (2009, 7(12):1438) was based on a maximum single application rate, default $DT_{50}$ values and the following data:

- Grapevine: a maximum application rate of 124 mg product/mL, i.e. 0.56 g a.s./ha, applied up to 4 times with a minimum interval of 4 days

**Table 9.2.1.1-1: Acute daily dietary dose of birds to heptamaloxyloglucan**

| Crop use pattern | Scenario | Indicator species | Shortcut value (SV) for acute assessment | Application rate (kg a.s./ha) | $MAF^{90}$ | Daily dietary dose (DDD) (mg/kg b.w.) |
|---|---|---|---|---|---|---|
| Grapes | Vineyard | Small omnivorous bird | 95.3 | 0.000560 | 2.1* | 0.11 |

* MAF for 4 applications with a minimum interval of 4 days

Since no toxicity data are available for birds, the applicant proposed to assess the margin of safety existing between mammalian toxicity data and the estimated theoretical exposure of birds in vines following applications of heptamaloxyloglucan in early spring.

RMS is not in favor of using mammalian data to assess the risk for avian species. However, considering the acute theoretical DDD of 0.11 mg/kg bw, the $LD_{50}$ should be lower than 1.1 mg/kg bw to reach an unacceptable acute risk (trigger = 10). Given the nature of the active substance that is extracted from apple pomace, this is considered unlikely. Therefore, RMS considered that no unacceptable acute risk of heptamaloxyloglucan to birds can be concluded.

Furthermore, three publications were found in literature data which characterize the effects of xylo-oligosaccharides on the growth performance and immune function of broiler chickens (Morgan *and al*. 2018; Suo Hai-qing and *al*, 2015 and Yuan *and al*, 2018. These studies showed a beneficial effect on growth and immune performance of broiler chickens when they are supplemented in diet with xylo-oligosaccharides. Summaries of these publications are presented in RAR Vol3. B9.1.1.

Overall, RMS considered that no acute risk on birds is expected following applications of PEL101GV on vine.

### *B.9.2.1.2. Long-term dietary risk assessment*

No toxicity data on reproduction effects of heptamaloxyloglucan is available.

DDD calculation is presented in the table below

**Table 9.2.1.1-1: Acute daily dietary dose of birds to heptamaloxyloglucan**

| Crop use pattern | Scenario | Indicator species | Shortcut value (SV) for acute assessment | Application rate (kg a.s./ha) | $MAF_m$ | Daily dietary dose (DDD) (mg/kg b.w.) |
|---|---|---|---|---|---|---|
| Grapes | Vineyard | Small omnivorous bird | 38.9 | 0.000560 | 2.8* | 0.06 |

* MAF for 4 applications with a minimum interval of 4 days

Heptamaloxyloglucan (extracted from apple) is a natural component of dicotyledonous leaves and vegetal parts, As such, heptamaloxyloglucan could be considered as a natural component of bird diet. In addition, it has been demonstrated that polyxyloglucan had beneficial effect on growth of broilers as shown in the publications (Morgan *and al*. 2018 [CA 8.1/04]; Suo Hai-qing and *al*, 2015 [CA 8.1/05] and Yuan *and al*, 2018 [CA 8.1/06] See Volume 3 CA B.9, point B.9.1.1 for details).

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 in soil and water/sediment system (see section B.8).

Overall, chronic risk of birds to heptamaloxylglucan is not expected due to the nature of the compound and the expected low level in the environment following use of PEL101GV.

Overall, RMS considered that no long term dietary risk on birds is expected following applications of PEL101GV on vine.

### *B.9.2.1.3. Risk for birds through drinking water*

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

***Leaf scenario***
The 'Leaf scenario' is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since the proposed use of PEL101GV is for application to vineyard the leaf scenario does not apply.

***Puddle scenario***
This is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for all uses of PEL101GV and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:
"*Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50, in the case of less sorptive substances ($K_{OC}$ <500 L/kg), or 3000 in the case of more sorptive substances ($K_{OC}$ ≥500 L/kg).*"

When multiple spray applications are considered, a $MAF_m$ based on the $DT_{50}$ in soil may be applied to calculate the effective application rate $AR_{eff.}$

$AR_{eff} = AR \times MAF_m$

With: $MAF_m = 2.8$ (maximum of 4 applications of the product with a minimum interval of 4 days according to the GAP)

Then $AR_{eff}$ of heptamaloxyloglucan = 0.560 * 2.8 = 1.57 g a.s./ha.

The Koc of heptamaloxyloglucan is considered to be equal to 0. The trigger for the ratio of effective application rate to acute and long-term endpoints is less than 50.

Since no toxicity data are available for birds, the notifier proposed to assess the margin of safety existing between mammalian toxicity data and the estimated theoretical effective application rate of birds in vines following applications of heptamaloxyloglucan in early spring.
RMS is not in favor of using mammalian data to assess the risk for avian species. However, considering the acute theoretical AReff of 1.57 g a.s./ha, the $LD_{50}$ should be lower than 0.031 mg/kg bw to meet the trigger for performing risk assessment for less sorptive substance. Given the nature of the active substance, this is considered unlikely. Therefore, RMS considered that risk of heptamaloxyloglucan to birds through drinking water from puddle is not necessary.

### *B.9.2.1.4. Risk assessment for secondary poisoning*

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log $P_{OW}$ greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. The Log $P_{ow}$ of heptamaloxyloglucan (<0) is below the limit of 3 (see Vol. 3CA B2, point B.2.7). A risk assessment for secondary poisoning is not required and the risk of food chain bioaccumulation to fish-eating and worm-eating birds is negligible.

### *B.9.2.1.5. Biomagnification in terrestrial food chains*

The results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for heptamaloxyloglucan accumulation (Volume 3 CA-B.6.1).

#### *B.9.2.1.6. Overall conclusion on effect on birds*

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

No toxicity data are available on effects of heptamaloxyloglucan on birds. Heptamaloxyloglucan (extracted from apple) is a natural component of dicotyledone leaves and vegetal parts, As such, heptamaloxyloglucan could be considered as a natural component of bird diet. Literature data show that xylo-oligosaccharide, arabinoxylo-oligosaccharides and arabinoxylans which belong to the carbohydrates family and are compounds close to heptamaloxyloglucan are not toxic to broilers via the diet.

Moreover in the 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 in soil and water/sediment system (see section B.8.

Acute risk of heptamaloxyloglucan to birds following applications of PEL101GV on vines is not expected . Indeed, in view of the acute exposure expected after applications of PEL101GV on vines, the acute LD50 leading to an unacceptable risk should be lower than 1.1 mg/kg bw. This is considered unlikely for such compound. Therefore, no unacceptable acute risk is expected for birds following appliaction of PEL101GV on vines.
Chronic dietary risk of birds to heptamaloxylglucan is not expected due to the nature of the compound and the expected low level in the environment following use of PEL101GV.

No unacceptable acute risk assessment through diet and drinking consumption for birds is considered for the intended uses of heptamaloxyloglucan. No chronic risk is anticipated due to the nature of the compound and the expected low level in the environment following use of PEL101GV.

A secondary poisoning risk assessment for earthworm- and fish-eating birds is not required (Log $P_{ow}$ heptamaloxyloglucan <0, see Vol. 3CA B2, point B.2.7). The risks for earthworm- and fish-eating birds are not required.

No unacceptable risk on birds is expected following applications of PEL101GV on vineyards.

### B.9.2.2. Risk assessment for mammals

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

#### *B.9.2.2.1. Acute dietary risk assessment*

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 9.2.2.1-1.

**Table 9.2.2.1-1: Endpoints for the risk assessment for mammals**

| **Species** | **Product** | **Exposure System** | **Results** | **Reference** |
|---|---|---|---|---|
| Rat | EL101GV (Heptamaloxyloglucan) | Oral 14 d Acute | $LD_{50}$ > 5000 mg/kg bw | CA 5.5/01 ****** (2004), 20030812ST |
| Rat | EL101GV (Heptamaloxyloglucan) | Oral Short-term toxicity | NOAEL = 1000 mg/kg bw/d | CA 5.3.1/01 ******. (2006), 20060118TRB * |

* This study is performed according to OECD Test 407– Repeated dose 28-day oral toxicity in rodents. It is part of the dataset to be considered when setting the NOAEL for reproductive risk assessment in the EFSA guidance for risk assessment on birds and mammals (2009).

Acute toxicity exposure ratios ($TER_A$) for heptamaloxyloglucan following applications of PEL101GV are calculated in table below.

**Table 9.2.2.1-2: Screening step: Acute risk ($TER_A$) of Heptamaloxyloglucan to mammals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | | Vines | | | |
| **Active substance** | | Heptamaloxyloglucan | | | |
| **Application rate** | | 4 × 0.000560 kg of EL101GV/ha | | | |
| **Acute toxicity (mg a.s./kg bw)** | | > 5000 | | | |
| **TER criterion** | | 10 | | | |
| **Scenario** | **Indicator species** | **Shortcut value ($SV_{90}$)** | **$MAF_{90}$** | **Daily dietary dose (DDD) (mg/kg b.w.)** | **$TER_A$** |
| Vineyard | Small herbivorous mammal | 136.4 | 2.1 | 0.16 | > 31 170.8 |

The $TER_A$ value for heptamaloxyloglucan is greater than the trigger value of 10, indicating that application of PEL101GV is considered to pose no unacceptable acute risk to mammals when applied according to Good Agricultural Practice on vineyard.

### *B.9.2.2.2. Long-term dietary risk assessment*

**Table 9.2.2.2-1: Endpoints for the long-term risk assessment for mammals**

| **Species** | **Compound** | **Exposure System** | **Results** | **Reference** |
|---|---|---|---|---|
| Rat | Heptamaloxyloglucan | Oral Short-term toxicity | NOAEL = 1000 mg/kg bw/d | CA 5.3.1/01 *******. (2006), 20060118TRB |

A short-term study was done on rats (development study) for 28 days, which leads to a NOAEL = 1000 mg a.s./kg b.w./d. This study was performed according to OECD Test 407– Repeated dose 28-day oral toxicity in rodents. It is part of the dataset to be considered when setting the NOAEL for reproductive risk assessment. As this data is the only one available that allow assessment of chronic effects, it was used for chronic risk assessment.

**Table 9.2.2.2-2: Screening step assessment for the long-term risk of heptamaloxyloglucan to mammals**

| | | | | | |
|---|---|---|---|---|---|
| **Intended use** | | Vines | | | |
| **Active substance/product** | | heptamaloxyloglucan | | | |
| **Application rate** | | 4 × 0.000560 kg of heptamaloxyloglucan /ha | | | |
| **Reproductive toxicity (mg/kg bw/d)** | | NOAEL = 1000 (based on short term study performed according to OECD 407) | | | |
| **TER criterion** | | 5 | | | |
| **Scenario** | **Indicator species** | **Shortcut value (SV) for assessment** | **$MAF_m$** | **Daily dietary dose (DDD) (mg/kg b.w.)** | **TER** |

| | | | | | |
|---|---|---|---|---|---|
| Vineyard | Small herbivorous mammal | 72.3 | 2.8 | 0.11 | 8821 |

The $TER_{LT}$ value for heptamaloxyloglucan is greater than the trigger value of 5, indicating that application of PEL101GV poses no unacceptable long-term risk to mammals when applied according to Good Agricultural Practice on vineyard.

### *B.9.2.2.3. Risk for mammals through drinking water*

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

***Leaf scenario***
The 'Leaf scenario' is relevant for mammals taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract mammals. Since the proposed use of PEL101GV is for application to vineyard the leaf scenario does not apply.

***Puddle scenario***
This is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is therefore relevant for all uses of PEL101GV and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:
"*Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50, in the case of less sorptive substances ($K_{OC}$ <500 L/kg), or 3000 in the case of more sorptive substances ($K_{OC}$ ≥500 L/kg).*"

When multiple spray applications are considered, a $MAF_m$ based on the $DT_{50}$ in soil may be applied to calculate the effective application rate $AR_{eff.}$

$AR_{eff} = AR \times MAF_m$

With: $MAF_m = 2.8$ (maximum of 4 applications of the product with a minimum interval of 4 days according to the GAP)

Then $AR_{eff}$ of PEL101GV = 0.560 * 2.8 = 1.568 g a.s./ha.

The Koc of heptamaloxyloglucan is equal to 0. The trigger for the ratio of effective application rate to acute and long term endpoints is less than 50.

**Table 9.2.2.3-1 Ratio of AReff to acute/short-term toxicity endpoint – heptamaloxyloglucan**

| **$AR_{eff}$ (g a.s./ha)** | **Acute $LD_{50}$ (mammals) (mg /kg b.w.)** | **$Ratio_{acute}$** | **Short-term NOED (mammals) (mg /kg b.w./d)** | **$Ratio_{short\text{-}term}$** |
|---|---|---|---|---|
| 1.568 | > 5000 | 0.00031 | 1000 | 0.0016 |

The acute and reproductive ratio are below the trigger value. Therefore, the acute and reproductive risk to mammals via drinking water, following application of heptamaloxyloglucan according to the proposed use pattern, is not triggered.

### *B.9.2.2.4. Risk assessment for secondary poisoning*

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log $P_{OW}$ greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. The Log $P_{ow}$ of heptamaloxyloglucan (<0) is below the limit of 3 (see Vol. 3CA B2, point B.2.7). A risk assessment for secondary poisoning is not required and the risk of food chain bioaccumulation to fish-eating and worm-eating mammals is negligible.

#### *B.9.2.2.5. Biomagnification in terrestrial food chains*

The results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for heptamaloxyloglucan accumulation (Volume 3 CA-B.6.1).

#### *B.9.2.2.6. Overall conclusion on effect on mammals*

The risk assessment was carried out according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Studies performed on mammals to investigate the acute and long-term toxicity of heptamaloxyloglucan show a low toxicity of the active substance on mammals. Based on TER calculation, no unacceptable acute and long-term risk is expected for wild mammals exposed to heptamaloxylogucan after applications of PEL101GV on vineyard.

As regards potential exposure via drinking water, only the puddle scenario is relevant for the proposed uses. Since the ratios of the effective application rates to the relevant toxicity endpoints are below the relevant threshold value, it is not necessary to conduct a quantitative risk assessment for the proposed use pattern of PEL101GV. In conclusion, the proposed use pattern of PEL101GV does not pose an unacceptable risk to mammals via uptake of contaminated drinking water.

A secondary poisoning risk assessment for earthworm- and fish-eating mammals is not required (Log $P_{ow}$ Heptamaloxyloglucan < 0). The risks for earthworm- and fish-eating mammals are not required.

### B.9.3. EFFECTS ON AQUATIC ORGANISMS

The risk assessment is based on the current guidance: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

Acute toxicity studies have been performed with the active substance heptamaloxyloglucan on rainbow trout, daphnia and one algae species. 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 the formulation is identical to the technical active substance heptamaloxyloglucan (EL101GV).

**Table 9.3-1: Toxicity data for aquatic organisms exposed to heptamaloxyloglucan**

| Test substance | Design<br>Test species | Endpoint<br>(mg heptamaloxyloglucan/L) | Reference |
|---|---|---|---|
| Heptamaloxyloglucan | Fish acute<br>*Oncorhynchus mykiss* | 96-h $LC_{50}$ > 150 | CA 8.2.1/01<br>********. 2006a<br>Report N°. 30711 EAP |
| Heptamaloxyloglucan | Invertebrate, acute<br>*Daphnia magna* | 48-h $EC_{50}$ > 150 | CA 8.2.4/01<br>L'Haridon J. 2006b<br>Report N°. 30710 EAD |
| Heptamaloxyloglucan | Algae, growth inhibition<br>*Scenedesmus subspicatus* | 72-h $E_rC_{50}$ > 150<br>72-h $E_bC_{50}$ > 150 | CA 8.2.6/01<br>L'Haridon J., 2006c<br>Report N°. 30709 EAA |

PECsw calculations are detailed in Volume 3-B.8.(CP), point B.8.5. Step 1 PECsw values used in PEC/RAC ratio calculation are reported in the table below.

**Table 9.3-2: PECsw calculated for vines early with FOCUS Step 1**

| **Scenario FOCUS** | **Waterbody** | **Max $PEC_{sw}$ (µg heptamaloxyloglucan/L)** | **Max $PEC_{sed}$ (µg heptamaloxyloglucan/kg)** |
|---|---|---|---|
| Step 1 | --- | 0.77 | 5.35 |

### B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

No additional data necessary as the formulation PEL101GV is identical to the technical active substance heptamaloxyloglucan coded EL101GV.

### B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No data.
Not required.

### B.9.3.3. Further testing on aquatic organisms

No data.
Not required.

## B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

### B.9.4.1. Risk assessment for the parent compound, heptamaloxyloglucan

The TER values for heptamaloxyloglucan is reported in Table 9.4.1-1.

**Table 9.4.1-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for heptamaloxyloglucan for each organism group based on FOCUS Steps 1 calculations for the use of PEL101GV in vines (early)**

| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
|---|---|---|---|---|
| **Test species** | | ***Oncorhynchus mykiss*** | ***Daphnia magna*** | ***Scenedesmus subspicatus*** |
| **Endpoint (µg/L)** | | $LC_{50}$ > 150 000 | $EC_{50}$ > 150 000 | $E_rC_{50}$ > 150 000 |
| **AF** | | 100 | 100 | 10 |
| **RAC (µg/L)** | | > 1 500 | > 1 500 | > 15 000 |
| **FOCUS Scenario** | **PEC $_{gl\text{-}max}$ (µg/L)** | | | |
| **Step 1** | | | | |
| | 0.77 | < 0.000513 | < 0.000513 | < 0.0000513 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

### B.9.4.2. 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 2.24 g a.s./ha (equivalent to no degradation of heptamaloxyloglucan after 4 applications of 0.560 g as/ha) show that no unacceptable risk on aquatic organisms are expected following applications of PEL101GV.

## B.9.5. EFFECTS ON ARTHROPODS

### B.9.5.1. Effects on bees

Acute oral and contact toxicity tests are available with technical active substance heptamaloxyloglucan coded EL101GV which is identical to the formulation PEL101GV.

No chronic test on adult and larvae bees are available.

The notifier argued that chronic toxicity tests are not required since heptamaloxyloglucan is a sugar present in plants; it is a natural component of bee food. It is not acutely toxic to bees as shown with the acute oral and contact toxicity study with the active substance. Furthermore, the product PEL101GV is to be applied very early in seasons to protect vineyard against frost. The notifier considered that at this time of the year bees are not in foraging activity on vines. Thus no contact with the product PEL101GV is expected.

RMS did not agree that bees will not be exposed to the active substance/product as application is intended at BBCH 7-16 which may correspond to applications in April-May in certain EU regions, including France. RMS however agreed that exposure of bees (adults, and larvae) to xyloglucan, polysaccharides, or monosaccharides occurred naturally (see below in paragraph related to exposure under vol 3 CP B.9.6.1). Being derived from apple, the composition of heptamaloxyloglucan is a combination of sugars monomers that occurred naturally in plants, nectar/pollen or honey. As such bees are naturally exposed to xyloglucans, including heptamaloxyloglucan. Thus, no chronic toxicity tests are considered necessary in this particular case.
Furthermore, from the literature studies provided under Vol 3 CA B.9.3.1.2, galactose, which is one of the monomers of heptamaloxyloglucan was found to be toxic after 16 days of oral administration. Thus, a risk assessment is proposed for this monomer (see below under B.9.6.1)

### B.9.5.2. Effects on non-target arthropods other than bees

No tests are available with technical active substance heptamaloxyloglucan coded EL101GV which is identical to the formulation PEL101GV.

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 were deemed necessary during the initial EU review of heptamaloxyloglucan.

Considering the type of component (xyloglucan extracted from apple), its mode of action (plant elicitor to protect vine from freezing), its natural occurrence in plants, the low dose applied (0.560 g/ha), RMS is still of the opinion that testing on non target arthropods is not required in this particular case.

## B.9.6. RISK ASSESSMENT FOR ARTHROPODS

### B.9.6.1. Risk assessment for bees

**Toxicity**

**Table 9.6.1-1: Toxicity to bees**

| Species | Test item | Exposure System | Endpoint | Reference |
|---|---|---|---|---|
| *Apis mellifera* L. (honey bee, adult) | Technical heptamaloxyloglucan (EL101GV) | Acute, 48h contact | $LD_{50}$ > 100 µg a.s./bee | CA 8.3.1.1/01 Servajean E. *et al.*, 2006 |
| | | Acute, 48h oral | $LD_{50}$ > 100 µg a.s./bee | |
| | Galactose | Chronic, feeding | LDD50 > 1620 µg/bee/d | CA 8.3.1/03 Barker R.J. *et al.*, 1977 |

**Exposure**

According to GAP, PEL101GV is applied up to 4 times at a maximal rate of 0.56 g a.s./ha in vine. This was used for a first-tier, in-field exposure assessment.

**Argumentation on natural exposure of bees proposed by the notifier**

Several publications have been provided in Vol 3 CA B.9 that allow to consider that xyloglucans among which heptamaloxyloglucan can be found in the environment and can be used and assimilated by bees.

Heptamaloxyloglucan is part of the oligosaccharides within the carbohydrates family. It is composed by different molecules: xylose, glucose, fucose, galactose and glucitol (= sorbitol). “Sugars” are known to be attractive for bees and publications were found to demonstrate the importance of sugars in honey and nectar, which are used by bees for nutrition (Smessaert, 2019 [CA 8.3.1/04]; Pavlova, 2018 [CA 8.3.1/05]; Machado De-Melo, 2017 [CA 8.3.1/06] and Dmitruk 2019 [CA 8.3.1/08]).
Two reviews from Pavlova *et al*. (2018, CA 8.3.1/05) and from Machado De-Melo *et al*. (2017, CA 8.3.1/06) show that honey dry matter is mainly composed of fructose and glucose, which accounts for 95–99% and other polysaccharides and monosaccharides (4-5%).
A comparison of sugar compounds present in honey and in heptamaloxyloglucan has been done by the applicant. The table is presented in introduction of Volume 3 CA B.9. The monomeric sugars forming heptamaloxyloglucan are very close to monomeric sugars found in honey and nectar and illustrates that honey bees are naturally in contact with various structure of sugar compounds.

The applicant submitted several publications to show that honey bees are naturally exposed to monomeric sugars that composed heptamaloxyloglucan.
Dmitruk investigated in 2019 the flowering and nectar release in *Acer pseudoplatanus* between 2011 and 2013 (CA 8.3.1/08).
Somme *and al.* (2016) (CA 8.3.1/09) investigated the chemical composition of pollen and nectar as well as the amount of nectar produced by the nine major insect-pollinated tree species planted in cities of Western Europe. Sugar concentration in nectar varied between approximately 20% for *Aesculus* species to 66% for *Robinia pseudoacacia*. In consequence, sugar content per flower ranged from 0.16–0.28 mg in *Aesculus* species to 1.28 mg in *Robinia pseudiacacia*.
Tomcyk *and al.* (2019 (CA 8.3.1/10) compared 60 varietal honey samples including 10 multifloral, 5 tilia, 5 rape, 5 acacia and 5 forest per each country were collected in 2015 directly from local beekeepers operating in South-Eastern part of Poland and North-Eastern part of Slovakia, respectively. The sugar profile of tested honey samples from both countries was similar ($P$ > 0.05). The fructose content could exceed that of glucose.

Smessaert and al. (2019) (CA 8.3.1/04) monitored the pollinator activity in an apple and pear orchard after introducing managed bumblebee hives in relation with nectar composition. Given the attractiveness of the nectar of apple’s flowers, and the fact that hepatamaloxyloglucan is extracted from apple, natural exposure of honeybees and bumblebees are expected to occur.

Lee *and al.*, 2014 (CA 8.3.1/07) investigated the potential for the microbiota to digest macromolecules, take up sugars and ferment carbohydrates. This publication confirms that the honey bee microbiome contains bacteria available to digest different sugars such as xylose, glucose, sorbitol and sucrose, which are presents in heptamaloxyloglucan. Indeed, sucrose is formed by glucose and fructose. Therefore, bees are already exposed in the nature to sugars contained in heptamaloxyloglucan.

Among the hexose constituting Heptamaloxyloglucan, galactose, was found to induce lethal effects, when bees are fed for 16 days with this monomeric sugar, with a 16d-LDD50 value of >6% in the syrup, corresponding to a dose level of 1.62 mg/bee/day, when taking into account the daily syrup consumption of 27 mg/bee/day (Barker R.J, 1977, CA 8.3.1/03). In honey, the mean galactose concentration was 0.0086%, with a spread of values from 0.0052 to 0.0151% (Val *and al.*, 1998 [CA 8.3.1/11]).

**Risk assessment**

**Acute risk assessment based on SANCO/10329/2002 guidance document**

The Table 9.6.1-2 presents the acute oral and contact hazard quotient calculation for honey bees.

**Table 9.6.1-2: Acute hazard quotients for honey bees according to SANCO/10329/2002**

| Test substance | Exposure route | Endpoint (µg a.s./bee) | Maximum single application rate (g a.s./ha) | Hazard quotient (HQ) | HQ assessment trigger |
|---|---|---|---|---|---|
| Heptamaloxyloglucan | Oral | $LD_{50}$ > 100 | 0.560 | < 0.0056 | 50 |
| | Contact | $LD_{50}$ > 100 | 0.560 | < 0.0056 | 50 |

For heptamaloxyloglucan, the oral and contact hazard quotient values are below the trigger value of 50 for acute oral and contact exposure indicating an acceptable acute risk to bees following application of PEL101GV in vineyard.

Based on this assessment, no unacceptable acute risk to honeybees following application of PEL101GV in accordance with the proposed GAP in vineyard is expected.

**Acute risk assessment based on EFSA 2013 guidance document**

**Screening acute oral risk assessment for honeybees:**
According to the Guidance Document on risk assessment of bees from EFSA, the acute oral risk assessment for adult bees is based on the ETRacute adult oral using the following equation:

ETRacute adult oral = AR * SV/$LD_{50}$ oral

Where:
AR = application rate in kg/ha
SV = 10.6 for an application made *via* an upwards or sideward spray (case for grapes)
$LD_{50oral}$ is expressed in µg a.s./bee.

**Table 9.6.1-3 Screening acute oral risk assessment for honey bees**

| Test substance | Exposure route | Endpoint (µg a.s./bee) | Maximum single application rate (kg a.s./ha) | SV | ETR acute adult oral | Trigger |
|---|---|---|---|---|---|---|
| Heptamaloxyloglucan | Oral | $LD_{50}$ > 100 | 0.00056 | 10.6 | < 0.00006 | 0.2 |

The ETRacute adult oral value is below the trigger of 0.2 indicating an acceptable acute oral risk for bees after applications of PEL101GV in vineyard.

**Screening acute contact risk assessment for honeybees:**
According to the Guidance Document on risk assessment of bees from EFSA, the acute contact risk assessment for adult bees is based on the hazard quotient (HQ) contact (HQcontact) using the following equation:

$$HQcontact = AR/LD_{50}\ contact$$

Where:
AR = application rate in g a.s./ha
$LD_{50}$ contact is expressed in µg a.s./bee

**Table 9.6.1-4: Screening acute contact risk assessment for honey bees**

| Test substance | Exposure route | Endpoint (µg a.s./bee) | Maximum single application rate (g a.s./ha) | Hazard quotient (HQ) | HQ assessment trigger |
|---|---|---|---|---|---|
| Heptamaloxyloglucan | Contact | $LD_{50}$ > 100 | 0.560 | < 0.0056 | 42 |

The HQ contact for Heptamaloxyloglucan is below the trigger values of 42 (for upwards and/or sideward spray), indicating an acceptable acute contact risk for adult bees exposed to heptamaloxyloglucan when applied as intended in vineyard.

**Chronic risk assessment**

Heptamaloxyloglucan is a natural sugar, a component of bee food. The acute oral and contact toxicity studies showed a low toxicity of the active substance to bees. The acute risk assessment indicated an acceptable risk following to the application of the product PEL101GV.
As sugars are attractive for bees and as heptamaloxyloglucan contains different forms of sugars, a risk assessment is performed in case of bees could be exposed to heptamaloxyloglucan when foraging on flowering weeds in the field and edge of the field.

**Risk assessment for adult honeybees**

Screening chronic oral risk assessment for adult honeybees:
According to the Guidance Document on risk assessment of bees from EFSA, the chronic risk assessment for adult bees is based on the ETRchronic adult oral using the following equation:

$$ETRchronic\ adult\ oral = AR * SV/LDD_{50}\ oral$$

Where:
AR = application rate in kg/ha
SV = 10.6 for an application made *via* an upwards and/or sideward spray (for vineyards)
$LDD_{50oral}$ is expressed in µg a.s./bee per day

The notifier proposed the following calculation to determine the critical $LDD_{50}$ oral to have an acceptable risk.

**Table 9.6.1-5 Screening chronic risk assessment for honey bees (adults)**

| Test substance | Exposure route | Maximum single application rate (kg a.s./ha) | Type of spraying | SV | Trigger ETR chronic adult oral | Endpoint (µg a.s./bee/d) |
|---|---|---|---|---|---|---|
| Heptamaloxyloglucan | Oral | 0.00056 | upwards and/or sideward | 10.6 | ≤ 0.03 | Oral $LDD_{50}$ ≥ 0.198 µg a.s./bee/d |

To obtain an acceptable risk for adult bees when exposed in chronic to heptamaloxyloglucan, the $LDD_{50}$ oral should be ≥ 0.198 µg a.s./bee/d which seems realistic for such compound.

The notifier indicated that, according to the Guideline OECD 245 which is followed for the chronic toxicity studies on adults, all bees are fed with a 50% w/v aqueous sucrose solution *ad libitum*. Sucrose is formed by glucose and fructose molecules. Therefore, bees are exposed to glucose, one of sugars constituting of heptamaloxyloglucan in the standard chronic tests. The notifier therefore considered that this demonstrates that the chronic exposure to a solution of sucrose does not lead adverse effects on adult bees. Moreover, other sugars were demonstrated not toxic for bees as they have bacteria allowing digesting of sugars such as xylose, glucose, sorbitol, which are presents in heptamaloxyloglucan. Therefore, the notifier considered that no chronic risk is expected for adult bees when exposed to heptamaloxyloglucan.

However, data are available on galactose, one of the monomer of heptamaloxylglucan, that was found to be toxic to adult bees after oral administration. Thus, a risk assessment is proposed for this monomer.

Case of galactose:
Galactose is an element constitutive of heptamaloxyloglucan. However, galactose, was found to have some lethal effects when adult bees are exposed to it (Barker R.J. et al., 1977, CA 8.3.1/03 summarised under Vol 3 CA B.9.3.1.2). Therefore, a risk assessment for galactose is performed considering that that all heptamaloxylogucan is completely transformed in galactose as worst case. That means an application rate of 0.00056 kg galactose/ha and a $LDD_{50}$ > 1.62 mg/bee/day.

$$ETR_{chronic\ adult\ oral} = AR * SV/LDD_{50}\ oral$$

**Table 9.6.1-6 Screening chronic risk assessment for honey bees (adults) exposed to galactose**

| Test substance | Exposure route | Endpoint (µg/bee/d) | Maximum single application rate (kg a.s./ha) | Type of spraying | SV | ETR chronic adult oral | Trigger |
|---|---|---|---|---|---|---|---|
| Galactose | Oral | $LDD_{50}$ > 1620 µg/bee/d | 0.00056 | upwards and/or sideward | 10.6 | $3.7 \times 10^{-6}$ | 0.03 |

The ETR is below the trigger of 0.03 indicating an acceptable chronic risk for adult bees exposed to galactose contained in heptamaloxyloglucan considering that heptamaloxyloglucan will be entirely converted in galactose as worst case exposure conditions.

**Chronic risk assessment for larvae bees according to EFSA Journal 2013;11(7):3295**
Screening chronic oral risk assessment for larvae honeybees:
According to the Guidance Document on risk assessment of bees from EFSA, the chronic risk assessment for larvae bees is based on the $ETR_{larvae}$ using the following equation:

$$ETR_{larvae} = AR * SV/NOEL_{larvae}$$

Where:
AR = application rate in kg/ha
SV = 6.1 for an application made *via* an upwards and/or sideward spray (for vineyards)
$NOEL_{larvae}$ is expressed in µg a.s./larvae per developmental period.

The notifier proposed to calculate the critical NOEL for larvae that will lead to consider the risk to larvae as acceptable.

**Table 9.6.1-7 Screening chronic risk assessment for honey bees (larvae)**

| Test substance | Exposure route | Maximum single application rate (kg a.s./ha) | Type of spraying | SV | Trigger ETR larvae | Endpoint (µg a.s./larvae/ developmental period) |
|---|---|---|---|---|---|---|
| Heptamaloxyloglucan | Oral | 0.00056 | upwards and/or sideward | 6.1 | ≤ 0.2 | NOEL ≥ 0.0171 |

To obtain an acceptable risk for larvae when exposed chronically to heptamaloxyloglucan, the NOEL should be ≥ 0.0171 µg a.s./bee/d.

The notifier also proposed an approach considering the diet of the OECD guidance document 239 that contains monomer of sugars that are part of heptamaloxyloglucan.
Indeed, according to the Guidance document OECD 239 which is followed for the chronic toxicity studies on larvae, all larvae are fed with three following diets:

- Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.
- Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.
- Diet C (from D4 to D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.

All diets contain glucose and fructose and are delivered daily. Glucose is one of sugars constituting of heptamaloxyloglucan. It is expected to be non-toxic for larvae as used as food in the standard chronic tests. That means that the chronic exposure to a solution containing glucose and fructose does not lead adverse effects on larvae. Moreover, other sugars were demonstrated not toxic for bees as they have bacteria allowing digesting of sugars such as xylose, glucose, sorbitol, which are presents in heptamaloxyloglucan.

RMS expected that NOEL for larvae bees of heptamaloxyloglucan would be greater than the critical NOEL of 0.0171 µg a.s./bee/development period calculated to have an acceptable risk for bee larvae. Therefore, no risk to larvae is expected for larvae bees when exposed to heptamaloxyloglucan. However, data are available on galactose, one of the monomer of heptamaloxylglucan, that was found to be toxic to adult bees after oral administration. Thus a risk assessment is proposed for this monomer.

Case of galactose:
Galactose is an element constitutive of heptamaloxyloglucan. However, galactose, was found to be toxic, with a NOEL of 1.62 mg/bee/day (see CA 8.3.1.2) for adult bees. No toxicity data of galactose on larvae were found. However, to consider toxic effects observed on adult the notifier proposed the following assessment/argumentation for larvae bees:
Galactose is present in low concentrations in honey and that honey is the main source of carbohydrates for bees in a colony. Therefore, larvae are exposed to galactose by honey. As shown in CA 8.3.1.2, galactose contents of 46 honey samples were analyzed. The mean galactose concentration was 0.0086%, with a spread of values from 0.0052 to 0.0151%.

According the OECD 239, each larvae receive different diets adapted to the needs of the larvae at different stages of development: 20 µL of diet B on day 3, 30 µL of diet C on day 4, 40 µL of diet C on day 5 and 50 µL of diet C on day 6, for a total of 140 µL of diet within 4 days of administration, equivalent to 154 mg of diet knowing that the diets A, B and C prepared have a density of about 1.1 mg/µL (*e.g.* 20 µL diet correspond to 22 mg diet).

Based on assumption that larvae are fed with 100% honey and knowing the mean galactose concentration on 46 honeys is 0.0086%, the notifier assumed that the level of 13.2 µg galactose/larvae/ development period deduced from diet of larvae in OECD GD 239 will have no adverse effects on larvae.

The notifier proposed a risk assessment for galactose considering that all heptamaloxylogucan is completely transformed in galactose.

$$ETR_{larvae} = AR * SV/NOEL_{larvae}$$

**Table 9.6.1-8 Screening chronic risk assessment for honey bees (larvae) exposed to galactose**

| Test substance | Exposure route | Theoretical Endpoint | Maximum single application rate (kg a.s./ha) | Type of spraying | SV | ETR larvae | Trigger |
|---|---|---|---|---|---|---|---|
| Galactose | Oral | NOEL = 13.2 µg/larvae/ develomental period | 0.00056 | upwards and/or sideward | 6.1 | 0.00026 | 0.2 |

The ETR is below the trigger of 0.2 indicating that an acceptable risk for larvae exposed to galacatose contained in heptamaloxyloglucan considering worst case exposure conditions is expected.

Overall, RMS considered appropriate the assumption proposed by the notifier to provide some evidence that no unacceptable risks for adult and larvae bees are expected from heptamaloxyloglucan or its monomer galactose.

**Overall conclusion of risk assessment for bees**

Heptamaloxyloglucan was not acutely toxic to bees (oral and contact $LD_{50}$ > 100 µg a.s./bee).

Acute oral and contact Hazard Quotients (HQ) and Exposure Toxicity Ratio (ETR) were found to be below triggers defined in Regulation EU No 546/2011 and EFSA guidance (2013). Thus, the acute oral and contact risk to bees of heptamaloxyoglucan following application of PEL101GV on vine is considered to be acceptable.

No studies on chronic toxicity to adult bees and on effects on honey bee development were provided by the notifier. However, 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. The monomeric sugars forming heptamaloxyloglucan are very close to monomeric sugars found in honey and nectar. “Sugars” are known to be attractive for bees and publications were found to demonstrate the importance of sugars in honey and nectar, which are used by bees for nutrition (Smessaert, 2019; Pavlova, 2018; Machado De-Melo, 2017 and Dmitruk 2019). The honey bee microbiome contains bacteria available to digest different sugars such as xylose, glucose, sorbitol and sucrose (Lee *et al.*, 2014).
Theoretical NOEL for larvae and LDD50 for adult bees to reach unacceptable risk have been calculated and could be assumed as unrealistic for the active substance. Thus, the chronic risks to adult bees and larvae following exposure to the formulation in vineyard are not expected to be unacceptable.

Furthermore, a chronic risk assessment has been performed for adult bees exposed to galactose, one of the hexose constituting heptamaloxyloglucan, found to have statically significant lethal effects compared to the sucrose control at a dose level of 8%, 2.16 mg/bee/day, in a 16-days dietary test (Barker, 1977 [CA 8.3.1/03]). Based on a $LD_{50}$ value of greater than 1620 µg/bee/day, no unacceptable chronic risk to adult honey bees resulted from exposure to galactose is expected.

Overall, no unacceptable risk to bees resulted from exposure to the active substance heptamaloxyloglucan, following up to 4 applications of PEL101GV at 0.56 g/ha on grapes is expected.

Co-RMS agreed that no unacceptable risk of heptamaloxyloglucan to bees can be concluded. Nevertheless, taking into account the theoretical approach used in risk assessment for larvae bees, Co-RMS indicated that a larval toxicity would help in the determination of the NOEC-Ecx for risk assessment.

## B.9.6.2. Risk assessment for non-target arthropods other than bees

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 were deemed necessary during the initial EU review of heptamaloxyloglucan.
Considering the type of component (xyloglucan extracted from apple), its mode of action (plant elicitor to protect vine from freezing), its natural occurrence in plants, the low dose applied (0.56 g/ha), RMS is still of the opinion that testing on non target arthropods is not required in this particular case.

## B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

### B.9.7.1. Earthworms

No study was performed with the formulation.

### B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

No study was performed with the formulation.

## B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

### B.9.8.1. 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 0.0018 mg a.s./kg soil 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).

Using literature data (see Volume 3 CA B.9.4.2), it is considered that oligosaccharides such as heptamaloxyloglucan, which is a xyloglucan-derived oligosaccharide, could be degraded in soil by enzymatic action of the microorganisms (also refer to Volume 3 CA B.8.1.1.1.4). The assimilation and degradation of xyloglucan-like molecules by soil macro-organisms such as earthworms is facilitated by soil microorganisms that they ingest together with soil (see Vol. 3 CA B.9.7). The possible assimilation and degradation by soil micro- and macro-organisms is considered as well described.

The natural concentration of heptamaloxyloglucan in the soil of an apple orchard has been estimated by the applicant (see Volume 3 CA B.8.1.1.1.4) to be **1.1 g/ha**. This value is similar to the intended application rate on vines (0.56 g/ha). Thus, the very low amounts of residues due to the use of heptamaloxyloglucan (0.0018 mg a.s./kg soil) is not expected to change the qualitative composition of the organic matter which reaches the soil or to cause damage on soil macro-organisms.

Overall, considering the type of component (xyloglucan extracted from apple), its mode of action (plant elicitor to protect vine from freezing), its natural occurrence in plants (estimated around 1.1 g/ha in apple field, see Volume 3 CA B.8.1.1.1.4), the low dose applied (0.56 g/ha), RMS is still of the opinion that no unacceptable effects are expected following application of heptamaloxyloglucan in vine as intended.

### B.9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

Please also consider Volume 3 CA B.9.4.3.

The initial PECsoil of heptamaloxyloglucan was calculated considering the worst case of the four applications performed in one time, it correspond to the situation of no degradation of heptamaloxyloglucan between each application. PECsoil was found to be 0.0018 mg/kg dry soil.

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

Plant decay is the natural substrate for soil meso- and macrofauna. In addition, the application rate of heptamaloxyloglucan (0.56 g/ha) is 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 soil macro-organisms.

## B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

No study.
Not required. See further justification under B.9.10.

## B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

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.

In an apple field, heptamaloxyloglucan natural level was estimated to be 1.1 g/ha (see Volume 3 CA B.8.1.1.1.4 for details). This value is similar to the intended application rate on vines (0.56 g/ha). Additionally, 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 0.0018 mg/kg dry soil. Such low concentrations are not susceptible to change the qualitative composition of the organic matter which reaches the soil.

Furthermore, using literature data (see Volume 3 CA B.9.4.2), it is considered that oligosaccharides such as heptamaloxyloglucan, which is a xyloglucan-derived oligosaccharide, could be degraded in soil by enzymatic action of the microorganisms (also refer to Volume 3 CA B.8.1.1.1.4). The assimilation and degradation of xyloglucan-like molecules by soil macro-organisms such as earthworms is facilitated by soil microorganisms that they ingest together with soil (see Vol. 3 CA B.9.7).
Veras *et al.*, 2017 (CA 8.5/02) showed that yeasts (*Scheffersomyces stipitis*, *Spathaspora passalidarum*, *Spathaspora arborariae* and *Candida tenuis*) were able to use xylose as substrate of fermentation to produce ethanol.

Considering the type of component (xyloglucan extracted from apple), its mode of action (plant elicitor to protect vine from freezing), its natural occurrence in plants, the low dose applied (0.56 g/ha), heptamaloxyloglucan is not expected to have any adverse effects on the function of soil micro-organisms ecosystems.

## B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

### B.9.11.1. Summary of screening data

No study was performed with the formulation.

### B.9.11.2. Testing on non-target plants

Study is available with the technical active substance coded EL101GV which is strictly identical to the formulation PEL101GV.

### B.9.11.3. Extended laboratory studies on non-target plants

No study was performed.

### B.9.11.4. Semi-field and field tests on non-target plants

No study was performed.

## B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

A toxicity study with the substance heptamaloxyloglucan was performed on vegetative vigour of 3 plants species (red cover, wheat and mustard) in semi-field conditions (see Volume 3 CA B.9, point B.9.6.2.) There were no adverse effects on the vegetative vigour observed up to 20.0 g a.s./ha application rate.

**Table B.9.12-1. Heptamaloxyloglucan: effect on terrestrial non-target higher plants in semi-field test**

| Substance | Tested species | Endpoint | Reference |
|---|---|---|---|
| Heptamaloxyloglucan | *Triticum aestivum* (wheat)<br>*Sinapis alba* (mustard)<br>*Trifolium pratense* (red cover) | No adverse effects up to 20.0 g a.s./ha | Servajean E., (2006b) (CA 8.6/01) |

**Exposure**

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90$^{th}$ percentile estimates derived by the *BBA (2000)*[1] from the spray drift predictions of *Ganzelmeier & Rautmann (2000)*[2].
The off-field exposure ($PER_{off\text{-}field}$) is calculated using the following equation:

$$PER_{off-field} = Application\ rate\ (kg\ a.s./ha)\ *MAF\ *\ \%\ drift$$

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, $DT_{50}$ value and number of applications. According to Escort 2 guidance, for 4 applications, the foliar MAF is equivalent to 2.7.

For application to grapes, 2.70% of the application rate was assumed to reach areas at 3 m from the edge of the crop (scenario for early applications).

The $PER_{off\text{-}field}$ of uses is presented in the following Table.

**Table B.9.12-2. $PER_{off\text{-}field}$ values for non-target plants exposed to heptamaloxyloglucan**

| Intended uses | Application rate (kg a.s./ha) | MAF | Drift (%) | $PER_{off\text{-}field}$ (kg a.s./ha) |
|---|---|---|---|---|
| Grapes | 0.000 | 2.7 | 2.70 (3 m) | 0.000041 |

**Risk assessment**

**Table B.9.12-3. Assessment of the risk for non-target plants due to the use of PEL101GV in vineyards**

| | | | | |
|---|---|---|---|---|
| **Intended use** | **Vineyards** | | | |
| **Active substance/product** | Heptamaloxyloglucan / PEL101GV | | | |
| **Application rate (g a.s./ha)** | 4 × 0.437 | | | |
| **MAF** | 2.7 | | | |
| **Test species** | **$ER_{50}$ (g a.s./ha)** | **Drift value (%)** | **$PER_{off\text{-}field}$ (g a.s./ha)** | **TER criterion: TER ≥ 5** |

[1] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[2] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

| | | | | |
|---|---|---|---|---|
| *Triticum aestivum* (wheat)<br>*Sinapis alba* (mustard)<br>*Trifolium pratense* (red cover) | > 20 | 2.77 | 0.041 | > 487 |

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The risk to non-target plants following application of the product PEL101GV, containing heptamaloxyloglucan is considered acceptable as TER is greater than the trigger value of 5. Therefore, the use of PEL101GV is not expected to pose unacceptable risk to non target plants and no higher tier assessment or risk mitigation are necessary.

**Overall conclusion**

The product PEL101GV is not expected to pose unacceptable risk to non-target plants in off-crop areas following the proposed use. No mitigation measures are needed.

## B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No further data on other terrestrial organisms (flora and fauna) are available.

## B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not required.

## B.9.15. REFERENCES RELIED ON

The formulation PEL101GV is strictly identical to the technical active substance heptamaloxyloglucan coded EL101GV. Please refer to Volume 3 CP B.1.1 and Volume 1 point 1.4.4.1.