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SCIENTIFIC OPINION

Assessment of genetically modified sugar beet KWS20-1 (application GMFF-2023-14732)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Genetically modified sugar beet KWS20-1 was developed to confer tolerance to glyphosate-, dicamba- and glufosinate-ammonium-based herbicides. These properties were achieved by introducing the *cp4 epsps*, *dmo* and *pat* expression cassettes. The molecular characterisation data and bioinformatic analyses do not identify issues requiring further safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between sugar beet KWS20-1 and its conventional counterpart need further assessment, except for pectin in roots, which underwent additional evaluation and was found not to raise any safety or nutritional concerns. The GMO Panel does not identify safety concerns regarding the potential toxicity and allergenicity of the CP4 EPSPS, DMO and PAT proteins as expressed in sugar beet KWS20-1, and finds no evidence that the genetic modification would change the overall safety of sugar beet KWS20-1 as food and feed. In the context of this application, the consumption of food and feed from sugar beet KWS20-1 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that sugar beet KWS20-1 is as safe as the conventional counterpart and non-GM sugar beet reference varieties tested, and no post-market monitoring of food/feed is considered necessary. The scope of the application does not include cultivation and import of viable materials in the EU and the products would be expected to only contain residual amounts of DNA and protein. The environmental risk assessment was limited to the possible plant-to-bacteria horizontal gene transfer and the evaluation of potential interactions of KWS20-1 sugar beet products with biogeochemical cycles, and neither of them indicates a safety concern. The GMO Panel concludes that the sugar beet KWS20-1 is as safe as its conventional counterpart and the tested non-GM reference sugar beet varieties with respect to potential effects on human and animal health and the environment.

KEYWORDS

CP4 EPSPS, DMO, genetic engineering, GM, import and processing, KWS20-1, PAT, sugar beet (*Beta vulgaris* L.)

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SUMMARY

Following the submission of application GMFF-2023-14732 under Regulation (EC) No 1829/2003 from Bayer Agriculture BV and KWS SAAT SE & Co. KGaA (referred to hereafter as 'the applicants'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide-tolerant sugar beet (*Beta vulgaris* L.) KWS20-1 (Unique Identifier KB-KWS2Ø1–6) according to Regulation (EU) No 503/2013. The scope of the application is for food and feed produced from and food containing ingredients produced from sugar beet KWS20-1 for import and processing submitted within the framework of Regulation (EC) No 1829/2003.

In this scientific opinion, the GMO Panel reports on the outcome of its risk assessment of sugar beet KWS20-1 according to the scope of the application. The GMO Panel conducted the assessment of sugar beet KWS20-1 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of GM plants.

The molecular characterisation data establish that sugar beet KWS20-1 contains a single insert consisting of one copy of the *cp4 epsps, dmo* and the *pat* expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the newly expressed proteins (NEPs), the sequences corresponding to open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise any safety concerns. The stability of the inserted DNA and of the introduced traits is confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS, DMO and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and *Escherichia coli*-produced CP4 EPSPS, DMO and PAT proteins indicate that these proteins are equivalent, and the *E. coli*-derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic-phenotypic and compositional characteristics tested between sugar beet KWS20-1 and its conventional counterpart need further assessment, except for pectin in roots, which underwent additional evaluation and was found not to raise any safety or nutritional concerns.

The GMO Panel does not identify safety concerns regarding the potential toxicity and allergenicity of the CP4 EPSPS, DMO and PAT proteins as expressed in sugar beet KWS20-1 and finds no evidence that the genetic modification would change the overall safety of sugar beet KWS20-1, as food and feed. In the context of this application, the consumption of food and feed from sugar beet KWS20-1 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that sugar beet KWS20-1, as described in this application, is as safe as the conventional counterpart and non-GM reference sugar beet varieties tested, and no post-market monitoring of food/feed is considered necessary.

The scope of the application does not include cultivation and import of viable materials in the EU and the products would be expected to only contain residual amounts of DNA and protein. The environmental risk assessment was limited to the possible plant-to-bacteria horizontal gene transfer and the evaluation of potential interactions of KWS20-1 sugar beet products with biogeochemical cycles, and neither of them indicates a safety concern. The GMO Panel concludes that the sugar beet KWS20-1 is as safe as its conventional counterpart and the tested non-GM reference sugar beet varieties with respect to potential effects on human and animal health and the environment.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of sugar beet KWS20-1.

The risk assessment of sugar beet KWS20-1 was conducted using a hybrid variety where the KWS20-1 event is in hemizygous condition. A KWS20-1 hybrid line has been cultivated to produce the relevant materials for protein expression, agronomic and phenotypic evaluation, compositional analysis and for the toxicological studies. Based on the above, the GMO Panel concluded its risk assessment assuming the material imported in the EU is derived from KWS20-1 hybrid varieties.

The GMO Panel concludes that sugar beet KWS20-1 as assessed in the context of this application is as safe as its conventional counterpart and the tested non-GM sugar beet reference varieties with respect to potential effects on human and animal health and the environment.

1 | INTRODUCTION

The scope of the application GMFF-2023-14732 is for food produced from genetically modified sugar beet KWS20-1 or containing ingredients produced from sugar beet KWS20-1 and feed produced from sugar beet KWS20-1 for import and processing submitted within the framework of Regulation (EC) No 1829/2003 and does not include the cultivation or the import of viable material in the EU. Sugar beet KWS20-1 was developed to confer tolerance to glyphosate-, dicamba- and glufosinate-ammonium-based herbicides.

1.1 | Background

On 31 May 2023, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application GMFF-2023-14732 for authorisation of sugar beet KWS20-1 (Unique Identifier KB-KWS2Ø1-6), submitted by Bayer Agriculture BV and KWS SAAT SE & Co. KGaA (hereafter referred to as 'the applicants') according to Regulation (EC) No 1829/2003.[1]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,[2] and with the EFSA guidance documents.

On 23 October 2023, EFSA declared the application valid and informed EU Member States, the European Commission and it was published on Open EFSA.[3]

From validity date, EFSA and the Panel on genetically modified organisms of the EFSA (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2023-14732. Such time limit was extended whenever EFSA and/or the GMO Panel requested supplementary information to the applicants. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicants during the risk assessment was made available to the EU Member States and European Commission (for further details, see the section 'Documentation', below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application GMFF-2023-14732 as of date of validity.

1.2 | Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of sugar beet KWS20-1 in the context of its scope as defined in application GMFF-2023-14732.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation. In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[5]

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicants have submitted data in the form of a technical dossier[6] to comply with the specific provisions of Regulation (EU) No 503/2013[2] and EFSA (2021a, 2021b).

In accordance with Art. 38 of the Regulation (EC) No 178/2002[7] and taking into account the protection of confidential information and personal data in accordance with Articles 39 to 39e of the same Regulation, the non-confidential version of the application has been published on OpenEFSA.[8]

[1]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, p. 1–48.

[3]Available online: https://open.efsa.europa.eu/dossier/GMFF-2023-00378.

[4]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

[5]These particulars are available online at: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2023-00378.

[6]https://open.efsa.europa.eu/dossier/GMFF-2023-14732.

[7]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[8]https://open.efsa.europa.eu/questions/EFSA-Q-2022-00330.

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[9] EFSA carried out a public consultation on the non-confidential version of the application from 9 February 2024 to 1 March 2024, for which no comments were received.

The GMO Panel based its scientific assessment on the data provided by the applicants, relevant scientific comments submitted by EU Member States, and relevant scientific publications.

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017, 2018, 2019a, 2019b; EFSA GMO Panel, 2010b, 2018, 2021) for the risk assessment of GM plants.

For this application, the contractors performed preparatory work for the evaluation of the applicants' literature search (OC/EFSA/MESE/2022/03-01-SC17), the completeness and quality of DNA sequencing information (OC/EFSA/GMO/2020/01), the bioinformatic analyses (OC/EFSA/GMO/2021/06) and methods applied for the statistical analysis of the field trial data (OC/EFSA/AMU/2020/02 - SC 4) and the analysis of the 90-day toxicity study (EOI/EFSA/2022/01 - CT 17–2024 and EOI/EFSA/2022/01 – CT NIF 2023 02).

3 | ASSESSMENT[10]

3.1 | Introduction

Sugar beet KWS20-1 expresses genes encoding a CP4 5-enolpyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS) protein, the dicamba mono-oxygenase (DMO) protein and the phosphinothricin acetyltransferase (PAT) protein to confer tolerance to glyphosate, dicamba, and glufosinate-ammonium-based herbicides, respectively.

The scope of application GMFF-2023-14732 is for food produced from genetically modified sugar beet KWS20-1 or containing ingredients produced from sugar beet KWS20-1 and feed produced from sugar beet KWS20-1 for import and processing submitted within the framework of Regulation (EC) No 1829/2003 and does not include the cultivation or the import of viable material in the European Union (EU).

In sugar beet breeding programmes, the availability of cytoplasmic male sterility promoted the development of hybrid varieties and currently in the commercial sugar beet seed market almost the totality of the varieties are hybrid varieties. The risk assessment of sugar beet KWS20-1 was conducted using hybrid varieties where the KWS20-1 event is in hemizygous condition. A KWS20-1 hybrid line has been cultivated to produce the relevant materials for protein expression, agronomic and phenotypic evaluation, compositional analysis and for the toxicological studies. Based on the above, the GMO Panel concluded its risk assessment assuming the material imported in the EU is derived from KWS20-1 hybrid varieties.

3.2 | Systematic literature review[10,11]

The GMO Panel assessed the applicants' literature searches on sugar beet KWS20-1, which include a scoping review, according to the guidelines given in EFSA (2010, 2019).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support of the risk assessment of application GMFF-2023-14732. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for sugar beet KWS20-1 at present.

The GMO Panel considered the overall quality of the performed literature searches acceptable.

The literature searches identified four relevant non-peer-reviewed publications on sugar beet KWS20-1 from relevant key organisations, and one peer-reviewed publication. The relevant publications are listed in Appendix A.

The relevant publications identified through the literature searches did not report information pointing to safety issues associated with sugar beet KWS20-1 relevant to the scope of this application.

[9]Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.

[10]The application is available online at: https://open.efsa.europa.eu/dossier/GMFF-2023-14732.

[11]Additional information: 5/6/2024 and 28/2/2025.

3.3 | Molecular characterisation[10,12]

3.3.1 | Transformation process and vector constructs

Sugar beet KWS20-1 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Hypocotyl segments of sugar beet genotype 04E05B1DH05 were co-cultured with a disarmed *A. tumefaciens* strain AGL1 containing the vector PV-BVHT527462. The plasmid PV-BVHT527462 used for the transformation contains three expression cassettes between the right and left border of the T-DNA, containing the following genetic elements:

- The *cp4 epsps* expression cassette consists of the promoter, intron and 5'UTR of the S-adenosyl-L-methionine synthetase gene (*SAM2*) from *Cucumis melo*; the chloroplast transit peptide of the *ShkG* gene from *Arabidopsis thaliana*; the plant codon-optimised *aroA* gene from the *Agrobacterium* sp. strain CP4 coding for the CP4 EPSPS protein; the 3' UTR from an expressed gene (T-*guf-Mt1*) of unknown function from *Medicago truncatula*.
- The *dmo* expression cassette consists of the enhancer from a Dahlia Mosaic Virus (DaMV) promoter region; the promoter, leader and intron of a putative ubiquitin protein gene from *Cucumis melo* (*Ubq-Cm1)*; the chloroplast transit peptide and the sequence encoding the first 27 amino acids of the *rbcs* gene from *Pisum sativum*; the plant codon-optimised dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia*; the 3' UTR from an expressed gene (T-*guf-Mt2)* of unknown function from *Medicago truncatula*.
- The *pat* expression cassette consists of the promoter and leader (P-*Cab-At1*) of a chlorophyll a/b-binding (CAB) protein from *Arabidopsis thaliana*; the plant codon-optimised phosphinothricin N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*; the 3' UTR region of a 3' UTR region (T-*Hsp20-Mt1*) of a putative *Hsp20* gene from *Medicago truncatula*.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria.

3.3.2 | Transgene constructs in the GM plant

Molecular characterisation of sugar beet was performed by Southern blot analysis, polymerase chain reaction (PCR) and DNA sequence analysis in order to determine insert copy number, size and organisation of the inserted sequences and to confirm the absence of plasmid backbone sequences.

The EFSA GMO Panel assessed the data and found it compliant with the requirements listed in EFSA GMO Panel (2018), both in terms of the approach, the coverage and sensitivity.

Southern blot analysis indicated that sugar beet KWS20-1 contains a single insert, consisting of a single copy of the T-DNA with no rearrangements as compared to the PV-BVHT527462 transformation vector. The insert and copy number were confirmed by multiple restriction enzyme/probe combinations covering the T-DNA region and flanking regions. PCR analyses confirmed the results obtained by the Southern blot analyses. The absence of vector backbone sequences was demonstrated by Southern blot analysis using four overlapping backbone-specific probes.

The nucleotide sequence of the entire insert of sugar beet KWS20-1 together with 1000 bp of the 5' and 1000 bp of the 3' flanking regions was determined. The insert of 11,722 bp is identical to the T-DNA of PV-BVHT527462, except for the deletion of 316 bp of the right border region and 182 bp of the left border region.

A comparison with the pre-insertion locus indicated that 7 bp were deleted from the sugar beet genomic DNA. The possible interruption of known endogenous sugar beet genes by the insertion in sugar beet KWS20-1 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in sugar beet KWS20-1.

The results of segregation (see Section 3.3.5) and bioinformatics analyses are compatible with a single insertion in the nuclear genome.

Bioinformatics analyses of the amino acid sequence of the newly expressed DMO, PAT and CP4 EPSPS proteins reveal no significant similarities to toxins and allergens.

In addition, bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA also do not indicate significant similarities to toxins and allergens. In conclusion, these analyses indicated that the expression of any ORF showing significant similarities to toxins or allergens in sugar beet KWS20-1 is unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicants performed a sequence identity analysis for sugar beet KWS20-1, which consists of three expression cassettes containing plant codon-optimised NEPs to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.6.

[12]Additional information: 6/12/2023, 17/6/2024 and 19/12/2024.

3.3.3 | Protein characterisation and equivalence

Sugar beet KWS20-1 expresses three new proteins: CP4 EPSPS, DMO and PAT. Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Escherichia coli*. A set of biochemical methods was employed to demonstrate the equivalence between the sugar beet and *E. coli*-derived CP4 EPSPS, DMO and PAT. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

– CP4 EPSPS protein characterisation and equivalence.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that both plant and *E. coli*-produced CP4 EPSPS proteins had the expected molecular weight of ~43 kDa and were comparably immunoreactive to CP4 EPSPS protein specific antibodies. Glycosylation detection analysis demonstrated that none of the CP4 EPSPS proteins were glycosylated. Amino acid sequence analysis of the plant-derived and the previously analysed *E. coli*-produced CP4 EPSPS protein by MS methods showed that both proteins matched the deduced sequence as defined by the *cp4 epsps* gene that is identical to that assessed in previously assessed events such as maize NK603 and maize MON 87429 (EFSA, 2004; EFSA GMO Panel, 2022a). In addition, the mass spectrometry (MS) data showed that in a subpopulation of plant-produced CP4 EPSPS protein the N-terminal methionine of the protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000). Functional equivalence was demonstrated by a biochemical in vitro assay which showed that plant- and *E. coli*-produced CP4 EPSPS proteins had comparable enzymatic activity.

– DMO protein characterisation and equivalence.

The amino acid sequence of DMO expressed in the event KWS20-1 (reported as DMO + 27.1 in the dossier) referred to hereafter as DMO is the same as that of the proteins expressed in previously assessed events MON 87429 and MON 87419 (EFSA GMO Panel, 2022a, 2023), with the exception of the N-terminal 27 amino acids coming from the *rbcs* gene from *Pisum sativum*, which are the same as the N-terminal 27 amino acids of DMO expressed in events MON 87708 and MON 94100 (EFSA GMO Panel, 2013, 2022b).

SDS-PAGE and western blot analysis showed that both plant and *E. coli*-produced DMO proteins had the expected molecular weight of ~38 kDa and were comparably immunoreactive to DMO protein specific antibodies. Glycosylation detection analysis demonstrated that none of the DMO proteins were glycosylated. Amino acid sequence analysis of the plant-derived DMO protein and the previously analysed *E. coli*-produced DMO by MS methods showed that both proteins matched the deduced sequence as defined by the *dmo* gene. Functional equivalence was demonstrated by an in vitro assay which showed that plant- and *E. coli*-derived DMO proteins had comparable enzymatic activity. High specificity of the DMO protein for dicamba in the event MON 87708 has been previously demonstrated (EFSA GMO Panel, 2013).

– PAT protein characterisation and equivalence.

SDS-PAGE and western blot analysis showed that both plant and *E. coli*-produced PAT proteins had the expected molecular weight of ~22 kDa and were comparably immunoreactive to PAT protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the PAT proteins were glycosylated. Amino acid sequence analysis of the plant-derived PAT and the previously analysed *E. coli*-produced PAT protein by MS methods showed that both proteins matched the deduced sequence as defined by the *pat* gene that is identical to that assessed in previously assessed events such as maize 1507 and maize MON 87429 (EFSA, 2005; EFSA GMO Panel, 2022a). In addition, the MS data showed that the N-terminal methionine of the plant-produced PAT protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000). Functional equivalence was demonstrated by a biochemical in vitro assay which showed that plant and *E. coli*-produced PAT proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the biochemical, structural and functional properties of plant and *E. coli*-produced CP4 EPSPS, DMO and PAT proteins indicate that these proteins are equivalent, and the *E. coli*-derived proteins can be used in the safety studies.

3.3.4 | Information on the expression of the insert

Protein levels of CP4 EPSPS, DMO and PAT were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in the United States during the 2020 growing season. Samples analysed included tops (BBCH 49 growth stage[13]) and over-season roots 3 (OSR3) (BBCH 49), from plants treated and not treated with the intended herbicides. The mean values and standard errors of protein expression levels in tops ($n = 20$) and over-season

[13]BBCH scale describes phenological stages (Meier, 2001).

roots 3 ($n = 20$) of the CP4 EPSPS, DMO and PAT proteins used to estimate human and animal dietary exposure (see Section 3.5.4) are reported in Table 1.

TABLE 1 Mean values ($n = 20$) and standard error of newly expressed protein expression levels in tops [μg/g dry weight (dw) and μg/g fresh weight (fw)] and over-season roots (OSR3) (μg/g dw) from sugar beet KWS20-1.

	Dicamba, glyphosate and glufosinate treatment			
	Not treated		Treated	
Tissue	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Tops (BBCH 49)				
CP4 EPSPS	290[a] ± 18[b] (200-490)[c]	46 ± 2.9 (31–78)	310 ± 12 (230–400)	49 ± 1.9 (37–63)
DMO	60 ± 2.9 (41–82)	9.5 ± 0.45 (6.5–13)	59 ± 3.5 (26–88)	9.3 ± 0.56 (4.1–14)
PAT	4.8 ± 0.36 (2.5–7.1)	0.76 ± 0.056 (0.40–1.1)	5.1 ± 0.45 (2.6–8.3)	0.80 ± 0.071 (0.41–1.3)
OSR3 (BBCH 49)				
CP4 EPSPS	110 ± 3.6 (74–130)	24 ± 0.81 (17–29)	100 ± 5.9 (68–150)	24 ± 1.3 (16–35)
DMO	11 ± 0.57 (7.2–16)	2.5 ± 0.13 (1.6–3.7)	12 ± 0.53 (7.6–17)	2.7 ± 0.12 (1.7–3.8)
PAT	< LOQ[d,e]	< LOQ[d,e]	< LOQ[d,e]	< LOQ[d,e]

[a]Mean value.
[b]Standard error.
[c]Range.
[d]All samples were below the limit of quantification (LOQ for PAT in OSR3 = 0.125 μg/g dw).
[e]For dietary exposure estimations (see Section 3.5.4), the LOQ (0.125 μg/g) was used.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of sugar beet KWS20-1 insert was assessed by Southern blot analysis of genomic DNA from five consecutive generations (T_1, T_2, T_3, T_4 and T_5) and PCR-based segregation analysis from three generations (BC_0S_1, BC_1S_1 and BC_2S_1). For Southern blot analysis, the restriction enzyme/probe combinations used were sufficient to conclude that all the tested plants retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

These results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that sugar beet KWS20-1 contains a single insert consisting of one copy of the *cp4 epsps*, *dmo* and *pat* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins, the sequences corresponding to ORFs within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise any safety concerns. The stability of the inserted DNA is confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS, DMO and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and *E. coli*-produced CP4 EPSPS, DMO and PAT proteins indicate that these proteins are equivalent, and the *E. coli*-derived proteins can be used in the safety studies.

3.4 | Comparative analysis[10,14]

3.4.1 | Overview of studies conducted for the comparative analysis

Application GMFF-2023-14732 presents data on agronomic and phenotypic characteristics, as well as on roots and tops composition of sugar beet KWS20-1 (Table 2).

[14]Additional information: 6/12/2023.

TABLE 2 Overview of the comparative analysis studies to characterise the sugar beet KWS20-1 provided in application GMFF-2023-14732.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic, phenotypic and compositional analysis	Field study, US, 2019, eight sites[a]	04E05B1DH05	14[b]

Abbreviation: GM, genetically modified.

[a]Three field trials were located in Idaho, two in Minnesota, and one in Michigan, North Dakota and South Dakota.

[b]Non-GM sugar beet varieties were: Betaseed BTS 1965, Betaseed BTS 2045, Betaseed BTS 4190RHC, Betaseed BTS 8750N, KWS 8K838, KWS 8K840 FIAMMETTA, KWS B8138, KWS BETA EXP 676, KWS BETA EXP 687, KWS BRITNEY, KWS BRUNA, KWS GRANDIOSA, KWS LAREINA and KWS URSELINA.

3.4.2 | Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: sugar beet KWS20-1 not exposed to the intended herbicides, sugar beet KWS20-1 exposed to the intended herbicides, the comparator 04E05B1DH05 and four non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes, for each of the two treatments of sugar beet KWS20-1, the application of a difference test (between the GM sugar beet and the non-GM comparator) and an equivalence test (between the GM sugar beet and the set of non-GM reference varieties). The results of the equivalence test are categorised into four possible outcomes (I-IV, ranging from equivalence to non-equivalence).[15]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

The non-GM sugar beet line 04E05B1DH05 was transformed to obtain sugar beet KWS20-1 (see Section 3.3.1 for a full description of the transformation process), which was then crossed with a CMS (cytoplasmic male sterile) line to produce the hybrid KWS20-1 used in the comparative analysis. The comparator used in the field trials is the non-GM sugar beet line 04E05B1DH05 crossed with the same CMS line used with KWS20-1; therefore, it has a similar genetic background as sugar beet hybrid KWS20-1 (as documented by the pedigree and by the additional information) and is considered to be the conventional counterpart.

Sugar beet KWS20-1 and its conventional counterpart are considered appropriate for areas where the selected field trials were conducted.

Commercial non-GM reference varieties were selected by the applicants based on their genetic diversity, region of registration and suitability for the selected locations, and at each selected site, four reference varieties were tested (see Table 2). On the basis of the provided information, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

3.4.3.2 | *Seed production and quality*

Seeds of sugar beet KWS20-1 and the conventional counterpart used in the 2019 field trials were produced, harvested and stored under similar conditions before being sown in the field trial sites. The seed lots were verified for their health, purity and identity via event-specific polymerase chain reaction analysis.

The seeds were tested for their germination capacity under optimal and suboptimal temperature conditions.[16] Germination capacity of sugar beet KWS20-1 was compared with the conventional counterpart, and the differences observed[17] in seed germination were not statistically significant between the sugar beet KWS20-1 and its conventional counterpart.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel concludes that the sugar beet KWS20-1, as assessed in this application, the conventional counterpart and the non-GM sugar beet reference varieties are appropriate for the comparative analysis, and the test materials are of sufficient quality.

[15]In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

[16]Optimal temperature condition corresponds to approximately 16 h at 20°C and 8 h at 30°C for 10 days. Suboptimal temperature condition corresponds to constant temperature of 6°C for 14 days.

[17]KWS20-1 showed a mean germination of 98% and 74% while its conventional counterpart showed a mean of 91% and 84% under optimal and sub-optimal temperature conditions, respectively.

3.4.4 | Representativeness of the receiving environments

3.4.4.1 | *Selection of field trial sites*

The selected field trial sites were located in commercial sugar beet-growing regions of the United States. The soil and climatic characteristics of the selected fields were diverse,[18] representing regions of diverse environmental conditions for sugar beet cultivation. The GMO Panel considers that the selected sites reflect commercial sugar beet-growing regions in which the test materials are likely to be grown.

3.4.4.2 | *Meteorological conditions*

Maximum and minimum mean temperatures and sum of precipitations were provided on a weekly basis.

No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.

3.4.4.3 | *Management practices*

The field trials included plots containing sugar beet KWS20-1, plots with the conventional counterpart and plots with non-GM sugar beet reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing sugar beet KWS20-1 managed following the same agricultural practices, plus exposed to the intended dicamba-, glufosinate-ammonium- and glyphosate-containing herbicides. Dicamba- and glyphosate-containing herbicides were applied at the BBCH 13–15 growth stage,[13] while glufosinate-ammonium-containing herbicide was applied at BBCH 16–18.

The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the selected receiving environments. Despite not being considered a normal agricultural practice, thinning was applied at all field trial sites to achieve a more homogeneous plant density across plots. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were acceptable for the selected receiving environments.

3.4.4.4 | *Conclusion on representativeness*

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and most of the management practices are typical for receiving environments where the tested materials could be grown.

3.4.5 | Agronomic and phenotypic analysis

Ten agronomic and phenotypic endpoints[19] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trial sites (see Table 2). Two endpoints (bolter and root count) were not analysed with formal statistical methods because of the lack of variability in the data (values 0 and 1, respectively, for all the data points).

The statistical analysis (Section 3.4.2) was applied to eight endpoints, with the following results:

- For sugar beet KWS20-1 (not treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, canopy cover, leaf number, plant height, root weight, and yield. All these endpoints fell under equivalence category I except for canopy cover, which fell under equivalence category III.[20]
- For sugar beet KWS20-1 (treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, canopy cover, final stand count, leaf number, plant height, root weight and yield. All these endpoints fell under equivalence category I except for canopy cover, which fell under equivalence category III.[20]

Canopy cover for sugar beet KWS20-1 was reduced with respect to its conventional counterpart and the non-GM reference varieties. However, as no impact on the other growth-related endpoints was observed, the GMO Panel considers that this result does not affect the use of the field trials for the comparative analysis. Given the scope of the application,

[18]Soil types of the field trials were silt loam, clay loam, loam, sandy loam and sandy clay loam; soil organic carbon ranged from 0.7% to 2.5%; pH ranged from 5.8 to 8.3; average temperatures and sum of precipitations during the usual crop growing season ranged respectively from 13.5 to 17.9°C and from 121 to 757 mm.

[19]Early stand count, bolters, canopy cover, final stand count, leaf colour, leaf number, plant height, root count, root weight and yield.

[20]For canopy cover (%), the estimated mean values for the conventional counterpart, the GM sugar beet (untreated), the GM sugar beet (treated) and the reference varieties were 49.4%, 42.2%, 42.5% and 54.6%, respectively; the equivalence limits (%) were (43.7, 65.5).

which does not include the cultivation or the import of viable material in the EU, no implication for environmental impact is expected.

3.4.6 | Compositional analysis

Sugar beet KWS20-1 tops and roots harvested from eight sites (Table 2) were analysed for 36 constituents (six in tops and 30 in roots), including those recommended by OECD (2002). The statistical analysis was applied to all constituents analysed in tops[21] and roots.[22]

A summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For sugar beet KWS20-1 not treated with the intended herbicides, statistically significant differences with the conventional counterpart were found for five endpoints (one in tops and four in roots), which all fell under equivalence categories I or II.
- For sugar beet KWS20-1 treated with the intended herbicides, statistically significant differences with the conventional counterpart were found for five endpoints (one in tops and four in roots), which all fell under equivalence categories I or II, except for pectin in roots for which the test of equivalence was not applied, because of the lack of variation among the non-GM reference varieties.

TABLE 3 Outcome of the comparative compositional analysis in tops and roots for sugar beet KWS20-1. The table shows the number of endpoints in each category.

		Test of difference[a]			
		Not treated[b]		Treated[b]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence [c]	Category I/II	27	5[d]	28	4[d]
	Category III/IV	1[e]	–	1[e]	–
	Not categorised	3[f]	–	2[f]	1[g]
	Total endpoints	36		36	

[a]Comparison between sugar beet KWS20-1 and its conventional counterpart.

[c]Four different outcomes: Category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[b]Treated/not treated with the intended herbicide.

[d]Endpoints with significant differences between sugar beet KWS20-1 and its conventional counterpart falling in equivalence category I/II. For tops, not treated: total fat; treated: ash. For roots, not treated: phenylalanine, isoleucine, methionine; treated: moisture, alanine; both treated and not treated: total fat.

[e]Endpoints in tops and roots with no significant differences between sugar beet KWS20-1 and its conventional counterpart and falling under equivalence category III: moisture in tops (treated) and histidine in roots (not treated).

[f]Endpoints not categorised for equivalence and without significant differences between the sugar beet KWS20-1 and its conventional counterpart: pectin in roots (not treated), crude fibre in tops (both treated and not treated), crude fibre in roots (both treated and not treated).

[g]Endpoint not categorised for equivalence and with significant differences between sugar beet KWS20-1 and its conventional counterpart: pectin in roots (treated).

The GMO Panel assessed all significant differences between sugar beet KWS20-1 and its conventional counterpart, taking into account their potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties.

Table 4 shows the quantitative results for pectin in roots (treated plants) with significant differences as compared to the conventional counterpart; the equivalence test could not be applied because of the lack of variation among the non-GM reference varieties.

TABLE 4 Quantitative results (estimated means) for compositional endpoints in roots that are further assessed based on the results of the statistical analysis.

		Sugar beet KWS20-1[a]			Non-GM reference varieties	
	Endpoint	Not treated	Treated	Conventional counterpart	Mean	Equivalence limits
Roots	Pectin (% dw)	3.01	2.96*	3.11	3.00	–

Note: For the sugar beet KWS20-1, significantly different values are marked with an asterisk

Abbreviations: dw, dry weight; treated, treated with the intended herbicides; not treated: treated only with conventional herbicides (see Section 3.4.4.3).

[21]Moisture, protein, total fat, crude fibre, carbohydrates, ash.

[22]Moisture, total fat, carbohydrates, crude fibre, pectin, protein, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, leucine, lysine, serine, tryptophan, valine, isoleucine, methionine, proline, threonine, tyrosine, phenylalanine, histidine, ash, phosphorus, potassium, sodium, sucrose, oleanolic acid.

3.4.7 | Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics between sugar beet KWS20-1 and the conventional counterpart need further assessment.
- None of the differences identified in tops and roots composition between sugar beet KWS20-1 and the conventional counterpart need further assessment regarding food and feed safety except for pectin in roots (treated), which is further assessed in Sections 3.5.2 and 3.5.5.

3.5 | Food/feed safety assessment[10,23]

3.5.1 | Overview of overarching information for food and feed assessment

3.5.1.1 | *Compositional analysis*

The compositional analysis of sugar beet KWS20-1 and the conventional counterpart provided by the applicants and assessed by the GMO Panel is described in Section 3.4.6.

3.5.1.2 | *Newly expressed proteins*

Three proteins, CP4 EPSPS, DMO and PAT, are newly expressed in sugar beet KWS20-1.

The CP4 EPSPS and PAT proteins are identical to those previously assessed by the GMO Panel and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified (Section 3.3.3; EFSA, 2004, 2005; EFSA GMO Panel, 2022a). Therefore, the CP4 EPSPS and PAT proteins will not be further considered in Section 3.5.1.2.2.

The DMO protein expressed in sugar beet KWS20-1 has not been previously assessed by the GMO Panel (Section 3.3.3). However, other similar DMO proteins were previously assessed by the GMO Panel and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified (Section 3.3.3; EFSA GMO Panel, 2013, 2022a, 2022b).

3.5.1.2.1 | *Molecular characterisation*

The characterisation of the CP4 EPSPS, DMO and PAT proteins provided by the applicants and assessed by the GMO Panel is described in Section 3.3.3. Furthermore, the equivalence between the sugar beet KWS20-1 and the *E. coli*-derived proteins used in safety studies was demonstrated.

3.5.1.2.2 | *History of safe use for consumption as food and feed of the newly expressed proteins*.

a. *Information on the source organism*

The DMO protein was originally derived from a *Stenotrophomonas maltophilia* strain found at the site of a dicamba manufacturing plant (Krueger et al., 1989). The *S. maltophilia* is a Gram-negative bacterium, closely related to *Pseudomonas* species, that is ubiquitous in the environment. It is isolated from soil, water, animals and plants, where it is also found associated with the rhizosphere (Berg et al., 1999). In humans, *S. maltophilia* is an opportunistic pathogen that can develop multidrug resistance particularly among immune-compromised patients (Calza et al., 2003; Looney et al., 2009). However, *S. maltophilia* is not employed for the processing of foods or feeds and, therefore, dietary exposure to this bacterium or its products is to be considered incidental (Qureshi et al., 2005). This information has been previously assessed by the GMO Panel (EFSA GMO Panel, 2022a).

b. *Information on structure, function and mode of action*

The DMO protein is a mono-oxygenase that catalyses, in a substrate-specific reaction, the *O*-demethylation of the herbicide dicamba, thus converting dicamba to the non-herbicidal reaction products 3,6-dichlorosalicylic acid and formaldehyde. This protein belongs to a family of enzymes known as Rieske non-haem iron oxygenases (Wang et al., 2016). Members of this family of enzymes display well-conserved secondary and tertiary structures, which confer the enzymatic function, but vary substantially at the level of their primary amino acid sequences (Ferraro et al., 2005). Proteins homologous to DMO

[23]Additional information: 6/12/2023; 5/06/2024; 13/08/2024 and 19/12/2024.

are widespread in nature. Homologous proteins were identified in soil bacteria such as *Sphingobium* and *Sphingomonas* species, indicating a wide presence of such enzymes in soil microorganisms (Wang et al., 2016). However, as reported in the literature, even the closest known relative, i.e. vanillate-*O*-demethylase from *Pseudomonas* and *Acinetobacter* species, displays sequence identities to the DMO protein of only 42% or less (D'Ordine et al., 2009). This information has been previously assessed by the GMO Panel (EFSA GMO Panel, 2022a).

c. *Information on identity/homology of NEPs to wild-type protein and other proteins/constituents in conventional food and feed sources*

The DMO protein expressed in sugar beet KWS20-1 differs in its amino acid sequence from the wild-type DMO protein from *S. maltophilia* due to the presence of 27 additional amino-terminal residues coming from the *rbcs* gene from *Pisum sativum* and an extra leucine at amino acid position two of the DMO protein itself. The source organism (*S. maltophilia*) is a spurious food or feed contaminant rather than being used for the production of foods or feeds, and conventional foods and feeds do not contain enzymes with high sequence homology to the DMO proteins. The GMO Panel noted that the DMO protein expressed in sugar beet KWS20-1 is identical to two variants of the DMO protein expressed in maize MON 87429 (EFSA GMO Panel, 2022a), except for the additional 27 amino acids at the *N* terminus. These 27 amino acids were also present in the DMO proteins expressed in soybean MON 87708 and oilseed rape MON 94100 (EFSA GMO Panel, 2013, 2022b), for which no safety concerns were identified in human and animal health (Wang et al., 2024).

The GMO Panel concluded that the physicochemical and functional properties of the DMO protein expressed in sugar beet KWS20-1 can be considered equivalent to previously assessed DMOs.

d. *Overall conclusion of the history of safe use*

The GMO Panel considers the above information insufficient to duly document the history of safe use for the consumption of the DMO protein.

3.5.1.2.3 | *Substrate specificity*

The substrate specificity of the DMO protein, as expressed in soybean MON 87708, has been previously assessed by the GMO Panel indicating that this enzyme has a high specificity for dicamba (EFSA GMO Panel, 2013). This assessment included ferulic acid, sinapic acid, syringic acid and vanillic acid, which are structurally similar to dicamba and are found in plants, including sugar beet. Furthermore, the DMO proteins expressed in sugar beet KWS20-1 and in soybean MON 87708 (EFSA GMO Panel, 2013) only differ at the amino acid positions 2 and 112, which are sterically distant from the catalytic site and consequently do not have an impact on the interaction with the substrate (D'Ordine et al., 2009; Dumitru et al., 2009). Therefore, it is possible to derive the conclusions from studies previously performed in the frame of soybean MON 87708 to sugar beet KWS20-1.

The GMO Panel assessed the substrate specificity of other EPSPS proteins in the past (e.g. EFSA GMO Panel, 2022a). The mechanism of action of EPSPS proteins is a biochemical reaction involving the conversion of shikimate-3-phosphate and phosphoenolpyruvate to 5-enolpyruvylshikimate-3-phosphate. The GMO Panel is not aware of additional information that would change its previous assessments.

The PAT protein has been assessed by the GMO Panel in the past (e.g. EFSA, 2005; EFSA GMO Panel, 2022a). PAT enzyme activity is limited to the acetylation of the glufosinate-ammonium substrate (Hérouet et al., 2005). The GMO Panel is not aware of additional information that would change its previous assessments.

3.5.1.2.4 | *Stability of the newly expressed proteins*

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010c, 2011a, 2017, 2021). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown that a prominent trait attributed to food allergens and protein safety is protein stability (Breiteneder & Mills, 2005; Costa et al., 2022; Foo & Mueller, 2021; Helm, 2001).

a. Effect of temperature and pH on newly expressed proteins

The safety of CP4 EPSPS and PAT proteins has been previously evaluated by the GMO Panel (EFSA, 2004, 2005; EFSA GMO Panel, 2022a). The applicants provided studies on the DMO protein from an *E. coli*-recombinant system (see Section 3.3.3). DMO protein samples were incubated for ~15 or 30 min at 25, 37, 55, 75 and 95°C, followed by a functional activity bioassay. The studies showed that the DMO protein activity was highly reduced at 55°C and eliminated at temperatures ≥ 75°C. In relation to the effect of pH on the DMO protein at pH 1.2 and 7.5, the molecular mass (~38 kDa) of the protein was unchanged.

b. In vitro protein degradation by proteolytic enzymes

The safety of CP4 EPSPS and PAT proteins has been previously evaluated by the GMO Panel (EFSA, 2004, 2005; EFSA GMO Panel, 2022a). The applicants provided information on in vitro protein degradation (resistance to pepsin in solutions at pH ~1.2) of the DMO protein from an *E. coli* recombinant system (see Section 3.3.3). The integrity of the test protein in samples of the incubation mixture taken at various time points was analysed by SDS–PAGE followed by protein staining or by western blotting. The DMO protein was degraded by pepsin within 0.5 min of incubation. Furthermore, the applicants also studied the degradation of the DMO protein by pancreatin at pH 7.5. The integrity of the test protein in samples taken at various time points was analysed by western blotting. The DMO protein was degraded by pancreatin within 5 min of incubation.

3.5.1.2.5 | *Synergistic and antagonistic interactions among the newly expressed proteins*

The potential for a functional interaction among the three proteins has been assessed with regard to human and animal health. Based on current scientific knowledge on the biological function of the three proteins (Table 5), no synergistic or antagonistic interactions between these three proteins which could raise safety concerns for food and feed from sugar beet KWS20-1 is expected.

TABLE 5 Intended effects and modes of action of the NEPs in sugar beet KWS20-1.

Protein	Intended effect and mode of action in GM plant
CP4 EPSPS	The CP4 EPSPS protein confers tolerance to glyphosate-containing herbicides, acting on the shikimic acid pathway for the biosynthesis of aromatic amino acids in bacteria, fungi and plants.
DMO	The DMO protein confers tolerance to dicamba-containing herbicides acting by catalysing the demethylation of dicamba into the non-herbicidal compound 3,6-dichlorosalicylic acid and formaldehyde (Herman et al., 2005).
PAT	The PAT protein confers tolerance to glufosinate-ammonium-containing herbicides acting by acetylating L-phosphinothricin, the active isomer of the glufosinate-ammonium herbicide.

3.5.1.3 | *Effects of processing*

Sugar beet KWS20-1 will undergo existing production processes used for conventional sugar beet. Based on the outcome of the comparative assessment, processing of the sugar beet KWS20-1 into food and feed products is not expected to result in products being different from those of conventional non-GM reference varieties currently in the EU market.[24]

A typical processing line from beet to sugar is described by OECD (2002). Sugar beet plants are commercially grown for sugar production due to the high concentration of sucrose in their roots. During the sugar extraction process, sugar beet pulp and molasses are produced as by-products and used mainly as animal feed. In addition, beet syrup for human consumption is produced as a regional speciality in Central Europe.

3.5.2 | Toxicology assessment

The strategies to assess the toxicological impact of any changes on the whole genetically modified food and feed resulting from the genetic modification focus on the assessment of: (i) newly expressed proteins; (ii) new constituents other than NEPs; (iii) altered levels of food and feed constituents; and (iv) the whole genetically modified food and feed.

3.5.2.1 | *Assessment of newly expressed proteins*

Three proteins (CP4 EPSPS, DMO and PAT and) are newly expressed in sugar beet KWS20-1.

3.5.2.1.1 | *Previously assessed NEP*

The CP4 EPSPS and PAT proteins were previously assessed by the GMO Panel in the context of other applications (EFSA, 2004, 2005; EFSA GMO Panel, 2022a) and no safety concerns for humans and animals (i.e. farmed and companion animals) were identified. These proteins have been extensively characterised and found to match the expected deduced amino acid sequences (Section 3.3). Updated bioinformatics analyses revealed no similarities of the CP4 EPSPS and PAT proteins with known toxins. The GMO Panel is not aware of any new information that would change previous conclusions on the safety of the CP4 EPSPS and PAT proteins.

[24]The GMO Panel is aware of an on-going assessment of a novel food application on sugar beet leaf protein (https://food.ec.europa.eu/food-safety/novel-food/authorisations/summary-applications-and-notifications_en).

3.5.2.1.2 | *NEP never assessed before*

A weight-of-evidence approach was followed by the GMO Panel to assess the toxicological profile of the newly expressed DMO protein, taking into account all of the information relevant for its hazard assessment, including molecular characterisation, substrate specificity, history of safe use for consumption as food and feed of the NEP, stability of the NEP and synergistic or antagonistic interactions (Section 3.5.1.2), updated bioinformatic analyses for similarity to toxins, and in vivo toxicity studies.

3.5.2.1.3 | *Bioinformatic analyses*

Updated bioinformatics analyses of the amino acid sequence of the DMO protein revealed no significant similarities to known toxins (Section 3.3.2).

3.5.2.1.4 | *In vivo toxicity studies*

A 28-day study with the DMO protein was not provided by the applicants. The GMO Panel concluded that this study was unnecessary for the assessment of the DMO protein based on: (i) the molecular characterisation; (ii) information on identity/homology to other proteins previously assessed by the Panel; (iii) absence of significant similarities to known toxins; and (iv) lack of stability of the protein. In particular, the GMO Panel took into account its previous assessment on the DMO protein expressed in maize MON 87429, for which an acute toxicity study and a 28-day toxicity study in mice were assessed and no safety concerns were identified for human and animal health (EFSA GMO Panel, 2022a).

Based on the above information, the GMO Panel considers that there are no toxicological concerns for the DMO protein newly expressed in sugar beet KWS20-1.

3.5.2.2 | *Assessment of new constituents other than newly expressed proteins*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the newly expressed proteins have been identified in roots and tops from sugar beet KWS20-1. Therefore, no further food and feed safety assessment of new components other than the newly expressed proteins is required.

3.5.2.3 | *Information on altered levels of food and feed constituents*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no altered levels of food and feed constituents have been identified in tops and roots from sugar beet KWS20-1, except for pectin in roots. This change is considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the change; therefore, no further toxicological assessment is needed. Further information on the relevance of this finding is provided in Sections 3.5.5.1 and 3.5.5.2.

3.5.2.4 | *Assessment of the whole genetically modified food and feed*

Based on the outcome of the molecular characterisation, toxicological and comparative analysis assessment, no compositional differences or indications of possible unintended effects relevant to food and feed safety have been identified for sugar beet KWS20-1. Therefore, animal feeding studies with food/feed derived from sugar beet KWS20-1 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicants provided a 90-day feeding study in rats fed with diets containing beet pulp derived from sugar beet KWS20-1.

In this study, pair-housed Crl:CD(SD) rats (16 per sex per group; 2 rats per cage) were allocated to three groups using a randomised complete block design with eight replications per sex.

Groups were fed diets containing sugar beet KWS20-1 pulp from plants treated with the intended herbicides (glyphosate, dicamba and glufosinate-ammonium) at 5% and 2.5% inclusion levels (the latter supplemented with 2.5% of the non-GM comparator) and the non-GM comparator (inclusion level 5%).

The study was adapted from OECD test guideline 408 (OECD, 2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and EFSA Explanatory statement (EFSA, 2014) and complied with the principles of good laboratory practice (GLP) with some minor deviations described in the study report, not impacting the study results and interpretation.

The stability of the test and control materials was not verified; however, in accordance with product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considered this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them.

Event-specific PCR analysis confirmed the presence of the event in both the GM sugar beet pulp and diets and excluded the presence of the event in the respective controls. Both the GM and control sugar beet and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications for LabDiet® Certified Rodent Diet 5002. Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance with OECD TG 408 (1998/2018).

An appropriate range of statistical tests was performed on the results of the study. A detailed description of the methodology and of statistically significant findings identified in rats given diets containing beet pulp derived from sugar beet KWS20-1 is reported in Appendix B.

There were no test diet-related incidents of mortality or clinical signs. One top dose female was euthanised on day 85, having broken a leg. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted, but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[25] for the parameter in rats of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints.
- exhibited no consistency with increasing incorporation levels.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets containing sugar beet KWS20-1 pulp at 5% or 2.5% for 90 days.

Sugar beet pulp is considered a suitable test material for 90-day studies in rodents (EFSA, 2014). In the absence of any established specific incorporation rate recommendations as reference values for such studies, the applicants proposed a 5% incorporation rate, supported by available literature.[26] Studies have shown that including sugar beet pulp products in rat diets at levels of up to 10% leads to changes in lipid metabolism and other metabolic parameters within a relatively short period.

Based on the current knowledge, the GMO Panel considers that an incorporation rate of 5% can be considered acceptable in the context of this application.

If, in the future, additional information becomes available on the maximum level of sugar beet pulp that is suitable for use in rodent diets, the acceptability of an incorporation rate of 5% will be reviewed and any new studies submitted to support 'GM sugar beet uses' will be considered against the additional information.

3.5.3 | Allergenicity

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which are defined as the ability to enhance an allergic reaction.

3.5.3.1 | *Assessment of allergenicity of the newly expressed proteins*

A weight-of-evidence approach was followed, taking into account all of the information obtained on the newly expressed protein, as no single piece of information or experimental method yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU)No 503/2013).

The *cp4 epsps, dmo* and *pat* genes originate from *Agrobacterium* sp., *S. maltophilia* and *S. viridochromogenes* and, respectively, none of which are considered common allergenic sources. The safety of the CP4 EPSPS and PAT proteins has been previously assessed by the GMO Panel and no safety concerns were identified (EFSA, 2004, 2005; EFSA GMO Panel, 2022a). The DMO protein as expressed in sugar beet KWS20-1 has not previously been assessed by the GMO Panel. However, the GMO Panel considered the previous assessment of the DMO protein expressed in maize MON 87429, for which no safety concerns were identified for human and animal health (EFSA GMO Panel, 2022a).

Updated bioinformatic analyses of the amino acid sequences of the CP4 EPSPS, DMO and PAT proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens.

[25] Although animals used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group is treatment-related, account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

[26] Additional information: 19/12/2024.

The degradation by proteolytic enzymes of CP4 EPSPS, DMO and PAT proteins has been described in Section 3.5.1.2. In addition, the GMO Panel did not find indications that these newly expressed proteins at the levels expressed in sugar beet KWS20-1 might be adjuvants.

Furthermore, the applicants provided information on the safety of the CP4 EPSPS, DMO and PAT proteins regarding their potential hazard to cause a coeliac disease response. For such assessment, the applicants followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the CP4 EPSPS and DMO proteins identified no perfect or relevant partial matches with known celiac disease peptide sequences. The assessment of the PAT protein revealed partial matches containing the Q/E-X1-P-X2 motif and required further investigation. These partial matches have been previously assessed by the EFSA GMO Panel (EFSA GMO Panel, 2022a). Therefore, no indications of safety concerns were identified by the GMO Panel.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed CP4 EPSPS, DMO and PAT proteins in sugar beet KWS20-1 may be allergenic.

3.5.3.2 | *Assessment of allergenicity of the whole GM plant or crop*

The GMO Panel regularly reviews the available publications on food allergy to common foods. Sugar beet is not considered a common allergenic food (OECD, 2002). Therefore, the GMO Panel does not request experimental data on a routine basis to analyse the allergen repertoire of GM sugar beet.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (see Sections 3.3, 3.4.6, 3.5), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from this sugar beet KWS20-1 with respect to that derived from the conventional counterpart and the non-GM reference varieties tested.

3.5.4 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicants provided dietary exposure estimates to CP4 EPSPS, DMO and PAT proteins newly expressed in sugar beet KWS20-1. Dietary exposure was estimated based on protein expression levels reported in this application for sugar beet KWS20-1 treated with the intended herbicides, the currently available consumption data and feed practices, and by taking into account the foods and feeds currently available in the market and the described processing conditions.

3.5.4.1 | *Human dietary exposure*

The primary use of sugar beet is for processing to produce refined white sugar, which is composed almost entirely of sucrose. Therefore, no dietary exposure to CP4 EPSPS, DMO and PAT proteins is expected from the consumption of refined white sugar produced from sugar beet KWS20-1.

Around 3000 eating occasions for sugar beet syrup are reported in the EFSA Comprehensive European Food Consumption database (EFSA Comprehensive database),[27] almost exclusively in one Nordic country. Sugar beet syrup, obtained during the production of white sugar crystals, contains very small amounts of proteins and, therefore, its consumption would barely contribute to the dietary exposure to CP4 EPSPS, PAT and DMO proteins.

Few eating occasions for sugar beet roots ($n = 27$) were identified in the EFSA Comprehensive database.[27] The consumption of sugar beet roots in Europe seems to be minor as revealed by the low number of eating occasions, all of which were reported in one country across different age classes. The mean consumption ranged between 11.7 grams/day in infants and 66.4 grams/day in the elderly population. The highest chronic exposure would be in toddler average consumers, with estimates of 45.7 μg/g, 5.1 μg/g, and 0.24 μg/g for DMO, PAT and CP4 EPSPS proteins, respectively. The low number of consumers adds uncertainty to the exposure estimations, which should be interpreted with care and only allows the estimation of dietary exposure for average consumers.

Molasses, a by-product of the sugar extraction process, has a crude protein content between 6.6 and 11.1% (dw basis), and it is mainly used in animal feeding as a feed ingredient, pelleting aid or ensiling agent (OECD, 2002). In fact, no eating occasions are described for sugar beet molasses in the EFSA Comprehensive database, indicating that its consumption in Europe is either non-existent or very low. The other main by-product, sugar beet pulp, is mainly composed of soluble and insoluble fibre (~80%), with up to ~10% crude protein content (dw basis) (OECD, 2002; Tomaszewska et al., 2018). Sugar beet pulp is also primarily used as animal feed, although it has minor uses in the food chain, mainly due to its content in dietary fibre (EFSA NDA Panel, 2011; FDA, 2013). Therefore, the current exposure to CP4 EPSPS, DMO and PAT proteins in humans via the consumption of sugar beet pulp and molasses is considered negligible.

Due to the inherent biological characteristics of sugar beet crops and their intended cultivation practices,[28] the pollen from sugar beet KWS20-1 is not expected to contribute to the dietary exposure to CP4 EPSPS, DMO and PAT proteins.

[27] https://www.efsa.europa.eu/en/data-report/food-consumption-data.

[28] Sugar beet crop is a biennial plant, with the agronomically relevant part of the crop, the root, developed during the first year (vegetative stage) when flowers are not present. Additionally, bolters are usually removed or destroyed from the fields before flowering.

3.5.4.2 | *Animal dietary exposure*

Anticipated dietary exposure to CP4 EPSPS, DMO and PAT proteins in sugar beet KWS20-1 was estimated across different animal species, as below described, assuming the consumption of sugar beet products commonly entering the feed supply chain (i.e. pulp, molasses and tops).

A conservative scenario was considered, assuming a 100% replacement of conventional sugar beet products with those from sugar beet KWS20-1 products.

Mean levels (dry weight) of the newly expressed proteins in roots and tops from the sugar beet KWS20-1 (treated with the intended herbicides) used for animal dietary exposure are listed in Table 1. All root samples analysed for the presence of PAT protein in sugar beet KWS20-1 were below the limit of quantification (LOQ) (0.125 µg/g dw); for the purpose of estimating animal dietary exposure, the LOQ was used as the assumed mean amount of protein in roots.

Mean levels (dry weight) of the newly expressed proteins in pulp and molasses were calculated to be, respectively, 1.4-fold and 1.5-fold higher than in roots, based on conversion factors that take into account the protein content in these feed materials relative to sugar beet roots (OECD, 2002), and assuming that no protein is lost during their production or processing.

The applicants estimated dietary exposure to CP4 EPSPS, DMO and PAT proteins via the consumption of sugar beet pulp, molasses and tops in lactating dairy cows; sugar beet pulp and molasses in finishing pigs; and sugar beet pulp in broilers. Estimations were based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of sugar beet feedstuffs in diets or rations, as provided for the EU by OECD (2002).

Estimated dietary exposure in the concerned animals is reported in Appendix C.

3.5.5 | Nutritional assessment of endogenous constituents

The intended trait of sugar beet KWS20-1 is herbicide tolerance, with no intention to alter nutritional parameters. However, levels of pectin in roots (treated plants) were significantly different from its conventional counterpart and could not be categorised for equivalence (Section 3.4.6). The biological relevance of this compound, the role of sugar beet as a contributor to its total intake and the magnitude and direction of the observed change were considered during the nutritional assessment.

3.5.5.1 | *Human nutrition*

The main sugar beet product used for human consumption is sugar that is deprived of pectin. As mentioned above, sugar beet roots and beet syrup are consumed in negligible amounts in Europe. Therefore, the ~5% decrease in pectin content identified in roots from sugar beet KWS20-1 as compared to its conventional counterpart does not represent a nutritional concern in humans.

3.5.5.2 | *Animal nutrition*

Several processed fractions (e.g. molasses and pulp) of the sugar beet plant and, more rarely, whole sugar beets and tops may contribute to the animal diet in farmed and companion animals.

Sugar beet roots and tops, whether fresh or ensiled, are fed to ruminants on a limited basis, especially sugar beet roots due to the risk of acidosis, while their use is not common in pigs and companion animals.

Beet pulp and molasses are the main sugar beet products fed to farmed and companion animals. While beet pulp contains pectin among other components, in a significant amount (up to 30%), the content of pectins in the molasses as a by-product during sugar extraction is irrelevant for animal nutrition. Sugar beet pulp is more effectively used in ruminant than in pig feeding, due to the capacity of ruminants to digest the high fibre content. Molasses can be used in feeding ruminants and pigs, but only to a limited extent, to increase the palatability of feed and as an energy source. In companion animals, beet pulp represents a good source of fibre that is needed to maintain a proper digestive tract health. Molasses could be included in companion animal diets, although its use is limited and infrequent.

The limiting factors of the beet pulp obtained from sugar processing are the low protein content and the high content of fibre, which is known to have a low digestibility in monogastric (e.g. pigs) and companion animals.

The ~5% decrease in pectin content identified in roots from sugar beet KWS20-1 as compared to its conventional counterpart does not represent a nutritional concern in farmed and companion animals because pectin, that can be included in the diet of farmed and companion animals, is used at a low level and the magnitude of the decrease can be considered negligible.

3.5.6 | Post-market monitoring of GM food/feed

Sugar beet KWS20-1, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. The GMO Panel concludes that, based on the information considered in its safety assessment, a post-market monitoring plan for food and feed is not necessary.

3.5.7 | Conclusions on the food/feed safety assessment

The proteins CP4 EPSPS, DMO and PAT newly expressed in sugar beet KWS20-1 do not raise safety concerns for human and animal health. No interactions between the newly expressed proteins relevant for food and feed safety were identified. Moreover, the GMO Panel does not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in sugar beet KWS20-1. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of sugar beet KWS20-1 as food and feed. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of sugar beet KWS20-1 does not represent any nutritional concern in the context of the scope of this application. The GMO Panel concludes that sugar beet KWS20-1, as described in this application, is as safe as the conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.6 | Environmental risk assessment and monitoring plan[10,29]

The scope of this application includes the placing in the market of food and feed produced from sugar beet KWS20-1 or food containing ingredients produced from sugar beet KWS20-1, and excludes the placing in the market of food and feed containing or consisting of sugar beet KWS20-1 and cultivation in the EU. Thus, whole GM sugar beets or viable parts will not be imported. Additionally, nucleic acids and proteins are substantially degraded during the processing of raw sugar beet into refined sugar, pulp and molasses (Klein, Altenbuchner, & Mattes, 1998; OECD, 2002; Oguchi et al., 2009), so that imported sugar beet KWS20-1 products would be expected to only contain residual amounts of DNA and protein.

Considering the scope of the application, the environmental risk assessment (ERA) of sugar beet KWS20-1 mainly takes into account the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to manure and faeces of these animals (EFSA GMO Panel, 2010a). Taking this into consideration, the ERA focuses on the assessment of potential risks associated with plant-to-microbe gene transfer and interactions with biogeochemical cycles.

Regarding the potential for horizontal gene transfer (HGT) from GM sugar beet to microorganisms, the results of an additional study provided by the applicants indicated that, even in case of the presence of residual recombinant DNA in the imported food and feed products produced from KWS20-1 sugar beet, the risk of HGT to microorganisms is extremely low. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Environmental exposure to recombinant DNA and proteins in KWS20-1 sugar beet will be limited to manure and faeces of animals fed with the GM sugar beet. As previously indicated, proteins are substantially degraded during processing. Additionally, ingested proteins are typically degraded before entering the environment through manure and faeces of animals fed with GM sugar beet (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2.4) support that also the NEPs in KWS20-1 sugar beet will be degraded. Compared to non-GM sugar beet, the GMO Panel considers that potential interactions of sugar beet KWS20-1 products with biogeochemical cycles do not raise any safety concern.

In conclusion, the analysis of HGT to bacteria and the evaluation of potential interactions of KWS20-1 sugar beet products with biogeochemical cycles do not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure linked to the intended uses, the GMO Panel concludes that the environmental impacts of sugar beet KWS20-1 would not differ from those of conventional sugar beet.

The EFSA GMO Panel notes that, according to Articles 9(3) and 21(3) of the Regulation (EC) No 1829/2003, the authorisation holder should inform the European Commission of any new scientific or technical information which might influence the evaluation of the safety in use of the food or feed. Taking into consideration the assessment of potential impacts on the environment of sugar beet KWS20-1 in the section above, the GMO Panel recommends that the applicants also consider any information related to any potential unexpected adverse effects on the environment.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of sugar beet KWS20-1. The scope of the application is for food and feed produced from or food containing ingredients produced from sugar beet KWS20-1 for import and processing submitted within the framework of Regulation (EC) No 1829/2003.

The molecular characterisation data establish that sugar beet KWS20-1 contains a single insert consisting of one copy of the *cp4 epsps, dmo* and the *pat* expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the newly expressed proteins, the sequences corresponding to ORFs within the insert

[29]Additional information: 6/12/2023; 5/6/2024 and 11/09/2024.

or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the CP4 EPSPS, DMO and PAT proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and *E. coli*-produced CP4 EPSPS, DMO and PAT proteins indicate that these proteins are equivalent, and the *E. coli*-derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between hybrid KWS20-1 sugar beet and its conventional counterpart need further assessment, except for pectin in roots, which underwent additional evaluation and was found not to raise any safety or nutritional concerns.

The GMO Panel does not identify safety concerns regarding the potential toxicity and allergenicity of the CP4 EPSPS, DMO and PAT proteins as expressed in sugar beet KWS20-1, and finds no evidence that the genetic modification would change the overall safety of sugar beet KWS20-1, as food and feed. In the context of this application, the consumption of food and feed from KWS20-1 sugar beet does not represent a nutritional concern in humans and animals. The GMO Panel concludes that KWS20-1 sugar beet, as described in this application, is as safe as the conventional counterpart and non-GM reference sugar beet varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the scope of this application, that does not include cultivation and importation of viable material in the EU, the environmental risk assessment was limited to the possible plant to bacteria horizontal gene transfer and the evaluation of potential interactions of KWS20-1 sugar beet products with biogeochemical cycles and did not indicate a safety concern.

The GMO Panel recommends the applicants to consider information related to any potential unexpected adverse effects on the environment as part of the new scientific or technical information which might influence the evaluation of the safety in use of the food or feed, and which should be promptly communicated to the European Commission.

Based on the relevant publications identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the intended uses of sugar beet KWS20-1.

The GMO Panel concludes that sugar beet KWS20-1, as assessed in the context of this application, is as safe as its conventional counterpart and the tested non-GM sugar beet reference varieties with respect to potential effects on human and animal health and the environment.

5 | DOCUMENTATION AS PROVIDED TO EFSA

The documentation as provided to EFSA is available on Open EFSA.[30]

ABBREVIATIONS

BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemical industry
bp	base pair
bw	body weight
CAB	chlorophyll a/b-binding
CMS	cytoplasmic male sterile
CP4 EPSPS	CP4 5-enolpyruvyl-shikimate-3-phosphate synthase
DaMV	Dahlia Mosaic Virus
DMO	dicamba mono-oxygenase
dw	dry weight
ELISA	Enzyme-Linked Immunosorbent Assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
EU	European Union
FOB	functional observational battery
fw	fresh weight
GLP	good laboratory practice
GM	genetically modified
GMO	genetically modified organisms
HGT	horizontal gene transfer
HR	homologous recombination
IR	(%) percentage of inclusion rate
LOQ	Limit of Quantification
MS	mass spectrometry
NEP	newly expressed protein

[30] https://open.efsa.europa.eu/questions/EFSA-Q-2023-00378.

OECD	Organisation for Economic Co-operation and Development
ORFs	open reading frames
OSR3	over-season roots 3
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SES	standardised effect sizes
T-DNA	transfer- deoxyribonucleic acid
T3	triiodothyronine
TDI	total daily intake
TSH	Thyroid-Stimulating Hormone
US	United States

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REQUESTOR

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

List of relevant publications identified by the applicants through systematic literature searches (January 2012–November 2024)

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APPENDIX B

Statistical analysis and statistically significant findings in the 90-day toxicity study in rats

B.1 | Statistical analysis of the 90-day toxicity study on sugar beet KWS20-1 in rats

The following endpoints were statistically analysed: body weights, body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity and histopathological data.

For all continuous endpoints, mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval were reported.

The main statistical analysis compared rats consuming the test diets (at low dose 2.5% test substance KWS20-1 sugar beet pulp +2.5% control substance near-isogenic conventional sugar beet pulp in 5002 rodent diet and high dose - 5% test substance KWS20-1 sugar beet pulp in 5002 rodent diet) with those consuming the control diet. Continuous data were analysed separately for each variable and period or time interval, according to a linear mixed model (factor: diet, sex and interaction 'dose-sex'); then, pairwise comparisons, between each test and control group (separately for each sex) were performed using a *t*-test (at the 5% level of significance). For the locomotor activity data, a more complex model was set up taking into consideration the time effect and its interaction with the dose fixed effect as additional factors. Pairwise comparisons were made for the test group with the control group at each time interval through linear contrasts. Binomial category data and unordered multi-category data were analysed by Fisher's exact probabilities test.

Missing data were considered by the Panel and found not to have an impact on the results (Table B.1).

TABLE B.1 Statistically significant findings in the 90-day toxicity study on sugar beet KWS20-1 in rats.

Statistically significant parameter/endpoint	Finding (vs. control)	GMO panel interpretation
Prothrombin time	Increased (3%) at the high dose (sexes combined)	Low magnitude. Within normal variation. Not an adverse effect of treatment.
T4 levels	Decreased 14% at the low dose (sexes combined)	Low magnitude. Not seen at the high dose. Within normal variation. TSH/T3 unchanged. Not an adverse effect of treatment.
Thyroid/parathyroid weights (absolute and relative)	Decreased (15%) in both female groups	Low magnitude. No dose response. Within normal variation. No associated histopathological findings.

Note: Where changes are given as percentages (e.g. reduced (30%)), this indicates the magnitude of the change relative to the control value (e.g. 30% decrease in mean body weights means a value of 70 g in test group animals vs. 100 g in controls).

APPENDIX C

Animal dietary exposure

TABLE C.1 Dietary exposure to DMO, PAT and CP4 EPSPS proteins (mg/kg body weight (bw) per day) in livestock, based on the consumption of sugar beet pulp, molasses and tops.

Animal category	Bw (kg)	TDI feed (kg DM/animal)	IR (%)			Dietary exposure (mg/kg bw per day)		
			Pulp	Molasses	Tops	Pulp	Molasses	Tops
DMO								
Dairy	650	25	20	10	30	0.1292	0.0692	0.6807
Finishing swine	100	3	20	5	–	0.1008	0.0270	–
Broiler	1.7	0.12	5	–	–	0.0592	–	–
PAT								
Dairy	650	25	20	10	30	0.0013	0.0007	0.0588
Finishing swine	100	3	20	5	–	0.0010	0.0002	–
Broiler	1.7	0.12	5	–	–	0.0006	–	–
CP4 EPSPS								
Dairy	650	25	20	10	30	1.0769	0.5769	3.5769
Finishing swine	100	3	20	5	–	0.8400	0.2250	–
Broiler	1.7	0.12	5	–	–	0.49411	–	–

Notes: A 5% inclusion of sugar beet pulp was used for broiler, as a conservative approach, in line with OECD (2002). Furthermore, the reported dietary exposures based on molasses consumption represent a conservative approach, considering the assumption that no protein is lost during production or processing, despite the harsh conditions of the sugar beet processing.