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Heptamaloxyloglucan

Volume 3 – B.9 (AS)

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

Version History

When	What
2020-09	Initial RAR

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.

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B.9. ECOTOXICOLOGY DATA

Technical Heptamaloxyloglucan (coded EL101GV) is a branched xyloglucan molecule, part of hemicellulose extracted from apple pomace by enzymatic hydrolysis and deacetylation/reduction after fractioning and purification. Samples are then purified and conditioned by lyophilisation.

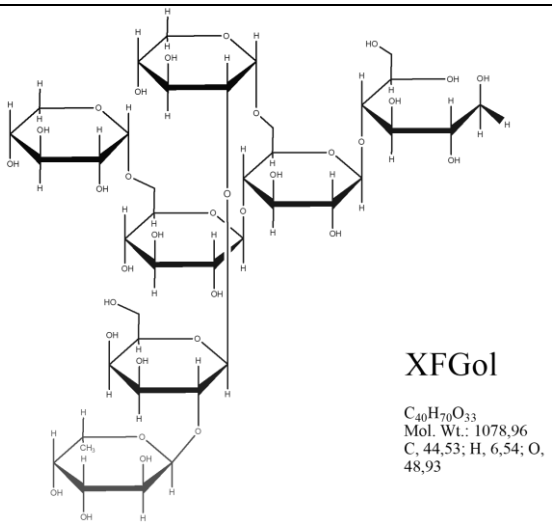
Xyloglucan is the principal hemicellulosic component of primary cell walls of dicotyledonous and non-graminaceous monocotyledonous plants. Xyloglucan plays a physiological key role in maintaining cell wall integrity by cross-linking individual cellulose microfibrils in the primary cell wall. Specific 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[1] CA 8.1/2; Buchanan et al., 2000[2] CA 8.1/1).

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) (Table 9.1).
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.

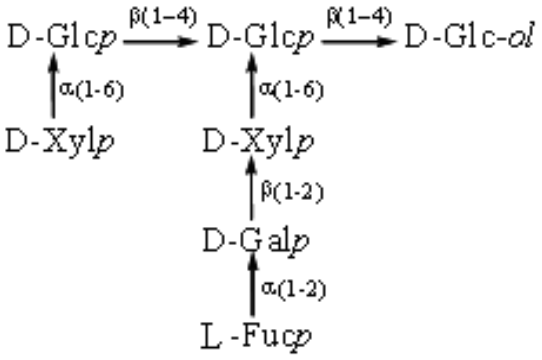
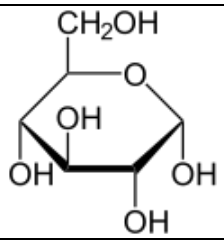
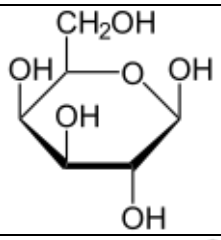
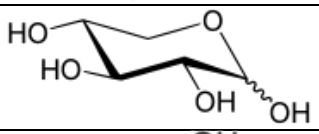
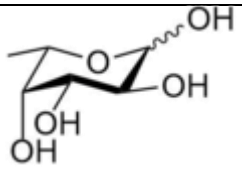
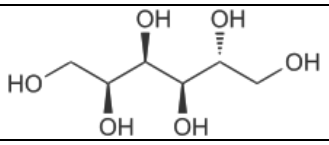
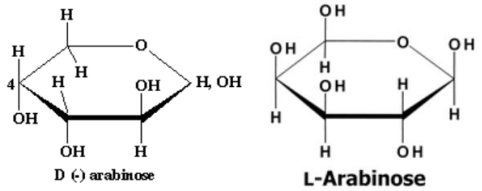
Heptamaloxyloglucan acts as a stimulator of plant defence natural mechanisms ("elicitor") which must preserve the chemical structure and conformation of the xyloglucan heptamer XFG in order to increase the cold resistance of the grapevine.

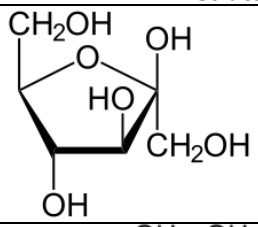
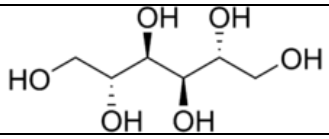
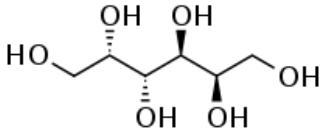
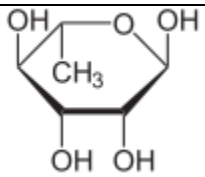
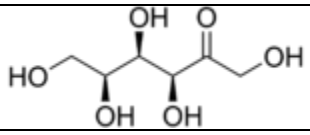
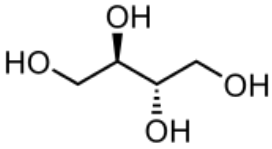
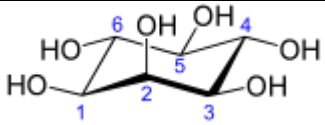
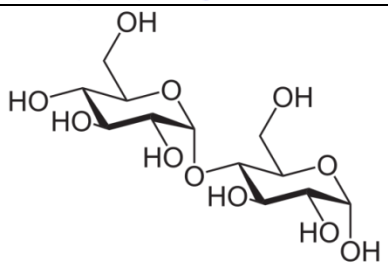
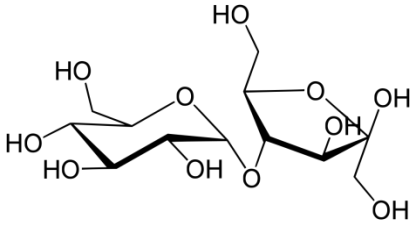
A comparison between the structure of heptamaloxyloglucan, the main hexoses present in heptamaloxyloglucan and the ones of sugar compounds on which literature studies are based is presented in the table below:

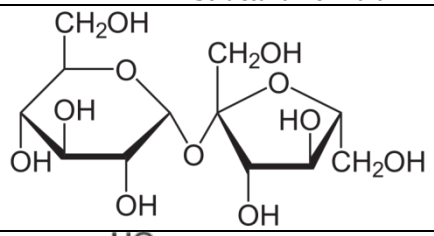
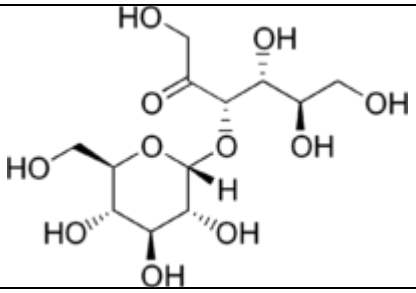
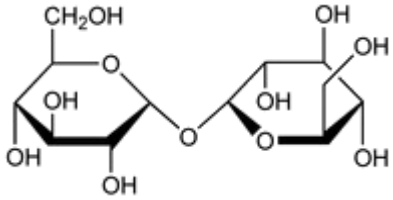
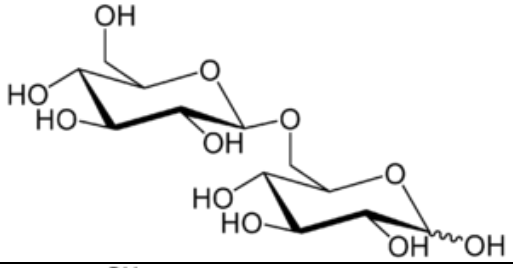
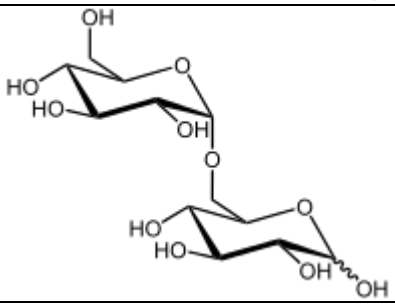
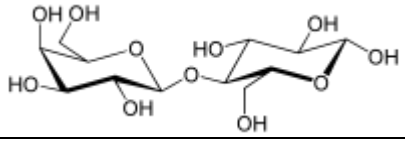
Table 9.1 : Compararison of sugar compounds and heptamaloxyloglucan

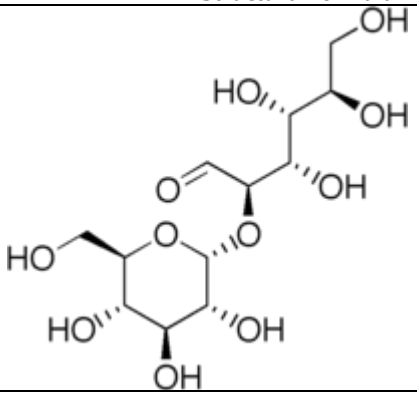
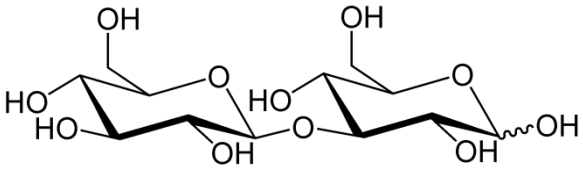
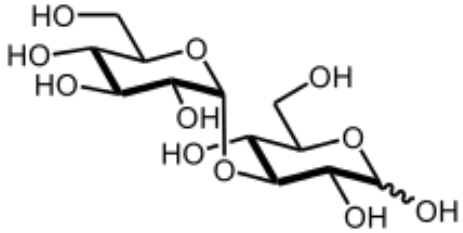
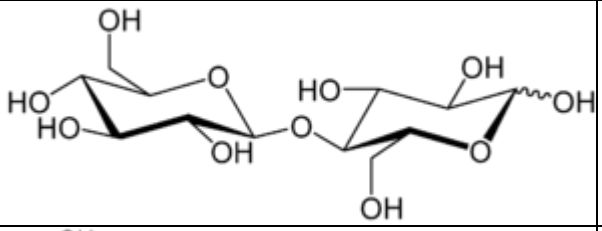
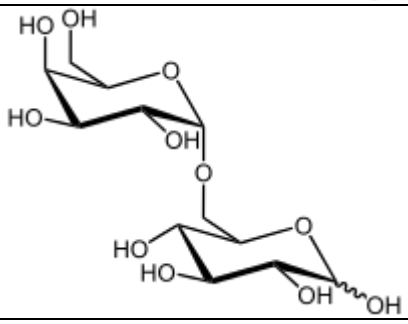
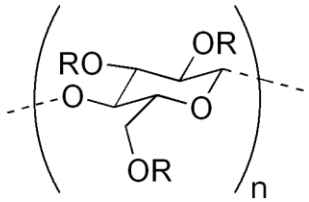
Name	Structural formula	Comments
Heptamaloxylogulcan		Following the IUPAC nomenclature, heptamaloxyloglucan is part of the oligosaccharides within the carbohydrates family. The IUPAC name of heptamaloxyloglucan is: *{[α-D-Xyl p-*(1→6)]-*β-D-Glc p-*(1→4)}{[*α-L- Fuc p-*(1→2)-*β-D-Gal p-*(1→2)-*α-D-Xyl p-*(1→6)]-*β-D-Glc p-*(1→4)}-*D-Glc-ol* Xyl p: xylopyranosyl Glc p: glucopyranosyl Fuc p: fucopyranosyl Gal p: galactopyranosyl Glc-ol: glucitol

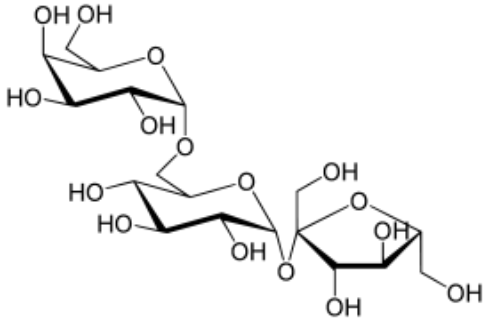
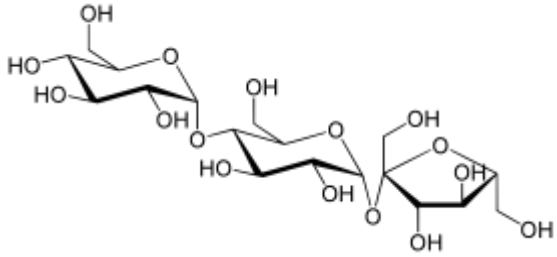
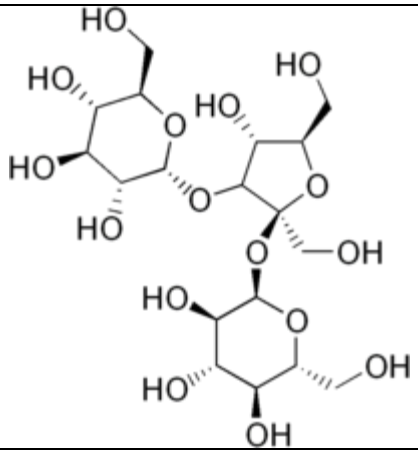
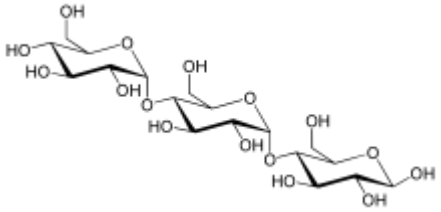
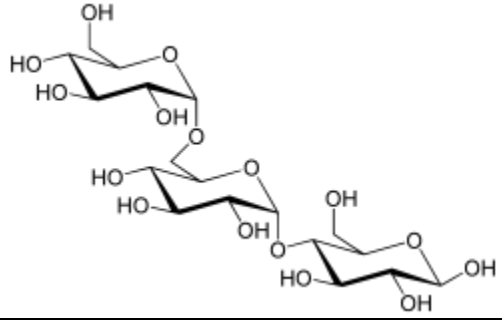
[1] Fry. S.C., Aldington S., Hetherington P.R., Aitken J., 1993. "Oligosaccharides as Signals and Substrates in the Plant Cell Wall".Plant Physiol., Vol. 103 (1993), pp. 1-5.
[2] Buchanan B.B. et al, 2000. "Chapter 2: The Cell Wall. Biochemistry and molecular Biology of Plants". B. Buchanan, W. Gruissem,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

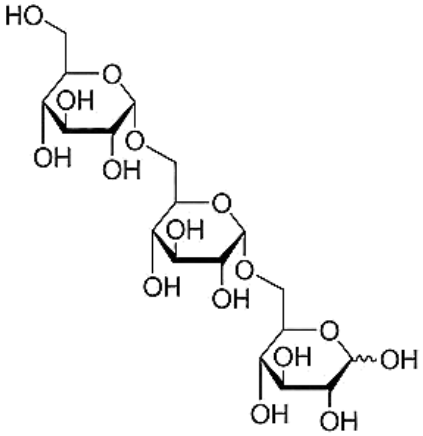
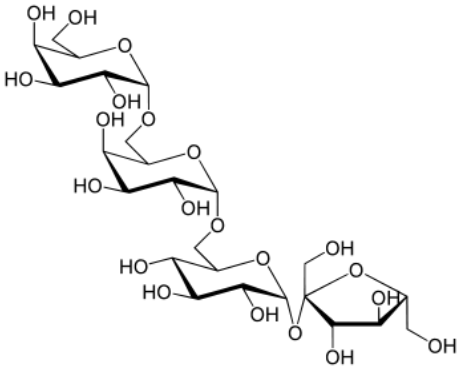
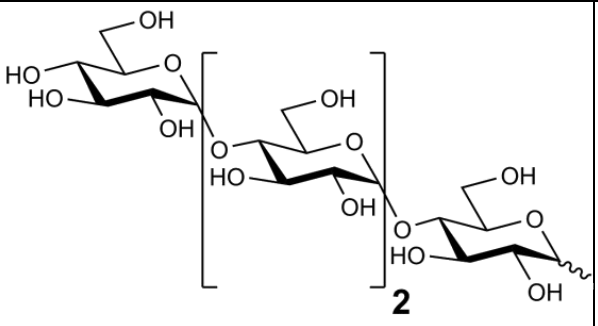
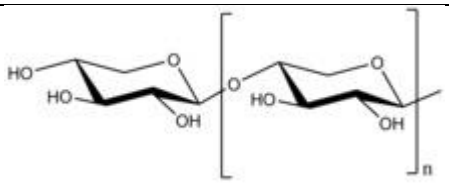
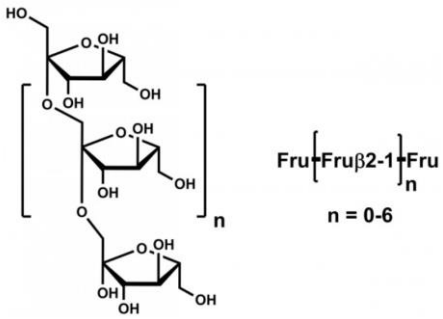
Name	Structural formula	Comments
		
Hexose	-	a hexose is a monosaccharide with six carbon atoms
Glucose (glucopyranose)		Most abundant carbohydrate Present in honey and heptamaloxyloglucan
Galactose (galactopyranose)		Present in honey and heptamaloxyloglucan
Xylose (xylopyranose)		Present in heptamaloxyloglucan
Fucose (fucopyranosyl)		Present in heptamaloxyloglucan
Glucitol (sorbitol)		Present in heptamaloxyloglucan
Arabinose		a monosaccharide containing five carbon atoms, and including an aldehyde (CHO) functional group.

Name	Structural formula	Comments
Fructose (fructofuranose)		Present in honey and in sucrose
Mannitol		Mannitol is classified as a sugar alcohol; that is, it can be derived from a sugar (mannose) by reduction.
Dulcitol (galactitol)		Galactitol (dulcitol) is a sugar alcohol, the reduction product of galactose.
Rhamnose		Rhamnose (Rha, Rham) is a naturally occurring deoxy sugar. It can be classified as either a methyl-pentose or a 6-deoxy-hexose
Sorbose		Sorbose is a ketose belonging to the group of sugars known as monosaccharides
Erythriol		It occurs naturally in some fruit and fermented foods
Inositol		Inositol, or more precisely myo-inositol, is a carbocyclic sugar
Maltose		Disaccharide: Two glucose monomers α(1→4) bond Present in honey
Maltulose		a glucose monomer and a fructose monomer α(1→4) Present in honey

Name	Structural formula	Comments
Sucrose		Disaccharide Two monosaccharides: glucose + fructose. Sucrose is produced naturally in plants Present in honey
Turanose		a glucose monomer and a fructose monomer α(1→3) Present in honey
Trehalose		Disaccharide: Two molecules of glucose (mycose or tremalose) Present in honey
Gentiobiose		Disaccharide Two units of D-glucose monomers β(1->6) linkage Present in honey
Isomaltose		Disaccharide Two units of glucose joined with a α-(1-6)-linkage Present in honey
Lactose		Disaccharide Galactose and glucose Present in honey

Name	Structural formula	Comments
Kojibiose		Disaccharide Two glucose monomers α(1→2) Present in honey
Laminaribiose		two glucose monomers β(1→3) Present in honey
Nigerose		two glucose monomers α(1→3) Present in honey
Cellobiose		two β-glucose molecules linked by a β(1→4)
Melibiose		a galactose monomer and a glucose monomer α(1→6)
Carboxymethylcellulose	 R = H or CH_2CO_2H	Carboxymethyl cellulose (CMC) or cellulose gum[1] is a cellulose derivative with carboxymethyl groups (-CH2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone

Name	Structural formula	Comments
Raffinose		Trisaccharide: Galactose + glucose + fructose Present in honey
Erlose glucosylsucrose		Triholoside One unit of fructose and two units of glucose Present in honey
Melezitose melicitose,		Trisaccharide Present in honey
Maltotriose		Trisaccharide three glucose molecules linked with α-1,4 glycosidic bonds Present in honey
Panose		Isomalto-oligosaccharides (IMO) are glucose oligomers with α-D-(1,6)-linkages Present in honey

Name	Structural formula	Comments
Isomaltotriose		Isomalto-oligosaccharides three isomaltose monomers with α-D-(1,6)-linkages Present in honey
Stachyose		Stachyose is a tetrasaccharide consisting of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit sequentially linked as gal(α1→6)gal(α1→6)glc(α1↔2β)fru
Maltotetraose		Isomalto-oligosaccharides (IMO) are glucose oligomers with α-D-(1,6)-linkages Present in honey
Xylooligosaccharides (XOS)		Xylooligosaccharides (XOS) are polymers of the sugar xylose. Molecular structure of an hypothetical xylooligosaccharide, where n is a variable number of xylose units.
Fructo-oligosaccharide (FOS)		Fructooligosaccharides (FOS) are oligomers of β-d-fructofuranosyl units linked (2 → 1).

Regarding literature data submitted by the notifier, RMS judged them acceptable when it is possible to derive useful and supportive information 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.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

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. Xyloglucans are synthesized in the ER-Golgi apparatus as soluble polymers, they are modified by acetylation and remain soluble until they can be cross-linked at the cell surface (Buchanan et al, 2000, CA 8.1/01).

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, CA 8.1/03). 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.

Heptamaloxyloglucan is intended to be used at very low dose rate (0.00069 to 0.560 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.

Supportive literature data already evaluated at EU-level during the first approval of Heptamaloxyloglucan was provided by the applicant and summarised by RMS focusing on xyloglucan information.

Data point:	CA 8.1/01
Report author	Buchanan B.B. *et al*.
Report year	2000
Report title	Chapter 2: The Cell Wall. Biochemistry and molecular Biology of Plants. B. Buchanan, W. Gruissem, R. Jones, Eds. 2000, pp 52-89. Published
Report No	-
Document No	
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR of Heptamaloxyloglucan (February 2005)and in the final Addendum (June 2009)
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes, provide justification on the natural occurrence of xyloglucan in plant cell wall

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Full summary of the study

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).

Assessment and conclusion

Assessment and conclusion by applicant:
This study provide justification on the natural occurrence of xyloglucan in plant cell wall

Assessment and conclusion by RMS:
The conclusion of RMS is the same as for the initial DAR (2009):
The literature data provided some qualitative information 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 information on the quantity of such molecules in a bird usual diet.

Data point:	CA 8.1/03
Report author	Stevens C.E., Hume I.D. 1998 *et al.*
Report year	1998
Report title	Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients Physiol.Rev., 1998 Apr, 78(2), pp 393-427 Published
Report No	-
Document No	
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the DAR of Heptamaloxyloglucan (February 2005)and in the final Addendum (June 2009)
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes, provide justification on the natural occurrence of xyloglucan in plant cell wall

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Full summary of the study

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).

Assessment and conclusion

Assessment and conclusion by applicant:
This study provide justification on the natural occurrence of xyloglucan in plant cell wall

Assessment and conclusion by RMS:
The conclusion of RMS is the same as for the initial DAR (2009):
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 information on the quantity of such molecules in a bird usual diet.

New supportive literature data not evaluated previously was provided by the applicant and summarised by RMS. These three publications characterise the effects of xylo-oligosaccharides on the growth performance and immune function of broiler chickens (Morgan *and al*. 2018 (CA 8.1/04); Suo Hai-qing and *all*, 2015 (CA 8.1/05) and Yuan *and al*, 2018 (CA 8.1/06)).

Data point:	CA 8.1/04
Report author	Morgan N. K., Keerqin C., Wallace A., Wu S.-B., Choct M.
Report year	2019
Report title	Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers Animal Nutrition 5 (2019) 56-62
Report No	-
Document No	
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive data (sugars tested with chemical structure differing to heptamaloxyloglucan but representative of hemicelluloses in the cell walls of plants (as heptamaloxyloglucan) and representativeness of data on broilers for risk assessment of wild birds)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Full summary of the study

Xylans, also known as arabinoxylans (AX) and pentosans, are the most abundant hemicelluloses in the cell walls of monocotyledonous plants, such as cereals. Arabinoxylo-oligosaccharides (AXOS) are hydrolytic degradation products of arabinoxylans (AX) that can be fermented by the gut microbiota, thus potentially displaying prebiotic

properties. This study examined the effects of AX and AXOS on net energy (NE) and nutrient utilization in broilers. 'Ross 308' broilers (n = 90, 30 birds per treatment) were fed wheat-soybean diets supplemented with pure AX, AXOS produced by exposing the AX to xylanase in vitro (AXOS), or AX with xylanase (AXþE) from 10 to 21 days. On day 15, 10 birds per treatment were allocated to assess the impact of AX and AXOS on dietary energy utilization, through assessment of both metabolisable energy (ME) and NE. Feed conversion ratio was numerically the lowest in birds fed the diet supplemented with AXOS, which is 1.26 compared to 1.37 and 1.30 for AX and AXþE, respectively. Ileal dry matter digestibility was higher in birds fed AXOS than those fed AX (P = 0.047). Ileal digestible energy and total tract dry matter digestibility were higher in birds fed AXOS than those fed AX or AXþE(P = 0.004 and P = 0.001,respectively). Ileal and caecal microbiota concentrations were numerically higher and pH was lower in birds fed AXOS and AXþE than those fed AX. Results from this study indicate that feeding AXOS directly is more efficient than AXOS generation in the gastrointestinal tract, and suggest that AXOS has a potential to be an efficacious prebiotic in broiler diets.

Materials and methods

This study examined the effects of diets supplemented in arabinoxylans (AX, 2%) and Arabinoxylo-oligosaccharides (AXOS, 2%), the hydrolytic degradation products of AX, on net energy (NE) and nutrient utilization in broilers. The broilers (n=90, 30 birds per treatment) were supplemented by wheat-soybean diets supplemented with pure AX, AXOS produced by exposing the AX to xylanase in vitro (AXOS), or AX with xylanase (AXþE) from day 10 to day 21. On day 15, 10 birds per treatment were allocated to assess the effects of the diets on growth performance, on ileal and total tract digestibility, energy balance and efficiency of energy utilization (in closed-circuit net energy chambers), on short-chain fatty acids (SCFA) and microflora concentrations in ileum and caeca.

Results

Performance and energy utilisation was lower in birds fed the diet containing AX, presumably because more digestive and metabolic effort was required for the birds to utilise this diet, meaning it was less efficient at providing energy for maintenance and production. This may be partly because the weight and relative proportion of energetically active organs, such as the gastrointestinal tract and pancreas, was greater in birds fed this diet (Wu et al.,2004), which increased the total cost of maintenance.

Effect of diets containing 2% AX, AXOS or AX + E on individual bird performance from d 10 to 21.

Item	FI, g	BWG, g	FCR
AX	1,015.69	746.67	1.37
AXOS	967.00	766.43	1.26
AX + E	987.55	760.36	1.30
SEM	11.52	4.77	0.02
P-value	0.267	0.818	0.167

AX = arabinoxylan; AXOS = arabinoxylo-oligosaccharides; AX + E = AX + xylanase; FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio.

Findings from this study suggested that microbial metabolites such short-chain fatty acids (SCFA) have the potential to be indicators of generation and prevalence of fermentative oligosaccharides and could hence be used to measure the effects of xylanase on nutrient digestibility and retention. Microbiota hydrolyse indigestible carbohydrates into oligosaccharides and then into monosaccharides, which they then ferment in the anaerobic environment of the gut. Arabinoxylooligosaccharides selectively stimulate beneficial bacteria, namely Bifidobacteria, and non-digestible carbohydrates act as the main source of energy during microbial proliferation in the hindgut (Mäkeläinen et al., 2010a, b). The impact of diet on microbiota was not significant in this study, likely due to the low number of replicates.

Effect of diets containing 2% AX, AXOS or AX + E on pH, SCFA concentration and log_{10} DNA enumeration of gut bacteria using 16S rDNA qPCR quantification in the ileum of broilers at d 21.

Item	pH	SCFA, μmol/g			Microbiota, log_{10} counts/g digesta		
		Total	Lactic acid	Formic acid	Total anaerobic	*Lactobacillus*	Enterobacteria
AX	6.64	21.25[b]	18.61[b]	0.51[b]	9.61	8.15	6.01
AXOS	6.46	49.18[a]	43.58[a]	1.42[a]	9.98	8.47	6.14
AX + E	6.53	38.79[ab]	35.59[ab]	0.70[ab]	10.03	8.51	6.19
SEM	0.04	6.65	6.01	0.23	0.11	0.09	0.04
P-value	0.831	0.011	0.012	0.023	0.088	0.375	0.499

AX = arabinoxylan; AXOS = arabinoxylo-oligosaccharides; AX + E = AX + xylanase; SCFA = short chain fatty acids.
[a,b] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

Effect of diets containing 2% AX, AXOS or AX + E on pH, SCFA concentration and log_{10} DNA enumeration of gut bacteria using 16S rDNA qPCR quantification in the caeca of broilers at d 21.

Item	pH	SCFA, μmol/g						Microbiota, log_{10} counts/g digesta		
		Total	Acetic acid	Propionic acid	Butyric acid	Isovaleric acid	Lactic acid	Total anaerobic	*Lactobacillus*	Enterobacteria
AX	6.29	46.42[b]	30.88[b]	1.79[b]	11.47[b]	0.05[b]	0.21[b]	10.51	8.69	7.70
AXOS	6.21	100.75[a]	65.47[a]	5.05[ab]	22.48[a]	0.21[a]	0.66[a]	10.53	8.90	7.80
AX + E	6.27	105.28[a]	67.87[a]	7.51[a]	24.39[a]	0.19[a]	0.30[b]	10.61	8.83	7.92
SEM	0.02	15.44	9.76	1.35	3.29	0.04	0.11	0.02	0.05	0.05
P-value	0.869	0.001	0.004	0.050	0.016	0.008	0.005	0.411	0.290	0.881

AX = arabinoxylan; AXOS = arabinoxylo-oligosaccharides; AX + E = AX + xylanase; SCFA = short chain fatty acids.
[a,b] Means within the same column with no common superscript differ significantly ($P \leq 0.05$).

Results from this study suggest that AXOS has the capacity to be an efficacious prebiotic in broiler diets, as highlighted by its positive effects on broiler performance, intestinal SCFA production and energy utilization.

Assessment and conclusion

Assessment and conclusion by applicant: Results from this study indicate that diet supplemented with AXOS could have beneficial effects on broilers and that AXOS has a potential to be an efficacious prebiotic in broiler diets.

Assessment and conclusion by RMS:

In conclusion, arabinoxylo-oligosaccharides (AXOS) appeared to be efficient prebiotics that have positive effects on net utilization of dietary energy and bird performance. This appears to be largely due to the ability of AXOS to stimulate beneficial bacteria and short-chain fatty acids (SCFA) production.

This study demonstrate that arabinoxylo-oligosaccharides (AXOS) and arabinoxylans (AX) are not toxic to broilers.

Broilers are not a common species used in ecotoxicological risk assessment. Moreover, xylan used in this study which are arabinoxylo-oligosaccharides (AXOS) and arabinoxylans (AX) are sugars that are close to heptamaloxyloglucan but the chemical structures of these compounds are not similar. These sugars are representative of hemicelluloses in the cell walls of plants (as heptamaloxyloglucan) .

Thus, the RMS considers this study as supportive information.

Data point:	CA 8.1/05
Report author	Suo, Hai-qing; Lu, Lin; Xu, Guo-hui; Xiao, Lin; Chen, Xiao-gang; Xia, Rui-rui; Zhang, Li-yang; Luo, Xu-gang
Report year	2015
Report title	Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional 2 corn-soybean meal diet community
Report No	-
Document No	
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information (representativeness of data on broilers for risk assessment of wild birds)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study reports the investigation of the effect on 450 1-day-old male broiler chicks of dietary supplementation of 10 xylo-oligosaccharides (XOS) (0, 25, 50, 75, 100 mg.kg^{-1} of diet for 42 days) on growth performance, meat quality, immune functions, duodenal morphology and intestinal microbial populations of broilers fed a conventional corn-soybean meal basal diet. Male broiler chicks were randomly 13 allocated by bodyweight to 1 of 5 treatments with 6 replicate cages (15 broilers per cage) for each 14 of 5 treatments in a completely randomized design.
The XOS tested have 2-7 xylose units and a purity of about 35 %.

Results

The results showed that supplementation of XOS improved feed conversion rate, drip loss in thigh muscle, and decreased duodenal crypt depth, but had no effect on all other measured indices (See Table below). The chicks fed the diet supplemented with 100 mg of XOS kg^{-1} had the lowest feed conversion rate and drip loss in thigh muscle. The drip loss in thigh muscle decreased linearly as the supplemented XOS increased. Duodenal crypt depth decreased at the supplemental level of 75 mg of XOS kg^{-1}.

Table : Effect of dietary XOS level on the growth performance and mortality of broiler chicks[1)]

Added XOS (mg kg^{-1})	Days 1-21				Days 22-42				Days 1-42			
	ADG (g d^{-1})	ADFI (g d^{-1})	F/G (g g^{-1})	Mortality (%)	ADG (g d^{-1})	ADFI (g d^{-1})	F/G (g g^{-1})	Mortality (%)	ADG (g d^{-1})	ADFI (g d^{-1})	F/G (g g^{-1})	Mortality (%)
0	34	48.4	1.41	1.11	78.9	151	1.91 a	0	56.9	99.8	1.75 a	1.11
25	33.5	48.7	1.45	5.56	80.3	151	1.89 a	0	57.3	100.1	1.75 a	5.56
50	34.6	50.3	1.46	0.00	78.4	149	1.90 a	0	56.9	99.6	1.75 a	0.00
75	35.5	51.3	1.44	1.11	79.3	152	1.92 a	0	57.9	101.7	1.76 a	1.11
100	34.6	49.4	1.43	3.33	79.6	145	1.83 b	0	57.6	97.4	1.69 b	3.33
Pooled SE	0.8	0.9	0.02	0.36	1.5	2	0.02	0	0.8	1.29	0.01	0.36
P-value	0.46	0.25	0.64	0.66	0.92	0.31	0.01	0	0.88	0.25	0.02	0.66
Linear[2)]							0.02				0.02	
Quadratic[3)]							0.08				0.12	

[1)] Data represent the means of 6 replicate cages (n=6).

[2)] Linear effects of added XOS levels.

[3)] Quadratic effects of added XOS levels.

Data with different letters within the same column differ significantly ($P<0.05$).

Average daily gain (ADG), average daily feed intake (ADFI), feed conversion rate (feed/gain, F/G)

Assessment and conclusion

Assessment and conclusion by applicant: This study supports that dietary supplementations of 75, 23 and 100 mg of XOS kg^{-1} are beneficial to broilers fed a conventional corn-soybean meal diet.

Assessment and conclusion by RMS:
In conclusion, the results from the present study indicate that the addition of 100 mg XOS kg^{-1} improves feed conversion rate and water-holding capacity of the thigh muscle of broilers; The addition of 75 mg of XOS kg^{-1} decreases duodenal crypt depth of broilers. Therefore, dietary supplementations with 75 and 100 mg of XOS kg^{-1} are beneficial to broilers fed a conventional corn-soybean meal diet.

This study demonstrate that xylo-oligosaccharides (XOS) are not toxic to broilers. XOS, which are polymers of the sugar xylose, are close to heptamaloxyglucan. Thus, the RMS considers this study as supportive information for risk assessment

Data point:	CA 8.1/06
Report author	Yuan, L.; Li, W.; Huo, Q.; Du, C.; Wang, Z.; Yi, B.; Wang, M.; *et al.*
Report year	2018
Report title	Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens
Report No	-
Document No	
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive information (representativeness of data on broilers for risk assessment of wild birds)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study investigated the effects of diets supplemented in 2 mg/kg of xylo-oligosaccharide (XOS) and 2 mg/kg of flavomycin (FLA) for 42 days on the growth performance and immune function of 150 broiler chickens (randomly divided into three groups of five replicates, 10 chickens each). At 21 and 42 days, the growth performance index values and short-chain fatty acid (SCFA) concentrations in the cecum were quantified. Furthermore, immunoglobulin G (IgG) and plasma interleukin 2 (IL-2) as well as mRNA expression of LPS-Induced TNF-alpha Factor (LITAF), Toll-like receptor-5 (TLR5) and interferon gamma (IFN) in the jejunum were quantified.

Results

The results showed that administration of XOS or FLA to chickens significantly improved the average daily gain (Cf. Table below). Supplementation with XOS increased acetate and butyrate in the cecum, while FLA supplementation increased propionate in the cecum. An increase in plasma IgG was observed in XOS-fed 21-day-old broilers, but FLA supplementation decreased IgG in the plasma of 42-day-old broilers and increased plasma IL-2. Furthermore, FLA or XOS supplementation downregulated mRNA expression of INFγ, LITAF and TLR5. The above data suggest that addition of XOS and FLA to the diet could improve the growth performance of broilers and reduce the expression of cytokine genes by stimulating SCFA.

At 1-21 days, supplementary FLA or XOS had no effect on the average daily feed intake (ADFI), the average daily gain (ADG) or the feed conversion ratio (FCR) (P >0.05). Compared to the control at 1-42 days, ADFI was significantly enhanced with XOS supplementation (P = 0.001); ADG was significantly enhanced with FLA (P

=0.047) or XOS (P = 0.001) supplementation; but supplementing FLA (P >0.08) or XOS (P = 0.074) had no effect on FCR compared to the control.

Table **Effect of FLA and XOS on performance of broilers.** Each value represents the mean SD of 5 replicates. In the same row, values with no superscript letter or the same superscript letter are not significantly different ($P > 0.05$); those with different superscript letters are significantly different ($P < 0.05$).

Parameter	CTL	FLA	XOS
1–21 days			
ADFI (g/d)	50.31 ± 1.23	51.31 ± 1.66	50.89 ± 1.35
ADG (g/d)	32.95 ± 0.79	33.81 ± 0.95	33.44 ± 1.07
FCR	1.53 ± 0.01	1.52 ± 0.01	1.52 ± 0.02
1–42 days			
ADFI (g/d)	96.46 ± 1.26[b]	98.36 ± 1.59[b]	101.06 ± 1.95[a]
ADG (g/d)	50.29 ± 1.04[b]	51.83 ± 1.32[a]	53.25 ± 0.88[a]
FCR	1.92 ± 0.02	1.90 ± 0.02	1.90 ± 0.01

Notes.
ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

Assessment and conclusion

Assessment and conclusion by applicant: This study supports that exposure of broilers to diets supplemented with XOS can improve the growth performance of chickens and reduce the expression of cytokine genes by stimulating short-chain fatty acid (SCFA).

Assessment and conclusion by RMS:
In this study, broilers with XOS- and FLA-supplemented diets had greater average daily feed intake (ADG) than those of the control group. That may be attributed to the regulation of XOS on intestinal microbes. These results show that adding XOS to the diet can improve the biological function of chickens, suggesting that XOS could be considered an emerging prebiotic, which is defined as a food ingredient that has no nutritional value but can improve the health of the host by regulating its microflora. Overall, the present study showed that the addition of XOS and FLA to the diet could improve the growth performance of broilers.

This study demonstrate that xylo-oligosaccharides (XOS) are not toxic to broilers. XOS, which are polymers of the sugar xylose, are close to heptamaloxyglucan. Thus, the RMS considers this study as supportive information for risk assessment

B.9.1.1.1. Acute oral toxicity to Birds

No acute avian toxicity studies on toxicity of heptamaloxyloglucan (EL101GV) have been performed. Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples. It is made of 7 glycosidic monomer units. As such, heptamaloxyloglucan could be considered as being part of usual bird diet.

B.9.1.1.2. Short-term dietary toxicity to birds

No short-term dietary toxicity study has been conducted.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples It is made of 7 glycosidic monomer units. As such, heptamaloxyloglucan could be considered as being part of usual bird diet. Moreover heptamaloxylglucan is expected to be rapidely degraded in the environment (please refer to CA B.8). Therefore, no subchronic and reproduction toxicity study has been provided.

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 technical heptamaloxyloglucan (coded EL101GV). They are reported in the relevant section of the Volume 3 CA B.6 and summarised in Table B 9.1.2.1-1 & 9.1.2.2-1.

B.9.1.2.1. Acute oral toxicity to mammals

Table 9.1.2.1-1: Acute toxicity of heptamaloxyloglucan to mammals

Test species	Test system	Results	References
Rat	Acute oral toxicity	LD_{50} > 5000 mg heptamaloxyloglucan/kg b.w.	*******, 2004 (B.6.2.1)

B.9.1.2.2. Long-term and reproduction toxicity to mammals

Table 9.1.2.2-1: Long-term and reproduction toxicity of heptamaloxyloglucan to mammals

Test species	Test system	Results	References
Rat	28-day oral toxicity	NOEL = 1000 mg heptamaloxyloglucan/kg b.w./d	********., 2006 (B.6.3.1)

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

According to EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation.

The log K_{ow} of heptamaloxyloglucan is low (-15.96) (for details please refer to Volume 3 (CA) Section 2). Therefore it is not expected that heptamaloxyloglucan accumulates along the food chains. Consequently study on bioconcentration in prey of birds and mammals is required.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the data requirements Commission Regulation (EU) No. 283/2013 available and relevant data regarding the potential effects to birds, mammals, reptiles and amphibians shall be presented. There are no specific requirements on specific experimental data to be generated.

Heptamaloxyloglucan is a branched xyloglucan molecule extracted from apples. Xyloglucans are synthesized in the ER-Golgi apparatus as soluble polymers. They are modified by acetylation and remain soluble until they can be cross-linked at the cell surface. Therefore, short-chain xyloglucans, similar or identical to Heptamaloxyloglucan are natural major components of dicotyledone plants. Xyloglucan molecules account for 10% of cell wall constituents in dicotyledones (Buchanan B.B., Gruissen W., and Jones R.L., Biochemistry and Molecular Biology of Plants, 2000, CA 8.1/01).
Heptamaloxyloglucan is a natural component of plant cell walls and therefore can enter as other natural component in vertebrate diet. Given the nature of the active substance (xyloglucan extracted from apple), the expected fast degradation of heptamaloxylogucan in the environment (see Volume 3 CA B.8 for details), its natural occurrence, its mode of action (anti-freezing) and its low application rate rate (0.00069 to 0.560 g/ha), RMS considered that no specific adverse effects of heptamaloxyloglucan are expected from exposure of terrestrial vertebrates including reptiles and amphibians.

In addition, there were no literature study found with indication of effects on other terrestrial vertebrate wildlife (reptiles and amphibians).

B.9.1.5. Potential for endocrine disruption

The notifier indicated that heptamaloxyloglucan, in unchanged form, cannot penetrate lipophilic membranes ($K_{ow} < 10^{-4}$) and thus not enter any host cell in animals and humans. Especially, crossing of the blood-brain barrier is not realistic.

He further argued that, as demonstrated under Volume 3 CA B.6, the absorbable metabolites of the Heptamaloxyloglucan are monosaccharides (glucose, fucose, xylose and galactose) and short-chain fatty acids (acetate, propionate and butyrate). They are naturally present in metabolic pathways of animals and humans and are known to be devoid of toxicity except when ingested in very large quantities.

He further argued that these compounds are also produced by degradation of oligosaccharides from vegetal food items or xyloglucans present in apples (from which Heptamaloxyloglucan is prepared), so the metabolites of the Heptamaloxyloglucan are already present in human and animal diet independently of vine treatment with the plant protection product.
The applicant indicated that neither xyloglucan nor Heptamaloxyloglucan metabolites are recognized as substance with established or potential endocrine disrupting activity. None are described in the "Suspected" Endocrine Disruptors List in EU. See: http://ec.europa.eu/environment/chemicals/endocrine/strategy/index_en.htm. [Annex 15 - List of 66 Category 1 substances with categorisation high, medium or low exposure concern and Annex 10 - List of 564 substances with their selection criteria]
Therefore, the applicant did not perform test concerning the endocrine disturbing properties of Heptamaloxyloglucan metabolites.

The general conclusion of the applicant for ED properties was as follows: “Using the EFSA Guidance for the identification of endocrine disruptors, by gathering all relevant information, after assessing their relevance and reliability, it is concluded that heptamaloyloglucan does not possess endocrine disrupting properties and therefore will not result in any “change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences”. No EATS-mediated adversity has been observed or is expected with the oligosaccharidic active substance heptamaloyloglucan.”

Effects on human health

For details of notifier proposal, please refer to Volume 3 CA B.6.8.3 and Appendix E.

RMS conclusions for human health (Volume 3 CA B.6.8.3.) are as follows:
Physico-chemical properties and ADME information suggest that no bioaccumulation potential relates to heptamaloxyloglucan as the unchanged molecule is not absorbed in the digestive tract, and as all metabolites are glucids or short-chain fatty acids which are involved in a large variety of physiological metabolic pathways occurring in Mammals.

The potential endocrine activity has not been investigated since no QSARs, in silico predictions, read across and no in vitro or in vivo assays providing data about selected endocrine mechanism have been provided.

Two in vivo short-term toxicity studies providing data on adverse effects on endocrine endpoints are available:

- The oral 28-day study in rat was performed with heptamaloxyloglucan treated by gavage at dose levels of 50, 200 or 1000 mg/kg b.w./day. In this study, relevant endocrine organs were weighed and examined histologically included testes, epididymides, prostate, seminal vesicles, ovaries, uterus and oviducts, vagina, thymus, adrenals. There was no toxicological effect observed of heptamaloxyloglucan.
- The oral 90-day study in mice is performed with tamarind seed. The results demonstrate that tamarind seed had no short-term toxicity. Contrary to the applicant opinion, RMS does not support the expect lack of short-term toxicity of heptamaloxyloglucan in mice. Indeed, even if the glucidic monomers structure of tamarind seed is similar to heptamaloxylogucan, the bridging is not considered acceptable based on the molecular weight gap (650,000 versus 1,078 g/mol).

The long-term toxicity and carcinogenicity studies provided are supportive studies performed with tamarind seed or protein-bound xyloglucan. RMS does not support the bridging between tamarind seed and heptamaloxyloglucan based on the molecular weight difference. The bridging between protein-bound xyloglucan and heptamaloxyloglucan is not supported based on the glucidic monomers structure.
No EATS mediated adversity has been observed but data are considered limited.

There is no indication of EATS mediated adversity of heptamaloxyloglucan observed in the oral 28-day study, which falls under level 4 of the OECD Conceptual Framework (CF) for endocrine disrupters. Considering additionally its physico-chemical properties and ADME information, and the unlikely potential for accumulation, it does not seem relevant to investigate further endocrine disrupting properties, as per Section 3.1 in "Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009".

Effects on wild mammals

Based on human health conclusion, RMS considered that based on its physico-chemical properties and ADME information, and the unlikely potential for accumulation, it does not seem relevant to investigate further endocrine disrupting properties for wild mammals, as per Section 3.1 in "Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009".

Effects on non-target species other than mammals

- In the effect on birds (CA B.9.1.1)

The notifier provide 3 publications which characterise the effects of xylo-oligosaccharides on the growth performance and immune function of broiler chickens (Morgan and al. 2018 (CA 8.1/04); Suo Hai-qing and all, 2015 (CA 8.1/05) and Yuan and al, 2018 (CA 8.1/06)). These studies showed a beneficial effect on growth and immune performance of broiler chickens when they are supplemented in diet with xylo-oligosaccharides. No investigation of EATS mediated effects have been performed.

- In the effect on fish (CA B.9.2.2)

The notifier provided 4 publications which characterise the effects of arabinoxylan-oligosaccharides on juvenile Siberian sturgeon (Geraylou and al., 2012, CA 8.2.1/02; Geraylou and al., 2013, CA 8.2.1/03) and the effects of fructo-oligosaccharide on common carp (*Cyprinus carpio*) (Hoseinifar and al., 2014, CA 8.2.1/04) and on the Japanese flounder *Paralichthys olivaceus* (Ye and al., 2011, CA 8.2.1/05). The supplementation in arabinoxylan- and fructo-oligosaccharides in their diet of these aquatic species showed no toxic effects and in some studies, beneficial effects in growth performance and immune response. The notifier concluded that oligosaccharides seem to act as prebiotic in fish organisms. No specific investigation of EATS mediated effects have been performed.

RMS conclusions for non-target organisms

Heptamaloxyloglucan has a molecular weight of >1078 daltons. According to EDSP of the US EPA, polymers with molecular weight greater than 1000 daltons are unlikely to interact with the hormone systems, as they are considered not able to cross biological membranes.

In addition, according to the Guidance for the identification of endocrine disruptors (EFSA, 2018): "There may be cases in which due to the knowledge on the physico-chemical and (eco)toxicological properties of the substance an ED assessment does not appear scientifically necessary or testing for this purpose not technically possible (BP Regulation1, Annex IV or PPP Regulation 2, Annex, Point 1.5)."

Therefore, as concluded for human health and as per Section 3.1 in "Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009", RMS considers that it does not seem relevant to investigate further endocrine disrupting properties for non target organisms.

B.9.2. EFFECT ON AQUATIC ORGANISMS

In the studies below, the code EL101GV corresponds to technical heptamaloxyloglucan.

B.9.2.1. Acute toxicity to fish

Data point:	CA 8.2.1/01
Report author	*******.
Report year	2006a
Report title	EL101GV: Acute toxicity in the Rainbow trout under semi-static conditions
Report No	Unpublished report 30711 EAP
Document No	-
Guidelines followed in study	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 from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the Corrigendum to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	Yes, conducted under GLP
Acceptability/Reliability:	Yes/Yes

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and 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 (total hardness 145-158 mg/L as $CaCO_3$ and pH 7.5-8.0).

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 $CaCO_3$.

Table 9.2.1-1. Concentration of EL101GV in the test solution (mg/L)

Nominal	Measured concentrations							
	0h (fresh)		24 h (old)		24 h (fresh)		48h (old)	
	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal
150	159	106	160	107	158	105	162	108
Nominal	Measured concentrations							
	48h (fresh)		72 h (old)		72 h (fresh)		96h (old)	
	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal
150	160	107	156	104	165	110	157	105

Concentrations of heptamaloxyloglucan (EL101GV) during the test were ranged from 157 mg/L (105%) and 165 mg/L (110%). Thus, the analytical results for the active substance concentrations in the test solution were within a range of $\pm$ 20% of the nominal values throughout the test. Hence the results are based on nominal concentrations.

Table 9.2.1-2. Acute toxicity (96 h) of EL101GV on rainbow trout (*Oncorhynchus mykiss*)

Group	Control	Treated
Concentration [mg a.s./L] nominal	0	150
Mortality [%]	0	0
Symptoms	none	None
Endpoint [mg a.s./L nominal]		
LC_{50}	> 150	

-: not measured

No mortality or clinical signs were observed in the control group at 150 mg/L. The pH of the control did not vary by more than 1 unit during the test and the dissolved oxygen concentration remained $\geq$ 60% of the air saturation value throughout the test. The study validity criteria were therefore met.
No mortality was observed in the treated group during the 96-hour observation period. No test item related effects were observed in the fish.

Assessment and conclusion

Assessment and conclusion by applicant:
In a semi-static acute toxicity study with EL101GV, the LC_{50} (96 h) on the rainbow trout was > 150 mg a.s./L (nominal). The NOEC was determined as 150 mg a.s./L, the highest tested concentration.

Assessment and conclusion by RMS:
The study is valid. Method of analysis is valid.

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

B.9.2.2. Long-term and chronic toxicity to fish

In environment, heptamaloxylglucan, a natural component, 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). Moreover, Heptamaloxyloglucan is a major natural component of dicotyledone leaves and vegetal parts, which are constantly and naturally brought to surface waters and sediments.
Overall, chronic exposure of fish to heptamaloxylglucan is not expected and thus chronic data on fish not necessary.

The notifier provided four publications which characterise the effects of arabinoxylan-oligosaccharides on juvenile Siberian sturgeon (Geraylou *and al*., 2012; Geraylou *and a*l., 2013) and the effects of fructo-oligosaccharide on common carp (*Cyprinus carpio*) (Hoseinifar *and al*., 2014) and on the Japanese flounder *Paralichthys olivaceus* (Ye *and al*., 2011). Summaries are provided thereafter.

Data point:	CA 8.2.1/02
Report author	Geraylou Z., Souffreau C., Rurangwa E., D'Hondt S., Callewaert L., Courtin C. M., Delcour Jan A.; Buyse J., Ollevier F. et al.
Report year	2012
Report title	Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (*Acipenser baerii*) performance, immune responses and gastrointestinal microbial community
Report No	Fish & Shellfish Immunology 33 (2012) 718e724
Document No	6
Guidelines followed in study	None (Public literature)
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	-
Acceptability/Reliability:	Yes/Supportive information. This literature study is not GLP, the sugar used in the design experiment is close to heptamaloxyloglucan but not identical and fish used are not common species used for risk assessment.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study reports the investigation of a diet of 12 weeks containing 2% of two kinds of Arabinoxylan-oligosaccharides (AXOS) obtained from wheat bran on growth performance (weigh gain, specific growth rate and feed conversion ratio), immune responses (cellular immune response: phagocytic activity, respiratory bursts activity; humoral immune response: alternative complement activity, serum oxidase, lysozyme activity), gut microbial fermentation (short-chain fatty acids, SCFAs) and gut bacterial composition of juvenile Siberian sturgeon (*Acipenser baerii*, mean weight 25.9±0.9 g). The results are compared to a control group feed with standard diet.

Results

Survival was high in all groups. Growth performance and feed utilization has not been significantly altered by the AXOS-enriched diet (Cf. Table below).

Table 9.2.2-1. Growth rate and survival of juvenile Siberian sturgeon fed with a control diet or diets supplemented with 2% AXOS-3-0.25 or AXOS-32-0.30.

Parameter	Control	AXOS-3-0.25	AXOS-32-0.30
Initial body weight (g)	25.8 ± 0.8	25.6 ± 0.7	26.2 ± 1.0
Final body weight (g)	133.8 ± 5.8	134.9 ± 6.7	145.1 ± 7.1
Weight gain rate (%)	416 ± 24	426 ± 26	464 ± 29
Specific growth rate	1.90 ± 0.02	1.99 ± 0.06	2.07 ± 0.05
Feed conversion rate	1.48 ± 0.05	1.51 ± 0.02	1.47 ± 0.05
Survival (%)	100 ± 0.0	96 ± 2.2	98 ± 1.4

Table values are group means ± SE (8 replicates per group).

AXOS preparation with high degree of polymerization significantly enhanced the immune responses measured except the lysozyme activity (Cf. Table below). The concentrations of acetate, butyrate and total SCFAs in fish fed AXOS preparation with high degree of polymerization was significantly higher than in the groups fed the control diet or AXOS preparation with low degree of polymerization. Both preparations of AXOS induced changes in the bacterial composition with stimulation of the growth of lactic acid bacteria and *Clostridium sp.*

Table 9.2.2-2. Immune responses of Siberian sturgeon fed a control diet or diets supplemented with 2% AXOS-3-0.25 or AXOS-32-0.30.

Parameter	Dietary group			*P* value
	Control	AXOS-3-0.25	AXOS-32-0.30	
Cellular immune responses				
Phagocitic activity (%)	56.0 ± 1.2^{a}	61.2 ± 1.4^{b}	62.0 ± 1.2^{b}	*0.044*
Respiratory burst activity (O.D. at 630 nm)	0.189 ± 0.08^{a}	0.238 ± 0.11^{a}	0.264 ± 0.11^{a}	*0.399*
Humoral immune responses				
Alternative complement activity (ACH_{50} U ml^{-1})	34.1 ± 4.5^{a}	50.2 ± 5.3^{ab}	58.0 ± 4.0^{c}	*0.039*
Serum peroxidase (Stimulation index)	1.30 ± 0.11^{a}	1.35 ± 0.14^{ab}	1.88 ± 0.24^{c}	*0.015*
Lysozyme activity (μg ml^{-1} serum)	8.62 ± 3.05^{a}	8.13 ± 3.08^{a}	9.01 ± 3.19^{a}	*0.373*

Values are presented as mean ± standard error ($n = 8$).
Values with a different superscript in the same line are significantly different ($P < 0.05$).
O.D. = Optical Density.

Assessment and conclusion

Assessment and conclusion by applicant: The two AXOS preparations showed an absence or an improvement on the different parameters evaluated in this study. The beneficial effect appears to be associated with the degree of polymerization of AXOS since a higher degree of polymerization of AXOS had a stronger beneficial impact in this sturgeon species.

Assessment and conclusion by RMS:

Comparison of the effects of two different preparations of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon indicated that AXOS-32-0.30, the preparation with a higher degree of polymerization, improves immune responses of fish. This observed enhancement of immune responses is probably related to the changes of the hindgut microbiota communities and the subsequent enhancement of short-chain fatty acid production. These results suggest that AXOS with a high degree of polymerization has the potential to be introduced as prebiotic in the feed of Siberian sturgeon.

Siberian sturgeon is not a specie usually used for risk assessment. The results of the study show that arabinoxylan-oligosaccharides (AXOS) which are sugars similar to heptamaloxyloglucan are not toxic to juvenile fish. Thus, this literature study can be used as supportive information.

Data point:	CA 8.2.1/03
Report author	Geraylou Z., Souffreau C., Rurangwa E., De Meester L., Courtin C. M., Delcour Jan A., Buyse J., Ollevier F., *et al*.
Report year	2013
Report title	Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*)
Report No	-
Document No	Fish & Shellfish Immunology 35 (2013) 766e775
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/ Supportive information. T his literature study is not GLP, the sugar used in the design experiment is close to heptamaloxyloglucan but not identical and fish used are not common species for risk assessment.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

The effect of administration of putative probiotics (*Lactococcus lactis spp. lactis* or *Bacillus circulans*) alone and in combination with arabinoxylan-oligosaccharides (AXOS) has been investigated in juvenile Siberian sturgeon (*Acipenser baerii*, mean weight 48.4±1.4 g) on growth performance (weigh gain, specific growth rate and feed conversion ratio), non-specific immunity parameters (phagocytic activity, respiratory bursts activity, alternative complement activity, serum oxidase, total immunoglobulin), hindgut microbiota diversity. Height diets of 4 weeks have been investigated: basal diet (Diet 1), basal diet supplemented with 2% AXOS (Diet 2), or *L. lactis* ST G81 (Diet 3), *L. lactis* ST G45 (Diet 4), *B. circulans* ST M53 (Diet 5), *L. lactis* ST G81 + 2% AXOS (Diet 6), *L. lactis* ST G45 + 2% AXOS (Diet 7), *B. circulans* ST M53 + 2% AXOS (Diet 8).

Results

The study shows that after 4 weeks of treatment, growth performance of fish was positively affected by AXOS diets ($p < 0.05$), independently to the supply of candidate probiotics (Table below). No interaction was found between the candidate probiotics and AXOS group treatments ($p > 0.05$).

Table 9.2.2-3. Growth performance, feed conversion ratio and survival of the Siberian sturgeon fed different experimental diets

Diet	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (% day^{-1})	FCR	Survival (%)
Diet 1 (Basal feed)	50.7 ± 0.4^{a}	87.8 ± 5.5^{ab}	37.0 ± 5.1^{a}	1.95 ± 0.15^{a}	1.31 ± 0.25^{a}	100
Diet 2 (Basal feed + AXOS)	47.8 ± 2.8^{a}	95.0 ± 4.9^{ab}	47.1 ± 7.0^{ab}	2.45 ± 0.27^{ab}	1.19 ± 0.35^{ab}	100
Diet 3 (Basal feed + *L. lactis* spp. *lactis* ST G81)	48.0 ± 1.2^{a}	87.75 ± 5.2^{ab}	39.6 ± 4.9^{ab}	2.14 ± 0.15^{ab}	1.18 ± 0.01^{ab}	100
Diet 4 (Basal feed + *L. lactis* spp. *lactis* ST G45)	48.0 ± 2.5^{a}	89.1 ± 1.1^{ab}	41.1 ± 3.4^{ab}	2.21 ± 0.17^{ab}	1.06 ± 0.04^{ab}	100
Diet 5 (Basal feed + *B. circulans* ST M53)	49.5 ± 0.6^{a}	91.3 ± 2.7^{ab}	41.7 ± 2.6^{ab}	2.18 ± 0.08^{ab}	1.24 ± 0.31^{ab}	100
Diet 6 (Basal feed + *L. lactis* spp. *lactis* ST G81 + AXOS)	46.0 ± 4.3^{a}	85.0 ± 4.8^{a}	39.0 ± 5.3^{ab}	2.19 ± 0.11^{ab}	1.17 ± 0.15^{ab}	100
Diet7 (Basal feed + *L. lactis* spp. *lactis* ST G45 + AXOS)	49.0 ± 1.3^{a}	105.9 ± 5.1^{b}	56.8 ± 4.7^{b}	2.74 ± 0.11^{b}	0.92 ± 0.01^{b}	100
Diet 8 (Basal feed + *B. circulans* ST M53 + AXOS)	48.1 ± 1.3^{a}	91.2 ± 14.3^{ab}	43.0 ± 13.4^{ab}	2.24 ± 0.38^{ab}	1.14 ± 0.22^{ab}	100
Two-way ANOVA						
***p*-Value**						
Probiotics	0.35	0.11	0.12	0.19	0.20	
AXOS	0.15	0.09	0.03	0.02	0.04	
Interaction probiotics * AXOS	0.46	0.13	0.15	0.25	0.29	

Data are means of triplicate. Means in the same column sharing a same superscript letter are not significantly different ($p < 0.05$) (by Two-way ANOVA). SGR: specific growth rate, FCR: feed conversion Ratio.

Innate immune responses were boosted with both AXOS and probiotic diets, however synergistic effects of AXOS and probiotic diets were only observed for phagocytic and alternative complement activity. AXOS improved the colonization or/and growth capacity of *L. lactis*, as a higher relative abundance of *L. lactis* was observed in fish receiving the diet composed of *L. lactis* ST G45 + AXOS (Diet 7). However, the other diets including *L. lactis* ST G81 and *B. circulans* ST M53 did not improved these parameters and diet 7 caused a decrease in gut bacteria diversity (Table below).

Table 9.2.2-4. Immune responses of juvenile Siberian sturgeon fed different experimental diets for 4 weeks.

Diet	Phagocytic activity (%)	Respiratory burst activity (O.D. at 630 nm)	Alternative complement activity (ACH 50 U ml^{-1})	Serum peroxidase (stimulation index)	Total immunoglobulin (mg ml^{-1})
Diet 1(Basal feed)	35.0 ± 1.55^{a}	0.143 ± 0.02^{a}	0.39 ± 0.05^{a}	1.24 ± 0.09^{a}	29.3 ± 3.2^{a}
Diet 2 (Basal feed + AXOS)	48.6 ± 3.01^{b}	0.258 ± 0.02^{b}	0.56 ± 0.04^{ab}	1.47 ± 0.11^{ab}	34.0 ± 4.3^{a}
Diet 3 (Basal feed + *L. lactis* spp. *lactis* ST G81)	36.0 ± 2.35^{ab}	0.174 ± 0.01^{ab}	0.42 ± 0.08^{a}	1.33 ± 0.13^{a}	33.3 ± 9.4^{a}
Diet 4 (Basal feed + *L. lactis* spp. *lactis* ST G45)	43.0 ± 4.24^{b}	0.221 ± 0.03^{ab}	0.41 ± 0.11^{a}	1.47 ± 0.09^{ab}	32.6 ± 5.4^{a}
Diet 5 (Basal feed + *B. circulans* ST M53)	41.3 ± 0.89^{ab}	0.146 ± 0.01^{a}	0.41 ± 0.07^{a}	1.29 ± 0.12^{a}	28.0 ± 5.4^{a}
Diet 6 (Basal feed + *L. lactis* spp. *lactis* ST G81 + AXOS)	41.0 ± 3.0^{ab}	0.210 ± 0.04^{ab}	0.45 ± 0.03^{ab}	1.40 ± 0.04^{ab}	31.3 ± 6.9^{a}
Diet 7 (Basal feed + *L. lactis* spp. *lactis* ST G45 + AXOS)	50.0 ± 2.94^{b}	0.275 ± 0.01^{b}	0.61 ± 0.03^{b}	1.98 ± 0.28^{b}	30.6 ± 5.6^{a}
Diet 8 (Basal feed + *B. circulans* ST M53 + AXOS)	42.6 ± 1.79^{ab}	0.210 ± 0.02^{ab}	0.42 ± 0.02^{a}	1.31 ± 0.27^{a}	31.6 ± 6.8^{a}
Two-way ANOVA					
***p*-Value**					
Candidate probiotics	0.01	0.08	0.06	0.01	0.53
AXOS	0.01	0.00	0.00	0.05	0.12
Interaction probiotics * AXOS	0.05	0.48	0.03	0.22	0.32

O.D. = optical density.
Values are presented as mean ± standard error ($n = 9$).
Values with a different superscript in the same column are significantly different ($p < 0.05$).

Assessment and conclusion

Assessment and conclusion by applicant: The present study supports that a AXOS-enriched diet improves growth performance, innate immune response and hindgut microbiota diversity in Siberian sturgeons. It also shows that in spite that AXOS and the candidate probiotic *L. lactis* ST G45 can a have a synergetic positive effect on *L. lactis* ST G81 colonisation the combination does not involve an improvement of bacterial diversity, growth performance and boosted immune responses of Siberian sturgeon compared to AXOS alone.

Assessment and conclusion by RMS:

In the present study, separate administration of arabinoxylan-oligosaccharides (AXOS) did not significantly affect the growth of juvenile Siberian sturgeon. Innate immune responses of fish were boosted with both AXOS and probiotic diets, however synergistic effects of AXOS and probiotic diets were only observed for phagocytic and alternative complement activity.

Outcome and conclusion of the study: The study can be used as supportive information for risk assessment. This study demonstrate that arabinoxylan-oligosaccharides (AXOS) did not affect the growth of juvenile Siberian sturgeon and did not increase the mortality of fish.

Data point: CA 8.2.1/04
Report author Hoseinifar, S. H.; Soleimani, N.; Ringo, E *et al.*
Report year 2014

Report title	Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry
Report No	-
Document No	British Journal of Nutrition (2014), 112, 1296–1302
Guidelines followed in study	-
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP (literature study)
Acceptability/Reliability:	Yes/Supportive information. T he common carp (*Cyprinus carpio*) is a specie used in risk assessment but Fructo-oligosaccharide (FOS) are close to Heptamaloxyloglucan but not identical

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study investigated the effects of diets supplemented in fructo-oligosaccharide (FOS) (0, 1, 2 and 3 % for 7 weeks) on the growth performance and survival rate, haemato-immunological parameters (including erythrocyte count, leucocyte counts, respiratory burst activity), cultivable autochthonous (non-adherent) intestinal microbiota and stress resistance of 480 common carp (*Cyprinus carpio*) fry (mean weight 3.23 ± 0.14 g).

Results

FOS supplementation had no significant effects on the growth performance and food intake of carp fry compared with the control treatment (Table below).

Table 9.2.2-5. Growth performance parameters and survival rate of common carp fry fed diets supplemented with varying levels of fructo-oligosaccharide for 7 weeks

(Mean values with their standard errors)

	Control		1 %		2 %		3 %	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial weight (g)	3·35	0·15	3·30	0·11	3·20	0·20	3·10	0·18
Final weight (g)*	9·79	0·29	9·35	0·62	9·57	0·25	10·10	0·41
Weight gain (%)†	192·20	6·03	178·68	14·46	199·61	13·65	225·22	18·57
SGR	1·91	0·11	1·85	0·16	1·95	0·12	2·05	0·15
CF	1·29	0·08	1·30	0·04	1·30	0·07	1·35	0·09
FCR‡	3·48	0·11	3·71	0·45	3·51	0·26	3·21	0·31
Survival (%)§	$61·60^{a}$	7·07	$83·30^{a,b}$	9·37	$78·40^{a,b}$	11·73	$98·30^{b}$	2·40

SGR, specific growth rates; CF, condition factor; FCR, feed conversion ratio.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P>0·05$).

* $\alpha = 0·05$; $\beta = 0·17$.

† $\alpha = 0·05$; $\beta = 0·15$.

‡ $\alpha = 0·05$; $\beta = 0·11$.

§ $\alpha = 0·05$; $\beta = 0·16$.

It also had no significant effects on the following haematological parameters: erythrocyte count; haematocrit; Hb; mean corpuscular volume; mean corpuscular Hb content; mean corpuscular Hb concentration. However, leucocyte

count and respiratory burst activity were significantly increased by dietary FOS supplementation (Table below). Evaluation of the cultivable autochthonous intestinal microbiota revealed a significant increase in the levels of total viable heterotrophic aerobic bacteria and lactic acid bacteria in fish fed diets supplemented with 2 and 3% FOS. Furthermore, FOS supplementation significantly increased the survival rate and stress resistance of carp fry compared with the control treatment.

Table 9.2.2-6. Differential leucocyte counts of common carp fry fed diets supplemented with different levels of fructo-oligosaccharide (FOS) for 7 weeks

(Mean values with their standard errors)

	Leucocyte count ($\times 10^3$ per μl)*		Lymphocytes (%)		Neutrophils (%)		Monocytes (%)	
Groups	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	32·66[a]	2·08	92·33[a]	2·08	5·66[a]	0·57	2·01[a]	0·32
1 % FOS	36·26[a,b]	3·55	93·03[a]	1·25	5·36[a]	1·52	1·61[a]	0·70
2 % FOS	37·33[a,b]	2·81	92·96[a]	1·15	5·33[a]	1·15	1·71[a]	0·41
3 % FOS	39·30[b]	1·15	92·60[a]	2·64	5·60[a]	1·63	1·80[a]	0·90

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P > 0·05$).
* $\alpha = 0·05$; $\beta = 0·12$.

Assessment and conclusion

Assessment and conclusion by applicant: This study supports that exposure of broilers to diets supplemented with FOS has no significant effects on the growth performance and haematological parameters of carp fry, but significantly increased leucocyte count, respiratory burst activity and modulated gut microbiota levels and improved stress resistance of carp fry.

Assessment and conclusion by RMS:
Fructo-oligosaccharide (FOS) is a fructan with a degree of polymerisation (2–20) that is obtained by enzymatic hydrolysis of inulin. FOS is present in a number of common foods such as garlic, onion, artichoke, and asparagus, and dietary FOS has received great attention as a prebiotic for aquatic animals.

In conclusion, the results of the present study showed that 1, 2 or 3% FOS supplementation had no significant effects on the growth performance of carp fry. FOS supplementation significantly increased the leucocyte counts and respiratory burst activity and modulated cultivable autochthonous gut microbiota levels and stress resistance.

RMS considers that this study can be used as supportive information. Indeed, the common carp (Cyprinus carpio) is a specie used in risk assessment. Fructo-oligosaccharide (FOS) are close to Heptamaloxyloglucan but not identical. Fructo-oligosaccharide (FOS) did not increase the mortality of fish and had no effect on growth.

Data point:	CA 8.2.1/05
Report author	Ye, J. D.; Wang, K.; Li, F. D.; Sun, Y. Z., *et al.*
Report year	2011
Report title	Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralichthys olivaceus.*
Report No	Aquaculture Nutrition 2011 17; e902–e911
Document No	-
Guidelines followed in study	None

Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive information. T he common carp (Cyprinus carpio) is a specie used in risk assessment but Fructo-oligosaccharide (FOS) are close to Heptamaloxyloglucan but not identical

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

In this study, the effects of diets supplemented in various concentrations of fructo-oligosaccharides (FOS), mannan oligosaccharides (MOS) and *Bacillus clausii* have been investigated on the fish Japanese flounder. Japanese flounder, initially weighing an average of 21 g, were distributed into 24 net cages at a stocking density of 20 fish per cage. Each diet was hand-fed to three groups of fish twice daily for 56 days. The effect of the different diets has been evaluated on growth performance, body composition, digestive enzyme activity, blood parameters, innate immune response and lipid metabolism.

Results

All fish maintained active ingestion, exhibited proper growth during the feeding period and survived each dietary treatment. Singe diet with MOS increased the final body weight. There were no significant differences ($P > 0.05$)in final body weight (BWG) and weight gain ration (WGR) among any FOS, MOS treatments (Table 1). In contrast, the fish that were fed diets with MOS had significantly lower feed conversion factor (FCR) than those fed the control diet ($P < 0.05$). Neither the feed intake (FI) nor the condition factor (CF) differ among any of the dietary treatments ($P > 0.05$).

Table 9.2.2-7. Growth performance of Japanese flounder fed the experimental diets

Diets[1]	IBW[2]	FBW[2]	FI[2]	WGR[2]	FCR[2]	CF[3]
Control	21.4 ± 0.1	73.5 ± 0.9^{b}	60.0 ± 0.4	242.7 ± 6.4^{b}	1.17 ± 0.01^{a}	0.85 ± 0.06
Diet F	21.5 ± 0.2	74.4 ± 2.1^{ab}	60.1 ± 0.9	245.8 ± 11.2^{ab}	1.15 ± 0.04^{ab}	0.82 ± 0.06
Diet M	21.5 ± 0.2	75.5 ± 0.6^{a}	60.7 ± 0.6	251.2 ± 2.8^{ab}	1.13 ± 0.01^{bc}	0.84 ± 0.04
Diet FM	21.4 ± 0.3	75.2 ± 0.5^{a}	60.5 ± 0.3	252.0 ± 5.3^{ab}	1.13 ± 0.01^{bc}	0.85 ± 0.05
Diet B	21.2 ± 0.2	73.9 ± 0.8^{ab}	61.0 ± 0.1	248.5 ± 5.8^{ab}	1.16 ± 0.02^{ab}	0.85 ± 0.02
Diet FB	21.3 ± 0.2	75.0 ± 0.4^{ab}	60.0 ± 0.3	251.8 ± 4.5^{ab}	1.12 ± 0.01^{c}	0.81 ± 0.03
Diet MB	21.2 ± 0.1	75.3 ± 0.7^{a}	61.0 ± 0.5	255.2 ± 2.5^{a}	1.13 ± 0.02^{bc}	0.82 ± 0.02
Diet FMB	21.2 ± 0.2	75.5 ± 0.9^{a}	60.7 ± 0.3	255.5 ± 1.8^{a}	1.12 ± 0.02^{c}	0.82 ± 0.01

[1] Control diet (no FOS, MOS and *Bacillus clausii*), diet F (5 g kg^{-1} FOS), diet M (5 g kg^{-1} MOS), diet FM (2.5 g kg^{-1} FOS + 2.5 g kg^{-1} MOS), diet B (10^{7} cells g^{-1} *B. clausii*), diet FB (5 g kg^{-1} FOS + 10^{7} cells g^{-1} *B. clausii*), diet MB (5 g kg^{-1} MOS + 10^{7} cells g^{-1} *B. clausii*) and diet FMB (2.5 g kg^{-1} FOS + 2.5 g kg^{-1} MOS + 10^{7} cells g^{-1} *B. clausii*).

[2] Data from each dietary treatment are mean ± SD of triplicate cages.

[3] Data from each dietary treatment are mean ± SD of 12 fish.

Values with different superscripts within a column indicate significant differences ($P < 0.05$).

FOS, fructo oligosaccharides; MOS, mannan oligosaccharides; IBW, initial body weight (g per fish); FBW, final body weight (g per fish); FI, feed intake (g); WGR, weight gain rate (%); FCR, feed conversion ratio; CF, condition factor (%).

There was an increase in body protein content in fish fed a FOS-, MOS-diet compared to control (Table below).

The phagocytic percentage (PP) and (phagocytic index (PI) of leucocytes in fish fed diets supplemented with FOS or MOS were similar to those of fish fed the control diet. Activities of protease and amylase is not modified by FOS- and MOS-diet, compared to control.

Table 9.2.2-8. Body composition of Japanese flounder fed the experimental diets

Diets[1]	Moisture	Crude protein	Crude lipid	Ash
Control	784.5 ± 6.8	142.0 ± 4.3^{b}	30.2 ± 3.6^{a}	37.8 ± 2.3
Diet F	781.0 ± 5.5	146.4 ± 6.1^{ab}	28.0 ± 1.7^{ab}	37.7 ± 1.1
Diet M	782.9 ± 4.5	144.7 ± 4.2^{ab}	30.1 ± 2.4^{a}	37.1 ± 3.6
Diet FM	781.4 ± 5.2	147.1 ± 6.0^{ab}	28.7 ± 4.5^{ab}	37.8 ± 1.9
Diet B	784.6 ± 1.3	145.3 ± 2.2^{ab}	26.0 ± 3.2^{b}	36.9 ± 2.5
Diet FB	779.3 ± 2.0	149.1 ± 1.3^{a}	26.9 ± 1.5^{ab}	37.3 ± 2.6
Diet MB	786.2 ± 5.5	145.0 ± 4.2^{ab}	26.5 ± 2.2^{b}	37.0 ± 2.9
Diet FMB	782.4 ± 5.0	147.7 ± 7.0^{a}	26.0 ± 2.9^{b}	36.3 ± 1.8

Values are expressed as wet body basis.
FOS, fructo oligosaccharides; MOS, mannan oligosaccharides.
[1] Control diet (no FOS, MOS and *Bacillus clausii*), diet F (5 g kg^{-1} FOS), diet M (5 g kg^{-1} MOS), diet FM (2.5 g kg^{-1} FOS + 2.5 g kg^{-1} MOS), diet B (10^7 cells g^{-1} *B. clausii*), diet FB (5 g kg^{-1} FOS + 10^7 cells g^{-1} *B. clausii*), diet MB (5 g kg^{-1} MOS + 10^7 cells g^{-1} *B. clausii*) and diet FMB (2.5 g kg^{-1} FOS + 2.5 g kg^{-1} MOS + 10^7 cells g^{-1} *B. clausii*).
Data from each dietary treatment are mean ± SD of triplicate cages.
Values are expressed on a wet weight basis (g per kg).
Values with different superscripts within a column indicate significant difference ($P < 0.05$).

Assessment and conclusion

Assessment and conclusion by applicant: This study shows that administration of FOS-, MOS-supplemented diets has no effect on growth, feed efficiency, nutrient deposition, digestive enzyme activity, non-specific immunity and lipid metabolism in the fish Japanese flounder.

Assessment and conclusion by RMS:
Fructo oligosaccharides (FOS) and mannan oligosaccharides (MOS) are two frequently used prebiotics with unique chemical structures that may improve the gut health and ecosystem of the host in different ways.

In this study, the effects of the following eight experimental diets, which varied in fructo oligosaccharides (FOS), mannan oligosaccharides (MOS) and *Bacillus clausii* concentrations, on the Japanese flounder were examined. Japanese flounder, initially weighing an average of 21 g, were distributed into 24 net cages at a stocking density of 20 fish per cage. Each diet was hand-fed to three groups of fish twice daily for 56 days.

The weight gain rate (WGR) in fish fed diets with MOS and FOS associated with *B. clausii* were significantly higher than in fish fed the control diet. Without exception, no diets affected feeding rate, condition factor, body moisture, ash contents, phagocytic activity of leucocytes or cholesterol or high-density lipoprotein cholesterol levels. This results suggest that diets supplemented with FOS, MOS and *B. clausii* improved growth performance and health benefits of the Japanese flounder more than other diets or the control diet.

RMS considers that this study can be used as supportive information. Indeed, the Japanese flounder (*Paralichthys olivaceus*) is not a specie used in risk assessment. Mannan oligosaccharides (MOS) and Fructo-oligosaccharide (FOS) are close to Heptamaloxyloglucan but not identical. FOS and MOS did not increase the mortality of fish and had no negative effect on growth.

B.9.2.2.1. Fish early life stage toxicity

No early life stage toxicity test in fish is required, since Heptamaloxyloglucan is a natural component of vegetal decays, undergoes natural degradation pathways in water and sediments, and displays no acute toxicity to fish.

B.9.2.2.2. Fish full life cycle test

No life cycle test in fish is required, since Heptamaloxyloglucan is a natural component of vegetal decays, undergoes natural degradation pathways in water and sediments, and displays no acute toxicity to fish. Moreover no adverse effects on juvenile fish were observed in the literature studies summarized above (Geraylou, 2012; Geraylou, 2013; Hoseinifar, 2014; Ye, 2011)

B.9.2.2.3. Bioconcentration in fish

The notifier considered that unchanged Heptamaloxyloglucan will not be 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 Regulation 283/2013 and the Guidance Document on Aquatic Ecotoxicology (EFSA Journal 2013;11(7):3290), 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 below 0 (see Vol. 3CA B2, point B.2.7), no study is required.

B.9.2.3. Potential for endocrine disruption

Refer to B.9.1.5.

B.9.2.4. Acute toxicity to aquatic invertebrates

Acute toxicity test on aquatic invertebrates has been performed with technical heptamaloxyloglucan (coded EL101GV).

Data point:	CA 8.2.4/01
Report author	*******.
Report year	2006b
Report title	EL101GV- Acute toxicity in *Daphnia magna* under static conditions.
Report No	30710 EAD
Document No	-
Guidelines followed in study	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 from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the *Corrigendum* to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	Yes, conducted under GLP
Acceptability/Reliability:	Yes/Yes

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and 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 $CaCO_3$.

For a test to be valid, the following performance criteria apply:
- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilized.
In the control test, 0% of the daphnids have been immobilized.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.
The concentration of $CaCO_3$. is 303 mg/L
Thus, the criteria of validity were fulfilled.

Concentration of heptamaloxyloglucan (EL101GV) at test initiation was 144 mg/L (96%) and 126 mg/L (84%) at test termination (Table 9.2.4-1).

Table 9.2.4-1. Concentration of EL101GV in the test solution (mg/L)

Nominal (mg/L)	Measured concentrations (mg/L)			
	0h		48 h	
	Meas. Conc.	% of nominal	Meas. Conc.	% of nominal
150	144	96	126	84

Therefore toxicity results (immobilisation) are based on nominal concentration.

There were no mortalities or clinical signs at nominal concentration of 150 mg/L (Table 9.2.4-2).

Table 9.2.4-2. Effect (48 h) of EL101GV on *Daphnia magna* immobility

Group	Control	EL101GV
Concentration (nominal) [mg a.s./L]	0	150
Immobile (24 h) [%]	0	0
Immobile (48 h) [%]	0	0
	Endpoints [mg a.s./L]	
EC_{50} (48 h)	> 150	
NOEC (48 h)	150	

Assessment and conclusion

Assessment and conclusion by applicant: In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of EL101GV was determined to be > 150 mg a.s./L. The NOEC was 150 mg a.s./L.

Assessment and conclusion by RMS:

The validity criteria were fulfilled. The methods of analysis are valid. *Daphnia magna* 48h static EC_{50} appears to be greater than 150 mg heptamaloxyloglucan/L. The NOEC was determined as 150 mg a.s./L (nominal), the highest tested concentration. This is equivalent to 117 mg/L pure heptamaloxyloglucan (nominal).

The study is valid and can be used for risk assessment.

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

In environment, heptamaloxylglucan, a natural component, 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). Moreover, Heptamaloxyloglucan is a major natural component of dicotyledone leaves and vegetal parts, which are constantly and naturally brought to surface waters and sediments. Heptamaloxyloglucan displays no acute toxicity to aquatic invertebrates.

Overall, chronic exposure of aquatic invertebrates to heptamaloxylglucan is not expected and thus chronic data on aquatic invertebrates not necessary.

B.9.2.6. Development and emergence in *Chironomus riparius*

Not required. See B.9.2.5.

B.9.2.7. Sediment dwelling organisms

Not required. See B.9.2.5.

B.9.2.8. Effects on algal growth

Data point:	CA 8.2.6/01
Report author	L'Haridon J.
Report year	2006c
Report title	EL101GV: Algal growth inhibition test
Report No	30709 EAD
Document No	-
Guidelines followed in study	OECD 201 (7th June 1984); Commission Directive 92/69/EEC C.3 (31st July 1992)
Deviations from current test guideline	pH varies from more than 1.5 units during the test. This deviation is not considered to impact results of the study
Previous evaluation	Yes, evaluated and accepted in the *Corrigendum* to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	Yes, conducted under GLP
Acceptability/Reliability:	Yes/Yes

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and 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 (Table 9.2.8-1).

Table 9.2.8-1. Concentration of EL101GV in the test solution (mg/L)

Nominal (mg/L)	Measured concentrations (mg/L)					
	0h		72 h			
	Meas. Conc.	% of nominal	Meas. Conc. (with algae)	% of nominal	Meas. Conc. (without algae)	% of nominal
150	155	103	165	110	158	105

For the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day^{-1}.
  The biomass in control cultures increased by a factor of 282.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.
  The mean coefficient of variation for section-by-section specific growth rates in the control cultures is 16.18%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests.
  The coefficient of variation of average specific growth rates in control culture is 0.88%.

Thus, the criteria of validity are fulfilled.

The table below summarised the main results obtained during the study.

Table 9.2.8-2. Cell densities, growth 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.

Assessment and conclusion

Assessment and conclusion by applicant:
In a 72-hour algae test with *Scenedesmus subspicatus*, the E_rC_{50} of EL101GV was determined to be > 150 mg a.s./L, the E_bC_{50} was > 150 mg a.s./L (nominal).

Assessment and conclusion by RMS:
The validity criteria were met. The methods of analysis are valid.

The study is valid and can be used for risk assessment.

The alga (*Scenedesmus subsipacatus*) 72h static E_rC_{50} and E_rC_{10} values are greater than 150 mg heptamaloxyloglucan/L.

According to Commission Regulation (EU) No 283/2013, studies on the effects on a second algal species are only required for herbicides. Since heptamaloxyloglucan does not belong to this class of products, a study on a second algal species is not required.

B.9.2.9. Effects on aquatic macrophytes

According to Commission Regulation (EU) No 283/2013, a "laboratory test with Lemna species shall be performed for herbicides and plant growth regulators and for substances where there is evidence from information submitted under point 8.6 of Part A of this Annex or point 10.6 of Part A of the Annex to Regulation (EU) No 284/2013 that the test substance has herbicidal activity."

Heptamaloxyloglucan is a plant elicitor and as such could be considered as a plant growth regulator. Nevertheless, no tests on aquatic plants have been submitted by the notifier. RMS agreed to consider that test on aquatic macrophytes are not deemed necessary as no phytotoxicity on terrestrial plants or on alga have been observed. Moreover in the environment, heptamaloxylglucan, a natural component, is degraded in smaller oligosaccharides, then to monomers and finally to CO2. It is soluble in water and is expected to be quickly degraded (see section B.8). Moreover, Heptamaloxyloglucan is a major natural component of dicotyledone leaves and vegetal parts, which are constantly and naturally brought to surface waters and sediments. Heptamaloxyloglucan displays no acute toxicity to alga and terrestrials non target plants.
Data on aquatic plants are not considered necessary.

B.9.2.10. Further testing on aquatic organisms

Because of the low toxicity of heptamaloxyloglucan on aquatic organisms observed in acute toxicity tests, no further testing is required on aquatic organisms.

B.9.2.11. Effects on aquatic plants

See B.9.2.9.

B.9.2.12. Microcosm or mesocosm study

Study not needed.

B.9.2.13. Residue data in fish

There is no indication for a bioaccumulation potential of heptamaloxyloglucan due to low log K_{ow} (-15.96) (for details please refer to Volume 3 (CA) Section 2).

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute toxicity

B.9.3.1.1.1. Acute contact and oral toxicity on honey bees (*A. mellifera*; laboratory studies)

Data point:	CA 8.3.1.1/01
Report author	Servajean E. *et al.*
Report year	2006
Report title	Laboratory determination of the contact and oral acute toxicity of a formulation to honey bees (*Apis mellifera*)
Report No	-
Document No	05-27-064-ES
Guidelines followed in study	OECD Method 213 OECD Method 214
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted *in the Corrigendum to the DAR (2009).*
GLP/Officially recognised testing facilities[1,2]	Yes, conducted under GLP
Acceptability/Reliability:	Yes/Yes

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Executive Summary

The purpose of the study was to determine the contact and oral acute effects, on mortality and behaviour, after 48 hours of exposure according to OECD 213&214 of the formulation to honey bees in a dose limit test at 100 µg/bee under laboratory conditions. The mortality and behaviour was assessed after 4, 24 and 48 hours of exposure.

Under laboratory test conditions, no oral toxicity of heptamaloxyloglucan has been observed. The acute oral and contact LD_{50} of EL101GV was estimated to be greater than 100 µg a.s./bee.

Materials and methods

Honeybees (*Apis mellifera*, unknown age) were subjected to contact and oral limit tests with technical heptamaloxyloglucan (EL101GV, batch AND0205, purity: 87.1%, storage in a room temperature protected from direct sun light).
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 (purity 99.9%, batch 3209X). 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 in round cage, at 24-27°C in darkness (except during observation). 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. Bee were starved for 1-2 hours before treatment. Bee were exposed to the product in cup and were allowed to feed during 4 hours.
For contact toxicity testing, treatment solution was prepared using 501.2 mg of the test item in 5mL of carrier (solution of water/acetone 50/50 v/v). Bees were immobilized. 20 bees were selected and individually dosed by delivering 1 µL of treatment and control solution on the thorax.
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

Validity of the oral toxicity test:
For a test to be valid the following conditions apply:
- the average mortality for the total number of controls must not exceed 10 per cent at the end of the test. The average mortality for controls is 5.3%.
- the LD50 of the toxic standard meets the specified range. 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.

Validity of the contact toxicity test:
For a test to be valid the following conditions apply:
- the average mortality for the total number of controls must not exceed 10 per cent at the end of the test. The average mortality for controls is 5.0%
- the LD50 of the toxic standard meets the specified range. 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.

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 9.3-1: 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 9.3-2: 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.

Assessment and conclusion

Assessment and conclusion by applicant:
Oral EL101GV LD_{50} 24 h and LD_{50} 48h > 100 µg/bee
Contact EL101GV LD_{50} 24 h and LD_{50} 48h > 100 µg/bee

Assessment and conclusion by RMS:
The study is valid.
In both oral and contact toxicity test, the 48h LD_{50} was > 100 µg heptamaloxyloglucan./bee.

B.9.3.1.1.2. Additionnal information on acute toxicity of carbohydrates to bees

Data point:	CA 8.3.1/01
Report author	Winston M.L. et al.
Report year	1987
Report title	The biology of the honey bee
Report No	Harvard University Press, 1987, 281 pp Published
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted *in the Corrigendum* to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive only because it is an extract of a book

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

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.

Assessment and conclusion by applicant: No comment from the applicant.

Assessment and conclusion by RMS:
This is an extract of a book. It provided only general information on the biology of the honey bee.

Data point:	CA 8.3.1/02
Report author	Haydak M.H. et al.
Report year	1970
Report title	Honey bee nutrition
Report No	Annu. Rev. Entomol., Vol. 15, pp. 143-156, 1970 Published
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted *in the Corrigendum* to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive only because it provided only general information on carbohydrates used by honey bee

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

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 cannot be used. Mannose which is poisonous to honeybees will be present in glycopeptide form in royal jelly to avoid detrimental effects.

Assessment and conclusion by applicant: No comment from the applicant.

Assessment and conclusion by RMS:
This article provided general information on the use of carbohydrates by honey bee.

B.9.3.1.2. Chronic toxicity to bees

According to Commission regulation (EU) No 283/2013, testing, including sub-lethal effects, shall be conducted where bees are likely to be exposed.

Exposure of bees (adults, and larvae) to xyloglucan, polysaccharides, or monosaccharides occurred naturally.
Heptamaloxyloglucan is derived from apple and thus its composition is a combination of sugars monomers that occurred naturally in plants, nectar/pollen or honey.
The notifier provided several studies to demonstrate that Heptamaloxyloglucan due to its composition and the composition of nectar/pollen and honey from different plants is not expected to have adverse effects on bees.

Despite Co-RMS agreed that no unacceptable risk of heptamaloxyloglucan to bees can be concluded, Co-RMS indicated that, taking into account the theoretical approach used in risk assessment for larvae bees, a larval toxicity would help in the determination of the NOEC-Ecx for risk assessment given. (See Volume 3 CP B.9.6).

The summaries of the studies from literature are presented below. Considering these studies, the applicant proposed an argumentation on natural exposure of bees to xyloglucans. This argumentation and RMS consideration are reported in Volume 3 CP B.9.6.1.

Data point:	CA 8.3.1/03
Report author	Barker R.J. *et al.*
Report year	1977
Report title	Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees
Report No	J. Nutr., Vol. 107, pp. 1859-1862, 1977 Published
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted *in the Corrigendum* to the DAR (2009).
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Yes

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

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.

Materials and methods

Groups of 60 workers honey bees (*Apis mellifera*) 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.

Table 9.3-3 percentage of bee mortality following 8 and 16 days exposure to hexose.

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)
	(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)**

Group (tested concentration)	% dead in 8-day test	% dead in 16-day test
(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).

Assessment and conclusion by applicant: 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.

Assessment and conclusion by RMS:
In the diet of bees, carbohydrates serve as a convenient source of energy. Nectar and honey conributes 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 Haydak 1970, CA 8.3.1/02).
The following sugar monomers of heptamaloxyloglucan could be 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.of Volume 3 CP B.9.6.1.
In this study, the 16d-NOEL was found to be 1.62 mg galactose/bee/day. At this statistical NOEL, 19% effects were observed. No LDD10 is available.
For purpose of risk assessment, a 16d-LDD50 value of greater than 1.62 mg galactose/bee/day is proposed.

Data point:	CA 8.3.1/04
Report author	J. Smessaert, O. Honnay and W. Keulemans
Report year	2019
Report title	Monitoring pollinator activity in an apple and pear orchard, linked with the analysis of the nectar composition
Report No	Acta Hortic. 1231. ISHS 2019. DOI 10.17660/ActaHortic.2019.1231.11 Proc. II Int. Workshop on Floral Biology and S-Incompatibility in Fruit Species Eds.: E. Ortega Pastor, J. Halász and P. De Franceschi
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information. Considering that heptamaloxyloglucan is derived from apple, the study provide information on the use of

	apple fields by pollinators that may be used for weight of evidence risk assessment.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

To ensure cross pollination, pollinizer cultivars are planted within the orchard, and pollinators such as honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.) are commonly introduced in apple orchards but rather exceptional in pear orchards (Bosch and Kemp, 2002; Klein et al., 2012).
Insect pollinators, such as bumblebees, pear and apple flowers are generally less pollinated by wild bumblebees since these are less abundant during the flowering period, but the use of commercially available bumblebee hives may improve the pollination. In this context, it is important to evaluate the attractiveness of flowers for bumblebees.
For honeybees it has already been observed that pear flowers are less attractive as compared to apple flowers due to the composition of the nectar, which contains less sugars (Farkas et al., 2002; Maccagnani et al., 2003). Also little information is available regarding sugar preferences in nectar for bumblebees (Cnaani et al., 2006; Pozo et al., 2014; Whitney et al., 2008).

Materials and methods

The field studies were conducted in orchards in Belgium. The experiments were done on *Pyrus communis* 'Conference' (6 000 m^2) and *Malus* × *domestica* Borkh. 'King Jonagold' (7 200 m^2).
This paper reports the analysis of nectar quantity and composition of apple flowers ('Jonagold') and pear flowers ('Conference'). The three main nectar sugars (fructose, glucose and sucrose) were quantified.

Results

The activity of the hives in the pear orchard was always sufficient and more than 10 bumblebees went in or out the nest except for one day.
The average nectar quantity per sample for apple differed significantly ($P \leq 0.05$) from pear (Figure 1A), respectively 6.7 and 8.6 µL. Furthermore there was high variation per day within the cultivar, especially for apple.
Sugar concentrations were significantly ($P \leq 0.05$) lower in the nectar of pear compared to the nectar of apple (Figure 1B).
The average of the total concentration of sugar were 8 g/mL of nectar in pear and 42 g/mL of nectar in apple. The relative amounts of glucose and fructose was 50% in apple and pears. There was a low amount of sucrose in pear (Figure 2).

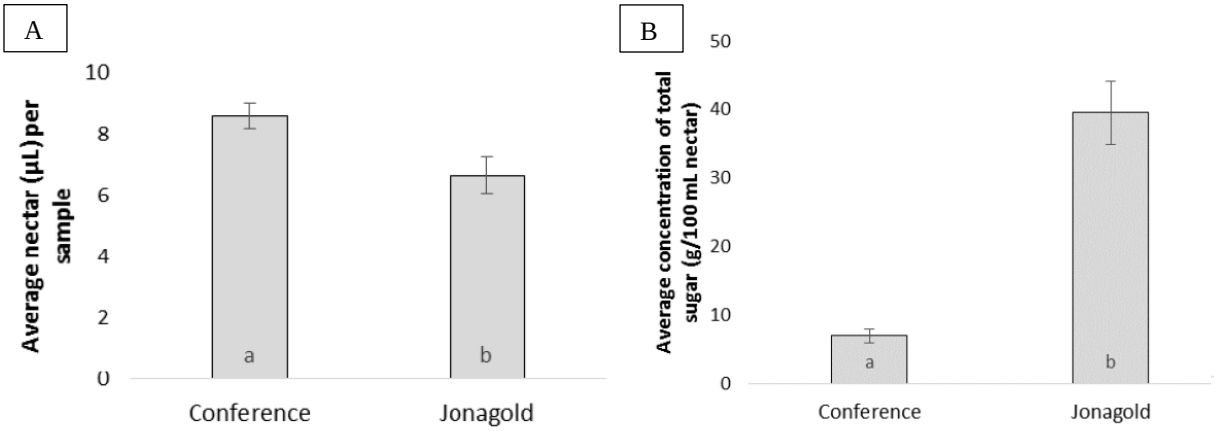


Figure 1. A) Average of the total nectar in µL (±SE) per sample from 1-day-old flowers for apple ("Jonagold", n=48) and pear ("Conference", n=36). B) Average of the total concentration of sugar (g 100 mL^{-1} nectar, ±SE) per sample from 1-day-old flowers for ("Jonagold", n=48) and pear ("Conference", n=36). Columns with a different letter are significantly different at $P \leq 0.05$).

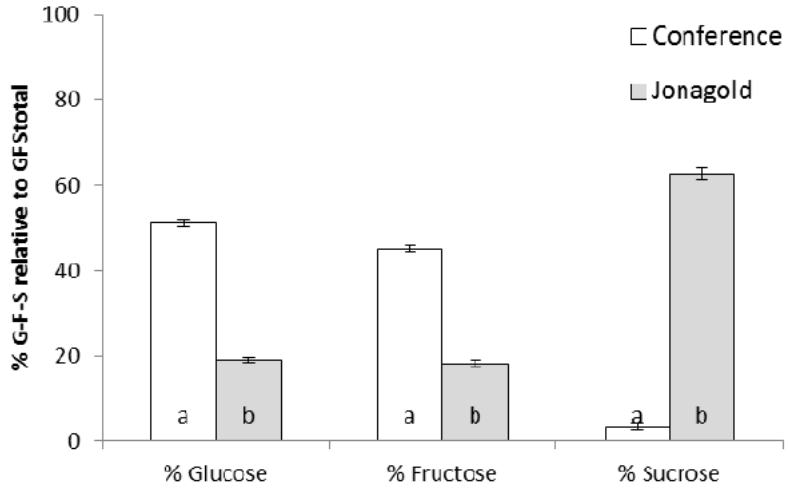


Figure 2. Percentage of glucose, fructise and sucrose of the toal sugar (±SE) per sample from 1-day-old flowers for apple ("Jonagold", n=48) and pear ("Conference", n=36). Columns with a different letter are significantly different at $P \leq 0.05$)

Assessment and conclusion

Assessment and conclusion by applicant: Flower nectars from Jonagold apple and Conference pear contain glucose and fructose. Sucrose is also present in the apple nectar and in very low quantities in the pear nectar.

Assessment and conclusion by RMS:

The summary of this study was amended by RMS. RMS agrees with the conclusion proposed by the notifier but the output of these results should be discussed as regard to information needed for risk assessment.

The following conclusions can be drawn from the study:

- The amount of sugar in the nectar of pear was relatively lower compared to the nectar of apple, what makes the last one more concentrated and attractive for pollinators.
- The nectar of apple is sucrose dominated, while the nectar of pear is more glucose and fructose dominated.

Sugars presents in apple flowers are attractive to honeybees and bumblebees. As apple nectar contains mainly sucrose, bees and bumblebees are naturally exposed to this sugar.

Considering that heptamaloxyloglucan is derived from apple, the study provide information on the use of apple fields by pollinators that may be used for weight of evidence risk assessment.

Data point:	CA 8.3.1/05
Report author	Pavlova T, Dimov I, Nakov G *et al.*
Report year	2018
Report title	Quality characteristics of honey: a review
Report No	Proceedings of university of ruse - 2018, volume 57, book 10.2.
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP

Acceptability/Reliability:	Supportive information. This study provided general information on chemical composition of honey

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

The review described the composition of honey. Honey is a supersaturated solution of sugars mainly comprised of D-fructose, D-glucose, sucrose, maltose and higher sugars (~80% of solid mass).

Materials and methods

This study is a review.

Results

The composition of honey varies from floral source to origin. Natural honey contains more than 300 bioactive substances, but it is mainly composed of water and sugars, primarily fructose and glucose, which accounts for 95–99% of honey dry matter, and about 4–5% of fructo-oligosaccharides. Besides fructose (38%) and glucose (31%), other identified sugars include maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose, gentiobiose and oligosaccharides.
A wide range of minor constituents is also present in honey such as alkaloids, flavonoids/isoflavones, glycosides, phenolics, peptides/proteins, certain enzymes (invertase, amylase and glucose oxidase), carotenoid-like substances, organic acids, Maillard reaction products, vitamins, and minerals.

Assessment and conclusion

Assessment and conclusion by applicant:
Honey dry matter is mainly composed of fructose and glucose, which accounts for 95–99% and other polysaccharides and monosaccharides (4-5%). They are identified as maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose, gentiobiose and oligosaccharides.

Assessment and conclusion by RMS:
This review described the chemical composition of honey. It is a mixture of carbohydrates mainly glucose and fructose. This study provides supportive information but cannot be used for a deterministic risk assessment. It may be used for weight of evidence risk assessment.

Data point:	CA 8.3.1/06
Report author	A. A. Machado De-Melo, L. Bicudo de Almeida-Muradiana, M. T. Sancho and A. Pascual-Mate
Report year	2017
Report title	Composition and properties of *Apis mellifera* honey: A review
Report No	Journal of Apicultural Research, 2017 - https://doi.org/10.1080/00218839.2017.1338444
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information (composition of honey)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study is a review of the literature.

Results

Honey is mainly composed of carbohydrates and water, parameters that influence its shelf life and some of its properties, including color, flavor, density, viscosity, hygroscopicity, and crystallization. Honey also contains small amounts of other components, such as nitrogen compounds, organic acids, minerals, vitamins, Maillard reaction products, volatile compounds, and several bioactive sub-stances that affect sensory and physical characteristics, as well as biological potential.
Honey is a supersaturated sugar solution, where carbohydrates are the main constituents accounting for about 95% dry matter. The monosaccharides (hexoses) fructose (32–44%) and glucose (23–38%) are the main honey sugars. Very small amounts of other monosaccharides, such as galactose, have been also identified in honeys. In almost all honey types, fructose is the main sugar, but there are exceptions honeys (rape, dandelion and blue curks), where glucose is present in higher amounts. They are produced by honeybees during the ripening process, by the transformation of nectar sucrose through the enzyme invertase from the bee's salivary glands. Furthermore, invertase has transglucosilation activity, producing more complex sugars from monosaccharides. Therefore the main disaccharides being present in honey are α-glucosyl derivatives of monosaccharides, being likely trisaccharides and tetrasaccharides α-glucosyl derivatives of the main disaccharides and trisaccharides, respectively. Other di- and trisaccharides in honey could be formed by microbial activity and enzymatic reactions in the intestinal tract of the plant-sucking insects (Hemiptera, mostly aphids) that excrete honeydew.
More than 45 di-, tri- and other oligo- and polysaccharides have been detected in honey in small amounts (5–15%), like maltose, sucrose, turanose, trehalose, gentiobiose, isomaltose, lactose, kojibiose, raffinose, erlose, melezitose, maltotriose, panose, isomaltotriose and maltotetraose.
Several sugars, such as galactose, lactose and raffinose have been described as toxic to honey bees because these insects do not have the appropriate enzymes for their digestion (Herbert, 1992).

Assessment and conclusion

Assessment and conclusion by applicant: This study shows that fructose, glucose, galactose are present in honey and thus, that bees can be naturally exposed to these carbohydrates. The carbohydrates identified are: maltose, sucrose, turanose, trehalose, gentiobiose, isomaltose, lactose, kojibiose, raffinose, erlose, melezitose, maltotriose, panose, isomaltotriose and maltotetraose.

Assessment and conclusion by RMS:
RMS agrees with the conclusion of the applicant. Bees can be naturally exposed mainly to glucose and fructose. Galactose, lactose and raffinose which can be described as toxic to honey bees are presented in small quantities in honey. Thus, bees are also exposed to these carbohydrates.
This study can be used in risk assessment for a weight of evidence approach to indicate that bees are exposed to carbohydrates when consuming honey.

Data point:	CA 8.3.1/07
Report author	Fredrick J. Lee, Douglas B. Rusch, Frank J. Stewart, Heather R. Mattila and Irene L. G. Newton
Report year	2014
Report title	Saccharide breakdown and fermentation by the honey bee gut microbiome
Report No	Environmental Microbiology (2014) - doi:10.1111/1462-2920.12526.
Document No	-
Guidelines followed in study	None

Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive information. This study focuses on the role of gut microbiome in the metabolism of carbohydrates of honeybees.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

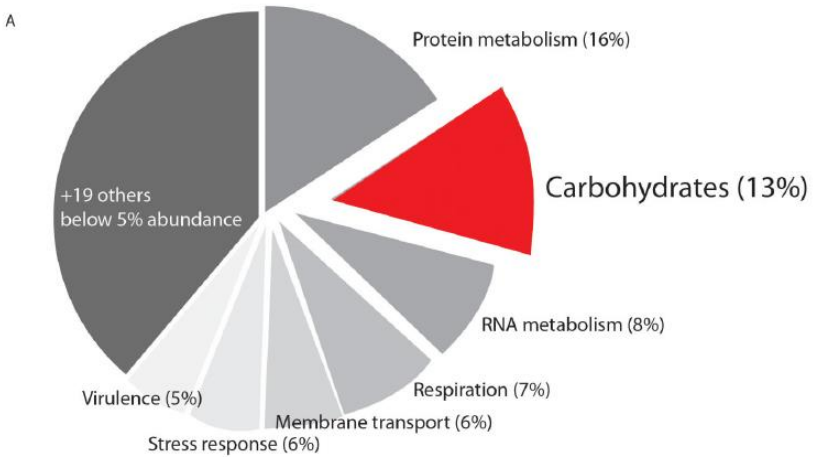
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study aims at better understanding the contribution of the microbial community to food processing in the honey bee. It reports the generation of a metatranscriptome of the honey bee gut microbiome.
Adult workers were collected from a single, healthy hive of *Apis mellifera* located in a research apiary at Wellesley College in Wellesley, MA during the summer 2011. Worker ages were not known, but collecting very young bees (slow and shiny-haired) or older bees (visible hair loss an wing wear) was avoided. They are collected only from the brood area of the colony.
Total RNA was extracted from three samples (complete digestive tracts from three individuals). Biolog Ecoplate analyses was used to confirm metatranscriptomic findings.

Results

Three major bacterial classes that are active in the gut are reported (γ-Proteobacteria, Bacilli and Actinobacteria), all of which are predicted to participate in the breakdown of complex macromolecules (e.g. polysaccharides and polypeptides), the fermentation of component parts of these macromolecules, and the generation of various fermentation products, such as short-chain fatty acids and alcohol.
The second most abundant functional class (based on MG-RAST subsystems hierarchical annotation) of transcripts is carbohydrate metabolism. Thirteen per cent of the annotated transcripts are associated with the uptake, export and metabolism of carbohydrates (Figure 1 A.). The most abundant bacterial classes (γ-Proteobacteria, Bacilli and Actinobacteria) contribute to this category in varying proportions and with emphasis on different functions (Figure 1 B). For example, the Bacilli are most prominent in metabolizing di- and oligosaccharides, fermentation, monosaccharides, and sugar alcohols while the γ-Proteobacteria are enriched for transcripts involved in anaplerotic reactions and central intermediary metabolism. Importantly, like many functional annotation categories, overlap exists between categories with regard to membership of particular transcripts. Moreover, evidence for the utilization of the sugars were tested on the Ecoplates (xylose, mannitol, NAG, glucose-1-phosphate, cellobiose and lactose).



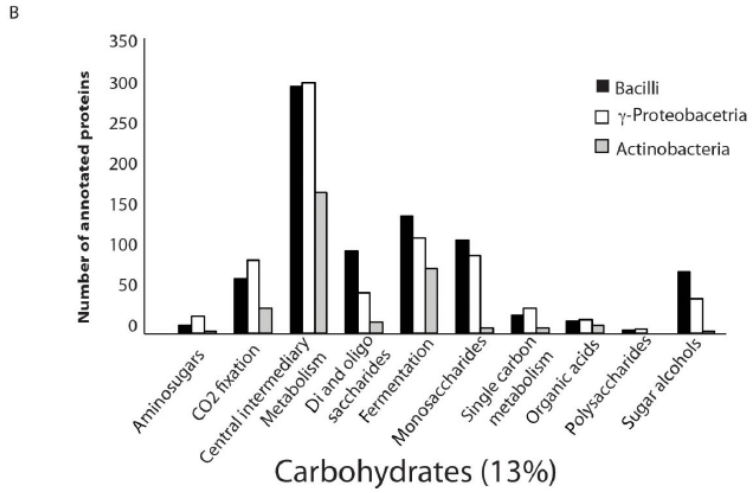


Figure 1. The second abundant functional class of transcripts is carbohydrate metabolism. A. Thirteen per cent of the annotated transcripts are associated with the uptake, export and metabolism of carbohydrates. B. The most abundant bacterial classes (γ-Proteobacteria, Bacilli and Actinobacteria) contribute to this category in varying proportions and with emphasis on different functions.

Assessment and conclusion

Assessment and conclusion by applicant: This study supports the major role of gut microbiome in the metabolism of carbohydrates by honeybees and thus, that bees are able to metabolize a wide diversity of carbohydrates encountered in nectar and pollen.

Assessment and conclusion by RMS:

Nectar is predominantly composed of sucrose and its component monosaccharides,fructose and glucose. In addition to these simple sugars, other trace monosaccharides are present in plant-derived nectar (mannose, arabinose, xylose), as well as the disaccharides maltose and melibiose, the oligosaccharide raffinose (on occasion), and the sugar alcohol sorbitol. However, not all of these sugars are equally utilized by the honeybee. Feeding experiments have determined that honey bee health declines when bee diets are restricted to certain carbohydrates, including rhamnose, fucose, mannose, sorbose, lactose, melibiose, dulcitol, erythritol and inositol. Indeed, mannose is poisonous to honey bees, presumably due to an imbalance between high hexokinase activity and low mannose-6-phosphate isomerase activity, leading to an accumulation of mannose-6-phosphate and a decrease in ATP. However, the honeybee gut microbiome may facilitate the metabolism of this sugar, potentially detoxifying some nectars for honeybees.

RMS agree with the conclusion of the applicant. However, this study focus on the role of gut microbiome in the metabolism of carbohydrates of honeybees which is only supportive for the risk assessment (weight of evidence).

Data point:	CA 8.3.1/08
Report author	Dmitruk M.
Report year	2019
Report title	Flowering, nectar secretion, and structure of the nectary in the fowers of *Acer pseudoplatanus L.*
Report No	Acta Agrobot. 2019;72(3):1787
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted

GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information. The subject of this study is the chemical composition of the nectar of Sycamore. This may be used together with other data on nectar composition.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

Flowering and nectar release in Sycamore (*Acer pseudoplatanus)* were investigated between 2011 and 2013 in the Czech Republic, Switzerland, and the United Kingdom, where dates of the different phases of foliage development and flowering of this species have been recordedThe weight and the content of sugars of nectar from 10 flowers has been investigated. The micromorphology of the floral and nectary elements was observed using a scanning electron microscope and the anatomy of nectaries was examined by light microscopy. The percentage of sugars in the nectar was determined using an Abbe refractometer.

Results

The average weight of nectar from 10 flowers was 16.54 mg (range: 11.0–23.75 mg) and the content of sugars in the nectar was found to be in the range of 23.5–50%, with an average of 37.3%. The calculated weight of sugars in the nectar from 10 flowers was on average 6.11 mg and so the average sugar yield from one sycamore tree was estimated to be 0.65 kg (Table 9.3-4). . The lowest sugar yield was recorded in 2011 (0.56 kg) and the highest value in 2012 (0.73 kg) (Fig. 1).

Table Table 9.3-4. Amount of nectar, sugar content of nectar, and total sugar mass in nectar per 10 flowers of *Acer pseudoplatanus* in 2011-2013

Year	Nectar amount (mg)	Sugars content in nectar (%)	Weight of sugars in nectar (mg)
2011	14.54 [a]	39.83 [a]	5.73 [a]
2012	15.87 [a]	37.50 [a]	6.01 [a]
2013	18.27 [a]	34.67 [a]	6.48 [a]
Mean	16.23	37.33	6.10

Values are means ±*SD*. Means in columns with the same letters are not significantly different at α = 0.05; the HSD Tukey test was used.

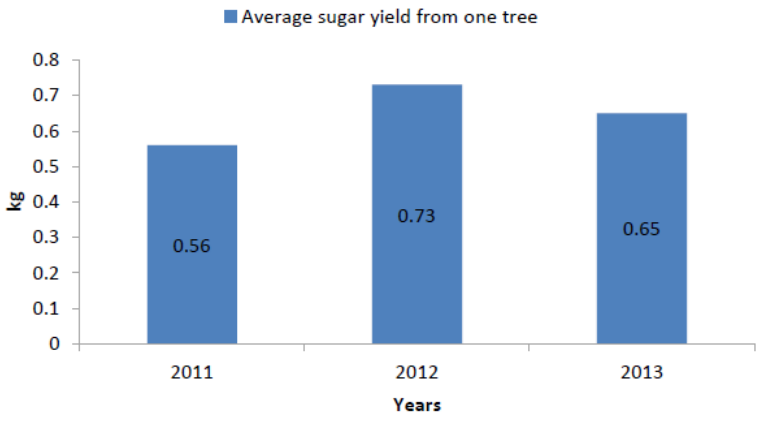


Figure 1. Sugars yiels of *Acer pseudoplatanus* in the years 2011-2013.

The nectar contains three sugars: sucrose, fructose, and glucose, with a significant predominance of sucrose. Since sycamore flowering coincides with colonization of trees by aphids, bees collect not only nectar from the flowers but also the honeydew deposited on pedicels.

Assessment and conclusion

Assessment and conclusion by applicant: This study supports that sugars are naturally present in sycamore flowers and thus, that bees can be exposed to theses carbohydrates.

Assessment and conclusion by RMS:
RMS agrees with the conclusion of the applicant. Bee can be naturally exposed to sucrose, fructose and glucose from sycamore flowers. However, *Acer pseudoplatanus* are barely presents in nearby vineyards which are the crops where the use of heptamaloxyloglucan is intended. Thus, this study could be used as supportive (weight of evidence). This data may be used together with other data on nectar composition from other plants.

Data point:	CA 8.3.1/09
Report author	Somme L., Moquet L., Quinet M., Vanderplanck M., Michez D., Lognay G., Jacquemart A.L.
Report year	2016
Report title	Food in a row : urban trees offer valuable floral resources to pollinating insects
Report No	Urban Ecosyst - DOI 10.1007/s11252-016-0555-z
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information. The subject of this study is the chemical composition of the nectar and pollen of trees present in cities.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Urbanization affects the availability and diversity of floral resources (pollen and/or nectar) for wild pollinating insects. To examine the suitability of urban trees as resources for pollinating insects, 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, namely *Acer pseudoplatanus, Aesculus carnea, A. hippocastanum, Robinia pseudoacacia, Tilia cordata, T. x euchlora, T. x europaea, T. platyphyllos and T. tomentosa* were investigated.

Materials and methods

This paper reports the analysis of 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, namely *Acer pseudoplatanus, Aesculus carnea, A. hippocastanum, Robinia pseudoacacia, Tilia cordata, T. x euchlora, T. x europaea, T. platyphyllos and T. tomentosa*. The total sugar content of nectar per flower was investigated.

Pollen sampling and chemical analyses

At the peak of flowering, branches with unopened flower buds were harvested from a minimum of five trees for each species. Branches, placed in tap water, were kept for one night at room temperature (approx. 20 °C). On the next morning, stamens were extracted from newly opened flowers. Stamens were stored at −20 °C and subsequently dried at room temperature for 12 h before pollen was removed using a sieve (Sieve 3 Brass-Stainless, Full Height, 80 µm). Pure pollen samples were pooled to obtain 200 mg samples sufficient for analyses and stored at −20 °C until use. All chemical analyses were performed in triplicate. The polypeptide content was quantified in 5 mg dry pollen for each species (n = 3).

Nectar sampling and chemical composition of nectar

Branches with unopened flower buds were harvested from a minimum of five trees for each species at the same time that pollen was collected. Branches, placed in tap water, were kept for one night at room temperature (approx. 20 °C). On the next morning, nectar was extracted from newly opened flowers. This method avoids differences due to climatic conditions (temperature and relative humidity) in the field and previous visits to flowers by insects. For each tree species, nectar was collected from at least 50 newly opened flowers with glass capillary tubes of 1 µL or 5 µL. The nectar volume was estimated by measuring the length of the nectar column in the capillary tube. Nectar samples were stored at −80 °C until analyses of sugar concentration and composition. Sugar composition was determined on pooled samples by gas chromatography. To determine the total sugar content of nectar per flower, sugar concentration was converted to mg/µl according to the formula: y = 0.00226 + (0.00937 x) + (0.0000585 x2) where y = sugar concentration (mg/µl) and x = sugar concentration (%). The total sugar content of nectar per flower (mg) was then calculated as volume of nectar (µl) x sugar concentration (mg/µl).

Data analyses
Prior to analyses of variance (ANOVA), homoscedasticity and normality were checked using Bartlett and Shapiro tests, respectively. One-way analyses of variance (ANOVA) were used to compare phytosterol content among tree species. To detect differences in pollen composition perMANOVA were conducted.

Results

Tree species produced pollen with polypeptide contents ranging from 21.0 ± 2.8 µg/mg (*Tilia cordata*) to 89.8 ± 11.6 µg/mg (*Aesculus carnea*). Pollen from Tilia species contained the lowest amounts of polypeptides, differing significantly from *Acer pseudoplatanus* and *Aesculus carnea* ($p < 0.001$; Table 1). *Robinia pseudoacacia* pollen contained the highest total amino acid content (375.1 ± 2.4 µg/mg), whereas *T. tomentosa* pollen contained the lowest (209.3 ± 5.6 µg/mg, $p = 0.002$; Table 9.3-5).

Table Table 9.3-5. Polypeptide, total amino acid and sterol content (µg/mg ; mean ± SD) in pollen from the nine studied tree species

Species	Polypeptide content (µg/mg)	Amino acid content (µg/mg)	Sterol content (µg/mg)
Acer pseudoplatanus	71.0 ± 11.1 ^{a}	290.2 ± 26.6 ^{ab}	9.04–13.74^{a}
Aesculus carnea	89.8 ± 11.6 ^{a}	318.2 ± 8.5 ^{ab}	3.90–9.48^{ab}
Aesculus hippocastanum	39.5 ± 7.0^{abc}	331.7 ± 27.1 ^{ab}	4.93–5.07^{b}
Robinia pseudoacacia	48.2 ± 5.1 ^{ab}	375.1 ± 2.4 ^{a}	9.99–10.35^{ab}
Tilia cordata	21.0 ± 2.8 ^{d}	243.1 ± 13.2 ^{ab}	3.39–4.97^{b}
Tilia x *euchlora*	36.3 ± 1.3^{abcd}	243.9 ± 0.8 ^{ab}	2.59–7.00^{b}
Tilia x *europaea*	33.4 ± 4.6^{bcd}	260.6 ± 8.4 ^{ab}	2.82–5.41^{b}
Tilia platyphyllos	26.5 ± 6.0 ^{cd}	321.4 ± 6.5 ^{ab}	3.74–5.66^{b}
Tilia tomentosa	25.6 ± 1.7 ^{cd}	209.3 ± 5.6 ^{b}	4.26–5.09^{b}

Different letters indicate significant difference among species (Tukey's post-hoc tests, $p < 0.05$)

The volume of nectar differed significantly among species; Tilia species (T. cordata, T. x euchlora and T. tomentosa) produced the highest quantities per flower compared with the other tree species (0.82–1.76 µl per flower, $p < 0.001$; Fig. 1a). Sugar concentration 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. Flower nectar from Tilia species presented sugar content comprised between 0.35 mg (T. platyphyllos) and 0.96 mg per flower (T. x euchlora). Nectar sugar content in Acer pseudoplatanus was about 0.67 mg per flower. Moreover, all Tilia nectars contained higher hexose (glucose + fructose) concentrations than the other species (Fig. 1b). Despite the variation in hexose and sucrose concentrations, all investigated species produced sucrose-rich nectar.

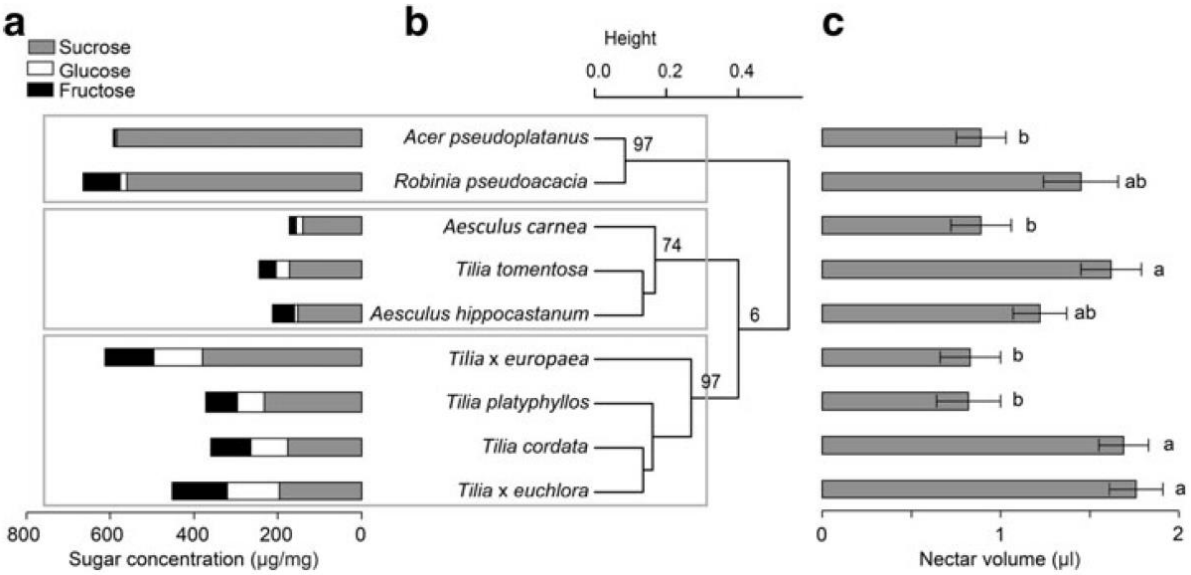


Figure 1. Nectar volume and composition for the studies tree species. a) Nectar sugar composition (concentration of fructose, glucose and sucrose). b) UPGMA cluster using Bray-Curtis dissimilarity index based on nectar sugar composition. c) Nectar volume per flower (µg; mean ± SD). The values near nodes are multiscale bootstrap resampling.

Assessment and conclusion

Assessment and conclusion by applicant: Despite the variation in hexose (glucose and fructose) and sucrose concentrations, all investigated species produced sucrose-rich nectar.

Assessment and conclusion by RMS:
Urban tree flowers offer abundant nectar with relatively high sugar contents (0.16–1.28 mg/flower); sucrose was the predominant sugar in all nectars.

Pollinating insects use nectar and/or pollen from foraged plants as nutrient resources. Nectar serves as an energy source while pollen is the major nutrient resource used for the development of larvae. Nectar consists mainly of sugars. Pollen consists mostly of lipids (including phytosterols) and nitrogen compounds. Nectar and pollen chemical composition are highly variable among plant species. Nectar that is sucrose-rich is highly attractive to honeybees and bumble bees. Thus, honeybees and bumble bees are naturally exposed to sucrose.
RMS considered that this kind of information can be considered suitable for the purpose of risk assessment based on weight of evidence.

Data point:	CA 8.3.1/10
Report author	Tomczyk M., Tarapatskyy M., Dzugan M.
Report year	2019
Report title	The influence of geographical origin on honey composition studied by Polish and Slovak honeys
Report No	Food Analysis, Food Quality and Nutrition Czech Journal of Food Sciences, 37, 2019 (4): 232–238 - doi.org/10.17221/40/2019-CJFS
Document No	-

Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information. The subject of this study is the chemical composition of Polish and Slovak honeys. May be useful for weight of evidence together with other studies on honey composition.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Honey composition is mainly affected by botanical origin, however geographical factors as well as beekeeping practice and storage conditions can also influence its quality. The aim of the study was to determine the impact of geographical origin on physicochemical quality and biological activity of honey.

Materials and methods

The aim of the study was to determine the influence of geographical origin on honey physicochemical quality and biological activity based on the comparison of Polish and Slovak varietal honeys. 60 varietal honey samples including 10 multifloral, 5 tilia, 5 rape, 5 acacia and 5 forest per each country were collected were compared according to their sugar profile (fructose, glucose and sucrose), physicochemical parameters (free acidity, pH, electrical conductivity, moisture content, and colour intensity), diastase activity, as well as antioxidant activity.

For comparison 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.

Among physicochemical properties, colour intensity was determined for 50% (g/l) aqueous solution of honey (homogenized and centrifuged at 14000 rpm for 5 min) and absorbance measured at 450 nm, using a spectrophotometer Biomate 3 (Thermo, USA).

Sugar content was determined by high-performance liquid chromatograph (Thermo Dionex Ultimate 3000; Thermo Fisher Scientific, USA) equipped with corona discharge detector (Corona Veo RS; ESA Chelmsford, USA) and Shodex Asahipak NH2P-504E (4.6 × 250 mm) chromatography column. Sample volume of 10 µl (2% g/l solution in ethanol) was injected. The separation was conducted at a temperature of 55°C with the mobile phase acetonitrile:water 78:22 (v/v), at a flow rate of 1 ml/min. The content of tested carbohydrates (fructose, glucose and sucrose) was calculated based on the external standard curve and expressed as g per 100 g of honey.

Statistical analysis of the results was performed with the software Statistica 13.1. (StatSoft Inc., USA). One-way ANOVA followed by Tukey's HSD test was used to investigate the differences between honey of the same varieties originating from different countries and $P < 0.05$ was accepted as significant. Correlations among analysed parameters were calculated by Pearson correlation coefficients (r) at a significance level of 95% ($P < 0.05$). Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method.

Results

The sugar profile of tested honey samples from both countries was similar (Fructose: from 36.35 to 39.23%; glucose: from 26.14 to 32.92% ; sucrose: from 3.53 to 6.26%; Table 1)The sucrose contents of the tested honey samples did not exceed the established European limits. According to Juan-Borras *et al*. (2014) even though honey contains an active sucrose splitting enzyme (sucrase and glucosidase), the sucrose level in honey never reaches zero. In all tested honey varieties fructose content was higher than glucose (Table 9.3-6).

Table Table 9.3-6. Physicochemical quality and biological activity of tested Polish (PL) and Slovak (SK) honey samples

	Multifloral		Tilia		Forest		Rape		Acacia	
	PL	SK	PL	SK	PL	SK	PL	SK	PL	SK
	(n = 10)						(n = 5)			
Moisture (%)	18.65 ± 1.34	18.53 ± 0.80	17.76 ± 0.57	18.35 ± 0.52	17.40 ± 1.12	17.98 ± 0.38	17.86 ± 1.14	17.45 ± 0.38	17.73 ± 0.51	17.86 ± 0.21
Conductivity (mS/cm)	0.35 ± 0.08	0.21 ± 0.12	0.53 ± 0.22*	0.23 ± 0.09*	0.82 ± 0.25	0.37 ± 0.23	0.23 ± 0.15	0.16 ± 0.08	0.42 ± 0.27*	0.20 ± 0.09*
Free acidity (mEq/kg)	37.0 ± 7.3*	23.3 ± 11.8*	34.2 ± 7.0*	21.6 ± 11.6*	33.6 ± 5.2	28.1 ± 8.5	18.6 ± 2.2	13.6 ± 2.8	25.6 ± 6.0	16.1 ± 7.1
Diastase (DN)	22.56 ± 5.35	13.14 ± 4.87	31.99 ± 9.60*	19.08 ± 6.93*	23.06 ± 6.05	21.09 ± 11.63	17.61 ± 6.80	12.38 ± 1.93	16.45 ± 1.19	14.15 ± 5.11
pH	3.57 ± 0.14	3.68 ± 0.13	3.81 ± 0.25	3.90 ± 0.18	4.16 ± 0.24	4.07 ± 0.14	3.88 ± 0.11	3.61 ± 0.06	3.79 ± 0.29	3.71 ± 0.23
Color intensity (mAU)	699 ± 259*	308 ± 186*	820 ± 241*	306 ± 74*	1098 ± 295	823 ± 173	427 ± 162	197 ± 37	422 ± 168	221 ± 220
Fructose (%)	39.23 ± 3.08	38.66 ± 2.52	36.89 ± 3.26	38.15 ± 2.35	34.67 ± 0.31	37.83 ± 0.68	36.35 ± 2.28	36.39 ± 1.44	37.64 ± 1.60	37.34 ± 1.66
Glucose (%)	31.82 ± 1.78	27.78 ± 3.32	28.43 ± 1.37	28.21 ± 1.81	29.10 ± 1.71	26.78 ± 1.83	32.92 ± 2.80	29.69 ± 1.57	31.75 ± 1.77	26.14 ± 0.88
Sucrose (%)	4.40 ± 1.95	4.47 ± 2.15	6.12 ± 1.32	4.68 ± 1.22	6.29 ± 1.07	5.06 ± 0.61	5.06 ± 0.54	4.61 ± 0.23	3.53 ± 0.76	5.27 ± 1.50
DPPH (% inhibition)	36.12 ± 12.67	22.24 ± 10.19	40.71 ± 13.30*	18.84 ± 1.75*	60.52 ± 3.83	46.53 ± 13.69	21.21 ± 3.76	11.76 ± 2.40	24.80 ± 7.98	14.47 ± 4.27
FRAP (mmol TE/kg)	1.32 ± 0.53	1.11 ± 0.51	0.95 ± 0.22	1.17 ± 0.33	2.32 ± 1.04	2.84 ± 0.55	0.68 ± 0.16	0.59 ± 0.06	1.59 ± 0.75	0.64 ± 0.11
ACW (mmol AA/kg)	16.10 ± 5.27	13.73 ± 5.53	12.62 ± 2.75	12.01 ± 2.16	19.09 ± 3.30	18.80 ± 4.61	9.93 ± 3.47	7.98 ± 1.03	15.77 ± 4.19	8.17 ± 1.28
ACL (mmol Trolox/kg)	2.19 ± 1.19	1.35 ± 0.58	1.48 ± 0.59	1.51 ± 0.54	2.34 ± 0.42	2.81 ± 0.58	0.67 ± 0.15	0.59 ± 0.16	1.73 ± 0.61	0.64 ± 0.13
TPC (g GAE/kg)	0.46 ± 0.11	0.34 ± 0.15	0.38 ± 0.04	0.35 ± 0.11	0.60 ± 0.24	0.68 ± 0.20	0.25 ± 0.05	0.21 ± 0.04	0.47 ± .011*	0.20 ± 0.05*
Flavonoids (mg QE/kg)	4.96 ± 2.37*	2.92 ± 1.23*	4.99 ± 1.90*	2.57 ± 1.18*	6.68 ± 1.54*	5.39 ± 0.95*	3.24 ± 0.87*	2.16 ± 0.46*	3.15 ± 1.47*	1.37 ± 0.70*

*significant differences (P < 0.05) between PL and SK honey within the counterparts

In order to study the influence of tested physicochemical parameters and bioactive compounds on honey quality, a PCA study was carried out. In the PCA analysis, PC1 was mainly related with antioxidant activity and physicochemical parameters and PC2 was positively associated with fructose, glucose and hydrophilic fraction of antioxidants (Figure 1). Regarding the influence of the parameters, it can be observed that antioxidant activity tested by different methods (FRAP, DPPH, ACW, ACL, TPC, and TFC) as well as colour intensity influence significantly the projection, while sugar profile (sucrose, fructose and glucose) as well as pH show no significant influence on the overall quality of honey.

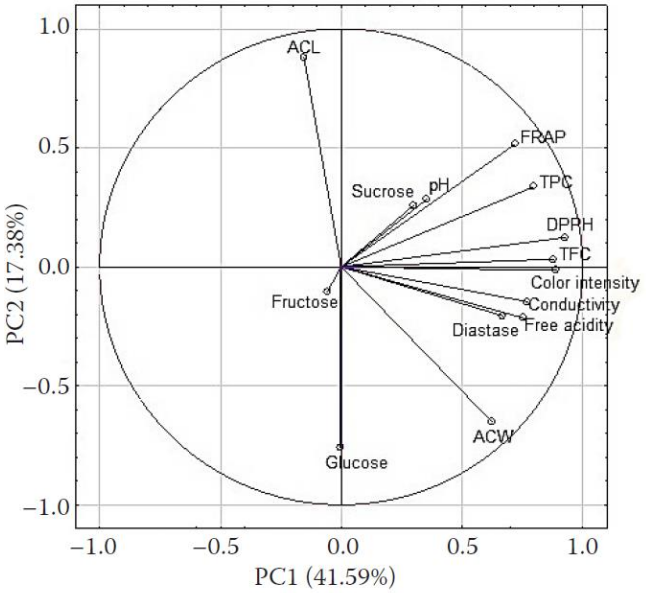


Figure 1. Principal Component Analysis (PCA) – biplot of scores and loadings of data obtained from physico-chemical and bioactive compounds determinations

Assessment and conclusion

Assessment and conclusion by applicant: Honey dry matter includes around 38% of fructose, 28% of glucose and 5% of sucrose.

Assessment and conclusion by RMS:
Honey of the same variety from different countries showed a variation in terms of antioxidant activity, diastatic activity and physicochemical properties. Among tested honey varieties, tilia honey from both countries differed the most in antioxidant and physicochemical parameters, while Polish and Slovak rape honey exhibited the most similar properties.

This study is supportive. Honey is composed of fructose, glucose and sucrose. Thus, honeybees are naturally exposed to these sugars. This study may be used in the context of weight of evidence together with other data on honey composition.

Data point:	CA 8.3.1/11
Report author	Val A, Huidobro JF, Sanchez MP, Muniategui S, Fernandez-Muino MA and Sancho MT
Report year	1998
Report title	Enzymatic Determination of Galactose and Lactose in Honey
Report No	J. Agric. Food Chem. 1998, 46, 1381-1385
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive information. This study provides information on galactose and lactose content in honey via the use of an enzymatic method. This study might be used together with other study on honey characterization.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study describes an enzymatic method for the analysis of galactose and lactose content naturally present in flower honeys. The method has been applied to 46 floral unpasteurized honeys commercially purchased from Galicia (in northwestern Spain) and harvested in autumn 1995 for analyzing these sugars.
The method of Boehringer-Mannheim GmbH (1995) was used for the determination of lactose and D-galactose (enzymatic test).

Results

The galactose content of the honeys analyzed ranged between 0.0052 and 0.0151%. The lactose content of the honeys analyzed ranged between 0.0062 and 0.0383%.

Table 9.3-7 Percent galactose and lactose contents of 46 honey samples

sample	botanical origin	% galactose	% lactose
1	*C. sativa*	0.0068	0.0132
2	*Eucalyptus* sp.	0.0088	0.0186
3	*Eucalyptus* sp.	0.0088	0.0383
4	*Eucalyptus* sp.	0.0083	0.0102
5	*Eucalyptus* sp.	0.0110	0.0115
6	*Eucalyptus* sp.	0.0089	0.0142
7	*Eucalyptus* sp.	0.0088	0.0119
8	*Eucalyptus* sp.	0.0091	0.0080
9	*Eucalyptus* sp.	0.0092	0.0077
10	*Eucalyptus* sp.	0.0082	0.0166
11	*Eucalyptus* sp.	0.0091	0.0114
12	*Eucalyptus* sp.	0.0094	0.0112
13	*Eucalyptus* sp.	0.0080	0.0070
14	*Eucalyptus* sp.	0.0085	0.0110
15	*Eucalyptus* sp.	0.0088	0.0135
16	*Eucalyptus* sp.	0.0088	0.0165
17	*Eucalyptus* sp.	0.0100	0.0132
18	*Eucalyptus* sp.	0.0067	0.0160
19	*Eucalyptus* sp.	0.0085	0.0120
20	*Eucalyptus* sp.	0.0074	0.0144
21	*Eucalyptus* sp.	0.0071	0.0124
22	*Eucalyptus* sp.	0.0094	0.0095
23	*Eucalyptus* sp.	0.0085	0.0114
24	*Eucalyptus* sp.	0.0093	0.0113
25	*Rubus* sp.	0.0093	0.0158
26	*Rubus* sp.	0.0091	0.0133
27	multifloral	0.0083	0.0127
28	multifloral	0.0096	0.0123
29	multifloral	0.0078	0.0129
30	multifloral	0.0089	0.0155
31	multifloral	0.0082	0.0159
32	multifloral	0.0085	0.0098
33	multifloral	0.0064	0.0165
34	multifloral	0.0100	0.0131
35	multifloral	0.0090	0.0062
36	multifloral	0.0085	0.0137
37	multifloral	0.0151	0.0137
38	multifloral	0.0099	0.0099
39	multifloral	0.0085	0.0198
40	multifloral	0.0081	0.0113
41	multifloral	0.0077	0.0180
42	multifloral	0.0060	0.0104
43	multifloral	0.0052	0.0179
44	multifloral	0.0104	0.0220
45	multifloral	0.0079	0.0194
46	multifloral	0.0064	0.0229
	mean	0.0086	0.0140
	SD	1.51×10^{-3}	5.21×10^{-3}
	V_{min}	0.0052	0.0062
	V_{max}	0.0151	0.0383

Assessment and conclusion

Assessment and conclusion by applicant: This study shows that galactose (and lactose) are present in honey and that bees can be naturally exposed to these carbohydrates.

Assessment and conclusion by RMS:
The purpose of this work has been to apply the Boehringer-Mannheim GmbH (1995) enzymatic method for determining galactose and lactose in honey. Thus, this study is considered only supportive by RMS.

In conclusion, enzymatic determination of galactose and lactose in honey, without previous chromatographic separation and avoiding the use of galactose oxidase, has been carried out for the first time. The method meets the conditions of precision, recovery, sensitivity, simplicity, and low cost required for an analytical method to be usable.

The galactose content of the honeys analyzed ranged between 0.0052 and 0.0151%. The lactose content of the honeys analyzed ranged between 0.0062 and 0.0383%.
Galactose and lactose are present in extraordinarily low concentrations in honey.

Both galactose and lactose (among other sugars such as mannose and raffinose) are toxic to honey bees because of their lack of proper enzymes for its digestion. The toxicity of lactose is due to the effect of galactose.
Both sugars reduce the longevity of honey bees even when fed in sucrose syrup (Barker and Lehner, 1974a,b; Herbert, 1992).

This study might be used in the context of weight of evidence together with other data on honey composition.

B.9.3.2. Effects on 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 (0.560 g/ha), RMS is still of the opinion that testing on non target arthropods is not required in this particular case.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

No study has been conducted since heptamaloxyloglucan is a xyloglucan molecule extracted from apples. Heptamaloxyloglucan is made of 7 glucidic monomer units, which are D-glucopyranosyl, D-glucitol, D-xylopyranosyl, D-galactopyranosyl and L-fucopyranosyl. 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. (Buchanan 2000,).
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.
Please also refer to the introduction of Volume 3 CA B.9 for more details.

Supportive literature data already evaluated at EU-level during first approval process of heptamaloxyloglucan (DAR of heptamaloxyloglucan (February 2008) and final Addendum (June 2009)) are presented below.

Data point	CA 8.4/01
Report author	Lattaud C. *et al.*
Report year	1997

Report title	Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (megascoloecidae)
Report No	Soil Biol. Biochem., Vol 29 (3/4), pp 335-339, 1997
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the *Corrigendum* to the DAR *(2009)*.
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive information. This study give general information on the digestive enzymes in the gut of earthworm.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Endogenic 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 endogenic 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 (Figure 1): 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.

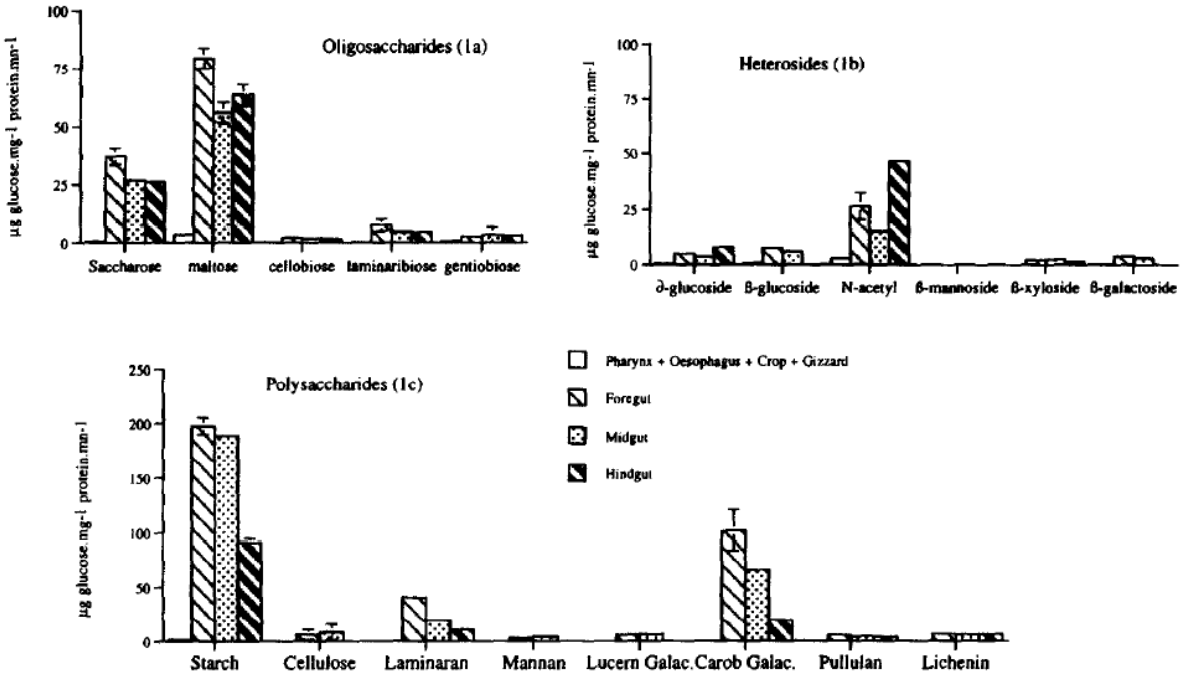


Figure 1. Specific glucosidic activities in the gut (wall and contents) of *Polypheretima elongata*. Mean of two independent assays ± standard error for (a) oligosaccharides, (b) heterosides and (c) polysaccharides.

Assessment and conclusion

Assessment and conclusion by applicant: The applicant did not present a conclusion on this study.

Assessment and conclusion by RMS:
RMS agrees with the summary presented by the applicant. It was already validated in the previous DAR. This study is supportive for risk assessment because it give general information on the use of sugars on the gut of earthworms. Thus, sugars present in heptamaloxyloglucan could be considered to have no detrimental adverse effects on earthworms.

Data point	CA 8.4/02
Report author	Zhang B.G. *et al.*
Report year	1993
Report title	Activity and origin of digestive enzymes in gut of tropical earthworm *Pontoscolex corethrurus*
Report No	Eur. J. Soil Biol., Vol 29 (1), pp 7-11, 1993
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the *Corrigendum* to the DAR *(2009).*
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive information. This study give general information on the digestive enzymes in the gut of earthworm.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

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 endogenic 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 endogenic 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.

Assessment and conclusion

Assessment and conclusion by applicant: The applicant did not present a conclusion on this study.

Assessment and conclusion by RMS:
RMS agrees with the summary presented by the applicant. It was already validated in the previous DAR. This study is supportive for risk assessment because it give general information on the activities of glucidic digestive enzymes in the gut of earthworms. It provided evidence that earthworms are able to use and degrade components such as oligosaccharides and polysaccharides. However, the earthworms used in this study is tropical. Thus, it might not be representative to the earthworms present in field where heptamaloxyloglucan will be applied.

Data point	CA 8.4/03
Report author	Garvin M.H. *et al.*
Report year	2000
Report title	Activity of glycolytic enzymes in the gut of *Hormogaster elisae* (Oligochaeta, Hormogastridae)
Report No	Soil Biology & Biochemistry, Vol. 32, pp 929-934, 2000
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted in the *Corrigendum* to the DAR *(2009).*
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive data. This study give general information on the activity of glycolytic enzymes in the gut of earthworm.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

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 endogenic 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 endogenic oligohumic species that feeds on soil low in organic matter.Moreover, sucrose is usually found in fresh litter but there was no activity on this substrate in the gut of *H. elisae*, which is consistent with this species not feeding on fresh material.

Assessment and conclusion

Assessment and conclusion by applicant: The applicant did not present a conclusion on this study.

Assessment and conclusion by RMS:

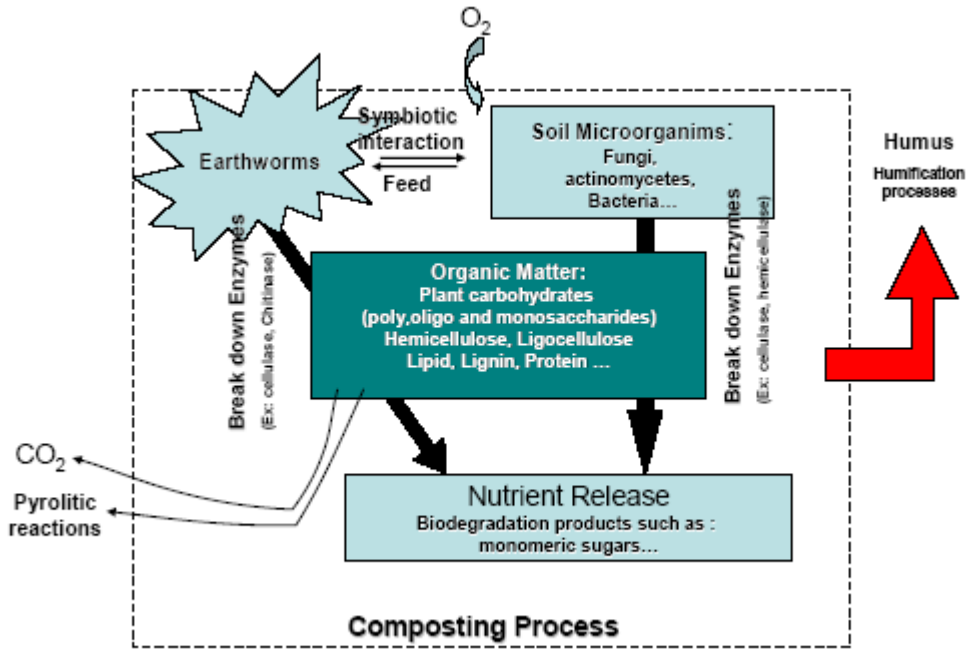
RMS agrees with the summary presented by the applicant. It was already validated in the previous DAR. This study is supportive for risk assessment because it give general information on the activities of glycolytic enzymes in the gut of *Hormogaster elisae*. The earthworms were exposed to sugars in soils and were able to degrade polysaccharides. No detrimental adverse effects on earthworms of these sugars are expected.

B.9.4.2. Conclusion on effects on earthworms

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.

Literature data showed 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). 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, Lattaud and al. 1997, Garvin and al. 2000).

Therefore, the notifier 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) 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.

For these reasons, no test on earthworms were deemed necessary during the initial EU review of heptamaloxyloglucan.

Using available literature, the applicant provided general information on the way 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. 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 testing on earthworms is not required in this particular case.

B.9.4.3. Effects on non-target soil meso- and macrofauna (other than earthworms)

No study has been conducted on effects on non-target soil meso- and macrofauna (other than eartworms).
Heptamaloxyloglucan is a xyloglucan molecule extracted from apples, made of 7 glucidic monomer units, which are D-glucopyranosyl, D-glucitol, D-xylopyranosyl, D-galactopyranosyl and L-fucopyranosyl. 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. (Buchanan 2000).
Furthermore heptamaloxyloglucan can be produced from xyloglucan by enzymatic degradation naturally occurring in plant. As such it could enter in the diet of soil 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 the function of soil macro-organisms ecosystems.

This conclusion is in line with the one reached 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 (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 testing on soil meso- and macro-organisms is not required in this particular case.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

The applicant provided two literature data.

Data point:	CA 8.5/01
Report author	Robertson G. P. and P. M. Groffman
Report year	2015
Report title	Nitrogen transformations.
Report No	Robertson, G. P. and P. M. Groffman. 2015. Nitrogen transformations. Pages 421-446 *in* E. A. Paul, editor. Soil microbiology, ecology and biochemistry. Fourth edition. Academic Press, Burlington, Massachusetts, USA.
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	No (book provided general information on Nitrogen Transformations)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

No other element essential for life takes as many forms in soil as nitrogen (N), and transformations among these forms are mostly mediated by microbes. Soil microbiology thus plays yet another crucial role in ecosystem function: in most terrestrial ecosystems N limits plant growth, and thus net primary production—the productive capacity of the ecosystem—can be regulated by the rates at which soil microbes transform N to plant-usable forms.

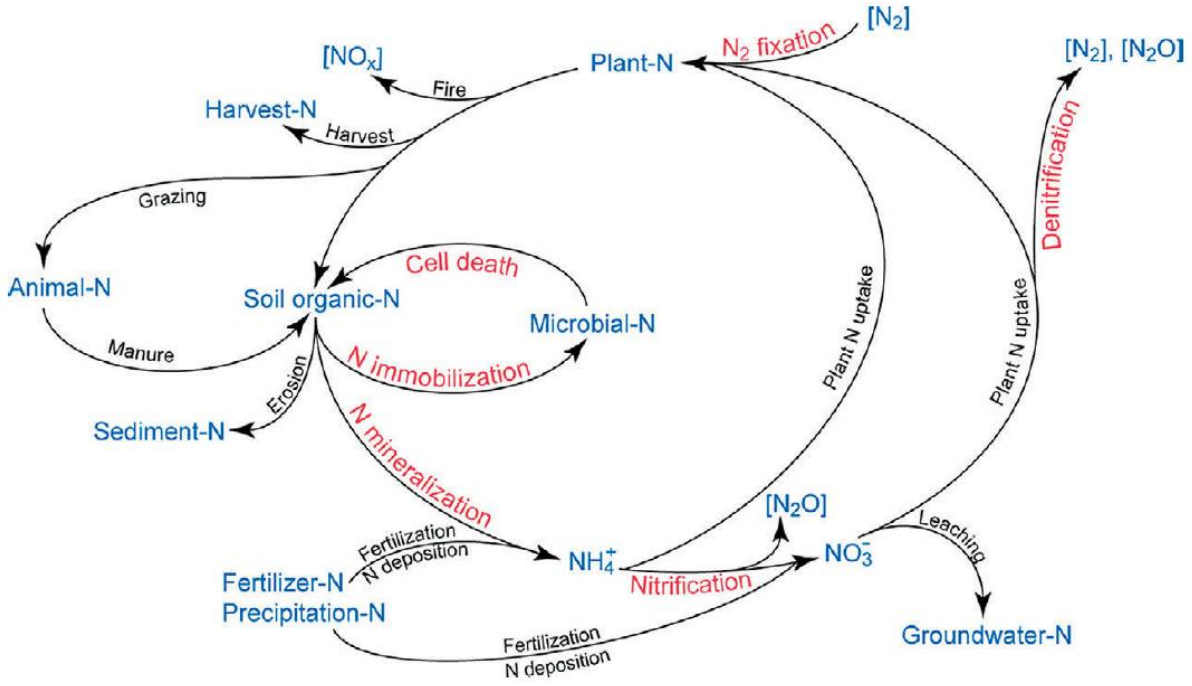


Figure 1. Schematic representation of the major elements of the terrestrial nitrogen cycle. Those processes mediated by soil microbes appear in red. Gases appear in brackets.

It has been shown that microbes invest more energy in the synthesis of enzymes (e.g., amidases to acquire N and phosphatases to acquire P) to obtain nutrients that they need when decomposing substrates of low quality. Microbial N uptake is also affected by organism growth efficiency. Fungi have wider C:N ratios in their tissues than bacteria and archaea and can grow more efficiently on low N substrates.
Nitrification is the microbial oxidation of ammonia to less reduced forms, principally NO_{-2} and NO_{-3}.
A wide variety of heterotrophic bacteria and fungi have the capacity to oxidize NH_{+4}. So-called heterotrophic nitrification is not linked to cellular growth, as it is for autotrophic nitrification.
Heterotrophic bacteria such as *Arthrobacter globiformis*, *Aerobacter aerogenes*, *Thiosphaera pantotropha*, *Streptomyces grisens*, and various *Pseudomonas* spp. have been found to nitrify.

Assessment and conclusion

Assessment and conclusion by applicant:
RMS comment: The applicant did not present a summary this book. The notifier indicated that nitrogen transformations are mostly mediated by soil microbes.

Assessment and conclusion by RMS:
The chapter of this book demonstrated that nitrogen transformations are mostly mediated by soil microbes. RMS considered that this publication is not considered suitable for assessing the impact of a compound on soil micro-organisms and nitrogen transformation.

Data point:	CA 8.5/02
Report author	Veras, H. C. T.; Parachin, N. S.; Almeida, J. R. M
Report year	2017
Report title	Comparative assessment of fermentative capacity of different xylose-consuming yeasts
Report No	Veras *et al.* Microb Cell Fact (2017) 16:153
Document No	-

Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive only because this study provide general information on the fermentative capacity of different xylose-consuming yeasts

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

Xylose is the second most abundant sugar in nature (Parachin *et al*., 2011 ; Moyses *et al*., 2016) In this work, xylose fermentative capacity of four naturally-xylose fermenting yeast strains (*Scheffersomyces stipitis, Spathaspora passalidarum, Spathaspora arborariae* and *Candida tenuis*) was compared under aerobic, oxygen-limited and anaerobic conditions to understand the effects of oxygen levels on yeast xylose metabolism for ethanol production. Xylose fermentation was evaluated in defined mineral medium by the determination of ethanol production from xylose. Xylose reductase (XR) and xylitol dehydrogenase (XDH) specific activities were determined for each strain in crude-cell extracts.

Results

The four yeast were able to use xylose as substrate of fermentation to produce ethanol. Performances of the four yeasts were greatly influenced by oxygen availability. *S. stipitis* and *S. passalidarum* showed the highest ethanol yields (above 0.44 g g−1) under oxygen limitation. However, *S. passalidarum* produced 1.5 times more ethanol than *S. stipitis* under anaerobiosis. While *C. tenuis* showed the lowest xylose consumption rate and incapacity to produce ethanol, *S. arborariae* showed an intermediate fermentative performance among the yeasts. NAD(P)H xylose reductase (XR) activity in crude cell extracts correlated with xylose consumption.

Assessment and conclusion

Assessment and conclusion by applicant: This study shows that the four yeast (*Scheffersomyces stipitis*, *Spathaspora passalidarum*, *Spathaspora arborariae* and *Candida tenuis*) were able to use xylose as substrate of fermentation to produce ethanol and that the performances of this activity of the four yeasts were greatly influenced by oxygen availability.

Assessment and conclusion by RMS:
RMS agrees with the conclusion proposed by the applicant.

Overall, the present work demonstrated that the availability of oxygen influences the production of ethanol by yeasts and indicates that the NADH-dependent XR activity is a limiting step on the xylose metabolism. *S.stipitis* and *S. passalidarum* have the greatest potential for ethanol production from xylose. Both yeasts showed similar ethanol yields near theoretical under oxygen-limited condition. Besides that, *S. passalidarum* showed the best xylose consumption and ethanol production under anaerobiosis.

This study demonstrated that xylose can be used as a substrate for fermentation thus it is not toxic for *Scheffersomyces stipitis, Spathaspora passalidarum, Spathaspora arborariae* and *Candida tenuis*.

Conclusion for 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-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).

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.

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 of the opinion that testing on soil micro-organisms is not required in this particular case.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

The notifier indicated that observations on adverse effects of heptamaloxyloglucan on other non-target organisms made during screening and developmental phase did not result in particular risks.

B.9.6.2. Testing on non-target plants

Considering that heptamaloxyloglucan is a plant growth regulator (elicitor), a study on side effect of heptamaloxyloglucan on the vegetative growth of terrestrial plants was performed and summarised below.

Data point:	CA 8.6/01
Report author	Servajean E. (final report)
Report year	2006
Report title	Semi-field assessment of the side-effect of a substance on the vegetative growth of terrestrial plants
Report No	CIT unpublished report number 05-27-072-ES
Document No	
Guidelines followed in study	Not specified
Deviations from current test guideline	-
Previous evaluation	Yes, evaluated and accepted *in the Corrigendum to the DAR (2009).*
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes despite no analytical check of applied rates

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and 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 0.25m² were planted with 14 plants each (16 plots per plant species). 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). Each plot (replicate) consisted of 14 seeded plugs. Each plug contained 3 seeds of wheat or 3 seeds of mustard or 5 to 7 seeds of red clover. The total of seeds per replicate were 42 seeds for wheat and mustard and 70 to 98 for red clover. 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 9.6-1.

Table 9.6-1: 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			

Assessment and conclusion

Assessment and conclusion by applicant: 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.

Assessment and conclusion by RMS:
The study was not conducted under GLP.

The study report did not mention if the test has been performed following an OECD guideline. The validity of the test has been checked by RMS in comparison to OECD 227.

Criteria of OECD 227	**Results**
the seedling emergence is at least 70 %.	No information in the raw data. Based on RMS assumption results from wheat and mustard can be considered to meet the emergence criteria. For red clover there is some uncertainties,

	that are not considered to affect the results. See below for details.
in the controls: the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.	There is no visible phytotoxic effects.
in the controls: the mean plant survival is at least 90 % for the duration of the study.	No information.
in the controls: environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.	This condition was achieved.

The emergence rate can not be calculated with the information provided. RMS assumed that the number of collected plants can be compared to the number of seeds. The resulting percentage is an estimate of both seed emergence rate and survival rate of emerged seeds. Considering the percentage presented in the tables as a surrogate of the minimum emergence rate, results in wheat and mustard are considered robust. There is some uncertainty on the ones obtained with red clover but, considering that the percentage did not allow to discriminate seed emergence rate and survival rate of emerged seeds, RMS considered that this may not affect the results particularly when considering the nature and profile of the active compound tested.

Wheat

	Replicate	**Number of seeds per replicate**	**number of collected plants**	**% of plants**
water	1	42	39.0	92.9
	2	42	40.0	95.2
	3	42	37.0	88.1
	4	42	35.0	83.3
	mean	42	37.8	89.9
0.2 g/ha	1	42	35.0	83.3
	2	42	31.0	73.8
	3	42	35.0	83.3
	4	42	36.0	85.7
	mean	42	34.3	81.5
2.0 g/ha	1	42	41.0	97.6
	2	42	40.0	95.2
	3	42	39.0	92.9
	4	42	36.0	85.7
	mean	42	39.0	92.9
20.0 g/ha	1	42	37.0	88.1
	2	42	35.0	83.3
	3	42	39.0	92.9
	4	42	36.0	85.7
	mean	42	36.8	87.5

mustard

	Replicate	**Number of seeds per replicate**	**number of collected plants**	**% of plants**
water	1	42	31.0	73.8
	2	42	38.0	90.5
	3	42	36.0	85.7
	4	42	34.0	81.0
	mean	42	34.8	82.7

0.2 g/ha	1	42	30.0	71.4
	2	42	34.0	81.0
	3	42	34.0	81.0
	4	42	37.0	88.1
	mean	42	33.8	80.4
2.0 g/ha	1	42	33.0	78.6
	2	42	33.0	78.6
	3	42	33.0	78.6
	4	42	32.0	76.2
	mean	42	32.8	78.0
20.0 g/ha	1	42	32.0	76.2
	2	42	32.0	76.2
	3	42	35.0	83.3
	4	42	32.0	76.2
	mean	42	32.8	78.0

red clover

	Replicate	**Number of seeds per replicate**		**number of collected plants**	**% of plants**	
		min	**max**		**min**	**max**
water	1	70	98	50.0	71.4	51.0
	2	70	98	52.0	74.3	53.1
	3	70	98	47.0	67.1	48.0
	4	70	98	44.0	62.9	44.9
	mean	70	98	48.3	68.9	49.2
0.2 g/ha	1	70	98	61.0	87.1	62.2
	2	70	98	48.0	68.6	49.0
	3	70	98	53.0	75.7	54.1
	4	70	98	54.0	77.1	55.1
	mean	70	98	54.0	77.1	55.1
2.0 g/ha	1	70	98	46.0	65.7	46.9
	2	70	98	48.0	68.6	49.0
	3	70	98	46.0	65.7	46.9
	4	70	98	51.0	72.9	52.0
	mean	70	98	47.8	68.2	48.7
20.0 g/ha	1	70	98	56.0	80.0	57.1
	2	70	98	50.0	71.4	51.0
	3	70	98	40.0	57.1	40.8
	4	70	98	55.0	78.6	56.1
	mean	70	98	50.3	71.8	51.3

RMS also noted that the study is not conducted under GLP and that no analytical check of applied rates were realized. Moreover only 3 species were tested.
However, considering the mode of action of heptamaloxyloglucan, the fact that it is extracted from apple and has a structure very similar to other polyxyloglucan, RMS still considered that the study is suitable for risk assessment purpose.
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.

Four new publications were found in the literature data which studied the oligosaccharides from fruits and vegetables.

Data point:	CA 8.6/02
Report author	Jovanovic-Malinovska R., Kuzmanova S., Winkelhausen E.,
Report year	2014
Report title	Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods
Report No	International Journal of Food Properties, 17:949–965, 2014
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Supportive only because this study focuses in oligosaccharide profile in fruits and vegetables. This may provide useful information for natural occurrence of oligosaccharides but did not provide specific data on their effects

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

Identification and quantification of low molecular weight carbohydrates (LMWC) content, i.e. monosaccharide and oligosaccharide contents has been investigated in a selection of fruits and vegetables. LMWC have been extracted with ethanol (80%, 50°C, 1 hour) from 32 fruits, including 3 apple species, and 41 vegetables from local green markets. The food samples were transferred to the laboratory and analyzed immediately.They were dried and analyzed by high performance chromatography (HPLC Agilent 1200 HPLC (USA) fitted with Zorbax carbohydrate analysis column Agilent, USA)). Monosaccharides including fructose, glucose, sorbitol, mannitol and xylitol and oligosaccharides including fructooligosaccharides (FOS) and raffinose family oligosaccharides (RFO) were identified and quantified thanks to standards. Descriptive statistics and one-way analysis of variance (ANOVA) was performed on the parameters to evaluate significant differences among the samples at 95% confidence interval ($p \leq 0.05$) according to Tukey's test using Minitab 15 statistical software.

Results

This study describes the monosaccharides and oligosaccharides profile of a wide range of fruits and vegetables. It shows that monosaccharides are more abundant in fruits than in vegetables and that most fruits and vegetables contain oligosaccharides (FOS and RFO). The respective amounts differ from a species of fruit or vegetable to another and different groups are proposed according to the oligosaccharide content. For example, Fructose concentration was the highest in grapes, 8.13 ±0.301 g/100 g FW for red grape Vranec and 7.58 ±0.273 g/100 g FW for white grape Smederevka.
The contents of sugars and sugar alcohols in 32 fruits and 41 vegetables are shown respectively in Table 1 and 2.

Table 9.6-2. Sugar and sugar alcohol content in selected fruits (*).

Sample	Moisture	Fructose	Glucose	Sucrose	Sorbitol	Mannitol	Xylitol
	g/100 g fresh weight of edible sample						
Apple, Golden Delicious	87 ± 0.67	0.45 ± 0.020^{a}	2.04 ± 0.110^{a}	1.18 ± 0.068^{a}	0.68 ± 0.020^{a}	nd^{a}	nd^{a}
Apple, Idared	85 ± 0.89	2.27 ± 0.111^{b}	3.47 ± 0.172^{b}	5.73 ± 0.299^{b}	nd^{b}	nd^{a}	nd^{a}
Apple, Petrovka	87 ± 0.21	3.96 ± 0.223^{c}	2.33 ± 0.108^{a}	1.93 ± 0.084^{c}	nd^{b}	nd^{a}	0.21 ± 0.018^{b}
Apricot	87 ± 0.38	0.30 ± 0.009^{a}	0.86 ± 0.060^{c}	3.46 ± 0.106^{d}	nd^{b}	nd^{a}	nd^{a}
Blackberry	71 ± 0.17	3.25 ± 0.214^{d}	3.55 ± 0.129^{b}	nd ^{e}	3.15 ± 0.169^{c}	nd^{a}	nd^{a}
Blueberry	85 ± 0.18	5.87 ± 0.317^{e}	10.64 ± 0.542^{d}	0.78 ± 0.049^{f}	0.96 ± 0.075^{d}	nd^{a}	nd^{a}
Cherry	86 ± 0.23	2.32 ± 0.167^{b}	4.63 ± 0.243^{e}	0.31 ± 0.024^{ef}	0.16 ± 0.012^{b}	nd^{a}	0.08 ± 0.007^{c}
Currant, black	78 ± 0.29	4.08 ± 0.123^{c}	5.17 ± 0.271^{f}	0.60 ± 0.042^{f}	1.21 ± 0.073^{e}	nd^{a}	0.13 ± 0.012^{d}
Currant, red	83 ± 0.66	2.29 ± 0.083^{b}	3.24 ± 0.173^{b}	0.51 ± 0.030^{f}	nd^{b}	nd^{a}	nd^{a}
Fig, common	73 ± 1.74	5.79 ± 0.223^{e}	7.47 ± 0.348^{g}	1.69 ± 0.091^{c}	0.09 ± 0.007^{b}	nd^{a}	0.21 ± 0.019^{b}
Fig, wild green	88 ± 2.12	1.00 ± 0.061^{f}	1.07 ± 0.063^{c}	0.50 ± 0.034^{f}	0.12 ± 0.009^{b}	nd^{a}	0.19 ± 0.018^{b}
Grape, red Vranec	74 ± 0.23	8.13 ± 0.301^{g}	13.75 ± 0.513^{h}	0.83 ± 0.058^{f}	nd^{b}	nd^{a}	nd^{a}
Grape,white Smederevka	74 ± 1.71	7.58 ± 0.273^{h}	11.72 ± 0.582^{i}	0.70 ± 0.042^{f}	nd^{b}	nd^{a}	nd^{a}
Medlar	67 ± 1.34	1.96 ± 0.094^{bi}	3.22 ± 0.175^{b}	nd^{e}	nd^{b}	nd^{a}	nd^{a}
Melon, honeydew	87 ± 0.62	3.05 ± 0.141^{d}	4.53 ± .0.194^{f}	6.48 ± 0.392^{g}	0.08 ± 0.006^{b}	nd^{a}	nd^{a}
Melon, Polidor	88 ± 1.46	2.48 ± 0.083^{bi}	3.20 ± 0.138^{b}	3.65 ± 0.199^{d}	nd^{b}	nd^{a}	nd^{a}
Mulberry, black	82 ± 1.33	4.00 ± 0.291^{c}	6.70 ± 0.363^{g}	0.91 ± 0.051^{af}	nd^{b}	nd^{a}	nd^{a}
Mulberry, white	79 ± 0.78	4.35 ± 0.301^{c}	6.56 ± 0.371^{g}	1.08 ± 0.064^{af}	nd^{b}	nd^{a}	nd^{a}
Nectarine	88 ± 0.44	1.15 ± 0.052^{f}	1.50 ± 0.083^{ac}	3.50 ± 0.198^{d}	1.08 ± 0.079^{de}	nd^{a}	0.28 ± 0.026^{e}
Peach, yellow-green	86 ± 0.14	0.86 ± 0.050^{af}	1.26 ± 0.063^{ac}	5.36 ± 0.251^{i}	0.14 ± 0.010^{b}	nd^{a}	nd^{a}
Pear	83 ± 0.01	4.50 ± 0.193^{c}	4.21 ± 0.243^{be}	2.44 ± 0.180^{h}	2.45 ± 0.192^{f}	nd^{a}	0.12 ± 0.012^{d}
Plum, cherry	83 ± 0.21	2.14 ± 0.121^{b}	6.95 ± 0.384^{g}	0.95 ± 0.047^{af}	0.06 ± 0.005^{b}	nd^{a}	0.18 ± 0.017^{b}
Plum, Ciruela	88 ± 0.54	2.99 ± 0.125^{d}	5.02 ± 0.269^{ef}	0.72 ± 0.050^{f}	tr^{b}	nd^{a}	0.16 ± 0.015^{d}
Plum, red	86 ± 1.63	1.63 ± 0.103^{bif}	5.53 ± 0.274^{f}	1.11 ± 0.081^{a}	0.12 ± 0.009^{b}	nd^{a}	0.09 ± 0.009^{c}
Pomegranate	76 ± 0.16	0.66 ± 0.041^{af}	14.82 ± 0.823^{j}	0.34 ± 0.023^{ef}	0.08 ± 0.007^{b}	nd^{a}	nd^{a}
Pumpkin	87 ± 0.52	0.41 ± 0.027^{a}	0.82 ± 0.054^{c}	4.79 ± 0.26^{j}	0.06 ± 0.006^{b}	nd^{a}	0.09 ± 0.008^{c}
Quince	75 ± 1.35	2.64 ± 0.124^{bd}	4.52 ± 0.231^{ef}	0.79 ± 0.060^{f}	0.12 ± 0.009^{b}	nd^{a}	0.10 ± 0.010^{cd}
Raspberry	84 ± 1.43	2.46 ± 0.117^{bd}	3.80 ± 0.193^{be}	0.32 ± 0.019^{ef}	nd^{b}	nd^{a}	0.22 ± 0.021^{b}
Sour cherry	77 ± 0.03	3.57 ± 0.129^{cd}	9.24 ± 0.484^{j}	0.82 ± 0.054^{f}	nd^{b}	nd^{a}	nd^{a}
Strawberry, common	91 ± 1.26	1.38 ± 0.062^{f}	1.64 ± 0.086^{ac}	0.60 ± 0.040^{f}	0.14 ± 0.011^{b}	nd^{a}	0.32 ± 0.031^{f}
Strawberry, woodland	82 ± 0.73	2.16 ± 0.131^{bd}	3.22 ± 0.170^{b}	1.19 ± 0.094^{a}	0.08 ± 0.007^{b}	nd^{a}	0.14 ± 0.013^{d}
Watermelon	90 ± 1.46	2.37 ± 0.142^{bd}	2.49 ± 0.133^{ab}	1.68 ± 0.082^{c}	nd^{b}	0.12 ± 0.011^{b}	nd^{a}

*Data are expressed as mean ± standard deviation ($n = 3$); nd: not detected; tr: trace amount; means with different superscript letters within a same column are significantly different ($p < 0.05$).

Table 2. Sugar and sugar alcohol content in selected vegetables (*).

Sample	Moisture	Fructose	Glucose	Sucrose	Sorbitol	Mannitol	Xylitol
	g/100 g fresh weight of edible sample						
Artichoke, Jerusalem	63 ± 0.86	1.36 ± 0.079^{a}	1.20 ± 0.067^{a}	3.15 ± 0.184^{a}	nd^{a}	nd^{a}	nd^{a}
Beans, yellow	92 ± 0.19	1.19 ± 0.070^{a}	1.87 ± 0.099^{a}	0.65 ± 0.042^{b}	0.15 ± 0.013^{b}	0.08 ± 0.007^{a}	tr^{a}
Beetroot	90 ± 0.05	0.13 ± 0.011^{be}	0.08 ± 0.005^{b}	2.37 ± 0.092^{c}	nd^{a}	nd^{a}	0.17 ± 0.014^{b}
Broccoli	86 ± 0.14	0.88 ± 0.053^{ac}	1.27 ± 0.073^{a}	0.65 ± 0.048^{b}	0.20 ± 0.017^{d}	nd^{a}	nd^{a}
Brussel sprouts	81 ± 1.40	0.86 ± 0.040^{c}	1.63 ± 0.084^{a}	0.80 ± 0.051^{b}	0.12 ± 0.010^{c}	nd^{a}	0.08 ± 0.007^{c}
Cabbage, common	88 ± 0.46	2.16 ± 0.131^{d}	3.51 ± 0.187^{c}	0.61 ± 0.035^{b}	0.18 ± 0.016^{d}	nd^{a}	tr^{a}
Cabbage, red	91 ± 0.53	1.14 ± 0.052^{ac}	2.05 ± 0.078^{a}	0.39 ± 0.027^{bd}	nd^{a}	nd^{a}	nd^{a}
Carrot	90 ± 0.21	1.17 ± 0.044^{ac}	2.83 ± 0.133^{c}	4.12 ± 0.186^{e}	nd^{a}	nd^{a}	0.08 ± 0.006^{c}
Cauliflower	90 ± 0.53	1.11 ± 0.073^{ac}	1.18 ± 0.064^{ab}	0.73 ± 0.044^{b}	nd^{a}	1.98 ± 0.105^{b}	0.26 ± 0.021^{d}
Celery, bulb	89 ± 1.47	0.17 ± 0.014^{b}	0.33 ± 0.008^{b}	1.30 ± 0.068^{f}	nd^{a}	2.39 ± 0.116^{c}	tr^{a}
Celery, leaves	84 ± 0.77	nd^{e}	1.36 ± 0.085^{a}	0.47 ± 0.032^{bd}	nd^{a}	1.65 ± 0.082^{d}	0.25 ± 0.023^{d}
Chard	91 ± 1.18	0.36 ± 0.016^{b}	0.56 ± 0.031^{ab}	0.03 ± 0.002^{bd}	nd^{a}	nd^{a}	nd^{a}
Chicory	59 ± 0.40	1.10 ± 0.049^{ac}	0.52 ± 0.040^{ab}	1.98 ± 0.079^{c}	nd^{a}	0.20 ± 0.015^{e}	nd^{a}
Daikon	95 ± 0.14	0.56 ± 0.022^{bc}	0.75 ± 0.049^{ab}	0.15 ± 0.013^{bd}	nd^{a}	tr^{a}	nd^{a}
Dandelion, bulb	62 ± 0.16	0.76 ± 0.05^{c}	0.65 ± 0.021^{ab}	2.79 ± 0.138^{ac}	nd^{a}	nd^{a}	0.81 ± 0.042^{e}
Eggplant	93 ± 0.17	1.56 ± 0.19^{ad}	2.72 ± 0.108^{c}	0.79 ± 0.065^{b}	0.15 ± 0.013^{b}	nd^{a}	nd^{a}
Fennel, bulb	92 ± 0.12	0.75 ± 0.08^{c}	1.13 ± 0.051^{ab}	0.70 ± 0.053^{b}	0.10 ± 0.009^{c}	0.24 ± 0.022^{e}	nd ^{a}
Fennel, leaves	88 ± 0.11	0.22 ± 0.14^{b}	0.21 ± 0.017^{ab}	0.36 ± 0.021^{bd}	0.18 ± 0.017^{d}	0.15 ± 0.011^{e}	0.06 ± 0.004^{c}
Garlic	63 ± 1.65	0.64 ± 0.28^{c}	0.82 ± 0.065^{ab}	2.05 ± 0.126^{c}	nd^{a}	nd^{a}	nd^{a}
Garlic, spring	70 ± 1.19	0.53 ± 0.08^{bc}	1.44 ± 0.087^{a}	1.54 ± 0.068^{f}	nd^{a}	nd^{a}	nd^{a}
Kale	88 ± 0.77	1.10 ± 0.07^{ac}	2.38 ± 0.98^{cd}	0.59 ± 0.01^{b}	0.16 ± 0.015^{bd}	nd^{a}	nd^{a}
Kohlrabi	90 ± 0.65	1.13 ± 0.09^{ac}	1.93 ± 0.77^{ad}	0.68 ± 0.05^{b}	nd^{a}	nd^{a}	nd^{a}
Leek	90 ± 1.25	0.93 ± 0.05^{ac}	1.41 ± 0.64^{a}	0.38 ± 0.01^{bd}	nd^{a}	nd^{a}	tr^{a}
Lettuce, green	91 ± 1.21	0.92 ± 0.06^{ac}	1.47 ± 0.52^{a}	0.24 ± 0.01^{bd}	nd^{a}	0.07 ± 0.010^{a}	0.05 ± 0.004^{c}
Lettuce, red	91 ± 1.15	0.55 ± 0.02^{bc}	0.30 ± 0.02^{ab}	0.21 ± 0.01^{bd}	nd^{a}	nd^{a}	nd^{a}
Mushroom, button	92 ± 0.32	0.27 ± 0.01^{b}	nd^{b}	0.42 ± 0.02^{b}	0.21 ± 0.018^{d}	1.33 ± 0.047^{f}	0.16 ± 0.013^{b}
Mushroom, oyster	92 ± 0.67	0.24 ± 0.01^{b}	nd^{b}	0.34 ± 0.01^{bd}	0.11 ± 0.009^{c}	0.41 ± 0.036^{g}	nd^{a}
Mushroom, Lisicarka	88 ± 0.93	0.05 ± 0.01^{be}	nd^{b}	0.65 ± 0.03^{b}	0.26 ± 0.022^{e}	0.35 ± 0.041^{g}	nd^{a}
Okra	87 ± 0.02	1.11 ± 0.26^{ac}	0.83 ± 0.04^{ab}	1.37 ± 0.06^{f}	nd^{a}	nd^{a}	nd^{a}

Onion, white	88 ± 0.93	1.21 ± 0.34^{ac}	0.42 ± 0.01^{ab}	0.43 ± 0.02^{bd}	nd^{a}	nd^{a}	nd^{a}
Parsnip	83 ± 0.70	0.73 ± 0.15^{c}	0.78 ± 0.02^{ab}	2.95 ± 1.04^{ac}	nd^{a}	nd^{a}	nd^{a}
Peas	67 ± 0.71	0.09 ± 0.02^{be}	1.04 ± 0.08^{ab}	0.89 ± 0.09^{b}	nd^{a}	0.56 ± 0.049^{h}	n^{a}
Pepper, red	88 ± 0.95	1.93 ± 0.18^{d}	3.74 ± 0.86^{c}	0.88 ± 0.05^{b}	nd^{a}	nd^{a}	nd^{a}
Radish	96 ± 1.11	0.19 ± 0.02^{b}	0.52 ± 0.02^{ab}	0.16 ± 0.01^{bd}	nd^{a}	nd^{a}	0.10 ± 0.009^{c}
Rucola	88 ± 0.78	0.20 ± 0.01^{b}	nd^{b}	0.53 ± 0.02^{b}	0.28 ± 0.026^{e}	0.37 ± 0.029^{g}	0.14 ± 0.012^{b}
Scallion	84 ± 1.76	0.74 ± 0.27^{c}	2.49 ± 1.03^{cd}	1.67 ± 0.53^{f}	nd^{a}	nd^{a}	nd^{a}
Tomato, cherry	92 ± 0.54	0.83 ± 0.08^{c}	1.35 ± 0.08^{a}	0.41 ± 0.02^{bd}	nd^{a}	nd^{a}	nd^{a}
Tomato, common	92 ± 0.11	1.28 ± 0.33^{acd}	1.66 ± 0.05^{a}	0.17 ± 0.02^{bd}	nd^{a}	nd^{a}	nd^{a}
Tomato, strawberry	81 ± 1.17	1.33 ± 0.16^{acd}	3.25 ± 0.86^{c}	2.21 ± 0.67^{c}	nd^{a}	nd^{a}	nd^{a}
Zucchini, common	96 ± 0.56	0.39 ± 0.02^{b}	0.59 ± 0.02^{ab}	0.09 ± 0.01^{bd}	nd^{a}	nd^{a}	tr^{a}
Zucchini, green	93 ± 0.51	0.92 ± 0.06^{ac}	1.47 ± 0.09^{a}	0.24 ± 0.01^{bd}	nd^{a}	nd^{a}	nd^{a}

*Data are expressed as mean ± standard deviation ($n = 3$); nd: not detected; tr: trace amount; means with different superscript letters within a same column are significantly different ($p < 0.05$).

Assessment and conclusion

Assessment and conclusion by applicant: Oligosaccharides have been identified and quantified in a large and diverse collection of fruits. This study support that they are naturally present in fruits. With monosaccharides and polysaccharides, they represent a fraction of the total carbohydrate content of fruits.

Assessment and conclusion by RMS:

RMS agrees with the conclusion proposed by the applicant.

Low molecular weight carbohydrates including sugar alcohols and mono-, di- and oligosaccharides, in particular fructooligosaccharides and raffinose-family oligosaccharides, were determined in 32 fruits and 41 vegetables. Vegetables generally contained less monosaccharides than fruits. Sorbitol was detected in 18 fruits, xylitol in 15, while mannitol was found only in watermelon. On the other hand, sorbitol was found in 12, xylitol in 16, and mannitol in 14 vegetables.

This study demonstrated that most plants are composed of sugars present in heptamaloxyloglucan.

This may provide useful information for natural occurrence of oligosaccharides but did not provide specific data on their effects

Data point:	CA 8.6/03
Report author	Jovanovic-Malinovska R., Kuzmanova S., Winkelhausen E.
Report year	2015
Report title	Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables
Report No	Ultrasonics Sonochemistry 22 (2015) 446–453
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	No (analytical method for determination of oligosaccharide)

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

This study presents the use of ultrasound assisted extraction (UAE) for extraction of oligosaccharides from fruits and vegetables. It compares the yield of extraction obtained through conventional extraction with ethanol (85%, 50°C, 1h) and UAE in different conditions of solvent, time and temperatures. Identification and quantification of oligosaccharides is performed by high performance chromatography (HPLC). The analysis is made for fructooligosaccharides (FOS) and raffinose family oligosaccharides (RFO). The results were expressed as g per 100 g fresh weight (FW), and represent mean values ± standard deviation (n= 3). Descriptive statistics and one way analysis of variance (ANOVA) were performed on the parameters to evaluate the significant differences among the samples at 95% confidence interval (p 6 0.05) according to Tukey's test using Minitab 15 statistical software.

Results

UAE is found as an efficient method for extraction of oligosaccharides from fruits and vegetables. Higher yields of FOS and RFO extraction are obtained thanks to UAE compared to conventional extraction with ethanol. The total amount of oligosaccharides extracted with UAE is almost 2-fold higher. For example, in nectarine, around 2 g of oligosaccharides are obtained from 100 g of fresh weight with UAE vs. around 1 g with ethanol extraction.

Assessment and conclusion

Assessment and conclusion by applicant: Oligosaccharides are natural components of fruits and UAE is an efficient method of extraction of these components.

Assessment and conclusion by RMS:

Ultrasound assisted extraction (UAE) was used to extract oligosaccharides from selected fruits (blueberry, nectarine, raspberry, watermelon) and vegetables (garlic, Jerusalem artichoke, leek, scallion, spring garlic and white onion).
UAE increased the concentration of extracted oligosaccharides in all fruits and vegetables from 2 to 4-fold compared to conventional extraction. UAE became a good alternative extraction method when compared to classical extraction methods because of its high efficiency, low energy requirement and low water consumption (no reflux or refrigeration are needed).

However, the aim of this study is a methological development with the comparison of ultrasound and conventional extraction of oligosaccharides. The results give no information that could be used for risk assessment. RMS therefore considered that this study is not useful for risk assessment purpose.

Data point:	CA 8.6/04
Report author	Kollárová Karin *et al.*
Report year	2009
Report title	Impact of galactoglucomannan oligosaccharides on elongation growth in intact mung bean plants
Report No	Plant Science 177 (2009) 324–330
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP

Acceptability/Reliability:	Yes/Supportive only because extrapolation of the results on galactoglucomannan oligosaccharides to heptamaloxyloglucan is tricky.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

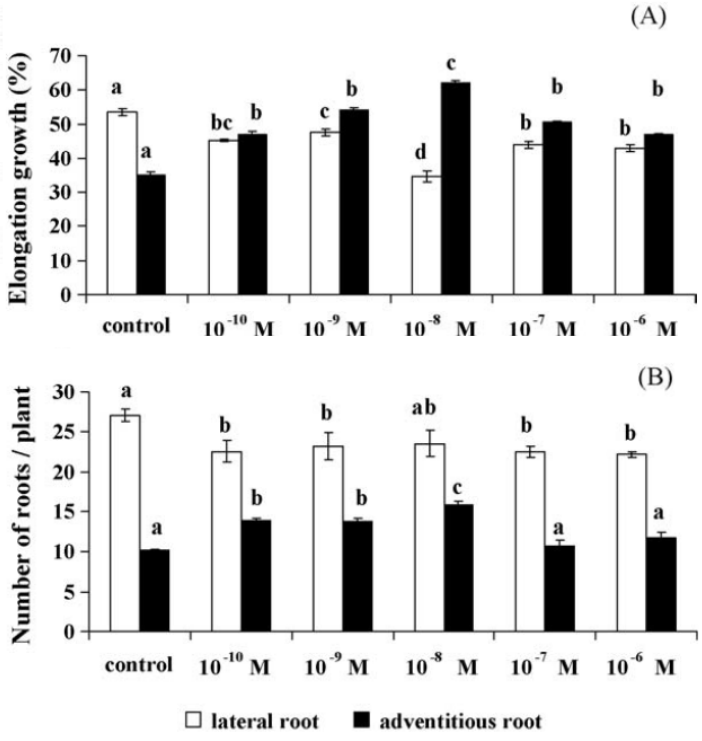
[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Materials and methods

Galactoglucomannan is a structural constituent of both, primary and secondary cell walls of higher plants (Lundqvist *et al.*, 2002 ; Schröder *et al.*, 2004 ; Willför *et al.*, 2008). The effect of galactoglucomannan oligosaccharides (GGMOs) and their structurally modified forms (GGMOs-rgalactoglucomannosyl alditols, GGMOs-g—with reduced galactose content) has been examined on the growth of mung bean (Vigna radiata (L.) Wilczek) intact plants. Plants were cultivated in hydroponic solution containing GGMOs or structurally modified oligosaccharides (concentrations ranging from 10^{-10} to 10^{-6} M) and the number and the length of lateral and adventitious roots, and hypocotyls and seminal root length were determined after seven days of culture. The elongation growth was evaluated.

Results

GGMOs, GGMOs-r, GGMOs-g influenced (with stimulation and/or inhibition effect) hypocotyl and seminal root elongation, adventitious and lateral roots formation and elongation in dependency on their concentration used (Figure 1).



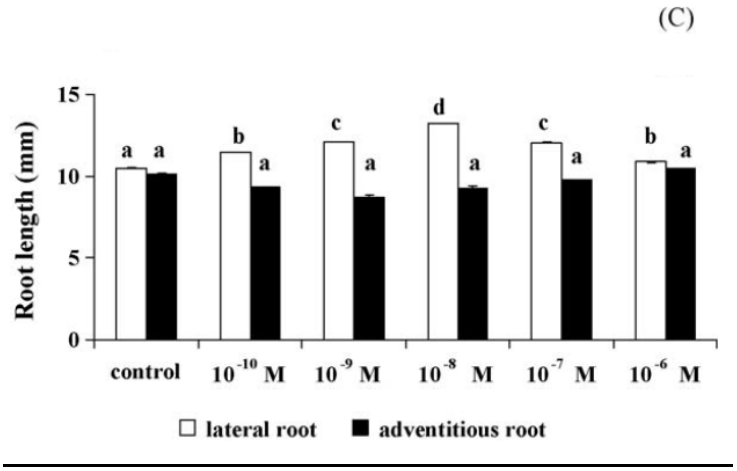


Figure 1. Effect of GGMOs alone (10^{-10} to 10^{-6} M) on elongation growth of hypocotyls and seminal roots (A), on formation (B) and elongation (C) of lateral and adventitious roots of mung bean seedlings. Elongation growth is expressed in %. Values are the means ±S.E. of three replicates with 15 samples. Different letters above bars indicate significant differences of identical parts of seedlings ($p < 0.05$).

Assessment and conclusion

Assessment and conclusion by applicant:
Data presented in this paper supports the hypothesis that exogenously added GGMOs may have anti-auxin activity and may interact also with endogenous growth regulators. Certain monosaccharide sequences with terminal galactose in the side chain of GGMOs probably play important role in their biological activity in intact plants.

Assessment and conclusion by RMS:

In this study, the impact of exogenously applied galactoglucomannan oligosaccharides (GGMOs) and their structurally modified (GGMOs-r—galactoglucomannosyl alditols, GGMOs-g—with reduced galactose content) on the growth of mung bean (Vigna radiata (L.) Wilczek) intact plants cultured inhydroponics has been determined.

RMS agrees with the conclusion proposed by the applicant.

This study suggests that the effects of galactoglucomannan oligosaccharides (GGMOs) and their structurally modified forms on plant growth is positive. These oligosaccharides are not exactly similar to heptamaloxyloglucan thus extrapolation of these results is difficult. However, it demonstrated that these sugars are not toxic to mung bean. This study may be used as a supportive (weight of evidence).

Data point:	CA 8.6/05
Report author	Kollárová et al.
Report year	2018
Report title	Impact of galactoglucomannan oligosaccharides and Cd stress on maize root growth parameters, morphology, and structure
Report No	Journal of Plant Physiology 222 (2018) 59–66
Document No	-
Guidelines followed in study	None

Deviations from current test guideline	-
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	No, not conducted under GLP
Acceptability/Reliability:	Yes/Supportive only because extrapolation of the results on galactoglucomannan oligosaccharides to heptamaloxyloglucan is tricky.

[1] See Art.3 of Annex of Regulation No 283/2013 and 284/2013

[2] RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).

Galactoglucomannan is a minority hemicellulosic component of the plant cell wall. Despite varying content and some structural differences in distinct plant species (Kubačková et al., 1992; Capek et al., 2000), the signalling properties of GGM-derived oligosaccharides, with some species specific activity, seem to be involved in the regulation systems of plants in general.

Materials and methods

The aim of this paper is to ascertain the response of maize (*Zea mays L.*) plants, considered here as a representative of grasses, to treatment by galactoglucomannan oligosaccharides (GGMOs). Plants were cultivated in hydroponic solution containing GGMOs (concentrations ranging from 10^{-10} to 10^{-6} M) for seven days. Growth parameters were measured (total length of the primary root, length of the branched part of the primary root and number and length of lateral roots) and the effect on the development of xylem and apoplasmic barriers has been determined (by the monitoring of casparian bands, suberin lamellae). Each experimental group, consisting of 15 samples, was characterized by basic statistical parameters: mean value ± standard error (SE) of three separate experiments, when not stated otherwise. The data were analysed by analysis of variance (ANOVA). The differences between separate experimental groups were evaluated by LSD test (least significant difference) at $P < 0.05$, using the Statistica, statistical program, Version 9.1, Series 1009 (StatSoft, Tulsa, USA).

Results

GGMOs stimulated primary root elongation, induction and elongation of lateral roots, and biomass production. Their effect was dependent on the concentration used. Besides, GGMOs caused an earlier development of protoxylem and early metaxylem compared to control, sown in the earlier development of xylem and casparian bands, but not of suberin lamellae (Figure 1).

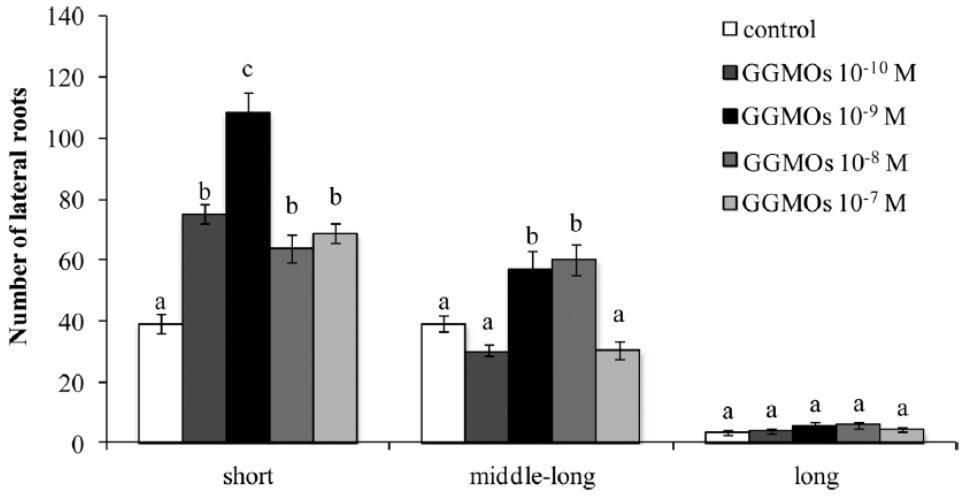


Figure 1. The number of lateral roots sorted into three groups by length: short (< 10 mm), middle-long (10–40 mm), and long (> 40 mm). The plants were cultured in Hoagland solution (control) and supplemented with GGMOs in various concentrations. Different letters show statistically significant differences between individual variants at $p < 0.05$ for each group of roots according to LSD test (n=45).

3. Assessment and conclusion

Assessment and conclusion by applicant: This study shows that GGMOs positively affected all root growth parameters of maize plants, a representative of grasses, and affected the primary root structure with an earlier development of xylem and apoplasmic barriers.

Assessment and conclusion by RMS:

RMS agrees with the conclusion of the applicant.

In conclusion, GGMOs positively affected the primary root elongation, the induction and elongation of lateral roots, biomass production, and determined the root structure in the monocot, *Zea mays*. Some differences were observed in comparison to dicots, depending on their concentrations. GGMOs affected the primary root growth, the structure (the differentiation of xylem, Casparian bands, suberine lamellae) and the morphology of the root system.

GGMOs increase plant vitality, also expressed in higher biomass production shows the potential of GGMOs to not be toxic to plants. This study may be used as a supportive (weight of evidence) given that these oligosaccharides are not exactly similar to heptamaloxyloglucan thus extrapolation of these results is difficult.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Not required.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR 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 was therefore 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 effects on biological methods for sewage treatment is not required in this particular case.

B.9.9. MONITORING DATA

No monitoring data on the effects of the active substance in the EU are available.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No metabolites identified in e-fate (Volume 3 CA and CP B.8.)

B.9.11. REFERENCES RELIED ON

LITERATURE REVIEW ON HEPTAMALOXYLOGLUCAN

RMS comment: 12 studies were considered relevant by the applicant for effects on non-target species. Search parameters and databases used seem exhaustive to RMS. Excluded studies based on full text (31) presented in the table below were not provided by the applicant. However, 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), these excluded studies would not have an impact on the outcome of the risk assessment of heptamaloxyloglucan. RMS considered the literature search provided acceptable.

The text below presents the strategy followed by the applicant to perform the literature review for heptamaloxyloglucan.

1-Summary

The applicant indicated that the literature data search of the active substance heptamaloxyloglucan, as required by Article 8(5) of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market, has been written according to:
- EFSA (2011). Guidance of EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092.
- AGES (2013). External scientific report, Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010, EFSA supporting publication 2013:EN-511.

In this literature data, aim was to find scientific peer-reviewed open literature on the active substance heptamaloxyloglucan dealing with toxicological and toxicokinetic studies, residues, fate and behavior in the environment and ecotoxicological studies which are published within the last ten years (>2008/01/01) from following data sources:
Pubmed, ScienceDirect, Europe PMC, Agricola,

The results of this search were as follows:

	HEPTAMALOXYLOGLUCAN			
Study selection process	**Toxicology**	**Residues**	**Fate**	**Ecotoxicology**
Total number of publications retrieved (with duplicates) (global search results)	17 611			
Total number of publications retrieved after removing too old literature (before 2008)	14 941			
Total number of publications retrieved after removing of duplicates	5 964			
Number of publications excluded after rapid assessment for relevance according to title (irrelevant literature)	5 731			
Number of publications further assessed in detail (possible relevant literature)	233			
Number of publications excluded according to irrelevance of title for respective section (excluded literature for this section)	151	163	198	129
Number of publications further assessed according to abstract for respective section (possible relevant literature for this section)	82	70	35	104
Number of publications excluded according to irrelevance of abstract for respective section (excluded literature for this section)	82	64	22	61

Number of publications further assessed according to full-text for respective section (possible relevant literature for this section)	0	6	13	43
Number of publications excluded according to irrelevance of full-text for respective section (excluded literature for this section)	0	2	10	31
Number of publications not excluded for relevance after detailed assessment (i.e. relevant publications) (included literature)	0	4	3	12

For ecotoxicology, 12 publications have been implemented in Volume 3 CA B.9.

2-Detailed strategy and search

BIBLIOGRAPHIC DATABASE SELECTION

Aim is to find scientific peer-reviewed open literature, as required by Article 8(5) of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market, on Heptamaloxyloglucan and its relevant metabolites dealing with toxicological and toxicokinetic studies, residues, fate and behavior in the environment and ecotoxicological studies which are published within the last ten years from various data sources.
EFSA and AGES agreed that it is sufficient to use only one bibliographic database. PubMed is a free search engine which has over 23 million records going back to 1966 and a database of citations and abstracts for biomedical literature from MEDLINE and additional life science journals. However in order to scan a broad spectrum of research, other various database have also been consulted at the occasion of this literature data review.
Therefore the following databases have been consulted to complete the scientific peer-reviewed open literature on Heptamaloxyloglucan and its relevant metabolites:
1. Pubmed, 2. ScienceDirect, 3. Europe PMC, 4. Agricola

The search was analyzed manually for each relevant reference without using dedicated software. Any removal of ambiguity or de-duplication was done manually based on the literature unique ID (e.g., PMID) or title of the literatures.

SEARCH STRATEGY

The main parameters that allow the characterization of the literature search are listed below. Trade names were not considered in the literature search as they are covered by the search on Heptamaloxyloglucan: any trade name should be found without reference to the active substance.

Date span of the literature search

The search identified scientific peer-reviewed open literatures published in last 10 years (≥ 2008).

Databases used in the literature review

The literature review has been performed using a broad collection of relevant databases for the literature search (see Table CA 9.11/01).

Table CA 9.11/01: List of databases for the literature search of Heptamaloxyloglucan

Database	Date of the Latest Database Update Included in the Search
1. Pubmed	2018/09
2. ScienceDirect	2018/09
3. Europe PMC	2018/09
4. Agricola	2018/09

Justifications for choosing these databases as literature sources are detailed in Appendix II (see Section CA 9.6.2).

Search terms for the literature review

The following Table CA 9.11/02 presents the General Search terms and Section Specific Search terms for the database search on Heptamaloxyloglucan. The information used for screening the selected databases to identify all relevant publications consists of common names, as far as available.
The General Search terms are intended to cover all data requirements whereas the Section Specific Search terms are keywords dedicated to their respective sections (Toxicology, Residues, Ecotoxicology, Fate and Environment).
Active substance EL101GV is an oligosaccharide made of 7 glucidic monomer units. 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). Therefore these terms have been used for the literature search.

Table CA 9.11/02: List of Search terms for the database search

Heptamaloxyloglucan	
Common names / ISO name	Heptamaloxyloglucan
General Search terms:	In all fields: Heptamaloxyloglucan - Oligoxyloglucan - Heteroglycan – Xyloglucan Only in title: Saccharide - Oligosaccharide - Monosaccharide - Sorbitol - Xylose Glucopyranosyl - Glucopyranose - Glucitol - Xylopyranosyl - Xylopyranose Galactopyranosyl - Galactopyranose - Fucopyranosyl – Fucopyranose
Section Specific Search terms:	See Section CA 9.6.3 Appendix III

SEARCH RESULTS

A total of 17 611 summary records was retrieved before removing duplicates, divided as:

Table CA 9.11/03: List of publications after first search and compilation of all databases

Database	Specific Search terms	First search
1_Pubmed Hepta-sorbi	In abstract: Heptamaloxyloglucan OR Oligoxyloglucan OR Heteroglycan OR Xyloglucan OR In title: Saccharide OR Oligosaccharide OR Monosaccharide OR Sorbitol AND 01/01/2008 – 23/10/2018	2 731
2_Pubmed glucitolxylose	In title: Xylose OR Glucitol AND 01/01/2008 – 23/09/2018	1 136
3_Pubmed Pyran	In title Glucopyranosyl OR Glucopyranose OR Xylopyranosyl OR Xylopyranose OR Galactopyranosyl OR Galactopyranose OR Fucopyranosyl OR Fucopyranose AND 01/01/2008 – 07/09/2018	352
4_ScienceDirect Xyloglyca	In title, abstract, or author-specified keywords Heptamaloxyloglucan OR Oligoxyloglucan OR Heteroglycan OR Xyloglucan AND 2008-2018	368
5_ScienceDirect Sacch	In title Saccharide OR Oligosaccharide OR Monosaccharide OR Sorbitol OR Xylose OR Glucopyranosyl OR Glucopyranose OR Glucitol AND 2008-2018	3 032

Database	Specific Search terms	First search
6_ScienceDirect pyran	In title Xylopyranosyl OR Xylopyranose OR Galactopyranosyl OR Galactopyranose OR Fucopyranosyl OR Fucopyranose AND 2008-2018	56
7_EuropePMChepta	(ABSTRACT:"oligoxyloglucan" OR ABSTRACT:"heptamaloxyloglucan" OR ABSTRACT:"heteroglycan" OR ABSTRACT:"xyloglucan" OR TITLE:"saccharide" OR TITLE:"oligosaccharide" OR TITLE:"monosaccharide") AND (SRC:"AGR" OR SRC:"CTX" OR SRC:"PAT" OR SRC:"PPR" OR SRC:"MED")[3] AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])	2 949
8_EuropePMCxylose	(TITLE:"sorbitol" OR TITLE:"xylose" OR TITLE:"glucopyranosyl" OR TITLE:"glucopyranose" OR TITLE:"glucitol" OR TITLE:"xylopyranosyl" OR TITLE:"xylopyranose" OR TITLE:"galactopyranosyl" OR TITLE:"galactopyranose" OR TITLE:"fucopyranosyl" OR TITLE:"fucopyranose") AND (FIRST_PDATE:[2008-01-01 TO 2018-09-24])	2 136
9_NAL hept	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan In title: Saccharide Oligosaccharide Monosaccharide Sorbitol (search done 09/2018)	23
10_NAL pyran	In title Xylose glucitol Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose (search done 09/2018)	12
11_NAL articles hepta	In any field: Heptamaloxyloglucan Oligoxyloglucan Heteroglycan Xyloglucan (search done 09/2018)	1 073
12_NAL articles sacc	In title: Saccharide Oligosaccharide Monosaccharide Sorbitol glucitol (search done 09/2018)	1 986
13_NAL articles pyran	In title Xylose Glucopyranosyl Glucopyranose Xylopyranosyl Xylopyranose Galactopyranosyl Galactopyranose Fucopyranosyl Fucopyranose (search done 09/2018)	1 757
Total	**(Total search in Excel sheet KCA 9.3/01)**	**17 611**

[3] AND Sources: Agricola (USDA/NAL), CiteXplore records, Patents, Preprint records, PubMed/MEDLINE (NKM)

All references have been compiled by the applicant under an Excel sheet referenced KCA 9.3/01.

The total of publications before removing duplicates was summarised in the Table below:

Table CA 9.11/04: List of publications after removing of too old literature (before 2007)

	Heptamaloxyloglucan
Total number of publications retrieved (with duplicates) (global search results)	17 611
Total number of too old publications (Before 2008 in Excel sheet KCA 9.3/01)	2 670
Total number of publications retrieved removing too old literature	**14 941**

After removing duplicates, the total number of publications is presented below:

Table CA 9.11/05: List of publications after removing duplicates/triplicates

	Heptamaloxyloglucan
Total number of publications retrieved removing too old literature	14 941
Total removed publications (duplicates/triplicates – Duplicate title in Excel sheet KCA 9.3/01)	8 977
Total number of publications retrieved after removing of duplicates	**5 964**

EVALUATION

The evaluation of the search results was performed according to the EFSA Guidance Document[4] and following the workflow described in Appendix I (see Section CA 9.6.1).
The numbering of the Tables in the following corresponds to the numbering system of the EFSA Guidance Document. The criteria for the relevance assessments are described in Appendix IV (see Section CA 9.6.4).

Rapid assessment on the literature review

A rapid assessment based on the reading of the titles allows performing a first selection.
The number of obviously irrelevant publications appears in Table CA 9.11/06, documenting the study selection process.

[4] Guidance of EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092

Table CA 9.11/06: List of publications after rapid assessment

	HEPTAMALOXYLOGLUCAN			
Study selection process	Toxicology	Residues	Fate	Ecotoxicology
Number of publications excluded after rapid assessment for relevance according to title (not relevant according to title in Excel sheet KCA 9.3/01)	5 731			
Number of publications further assessed in detail (possible relevant literature for at least one section according to title – Relevant according to title in Excel sheet KCA 9.3/01) Table CA 9.4.2/06	**233**			

Detailed assessment on the literature review

Those publications, which have passed the rapid assessment, have been evaluated based on abstract and full text versions.

The criteria for the relevance assessments of ecotoxicological studies ((OECD IIA 8.1 to 8.3) are :

1. Well defined test material (including its purity and impurity profile)
2. Relevant test species (terrestrial vertebrates (e.g. mammals, birds), aquatic organisms (fish, invertebrate, algae), honey bees, arthropod species, earthworms, soil microorganisms)
3. Several dose levels tested
4. Description of the observations, examinations, analysis performed

Table CA 9.11/07: Results of the study selection process

Study selection process	**Ecotoxicology**
Number of possible relevant publications (title relevant for at least one section) Table CA 9.4.2/06 ("yes")	104
Number of publications further assessed according to abstract for respective section (possible relevant literature for this section) Table CA 9.4.2/06 ("yes")	104
Number of publications excluded according to irrelevance of abstract for respective section (excluded literature for this section) Table CA 9.4.2/05	61
Number of publications further assessed according to full-text for respective section (possible relevant literature for this section) Table CA 9.4.2/04 + Table CA 9.4.2/02	43
Number of publications excluded according to irrelevance of full-text for respective section (excluded literature for this section) Table CA 9.4.2/04	31
Number of publications not excluded for relevance after detailed assessment (i.e. relevant publications) (included literature) Table CA 9.4.2/02 (Table CA 9.4.2/03)	12

Relevant literature:
In Table CA 9.11/08: Report of all relevant studies after detailed assessment of full-text documents: ordered by data requirement
In Table CA 9.11/09: Report of all relevant studies after detailed assessment of full-text documents: ordered by author(s) (F)

No relevant literature according to full-text:
In Table CA 9.11/10: Report of studies relevant according to abstract but excluded after detailed assessment of full-text

No relevant literature according to abstract:
In Table CA 9.11/11: Report of studies relevant according to title but excluded after detailed assessment of abstract (C)

In Table CA 9.11/12 is presented the study selection process: publications inclued according to relevance of title for at least one section ("yes")

Table CA 9.11/08: Report of all relevant studies after detailed assessment of full-text documents: ordered by data requirement

KCA - SANCO Data Point	Author(s)	Year	Title	Source	Classification of study
ECOTOX					
KCA 8.1	Morgan, Natalie K.; Keerqin, Chake; Wallace, Andrew; Wu, Shu-Biao; Choct, Mingan	2018	Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers	Animal Nutrition	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.1	Suo, Hai-qing; Lu, Lin; Xu, Guo-hui; Xiao, Lin; Chen, Xiao-gang; Xia, Rui-rui; Zhang, Li-yang; Luo, Xu-gang	2015	Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean meal diet	Journal of Integrative Agriculture Vol 14 Issue 10 Pages 2050-2057	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.1	Yuan, L.; Li, W.; Huo, Q.; Du, C.; Wang, Z.; Yi, B.; Wang, M.	2018	Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens	PeerJ Vol 6 Pages e4435	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.2.1	Geraylou, Zahra; Souffreau, Caroline; Rurangwa, Eugene; D'Hondt, Sofie; Callewaert, Lien; Courtin, Christophe M.; Delcour, Jan	2012	Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (Acipenser baerii) performance, immune responses and gastrointestinal microbial community	Fish & Shellfish Immunology Vol 33 Issue 4 Pages 718-724	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

KCA - SANCO Data Point	Author(s)	Year	Title	Source	Classification of study
	A.; Buyse, Johan; Ollevier, Frans				

KCA - SANCO Data Point	Author(s)	Year	Title	Source	Classification of study
KCA 8.2.1	Geraylou, Zahra; Souffreau, Caroline; Rurangwa, Eugene; De Meester, Luc; Courtin, Christophe M.; Delcour, Jan A.; Buyse, Johan; Ollevier, Frans	2013	Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (Acipenser baerii)	Fish & Shellfish Immunology Vol 35 Issue 3 Pages 766-775	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.2.1	Hoseinifar, S. H.; Soleimani, N.; Ringo, E.	2014	Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (Cyprinus carpio) fry	Br J Nutr Vol 112 Issue 8 Pages 1296-302	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.2.1	Ye, J. D.; Wang, K.; Li, F. D.; Sun, Y. Z.	2011	Single or combined effects of fructo- and mannan oligosaccharide supplements and Bacillus clausii on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus	Aquaculture nutrition Vol 17 Issue 4 Pages e902-e911	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.5	Veras, H. C. T.; Parachin, N. S.; Almeida, J. R. M.	2017	Comparative assessment of fermentative capacity of different xylose-consuming yeasts	Microb Cell Fact Vol 16 Issue 1 Pages 153	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.6	Jovanovic-Malinovska, Ruzica; Kuzmanova, Slobodanka; Winkelhausen, Eleonora	2014	Oligosaccharide Profile in Fruits and Vegetables as Sources of Prebiotics and Functional Foods	International journal of food properties Vol 17 Issue 5 Pages 949-965	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

KCA - SANCO Data Point	Author(s)	Year	Title	Source	Classification of study
KCA 8.6	Jovanovic-Malinovska, Ruzica; Kuzmanova, Slobodanka; Winkelhausen, Eleonora	2015	Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables	Ultrasonics Sonochemistry Vol 22 Pages 446-453	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.6	Kollárová, Karin; Richterová, Danica; Slováková, Ľudmila; Henselová, Mária; Capek, Peter; Lišková, Desana	2009	Impact of galactoglucomannan oligosaccharides on elongation growth in intact mung bean plants	Plant Science Vol 177 Issue 4 Pages 324-330	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
KCA 8.6	Kollárová, Karin; Kamenická, Viktória; Vatehová, Zuzana; Lišková, Desana	2018	Impact of galactoglucomannan oligosaccharides and Cd stress on maize root growth parameters, morphology, and structure	Journal of Plant Physiology Vol 222 Pages 59-66	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

Table CA 9.11/09: Report of all relevant studies after detailed assessment of full-text documents: ordered by author(s)

Author(s)	KCA - SANCO Data Point	Year	Title	Source	Classification of study
ECOTOX					
Geraylou, Zahra; Souffreau, Caroline; Rurangwa, Eugene; D'Hondt, Sofie; Callewaert, Lien; Courtin, Christophe M.; Delcour, Jan A.; Buyse, Johan; Ollevier, Frans	KCA 8.2.1	2012	Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (Acipenser baerii) performance, immune responses and gastrointestinal microbial community	Fish & Shellfish Immunology Vol 33 Issue 4 Pages 718-724	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Geraylou, Zahra; Souffreau, Caroline; Rurangwa, Eugene; De Meester, Luc; Courtin, -Christophe M.; Delcour, Jan A.; Buyse, Johan; Ollevier, Frans	KCA 8.2.1	2013	Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (Acipenser baerii)	Fish & Shellfish Immunology Vol 35 Issue 3 Pages 766-775	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Hoseinifar, S. H.; Soleimani, N.; Ringo, E.	KCA 8.2.1	2014	Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut	Br J Nutr Vol 112 Issue 8 Pages 1296-302	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested.

Author(s)	KCA - SANCO Data Point	Year	Title	Source	Classification of study
			microbiota and stress resistance of common carp (Cyprinus carpio) fry		The data are considered as supplemental information.
Jovanovic-Malinovska, Ruzica; Kuzmanova, Slobodanka; Winkelhausen, Eleonora	KCA 8.6	2014	Oligosaccharide Profile in Fruits and Vegetables as Sources of Prebiotics and Functional Foods	International journal of food properties Vol 17 Issue 5 Pages 949-965	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

Author(s)	KCA - SANCO Data Point	Year	Title	Source	Classification of study
Jovanovic-Malinovska, Ruzica; Kuzmanova, Slobodanka; Winkelhausen, Eleonora	KCA 8.6	2015	Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables	Ultrasonics Sonochemistry Vol 22 Pages 446-453	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Kollárová, Karin; Richterová, Danica; Slováková, Ľudmila; Henselová, Mária; Capek, Peter; Lišková, Desana	KCA 8.6	2009	Impact of galactoglucomannan oligosaccharides on elongation growth in intact mung bean plants	Plant Science Vol 177 Issue 4 Pages 324-330	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Kollárová, Karin; Kamenická, Viktória; Vatehová, Zuzana; Lišková, Desana	KCA 8.6	2018	Impact of galactoglucomannan oligosaccharides and Cd stress on maize root growth parameters, morphology, and structure	Journal of Plant Physiology Vol 222 Pages 59-66	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Morgan, Natalie K.; Keerqin, Chake; Wallace, Andrew; Wu, Shu-Biao; Choct, Mingan	KCA 8.1	2018	Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers	Animal Nutrition	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Suo, Hai-qing; Lu, Lin; Xu, Guo-hui; Xiao, Lin; Chen, Xiao-gang; Xia, Rui-rui; Zhang, Li-yang; Luo, Xu-gang	KCA 8.1	2015	Effectiveness of dietary xylo-oligosaccharides for broilers fed a conventional corn-soybean meal diet	Journal of Integrative Agriculture Vol 14 Issue 10 Pages 2050-2057	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Veras, H. C. T.; Parachin, N. S.; Almeida, J. R. M.	KCA 8.5	2017	Comparative assessment of fermentative capacity of different xylose-consuming yeasts	Microb Cell Fact Vol 16 Issue 1 Pages 153	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

Author(s)	KCA - SANCO Data Point	Year	Title	Source	Classification of study
Ye, J. D.; Wang, K.; Li, F. D.; Sun, Y. Z.	KCA 8.2.1	2011	Single or combined effects of fructo- and mannan oligosaccharide supplements and Bacillus clausii on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus	Aquaculture nutrition Vol 17 Issue 4 Pages e902-e911	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.
Yuan, L.; Li, W.; Huo, Q.; Du, C.; Wang, Z.; Yi, B.; Wang, M.	KCA 8.1	2018	Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens	PeerJ Vol 6 Pages e4435	b) EFSA guidance point 5.4.1: The study results are in line with the available regulatory data requested. The data are considered as supplemental information.

Table CA 9.11/10: Report of studies relevant according to abstract but excluded after detailed assessment of full-text

Author(s)	Year	Title	Source	Reason for not including in dossier
ECOTOXICOLOGY				
Akter, Mst Nahid; Sutriana, Amalia; Talpur, Allah Dad; Hashim, Roshada	2016	Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish, Pangasianodon hypophthalmus	Aquaculture international Vol 24 Issue 1 Pages 127-144	According to the full-text, the mannan-oligosaccharides tested in this study are derived from cell walls of the yeast *Saccharomyces cerevisiae*. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Ali, Syed Raffic; Ambasankar, Kondusamy; Praveena, Ezhil; Nandakumar, Sambasivam; Syamadayal, Jagabatula	2017	Effect of dietary mannan oligosaccharide on growth, body composition, haematology and biochemical parameters of Asian seabass (Lates calcarifer)	Aquaculture research Vol 48 Issue 3 Pages 899-908	According to the full-text document, the mannan-oligosaccharides tested in this study are derived from cell walls of yeast. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Andrews, Simi Rose; Sahu, Narottam P.; Pal, Asim K.; Kumar, Shivendra	2009	Haematological modulation and growth of Labeo rohita fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella	Aquaculture research Vol 41 Issue 1 Pages 61-69	According to the full-text document, the mannan-oligosaccharides tested in this study are derived from cell walls of yeast. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Battaglia, M.	2017	Assessment of a Registered Dietitian Administered Fermentable Oligosaccharide Disaccharide, Monosaccharide, and Polyol Elimination Diet Experience	Journal of the Academy of Nutrition and Dietetics Vol 117 Issue 9, Supplement Pages A17	According to full-text (only a poster), not relevant for ecotoxicology data. Indeed, the test was done on humans. So not relevant for ecotox purpose.
Bozkurt, M.; Küçükyilmaz, K.; Çatli, A. U.; Çinar, M.	2008	Growth Performance and Slaughter Characteristics of Broiler Chickens Fed with Antibiotic, Mannan Oligosaccharide and Dextran Oligosaccharide Supplemented Diets	International journal of poultry science Vol 7 Issue 10 Pages 969-977	According to the full-text document, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan

Author(s)	Year	Title	Source	Reason for not including in dossier
Bozkurt, M.; Kucukyilmaz, K.; Catli, A. U.; Cinar, M.; Bintas, E.; Coven, F.	2012	Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions	Poult Sci Vol 91 Issue 6 Pages 1379-86	According to the full-text, the mannan-oligosaccharides tested in this study are derived from the yeast *Saccharomyces cerevisiae*. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Bozkurt, M.; Bintas, E.; Kirkan, S.; Aksit, H.; Kucukyilmaz, K.; Erbas, G.; Cabuk, M.; Aksit, D.; Parin, U.; Ege, G.; Kocer, B.; Seyrek, K.; Tuzun, A. E.	2016	Comparative evaluation of dietary supplementation with mannan oligosaccharide and oregano essential oil in forced molted and fully fed laying hens between 82 and 106 weeks of age	Poult Sci Vol 95 Issue 11 Pages 2576-2591	According to the full-text document, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Cabrera, J. C.; Wégria, G.; Onderwater, R. C. A.; González, G.; Nápoles, M. C.; Falcón-Rodríguez, A. B.; Costales, D.; Rogers, H. J.; Diosdado, E.; González, S.; Cabrera, G.; González, L.; Wattiez, R.	2012	Practical use of oligosaccharins in agriculture	Acta horticulturae Issue 1009 Pages 195-212	This publication explains the mode of action of xyloglucan but doesn't describe the ecotox effects.
Daniels, Carly L.; Merrifield, Daniel L.; Boothroyd, Dominic P.; Davies, Simon J.; Factor, Jan R.; Arnold, Katie E.	2010	Effect of dietary Bacillus spp. and mannan oligosaccharides (MOS) on European lobster (Homarus gammarus L.) larvae growth performance, gut morphology and gut microbiota	Aquaculture Vol 304 Issue 1 Pages 49-57	According to the full-text document, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Dimitroglou, Arkadios; Merrifield, Daniel Lee; Spring, Peter; Sweetman, John; Moate, Roy; Davies, Simon John	2010	Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (Sparus aurata)	Aquaculture Vol 300 Issue 1 Pages 182-188	According to the full-text document, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.

Author(s)	Year	Title	Source	Reason for not including in dossier
Duan, X. D.; Chen, D. W.; Zheng, P.; Tian, G.; Wang, J. P.; Mao, X. B.; Yu, J.; He, J.; Li, B.; Huang, Z. Q.; Ao, Z. G.; Yu, B.	2016	Effects of dietary mannan oligosaccharide supplementation on performance and immune response of sows and their offspring	Animal Feed Science and Technology	According to the full-text document, the mannan-oligosaccharides tested in this study are Actigen, which was provided by Alltech, Inc.(Nicholasville, USA). Actigen is a high concentrated MOS product and derived from the mannan fraction of a specific strain of *Saccharomyces cerevisae* It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Forsatkar, Mohammad Navid; Nematollahi, Mohammad Ali; Rafiee, Gholamreza; Farahmand, Hamid; Martínez-Rodríguez, Gonzalo	2017	Effects of prebiotic mannan oligosaccharide on the growth, survival, and anxiety-like behaviors of zebrafish (Danio rerio)	Journal of applied aquaculture Vol 29 Issue 2 Pages 183-196	According to the full-text document, the mannan-oligosaccharides tested in this study are Agrimos®, which is a specific combination of mannan oligosaccharides and glucose (B-glucans) extracted from the yeast cell walls of Saccharomyces cerevisiae (Lallemand AnimalNutrition, France). It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan. The prebiotic used
Galloway, Andrew F.; Pedersen, Martin J.; Merry, Beverley; Marcus, Susan E.; Blacker, Joshua; Benning, Liane G.; Field, Katie J.; Knox, J. Paul	2017	Xyloglucan is released by plants and promotes soil particle aggregation	new phytologist Vol 217 Pages 1128-1136	According to the full-text document, the publication shows that there is xyloglucan in roots of several species but doesn't show the effects of xyloglucan on non-target plants. It is already known that xyloglucan is present in plants. It doesn't give additional information for ecotoxicological purpose.
Ghasemian, M.; Jahanian, R.	2016	Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility in aged laying hens	Animal Feed Science and Technology Vol 213 Pages 81-89	According to the full-text, the mannan-oligosaccharides tested in this study are ActiveMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Gu, Hao; Lu, Man; Zhang, Zhiping; Xu, Jinjin; Cao, Wenhua; Miao, Minmin	2018	Metabolic process of raffinose family oligosaccharides during cold stress and recovery in cucumber leaves	Journal of Plant Physiology Vol 224-225 Pages 112-120	According to full-text document, not relevant for ecotoxicology data, because it focuses on metabolic process against cold stress and not on effects of oligosaccharides on cucumber.

Author(s)	Year	Title	Source	Reason for not including in dossier
Hajiaghapour, M.; Rezaeipour, V.	2018	Comparison of two herbal essential oils, probiotic, and mannan-oligosaccharides on egg production, hatchability, serum metabolites, intestinal morphology, and microbiota activity of quail breeders	Livestock Science Vol 210 Pages 93-98	According to the full-text document, the mannan-oligosaccharides tested in this study are derived from yeas *S. cerevisiae* t. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Hill, T. M.; Bateman, H. G.; Aldrich, J. M.; Schlotterbeck, R. L.	2008	Oligosaccharides for Dairy Calves	The Professional Animal Scientist Vol 24 Issue 5 Pages 460-464	According to the full-text document, the mannan-oligosaccharides tested in this study are derived from BioMoS which is derived from yeast cells.. The fructo-oligosaccharides were derived from Ultra-FOS (derived from chicory inulin). It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan. Moreover, the studied parameters are not of ecotox relevance.
Hsieh, Y. S.; Harris, P. J.	2009	Xyloglucans of monocotyledons have diverse structures	Mol Plant Vol 2 Pages 943-65	According to the full-text document, this publication focuses on different structures of xyloglucan in monocotydedoneous. But this information is not relevant for ecotox purpose. It just proves that xyloglucans are constitutive of cell walls of monocotyledoneous.
Hsieh, Y. S.; Harris, P. J.	2012	Structures of xyloglucans in primary cell walls of gymnosperms, monilophytes (ferns sensu lato) and lycophytes	Phytochemistry Vol 79 Pages 87-101	According to full-text document, not relevant for ecotoxicology data
Lee, F. J.; Rusch, D. B.; Stewart, F. J.; Mattila, H. R.; Newton, I. L.	2014	Saccharide breakdown and fermentation by the honey bee gut microbiome	Environ Microbiol Vol 17 Issue 3 Pages 796-815	According to the full-text document, this publication describes the honey bee gut microbiome and its functions. But this information cannot be used for ecotox purpose in terms of risk assessment.
Mao, X.; Xiao, X.; Chen, D.; Yu, B.; He, J.; Chen, H.; Xiao, X.; Luo, J.; Luo, Y.; Tian, G.; Wang, J.	2017	Dietary apple pectic oligosaccharide improves gut barrier function of rotavirus-challenged weaned pigs by increasing antioxidant capacity of enterocytes	Oncotarget Vol 8 Issue 54 Pages 92420-92430	According to the full-text document, piglets were contaminated with rotavirus, which is not representative for ecotox purpose .This publication describes toxicological parameters which are not fully relevant for ecotox purpose.

Author(s)	Year	Title	Source	Reason for not including in dossier
Mirzapour-Rezaee, S. S.; Farhangi, M.; Rafiee, G.	2017	Combined effects of dietary mannan- and fructo-oligosaccharide on growth indices, body composition, intestinal bacterial flora and digestive enzymes activity of regal peacock (Aulonocara stuartgranti)	Aquaculture nutrition Vol 23 Issue 3 Pages 629-636	According to the full-text, the mannan-oligosaccharides tested in this study are ActiveMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Najdegerami H., Ebrahim; Tokmachi, Amir; Bakhshi, Farideh	2017	Evaluating the Effects of Dietary Prebiotic Mixture of Mannan Oligosaccharide and Poly-β-Hydroxybutyrate on the Growth Performance, Immunity, and Survival of Rainbow Trout, Oncorhynchus mykiss (Walbaum 1792), Fingerlings	Journal of the World Aquaculture Society Vol 48 Issue 3 Pages 415-425	According to the full-text document, the mannan-oligosaccharides tested in this study are from ActiveMOS which is derived from the yeast *Saccharomyces cerevisiae*. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Pepe-Ranney, C.; Campbell, A. N.; Koechli, C. N.; Berthrong, S.; Buckley, D. H.	2016	Unearthing the Ecology of Soil Microorganisms Using a High Resolution DNA-SIP Approach to Explore Cellulose and Xylose Metabolism in Soil	Front Microbiol Vol 7 Pages 703	According to the full-text document, the information from this publication are highly complex, with biochemical processus, extraction of DNA…. This is not really useful for ecotox purpose.
Razeghi Mansour, M.; Akrami, R.; Ghobadi, S. H.; Amani Denji, K.; Ezatrahimi, N.; Gharaei, A.	2012	Effect of dietary mannan oligosaccharide (MOS) on growth performance, survival, body composition, and some hematological parameters in giant sturgeon juvenile (Huso huso Linnaeus, 1754)	Fish physiology and biochemistry Vol 38 Issue 3 Pages 829-835	According to the full-text document, the mannan-oligosaccharides tested in this study are from ActiveMOS which is derived from the yeast *Saccharomyces cerevisiae*. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Safari, Omid; Shahsavani, Davar; Paolucci, Marina; Atash, Masoomeh Mehraban Sang	2014	Single or combined effects of fructo- and mannan oligosaccharide supplements on the growth performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish, Astacus leptodactylus leptodactylus Eschscholtz, 1823	Aquaculture Vol 432 Pages 192-203	According to the full-text document, this publication describes highly specifics parameters not directly for ecotox purpose and rather combined effects studied than single.
Stick, Robert V.; Williams, Spencer J.	2009	Chapter 9 - Disaccharides, Oligosaccharides and Polysaccharides	Elsevier Pages 321-341	According to full-text documents, the information is too generalist and not fully relevant for ecotoxicology data.

Author(s)	Year	Title	Source	Reason for not including in dossier
Szklany, K.; De Theije, C. G. M.; De Waard, C.; Van Staveren, N. G.; Van Wageningen, T. A.; Wu, J.; Verdouw, M.; Van Limpt, K.; Wopereis, H.; Groenink, L.; Oozeer, R.; Garssen, J.; Knippels, L. M. J.; Kraneveld, A. D.	2016	P.1.c.008 - Effect of early life supplementation of non-digestible oligosaccharides on brain development and behaviour in healthy mice	European Neuropsychopharmacology Vol 26 Pages S191	non relevant according to review of full text (not enough information)
Torrecillas, Silvia; Montero, Daniel; Izquierdo, Marisol	2014	Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action	Fish & Shellfish Immunology Vol 36 Issue 2 Pages 525-544	According to the full-text document, the mannan-oligosaccharides tested in this study are derived from cell walls of yeast (BioMoS, Active MOS…). It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan. Moreover, it is a review of several publications, so it is not relevant.
Torrecillas, Silvia; Montero, Daniel; Caballero, Maria José; Robaina, Lidia; Zamorano, Maria Jesús; Sweetman, John; Izquierdo, Marisol	2015	Effects of dietary concentrated mannan oligosaccharides supplementation on growth, gut mucosal immune system and liver lipid metabolism of European sea bass (Dicentrarchus labrax) juveniles	Fish & Shellfish Immunology Vol 42 Issue 2 Pages 508-516	According to full-text document, the mannan-oligosaccharides tested in this study are from Actigen® (cMOS; Alltech, Nickolasville, KY, USA), which is a second-generation yeast outer cell wall compound developed using nutrigenomics technology. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Zhang, Jian; Liu, Yongjian; Tian, Lixia; Yang, Huijun; Liang, Guiying; Xu, Donghui	2012	Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, Litopenaeus vannamei	Fish and Shellfish Immunology Vol 33 Issue 4 Pages 1027-1032	According to full-text document, not relevant for ecotoxicology data because the species Pacific white shrimp *Litopenaeus vannamei* is not relevant of European species. Moreover, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.

Table CA 9.11/11: Report of studies relevant according to title but excluded after detailed assessment of abstract

Author(s)	Year	Title	Source	Reason for not including in dossier
ECOTOXICOLOGY				
	2016	Fructose and sorbitol	Elsevier Title: Meyler's SideEffects of Drugs Pages 460-461	Information from a book. Abstract not available. So it couldn't be assessed. Information should be likely too generalist.
Agbogbo, Frank K.; Coward-Kelly, Guillermo	2008	Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, Pichia stipitis	Biotechnology letters Vol 30 Issue 9 Pages 1515-1524	According to abstract, not relevant for ecotoxicology data.
Andres-Barranco, S.; Vico, J. P.; Grillo, M. J.; Mainar-Jaime, R. C.	2015	Reduction of subclinical Salmonella infection in fattening pigs after dietary supplementation with a ss-galactomannan oligosaccharide	J Appl Microbiol Vol 118 Issue 2 Pages 284-94	According to abstract, not relevant for ecotoxicology data.
Bonos, Eleftherios; Christaki, Efterpi; Abrahim, Amin; Soultos, Nikolaos; Florou-Paneri, Panagiota	2011	The influence of mannan oligosaccharides, acidifiers and their combination on caecal microflora of Japanese quail (Coturnix japonica)	Anaerobe Vol 17 Pages 436-439	According to the abstract, the mannan-oligosaccharides tested in this study are derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Courtois, Josiane	2009	Oligosaccharides from land plants and algae: production and applications in therapeutics and biotechnology	Current Opinion in Microbiology Vol 12 Issue 3 Pages 261-273	According to abstract, it concerns the production of oligosaccharides. So it is not relevant for ecotoxicology data.
Dimitroglou, Arkadios; Davies, Simon J.; Sweetman, John; Divanach, Pascal; Chatzifotis, Stavros	2010	Dietary supplementation of mannan oligosaccharide on white sea bream (Diplodus sargus L.) larvae: effects on development, gut morphology and salinity tolerance	Aquaculture research Vol 41 Issue 9 Pages e245-e251	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Do Huu, Hoang; Jones, Clive M.	2014	Effects of dietary mannan oligosaccharide supplementation on juvenile spiny lobster Panulirus homarus (Palinuridae)	Aquaculture Vol 42 Pages 258-264	According to the abstract, this publication was not deemed relevant because it concerns a tropical marine species, thus not relevant for EU species. Moreover, according to the uses of product, no exposure of marine species is expected. So this publication is not relevant.

Author(s)	Year	Title	Source	Reason for not including in dossier
Fettke, Joerg; Malinova, Irina; Eckermann, Nora; Steup, Martin	2009	Cytosolic heteroglycans in photoautotrophic and in heterotrophic plant cells	Phytochemistry Vol 70 Issue 6 Pages 696-702	According to abstract, not relevant for ecotoxicology data.
Filip, Miuța; Vlassa, Mihaela; Coman, Virginia; Halmagyi, Adela	2016	Simultaneous determination of glucose, fructose, sucrose and sorbitol in the leaf and fruit peel of different apple cultivars by the HPLC–RI optimized method	Food Chemistry Vol 199 Pages 653-659	According to abstract, description of analytical method, not relevant for ecotoxicological data
Geigerová, Martina; Bunešová, Věra; Vlková, Eva; Salmonová, Hana; Rada, Vojtěch	2017	Selection of prebiotic oligosaccharides suitable for synbiotic use in calves	Animal Feed Science and Technology Vol 229 Pages 73-78	According to abstract, not relevant for ecotoxicology data because the oligosaccharides are derived from industry and are not representatives of natural oligosaccharides.
Gelsinger, S. L.; Pino, F.; Jones, C. M.; Gehman, A. M.; Heinrichs, A. J.	2016	Effects of a dietary organic mineral program including mannan oligosaccharides for pregnant cattle and their calves on calf health and performance	The Professional Animal Scientist Vol 32 Issue 2 Pages 205-213	According to the abstract, the animals were supplemented with mannan oligosaccharides and products of yeast and bacterial fermentation in combination. The mannan-oligosaccharide were not tested alone in the diet. So it is not relevant for ecotox purpose.
Giannenas, I.; Doukas, D.; Karamoutsios, A.; Tzora, A.; Bonos, E.; Skoufos, I.; Tsinas, A.; Christaki, E.; Tontis, D.; Florou-Paneri, P.	2016	Effects of Enterococcus faecium, mannan oligosaccharide, benzoic acid and their mixture on growth performance, intestinal microbiota, intestinal morphology and blood lymphocyte subpopulations of fattening pigs	Animal Feed Science and Technology Vol 220 Pages 159-167	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Gómez, Belén; Gullón, Beatriz; Yáñez, Remedios; Schols, Henk; Alonso, José L.	2016	Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation	Journal of Functional Foods Vol 20 Pages 108-121	According to abstract, not relevant for ecotoxicology data.
Gomez-Verduzco, G.; Cortes-Cuevas, A.; Lopez-Coello, C.; Avila-Gonzalez, E.; Nava, G. M.	2009	Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to Eimeria spp	Acta Vet Scand Vol 51 Pages 11	According to the abstract, the mannan-oligosaccharides tested in this study are derived from yeasts. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Harcus, D.; Dignard, D.; Lepine, G.; Askew, C.; Raymond, M.; Whiteway, M.; Wu, C.	2013	Comparative xylose metabolism among the Ascomycetes C. albicans, S. stipitis and S. cerevisiae	PLoS One Vol 8 Issue 11 Pages e80733	According to abstract, engineered strains of ascomycetes were tested, which is not relevant for ecotox purpose.

Author(s)	Year	Title	Source	Reason for not including in dossier
Kučerová, Danica; Kollárová, Karin; Zelko, Ivan; Vatehová, Zuzana; Lišková, Desana	2014	Galactoglucomannan oligosaccharides alleviate cadmium stress in Arabidopsis	Journal of Plant Physiology Vol 171 Issue 7 Pages 518-524	According to the abstract, the role of galactoglucomannan oligosaccharides in plant protection against cadmium stress was examined. This is not relevant for ecotox purpose.
Kumar, Vineet; Naudiyal, Meenakshi; Dubey, Pallavi	2017	Acidic and Neutral Monosaccharide Analysis of Cold Water Soluble Polysaccharide from Hippophae salicifolia D. Don Leaves	Journal of Biologically Active Products from Nature Vol 7 Issue 1 Pages 27-33	According to abstract, not relevant for ecotoxicology data.
Lange, M.; Lee, H.; Dallas, D.; Le Parc, A.; de Moura Bell, J. M. L. N.; Barile, D.	2014	Determining Functional Properties and Sources of Recently Identified Bioactive Food Components: Oligosaccharides, Glycolipids, Glycoproteins, and Peptides	Academic Press Pages 441- 461	Information from a book which is not fully useful for the purpose of this bibliography.
Le Gall, H.; Philippe, F.; Domon, J. M.; Gillet, F.; Pelloux, J.; Rayon, C.	2015	Cell Wall Metabolism in Response to Abiotic Stress	Plants (Basel) Vol 4 Issue 1 Pages 112-66	According to abstract, not relevant for ecotoxicology data.
Li, Zongjun; Bai, Hanxun; Zheng, Lixin; Jiang, Huai; Cui, Huiying; Cao, Yangchun; Yao, Junhu	2018	Bioactive polysaccharides and oligosaccharides as possible feed additives to manipulate rumen fermentation in Rusitec fermenters	International Journal of Biological Macromolecules Vol 109 Pages 1088-1094	According to abstract, not relevant for ecotoxicology data. No source about the tested polysaccharides and oligosaccharides. A rumen simulation technique as used which is not relevant in comparison to in vivo test.
Lim, E.; Lim, J. Y.; Shin, J. H.; Seok, P. R.; Jung, S.; Yoo, S. H.; Kim, Y.	2015	D-Xylose suppresses adipogenesis and regulates lipid metabolism genes in high-fat diet-induced obese mice	Nutr Res Vol 35 Issue 7 Pages 626-36	The abstract presents highly specific mechanistic information which is not requested by the ecàtoxicological data requirements for active substances per se.
Linneen, S. K.; Mourer, G. L.; Sparks, J. D.; Jennings, J. S.; Goad, C. L.; Lalman, D. L.	2014	Effects of mannan oligosaccharide on beef-cow performance and passive immunity transfer to calves	The Professional Animal Scientist Vol 30 Issue 3 Pages 311-317	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.

Author(s)	Year	Title	Source	Reason for not including in dossier
Lucyszyn, Neoli; Lubambo, Adriana F.; Ono, Lucy; Jó, Tatiane A.; de Souza, Clayton F.; Sierakowski, Maria Rita	2011	Chemical, physico-chemical and cytotoxicity characterisation of xyloglucan from Guibourtia hymenifolia (Moric.) J. Leonard seeds	Food hydrocolloids Vol 25 Issue 5 Pages 1242-1250	According to abstract, not relevant for ecotoxicology data.
Luzardo-Ocampo, I.; Campos-Vega, R.; Gaytán-Martínez, M.; Preciado-Ortiz, R.; Mendoza, S.; Loarca-Piña, G.	2017	Bioaccessibility and antioxidant activity of free phenolic compounds and oligosaccharides from corn (Zea mays L.) and common bean (Phaseolus vulgaris L.) chips during in vitro gastrointestinal digestion and simulated colonic fermentation	Food Research International Vol 100 Pages 304-311	According to abstract, not relevant for ecotoxicology data. Indeed, in vitro tests were performed instead of in vivo tests which are more relevant for ecotoxicology. No sufficient information for ecotox purpose.
Nishinari, K.; Takemasa, M.; Yamatoya, K.; Shirakawa, M.	2009	19 - Xyloglucan	Woodhead Publishing Pages 535-566	According to abstract, not relevant for ecotoxicology data.
Nochta, I.; Tuboly, T.; Halas, V.; Babinszky, L.	2009	Effect of different levels of mannan-oligosaccharide supplementation on some immunological variables in weaned piglets	Journal of animal physiology and animal nutrition Vol 93 Issue 4 Pages 496-504	According to the abstract, the mannan-oligosaccharides were tested in this study. It is quite different of heptamaloxyloglucan derived from fruits. Moreover, only immune parameters were studied which are not fully relevant for ecotox purpose.
Nochta, I.; Halas, V.; Tossenberger, J.; Babinszky, L.	2010	Effect of different levels of mannan-oligosaccharide supplementation on the apparent ileal digestibility of nutrients, N-balance and growth performance of weaned piglets	Journal of animal physiology and animal nutrition Vol 94 Issue 6 Pages 747-756	According to the abstract, the mannan-oligosaccharides were tested in this study. It is quite different of heptamaloxyloglucan derived from fruits. Moreover, only immune parameters were studied which are not fully relevant for ecotox purpose.
Nodeh, H.; Mansoori, B.; Rahbari, S.; Modirsanei, M.; Aparnak, P.	2008	Assessing the effect of diclazuril on the intestinal absorptive capacity of broilers infected with experimental coccidiosis, using d-xylose absorption test	Journal of veterinary pharmacology and therapeutics Vol 31 Issue 3 Pages 265-267	According to abstract, not relevant for ecotoxicology data. IT concerns the effects of another active substance than heptamaloxyloglucan.
Nugroho, Rudy Agung; Fotedar, Ravi	2014	Comparing the effects of dietary selenium and mannan oligosaccharide supplementation on the growth, immune function, and antioxidant enzyme activity in the cultured marron Cherax cainii (Austin, 2002)	Aquaculture international Vol 22 Issue 2 Pages 585-596	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.

Author(s)	Year	Title	Source	Reason for not including in dossier
Pantophlet, A. J.; Gilbert, M. S.; van den Borne, J. J. G. C.; Gerrits, W. J. J.; Priebe, M. G.; Vonk, R. J.	2016	Insulin sensitivity in calves decreases substantially during the first 3 months of life and is unaffected by weaning or fructo-oligosaccharide supplementation	Journal of Dairy Science Vol 99 Issue 9 Pages 7602-7611	According to abstract, not relevant for ecotoxicology data.
Park, M. H.	2016	Sucrose delays senescence and preserves functional compounds in Asparagus officinalis L	Biochem Biophys Res Commun Vol 480 Issue 2 Pages 241-247	According to abstract, not relevant for ecotoxicology data, because concerns the effects of sucrose on storage of asparagus
Pena, M. J.; Darvill, A. G.; Eberhard, S.; York, W. S.; O'Neill, M. A.	2008	Moss and liverwort xyloglucans contain galacturonic acid and are structurally distinct from the xyloglucans synthesized by hornworts and vascular plants	Glycobiology Vol 18 Issue 11 Pages 891-904	According to abstract, this publication gives structural information on the composition in xyloglucans of several species, but it is not relevant for ecotoxicology data.
Peng, B.; Huang, S.; Liu, T.; Geng, A.	2015	Bacterial xylose isomerases from the mammal gut Bacteroidetes cluster function in Saccharomyces cerevisiae for effective xylose fermentation	Microb Cell Fact Vol 14 Pages 70	According to abstract, not relevant for ecotoxicology data.
Qian, Li; Zhou, Yan; Teng, Zhaolin; Du, Chun-Ling; Tian, Changrong	2014	Preparation and antibacterial activity of oligosaccharides derived from dandelion	International Journal of Biological Macromolecules Vol 64 Pages 392-394	According to abstract, not relevant for ecotoxicology data.
Qian, Zhi-Gang; Jiang, Long-Fa	2014	Preparation and antibacterial activity of the oligosaccharides derived from Rhizoma Phragmites	Carbohydrate Polymers Vol 111 Pages 356-358	According to abstract, not relevant for ecotoxicology data because the species *Rhizoma Phragmites* is not relevant of European species.
Rivero, F.; Torrecillas, S.; Caballero, M. J.; Makol, A.; Izquierdo, M. S.; Montero, D.	2016	Combined effects of dietary mannan oligosaccharides and Pediococcus acidilactici and their combination in low fish meal and fish oil diets for European sea bass, Dicentrarchus labrax, juveniles	Fish & Shellfish Immunology Vol 53 Pages 69	According to abstract, the mannan-oligosaccharides tested in this study were derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Ruiz, Encarnación; Gullón, Beatriz; Moura, Patrícia; Carvalheiro, Florbela; Eibes, Gemma; Cara, Cristóbal; Castro, Eulogio	2017	Bifidobacterial growth stimulation by oligosaccharides generated from olive tree pruning biomass	Carbohydrate Polymers Vol 169 Pages 149-156	According to abstract, not relevant for ecotoxicology data.

Author(s)	Year	Title	Source	Reason for not including in dossier
Salmon, L.; Edwards, S. A.	2015	The effects of dietary fructo-oligosaccharide addition on boar taint compounds and performance in heavy slaughter weight boars and gilts	Animal Feed Science and Technology Vol 207 Pages 130-139	According to the abstract, not useful for ecotox purpose (specific to slauhtering and malodoreous faecal compunds)
Sang, H. M.; Ky le, T.; Fotedar, R.	2009	Dietary supplementation of mannan oligosaccharide improves the immune responses and survival of marron, Cherax tenuimanus (Smith, 1912) when challenged with different stressors	Fish Shellfish Immunol Vol 27 Issue 2 Pages 341-8	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Sang, Huynh Minh; Fotedar, Ravi	2010	Prebiotic mannan oligosaccharide diet improves health status of the digestive system of marron, Cherax tenuimanus (Smith 1912)	Journal of applied aquaculture Vol 22 Pages 240-250	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Sang, Huynh Minh; Fotedar, Ravi; Filer, Keith	2011	Effects of Dietary Mannan Oligosaccharide on Survival, Growth, Physiological Condition, and Immunological Responses of Marron, Cherax tenuimanus (Smith 1912)	Journal of the World Aquaculture Society Vol 42 Issue 2 Pages 230-241	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Sang, H. M.; Fotedar, R.; Filer, K.	2011	Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, Cherax destructor Clark (1936)	Aquaculture nutrition Vol 17 Issue 2 Pages e629-e635	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.
Sang, H. M.; Kien, N. T.; Thanh Thuy, N. T.	2014	Effects of dietary mannan oligosaccharide on growth, survival, physiological, immunological and gut morphological conditions of black tiger prawn (Penaeus monodon Fabricius 1798)	Aquaculture nutrition Vol 20 Issue 3 Pages 341-348	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not pertinent to assess the effects of heptmaloxyloglucan.

Author(s)	Year	Title	Source	Reason for not including in dossier
Singh, Ramkrishna D.; Banerjee, Jhumur; Arora, Amit	2015	Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides	Bioactive Carbohydrates and Dietary Fibre Vol 5 Pages 19-30	According to abstract, not relevant for ecotoxicology data.
Sparkman, O. David; Penton, Zelda E.; Kitson, Fulton G.	2011	Chapter 35 - Sugars (Monosaccharides)	Academic Press Pages 407-410	According to abstract, not relevant for ecotoxicology data. It concerns an analytical method review.
Takada, T.; Sato, R.; Kikuta, S.	2017	A mannitol/sorbitol receptor stimulates dietary intake in Tribolium castaneum	PLoS One Vol 12 Issue 10 Pages e0186420	According to abstract, not relevant for ecotoxicology data. It concerns the biochemistry of a pest of stored products.
Torrecillas, Silvia; Makol, Alex; Benítez-Santana, Tibiábin; Caballero, María José; Montero, Daniel; Sweetman, John; Izquierdo, Marisol	2011	Reduced gut bacterial translocation in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS)	Fish & Shellfish Immunology Vol 30 Issue 2 Pages 674-681	According to the abstract, the mannan-oligosaccharides tested in this study are derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Torrecillas, S.; Rivero-Ramírez, F.; Izquierdo, M. S.; Caballero, M. J.; Makol, A.; Suarez-Bregua, P.; Fernández-Montero, A.; Rotllant, J.; Montero, D.	2018	Feeding European sea bass (Dicentrarchus labrax) juveniles with a functional synbiotic additive (mannan oligosaccharides and Pediococcus acidilactici): An effective tool to reduce low fishmeal and fish oil gut health effects?	Fish & Shellfish Immunology Vol 81 Pages 10-20	According to the abstract, the mannan-oligosaccharides tested in this study are BioMOS, which is derived from yeast cells. It is quite different of heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Tuoping, L. I.; Suhong, L. I.; Na, Wang; Mei, G. U. O.	2009	Oligosaccharide probiotics	Not information	The abstract and the publication are not available. So it couldn't be assessed.
Valpotic, H.; Zura Zaja, I.; Samardzija, M.; Habrun, B.; Ostovic, M.; Duricic, D.; Macesic, N.; Mikulec, Z.; Kocila, P.; Sobiech, P.; Valpotic, I.; Vince, S.	2018	Dietary supplementation with mannan oligosaccharide and clinoptilolite modulates innate and adaptive immune parameters of weaned pigs	Pol J Vet Sci Vol 21 Issue 1 Pages 83-93	According to abstract, not relevant for ecotoxicology data.
Wang, Yan; Guo, Qingbin; Douglas Goff, H.; LaPointe, Gisèle	2018	Oligosaccharides: Structure, Function and Application	Elsevier Title: Reference Module in Food Science 31	According to the abstract, this publication could be relevant. But it comes from a book, which is not available. So the full-text cannot be assessed.

Author(s)	Year	Title	Source	Reason for not including in dossier
Wu, Sheng-Jun	2014	Preparation and antioxidant activity of the oligosaccharides derived from Laminaria japonica	Carbohydrate Polymers Vol 106 Pages 22-24	According to abstract, the oligosaccharides come from *Laminaria japonica*, which is not relevant for heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Wu, Shengjun; Huang, Xiaolian	2017	Preparation and antioxidant activities of oligosaccharides from Crassostrea gigas	Food Chemistry Vol 216 Pages 243-246	According to abstract, the oligosaccharides come from *Crassostrea gigas*, which is not relevant for heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Xia, Zhenqiang	2015	Preparation of the oligosaccharides derived from Flammulina velutipes and their antioxidant activities	Carbohydrate Polymers Vol 118 Pages 41-43	According to abstract, the oligosaccharides come from *Flammulina velutipes*, which is not relevant for heptamaloxyloglucan derived from fruits. So this publication is not relevant to assess the effects of heptmaloxyloglucan.
Xu, H.; Xiong, A. S.; Zhao, W.; Tian, Y. S.; Peng, R. H.; Chen, J. M.; Yao, Q. H.	2011	Characterization of a glucose-, xylose-, sucrose-, and D-galactose-stimulated beta-glucosidase from the alkalophilic bacterium Bacillus halodurans C-125	Curr Microbiol Vol 62 Issue 3 Pages 833-9	According to abstract, not relevant for ecotoxicology data. It is more specialized in genetics.
Yao, Xing-Cun; Cao, Yan; Wu, Sheng-Jun	2013	Antioxidant activity and antibacterial activity of peach gum derived oligosaccharides	International Journal of Biological Macromolecules Vol 62 Pages 1-3	According to abstract, not relevant for ecotoxicology data.
Zabotina, O. A.	2012	Xyloglucan and its biosynthesis	Front Plant Sci Vol 3 Pages 134	According to the abstract, not relevant for ecotoxicology data because of focus on biosynthtis of xyloglucan and not the observation of effects.
Zerillo, M. M.; Adhikari, B. N.; Hamilton, J. P.; Buell, C. R.; Levesque, C. A.; Tisserat, N.	2013	Carbohydrate-active enzymes in pythium and their role in plant cell wall and storage polysaccharide degradation	PLoS One Vol 8 Issue 9 Pages e72572	According to abstract, not relevant for ecotoxicology data. It gives mainly information on genetic and enzymatic processus, which are not fully relevant for ecotox purpose.
Zhang, Shanshan; Hu, Haijuan; Wang, Lufeng; Liu, Fengxia; Pan, Siyi	2018	Preparation and prebiotic potential of pectin oligosaccharides obtained from citrus peel pectin	Food Chemistry Vol 244 Pages 232-237	According to abstract, the oligosaccharides come from citrus, which is not relevant for heptamaloxyloglucan. So this publication is not relevant to assess the effects of heptmaloxyloglucan.

Author(s)	Year	Title	Source	Reason for not including in dossier
Zhao, X.; Moates, G. K.; Wellner, N.; Collins, S. R.; Coleman, M. J.; Waldron, K. W.	2014	Chemical characterisation and analysis of the cell wall polysaccharides of duckweed (Lemna minor)	Carbohydr Polym Vol 111 Pages 410-8	The abstract gives information about the structure of wall of *L. minor*. But polysaccharides constitutive of *L. minor* are not fruits extracts as heptamaloxyloglucan. So it is not relevant for the active substance.
Zhou, Xiao-Li; Kong, Xiang-Feng; Lian, Guo-Qi; Blachier, Francois; Geng, Mei-Mei; Yin, Yu-Long	2014	Dietary supplementation with soybean oligosaccharides increases short-chain fatty acids but decreases protein-derived catabolites in the intestinal luminal content of weaned Huanjiang mini-piglets	Nutrition Research Vol 34 Issue 9 Pages 780-788	According to the abstract, the oligosaccharides tested on piglets were extracted from soybean and are thus not relevant for the effects of heptamaloxyloglucan which is extracted from apples.

Table CA 9.11/12: Study selection process: publications inclued according to relevance of title for at least one section ("yes")

Table updated by RMS to present only studies selected for ecotoxicology

Author(s)	Year	Title	Source
	2016	Fructose and sorbitol	Elsevier 460-461
Agbogbo, Frank K.; Coward-Kelly, Guillermo	2008	Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, Pichia stipitis	Biotechnology letters 30 1515-1524
Akter, Mst Nahid; Sutriana, Amalia; Talpur, Allah Dad; Hashim, Roshada	2016	Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish, Pangasianodon hypophthalmus	Aquaculture international 24 127-144
Ali, Syed Raffic; Ambasankar, Kondusamy; Praveena, Ezhil; Nandakumar, Sambasivam; Syamadayal, Jagabatula	2017	Effect of dietary mannan oligosaccharide on growth, body composition, haematology and biochemical parameters of Asian seabass (Lates calcarifer)	Aquaculture research 48 899-908
Andres-Barranco, S.; Vico, J. P.; Grillo, M. J.; Mainar-Jaime, R. C.	2015	Reduction of subclinical Salmonella infection in fattening pigs after dietary supplementation with a ss-galactomannan oligosaccharide	J Appl Microbiol 118 284-94
Andrews, Simi Rose; Sahu, Narottam P.; Pal, Asim K.; Kumar, Shivendra	2009	Haematological modulation and growth of Labeo rohita fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella	Aquaculture research 41 61-69
Battaglia, M.	2017	Assessment of a Registered Dietitian Administered Fermentable Oligosaccharide Disaccharide, Monosaccharide, and Polyol Elimination Diet Experience	Journal of the Academy of Nutrition and Dietetics 117 A17
Bonos, Eleftherios; Christaki, Efterpi; Abrahim, Amin; Soultos, Nikolaos; Florou-Paneri, Panagiota	2011	The influence of mannan oligosaccharides, acidifiers and their combination on caecal microflora of Japanese quail (Coturnix japonica)	Anaerobe 17 436-439

Author(s)	Year	Title	Source
Bozkurt, M.; Bintas, E.; Kirkan, S.; Aksit, H.; Kucukyilmaz, K.; Erbas, G.; Cabuk, M.; Aksit, D.; Parin, U.; Ege, G.; Kocer, B.; Seyrek, K.; Tuzun, A. E.	2016	Comparative evaluation of dietary supplementation with mannan oligosaccharide and oregano essential oil in forced molted and fully fed laying hens between 82 and 106 weeks of age	Poult Sci 95 2576-2591
Bozkurt, M.; Küçükyilmaz, K.; Çatli, A. U.; Çinar, M.	2008	Growth Performance and Slaughter Characteristics of Broiler Chickens Fed with Antibiotic, Mannan Oligosaccharide and Dextran Oligosaccharide Supplemented Diets	International journal of poultry science 7 969-977
Bozkurt, M.; Kucukyilmaz, K.; Catli, A. U.; Cinar, M.; Bintas, E.; Coven, F.	2012	Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions	Poult Sci 91 1379-86
Cabrera, J. C.; Wégria, G.; Onderwater, R. C. A.; González, G.; Nápoles, M. C.; Falcón-Rodríguez, A. B.; Costales, D.; Rogers, H. J.; Diosdado, E.; González, S.; Cabrera, G.; González, L.; Wattiez, R.	2012	Practical use of oligosaccharins in agriculture	Acta horticulturae 195-212
Courtois, Josiane	2009	Oligosaccharides from land plants and algae: production and applications in therapeutics and biotechnology	Current Opinion in Microbiology 12 261-273
Daniels, Carly L.; Merrifield, Daniel L.; Boothroyd, Dominic P.; Davies, Simon J.; Factor, Jan R.; Arnold, Katie E.	2010	Effect of dietary Bacillus spp. and mannan oligosaccharides (MOS) on European lobster (Homarus gammarus L.) larvae growth performance, gut morphology and gut microbiota	Aquaculture 304 49-57
Dimitroglou, Arkadios; Davies, Simon J.; Sweetman, John; Divanach, Pascal; Chatzifotis, Stavros	2010	Dietary supplementation of mannan oligosaccharide on white sea bream (Diplodus sargus L.) larvae: effects on development, gut morphology and salinity tolerance	Aquaculture research 41 e245-e251

Author(s)	Year	Title	Source
Dimitroglou, Arkadios; Merrifield, Daniel Lee; Spring, Peter; Sweetman, John; Moate, Roy; Davies, Simon John	2010	Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (Sparus aurata)	Aquaculture 300 182-188
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CA 8.3.1/03	Barker R.J. *et al.*	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	N	N	-	-	Y
CA 8.3.1/04	Smessaert J. *et al.*	2019	Monitoring pollinator activity in an apple and pear orchard, linked with the analysis of the nectar composition Acta Hortic. 1231. ISHS 2019. DOI 10.17660/ActaHortic.2019.1231.11 Proc. II Int. Workshop on Floral Biology and S-Incompatibility in Fruit Species Eds.: E. Ortega Pastor, J. Halász and P. De Franceschi Not GLP, published	N	N	-	-	N
CA 8.3.1/05	Pavlova T. *et al.*	2018	Quality characteristics of honey: a review Proceedings of university of ruse - 2018, volume 57, book 10.2. Not GLP, published	N	N	-	-	N

CA 8.3.1/06	Machado De-Melo A. A. *et al.*	2017	Composition and properties of Apis mellifera honey: A review. Journal of Apicultural Research, 2017 - https://doi.org/10.1080/00218839.2017.1338444 Not GLP, published	N	N	-	-	N
CA 8.3.1/07	Lee F.J.*et al.*	2014	Saccharide breakdown and fermentation by the honey bee gut microbiome Environmental Microbiology (2014) - doi:10.1111/1462-2920.12526. Not GLP, published	N	N	-	-	N
CA 8.3.1/08	Dmitruk M. *et al.*	2019	Flowering, nectar secretion, and structure of the nectary in the fowers of *Acer pseudoplatanus L.* Acta Agrobot. 2019;72(3):1787 Not GLP, published	N	N	-	-	N
CA 8.3.1/09	Somme L. *et al.*	2016	Food in a row : urban trees offer valuable floral resources to pollinating insects Urban Ecosyst - DOI 10.1007/s11252-016-0555-z Not GLP, published	N	N	-	-	N
CA 8.3.1/10	Tomczyk M. *et al.*	2019	The influence of geographical origin on honey composition studied by Polish and Slovak honeys Food Analysis, Food Quality and Nutrition Czech Journal of Food Sciences, 37, 2019 (4): 232–238 - doi.org/10.17221/40/2019-CJFS Not GLP, published	N	N	-	-	N
CA 8.3.1/11	Val A. *et al.*	1998	Enzymatic Determination of Galactose and Lactose in Honey J. Agric. Food Chem. 1998, 46, 1381-1385 Not GLP, published	N	N	-	-	N
CA 8.4/01	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 Not GLP, published	N	N	-	-	Y

CA 8.4/02	Zhang B.G. *et 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 Not GLP, published	N	N	-	-	Y
CA 8.4/03	Garvin M.H. *et al*.	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	N	N	-	-	Y
CA 8.5/01	Robertson G.P. e*t al*.	2015	Nitrogen transformations. Pages 421-446 *in* E. A. Paul, editor. Soil microbiology, ecology and biochemistry. Fourth edition. Academic Press, Burlington, Massachusetts, USA Not GLP, published	N	N	-	-	N
CA 8.5/02	Veras H.C.T e*t al*.	2017	Comparative assessment of fermentative capacity of different xylose-consuming yeasts Veras *et al.* Microb Cell Fact (2017) 16:153 Not GLP, published	N	N	-	-	N
CA 8.6/01	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 CIT unpublished report number 05-27-072-ES 05-27-072-ES Not GLP, unpublished	N	N	-	Elicityl	Y
CA 8.6/02	Jovanovic-Malinovska R e*t al*.	2014	Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods. International Journal of Food Properties, 17:949–965, 2014 Not GLP, published	N	N	-	-	N
CA 8.6/03	Jovanovic-Malinovska R e*t al*.	2015	Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables Ultrasonics Sonochemistry 22 (2015) 446–453 Not GLP, published	N	N	-	-	N
CA 8.6/04	Kollárová R e*t al*.	2009	Impact of galactoglucomannan oligosaccharides on elongation growth in intact mung bean plants	N	N	-	-	N

			Plant Science 177 (2009) 324–330 Not GLP, published					
CA 8.6/05	Kollárová R e*t al*.	2018	Impact of galactoglucomannan oligosaccharides and Cd stress on maize root growth parameters, morphology, and structure Journal of Plant Physiology 222 (2018) 59–66 Not GLP, published	N	N	-	-	N

[1] In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
[2] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[3] The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).