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Request for a Regulatory Status Review for Dicamba, Glufosinate and Glyphosate-Tolerant KWS20-1 Sugar Beet

OECD Unique Identifier: KB-KWS2Ø1-6

The undersigned submits this Regulatory Status Review (RSR) request under 7 CFR § 340.4 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

August 30, 2022

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Ronald Schneider
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ABBREVIATIONS AND DEFINITIONS

APHIS	Animal and Plant Health Inspection Service
CMS	Cytoplasmic male sterile
CP4	*Agrobacterium* sp. strain CP4
cp4 epsps	Coding sequence for the CP4 EPSPS protein
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic acid
DMO	Dicamba mono-oxygenase
dmo	Coding sequence for the DMO protein
DCSA	Dichlorosalicylic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
GE	Genetically engineered
ILSI-CERA	International Life Sciences Institute – Center for Environmental Risk Assessment
OECD	Organisation for Economic Co-operation and Development
PAT	Phosphinothricin N-acetyltransferase
pat	Coding sequence for the PAT protein
PEP	Phosphoenolpyruvate
Pi	Inorganic phosphate
PPT	DL-phosphinothricin
RSR	Regulatory Status Review
S3P	Shikimate-3-phosphate
T-DNA	Transfer deoxyribonucleic acid
USDA	United States Department of Agriculture

I REQUESTOR

The submitters of this initial Regulatory Status Review request for KWS20-1 sugar beet are:

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II RATIONALE FOR THE DEVELOPMENT OF KWS20-1 SUGAR BEET

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulations at 7 CFR § 340.4, that are in effect on the date this Regulatory Status Review (RSR) request was filed, provide that an applicant may request a RSR of a plant developed using genetic engineering to evaluate whether the combination of the plant, introduced trait, and the trait's mechanism-of-action pose an increased plant pest risk relative to the comparator plant.

II.A Basis for the Request

Bayer and KWS are submitting this request for an initial RSR to APHIS for the agency to evaluate whether the genetically engineered (GE) product, KWS20-1 sugar beet, any progeny derived from crosses between KWS20-1 sugar beet and conventional sugar beet, and any progeny derived from crosses between KWS20-1 sugar beet and other GE sugar beet not subject to 7 CFR Part 340 regulations should continue to be regulated by APHIS.

II.B Rationale for the Development of Herbicide-Tolerant Sugar Beet

Bayer and KWS have jointly developed biotechnology-derived sugar beet KWS20-1 that is tolerant to in-crop applications of dicamba, glufosinate and glyphosate herbicides. Herbicide tolerance was conferred to KWS20-1 sugar beet via *Agrobacterium*-mediated insertion of a single gene cassette containing a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, a gene from *Streptomyces viridochromogenes* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate-ammonium herbicide, and the

cp4 epsps coding sequence isolated from *Agrobacterium* sp. strain CP4 that encodes the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein to confer tolerance to glyphosate herbicide.

KWS20-1 sugar beet will offer growers multiple choices for effective weed management including tough-to-control and herbicide-resistant broadleaf and grass weeds. The flexibility to use combinations of any of these three herbicides representing multiple mechanisms-of-action provides an effective and more durable weed management system for sugar beet production. The best management practices for minimizing the development of herbicide-resistant weeds involve implementing diversified weed management programs, which include using multiple herbicides with different mechanisms-of-action either in mixtures, sequencial application or in rotation and other recommended integrated weed management principles. Therefore, KWS20-1 sugar beet will provide sugar beet growers an efficient and flexible weed management system that enables: 1) an opportunity to delay selection for further resistance to glyphosate and other herbicides that are important in crop production; 2) excellent crop tolerance to dicamba, glufosinate and glyphosate; and 3) additional weed management tools and flexibility to enhance, maintain or improve sugar beet yield and quality to meet the growing needs of the food, feed and industrial markets.

III DESCRIPTION OF COMPARATOR PLANT

Sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) derived from breeding line 04E05B1DH05 was used as the conventional sugar beet comparator in the safety assessment of KWS20-1 sugar beet. Breeding line 04E05B1DH05 has a near-isogenic background to KWS20-1 sugar beet with the exception of the single T-DNA containing the *dmo, pat* and *cp4 epsps* expression cassettes, thus the effect of the *dmo, pat,* and *cp4 epsps* cassesttes could be assessed in an unbiased manner in the comparative safety assessment. Breeding line 04E05B1DH05 was developed *via* conventional breeding and is adapted to the U.S. sugar beet growing regions. In commercial sugar beet root production, growers plant hybrid seed. Similarly, some of the safety assessment data were generated on materials produced from hybrid seed. Molecular insert sequencing information described in Section IV was generated on KWS20-1 sugar beet in a cytoplasmic male sterile (CMS)-F1× 04E05B1DH05 hybrid.

IV GENOTYPE OF THE MODIFIED PLANT FOR KWS20-1 SUGAR BEET

This section describes information to understand the genetic differences between the modified plant and the comparator plant, including nucleotide sequence and annotation of the genetic material that has been inserted into and remains in the genome of the modified plant, as described in the "Guidance for Requesting a Regulatory Status Review under 7 CFR part 340" (USDA-APHIS Document ID BRS-GD-2020-003).

IV.A Sequence, Identity and Sources of the Genetic Material Inserted into KWS20-1 Sugar Beet

KWS20-1 sugar beet was generated through an *Agrobacterium*-mediated transformation in conventional sugar beet (genotype breeding line 04E05B1DH05) shoot segments cultured with the *A. tumefaciens* strain AGL1, containing plasmid PV-BVHT527462 vector DNA (~18.9 kb)

based on the methods described by Lindsey and Gallois (1990). After co-culturing with *Agrobacterium* AGL1 strain carrying the plasmid vector, calli were placed on selection medium containing DL-phosphinothricin (PPT) to favor selection of transgenic events and Timentin to inhibit the overgrowth of *Agrobacterium*.

PV-BVHT527462 contains one transfer-DNA (T-DNA), which is delineated by Right and Left Border regions and the vector backbone sequences. The T-DNA is approximately 12.2 kb and contains the *dmo, pat* and *cp4 epsps* expression cassettes. The *dmo* expression cassette contains the *dmo* coding sequence under the regulation of the *DaMV-1* enhancer, *Ubq-Cm1* promoter, 5′ untranslated sequence and intron, *RbcS (Ps)* targeting sequence, and the *guf-Mt2* 3′ untranslated sequence. The *pat* expression cassette consists of the *pat* coding sequence under the regulation of the *Cab-At1* promoter and 5′ untranslated sequence, and the *Hsp20-Mt1* 3′ untranslated sequence. The *cp4 epsps* expression cassette contains the *cp4 epsps* coding sequence under the regulation of the *SAM2-Cm1* promoter, intron and 5′ untranslated sequence, *CTP2* targeting sequence, and the *guf-Mt1* 3′ untranslated sequence. The vector backbone sequence is approximately 6.7 kb and contains the *aadA* expression cassette. During transformation, the T-DNA was inserted into the sugar beet genome. Subsequently, traditional breeding, selection and screening were used to identify those plants that contain the T-DNA expression cassettes and did not contain the *aadA* expression cassette or other backbone sequences from the transformation vector. KWS20-1 sugar beet was selected as the lead event based on superior agronomic, phenotypic and molecular characteristics. The nucleotide sequence of the inserted genetic material in KWS20-1 sugar beet is provided in 0, and an annotation of the different genetic elements is provided in Table IV-1.

Table IV-1. Annotation of the Inserted Genetic Material in KWS20-1 Sugar Beet

Genetic Element	Location in Plasmid Vector	Function (Reference)
B[1]-Right Border Region[r1]	1-41	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982). GenBank accession: JN400383, positions 10468 through 10508)
Intervening Sequence	42-85	"Synthetic[2] sequence" used in DNA cloning
T[3]-*guf-Mt1*	86-585	3' UTR from an expressed gene of *Medicago truncatula* of unknown function that directs polyadenylation of mRNA (Hunt, 1994). (NCBI Accession: MH931406)
Intervening Sequence	586-591	"Synthetic sequence" used in DNA cloning
CS[4]-*cp4 epsps*	592-1959	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance (Barry et al., 2001; Padgette et al., 1996). (GenBank accession: JN400383, positions 7133 through 8500)
TS[5]-*CTP2*	1960-2187	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Klee et al., 1987; Herrmann, 1995). (GenBank accession: JN400383, positions 8501 through 8728)
Intervening Sequence	2188-2196	"Synthetic sequence" used in DNA cloning
P[6]-*SAM2-Cm1*	2197-4200	Intron, 5′ UTR, and promoter from a *Cucumis melo* (melon) *SAM2* gene encoding S-adenosyl-L-methionine synthetase which directs transcription (Hernandez-Garcia and Finer, 2014). (NCBI Accession: OK149194)
Intervening Sequence	4201-4206	"Synthetic sequence" used in DNA cloning
E[7]-*DaMV-1*	4207-4538	Enhancer from a Dalia Mosaic Virus (DaMV) promoter region (Kuluev and Chemeris, 2007) that enhances transcription in plant cells. (NCBI Accession: EF513491, positions 1 through 332)
Intervening Sequence	4539-4548	"Synthetic sequence" used in DNA cloning
P-*Ubq-Cm1*	4549-7159	Promoter, leader and intron for a putative ubiquitin protein gene from *Cucumis melo* (melon) which directs and regulates transcription (Hernandez-Garcia and Finer, 2014). (NCBI Accession: OK149193)
Intervening Sequence	7160-7170	"Synthetic sequence" used in DNA cloning
TS-*RbcS (Ps)*	7171-7422	Targeting sequence and the first 27 amino acids from *Pisum sativum* (pea) *rbcs* gene family encoding the small subunit ribulose 1.5 bisphosphate carboxylase protein that is expressed in the chloroplast (Fluhr et al., 1986). (NCBI Accession: ON714500)
CS-*dmo*	7423-8445	Codon optimized coding sequence for the dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba resistance (Herman et al., 2005; Wang et al., 1997). (NCBI Accession: ON17003)
Intervening Sequence	8446-8451	"Synthetic sequence" used in DNA cloning

T-*guf-Mt2*	8452-8951	3' UTR from an expressed gene of *Medicago truncatula* of unknown function that directs polyadenylation of mRNA (Hunt, 1994). (NCBI Accession: OK149195)
Intervening Sequence	8952-8957	"Synthetic sequence" used in DNA cloning
P-*Cab-At1*	8958-10345	Promoter and leader from an *Arabidopsis thaliana* chlorophyll a/b-binding (CAB) protein that is involved in regulating gene expression (Ha and An, 1988). (NCBI Accession: OK149192)
Intervening Sequence	10346-10351	"Synthetic sequence" used in DNA cloning
CS-*pat*	10352-10903	Codon optimized coding sequence from *Streptomyces viridochromogenes* for the phosphinothricin N-acetyltransferase (PAT) protein that confers tolerance to glufosinate (Wehrmann et al., 1996; Wohlleben et al., 1988). (NCBI Accession: ON17004)
Intervening Sequence	10904-10911	"Synthetic sequence" used in DNA cloning
T-*Hsp20-Mt1*	10912-11411	3′ UTR sequence from *Medicago truncatula* (barrel medic) of a putative *Hsp20* gene encoding a heat shock protein that directs polyadenylation of the mRNA (Hunt, 1994). (NCBI Accession: OK149196)
Intervening Sequence	11412-11463	"Synthetic sequence" used in DNA cloning
B-Left Border Region [r1]	11464-11722	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983). (NCBI Accession: OK586894, positions 1 through 259)

[1] B, Border

[r1] Superscript in the Left and Right Border Regions indicates that the sequence in KWS20-1 sugar beet was truncated compared to the sequences in PV-BVHT527462.

[2] The term "synthetic" used in this table is defined and described in the USDA/APHIS-BRS Guidance Document BRS-GD-2020-0003. In the context of this table, the word synthetic does not indicate that the sequence was manufactured but rather that the sequence is not purposefully obtained from a known source and does not have an assigned function; although, some homology may exist to known DNA sequences.

[3] T, Transcription termination sequence

[4] CS, Coding sequence

[5] TS, Targeting sequence

[6] P, Promoter

[7] E, Enhancer

V DESCRIPTION OF THE NEW TRAIT FOR KWS20-1 SUGAR BEET

This section describes the intended KWS20-1 sugar beet traits, intended phenotype associated with the traits, and mechansim-of-action by which the intended phenotype will be conferred, as described in the Guidance for Requesting a Regulatory Status Review under 7 CFR part 340 (USDA-APHIS Document ID BRS-GD-2020-003).

V.A Description of the Intended KWS20-1 Sugar Beet Trait

KWS20-1 sugar beet is intended to provide herbicide tolerance.

V.B Intended Phenotype of KWS20-1 Sugar Beet

Herbicide-tolerant KWS20-1 sugar beet is intended to provide tolerance to dicamba, glufosinate and glyphosate herbicides, and will offer growers multiple choices for effective weed management, including tough-to-control and herbicide-resistant broadleaf and grass weeds.

V.C Description of the Mechanism-of-Action for KWS20-1 Sugar Beet

DMO Protein

KWS20-1 sugar beet contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein. As a mono-oxygenase protein, the DMO protein is part of the larger oxygenase family of enzymes that incorporate one or two oxygen atoms into substrates and are widely distributed in many universal metabolic pathways (Harayama et al., 1992). The DMO protein enzymatically catalyzes the demethylation of the broadleaf herbicide dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde, thus conferring dicamba tolerance (Chakraborty et al., 2005). Expression of the DMO protein in KWS20-1 sugar beet is targeted to the chloroplast by a chloroplast transit peptide (CTP), which facilitates its co-localization with endogenous reductase and ferredoxin enzymes required to supply electrons for the DMO demethylation reaction (Behrens et al., 2007).

The DMO protein expressed in KWS20-1 sugar beet is identical to the DMO variants in MON 87419 and MON 87429 corn (Petition Numbers 15-113-01p and 19-316-01p, respectively), aside from the residual amino acids remaining from the celluar processing of the CTP. Additionally, the 27 residual amino acids from the CTP in the KWS20-1 sugar beet DMO are identical to those present in DMO variants expressed in MON 87708 soybean and MON 94100 canola (Petition Numbers 10-188-01p and 20-078-01ext, respectively). The DMO protein in KWS20-1 sugar beet contains 367 amino acids and has an apparent molecular weight of ~38.3 kDa.

The KWS20-1 sugar beet DMO protein shares a high level of sequence identity with other DMO proteins previously assessed and present in biotechnology-derived crops that were deregulated by USDA-APHIS (e.g., MON 87708 soybean, USDA-APHIS Petition #10-188-01p; MON 88701 cotton, USDA-APHIS Petition #12-185-01p; and MON 87419 corn, USDA-APHIS Petition #15-113-01p). The minor amino acid substitutions between the wild-type DMO protein from the DI-6 strain of *S. maltophilia*, the KWS20-1 sugar beet DMO protein, and the DMO

proteins expressed in these other biotechnology-derived crops are localized to the N-terminus of the proteins and at positions 2 and 112 of the amino acid sequences (Herman et al., 2005) (Figure V-1). Additionally, based upon the crystal structure of the wild-type DMO proteins, these amino acid substitutions are structurally distant from the active site and are not expected to impact catalytic site coordination, functional activity, immunoreactivity or specificity (D'Ordine et al., 2009; Dumitru et al., 2009; Wang et al., 2016). Thus, prior evaluations of the DMO protein expressed in other biotechnology-derived crops are directly applicable to the DMO protein expressed in KWS20-1 sugar beet.

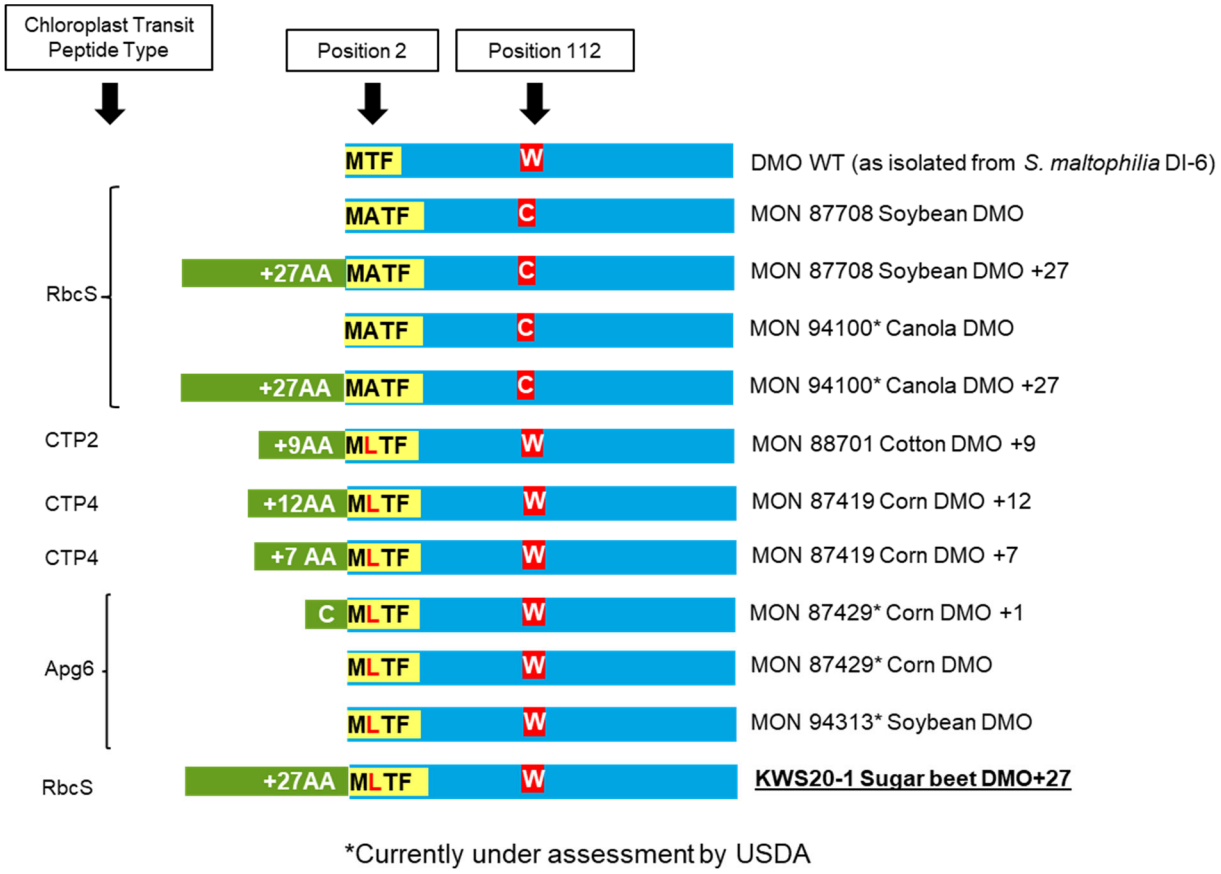

Figure V-1. Variants of DMO Protein and Their Relation to the Wild-Type DMO Protein
The diagram represents the wild-type DMO from *S. maltophilia* relative to various DMO variants discussed in this section. Position refers to amino acid residues as wild-type DMO and the N-terminal green region indicate residues from chloroplast transit peptides (CTPs). The blue regions indicate sequence of 100% amino acid identity, with the N-terminus region highlighted in yellow. The KWS20-1 sugar beet DMO protein is identical to wild-type DMO, except for the insertion of a leucine at position 2. The KWS20-1 sugar beet DMO protein is identical to DMO isoforms from other crop products with the exception of some minor differences at either position 2 or position 112. Other DMO variants also have additional N-terminal amino acids remaining from the cellular processing of the CTP.

The DMO protein is specific for the oxidative demethylation of dicamba, forming DCSA. Dicamba interacts with amino acids in the catalytic site of DMO through both the carboxylate moiety and the chlorine atoms of dicamba, which are primarily involved in orienting the substrate in the catalytic site. These chlorine atoms are required for catalysis (D'Ordine et al., 2009; Dumitru et al., 2009). Given the limited existence of chlorinated compounds with structures similar to dicamba in plants and other eukaryotes (Wishart, 2010; Wishart et al., 2009), it is unlikely that KWS20-1 sugar beet DMO will catalyze the metabolism of endogenous compounds. A previous assessment of MON 87429 corn DMO that contains a variant identical to the KWS20-1 sugar beet DMO, aside from the residual 27 amino acids from the CTP, evaluating the potential for DMO to catabolize dicamba and *o*-anisic acid, confirmed the specificity of the DMO protein expressed by KWS20-1 sugar beet. *O*-anisic acid was the natural plant metabolite chosen for a confirmatory substrate specificity assessment since it is the plant metabolite most structurally similar to dicamba (i.e., identical to dicamba, except for absence of chlorine atoms) (Dumitru et al., 2009). The assessment demonstrates that the minor differences in amino acid sequences present in the KWS20-1 sugar beet DMO protein relative to other DMO proteins expressed in previous biotechnology-derived crops do not impact the activity or selectivity for dicamba herbicide as compared to potential endogenous substrates.

The data and information summarized in this section confirm that the molecular mechanism-of-action of the KWS20-1 sugar beet DMO protein that confers dicamba tolerance is well understood, that the KWS20-1 sugar beet DMO protein is specific for dicamba, and that aside from dicamba tolerance, no changes to metabolism, physiology or development of the KWS20-1 sugar beet plant are expected. Furthermore, the KWS20-1 sugar beet DMO protein is structurally and functionally homologous to the DMO proteins present in biotechnology-derived crops that have been previously deregulated by USDA-APHIS.

PAT Protein

KWS20-1 sugar beet contains an acetyltransferase gene from *Streptomyces viridochromogenes* that expresses phosphinothricin N-acetyltransferase (PAT) protein. The molecular mechanism of the PAT protein, which acetylates glufosinate in the presence of acetyl CoA to form N-acetyl glufosinate, is well understood (Thompson et al., 1987). Glufosinate is a racemic mixture of the D- and L-forms of the amino acid phosphinothricin. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthetase in plants (OECD, 1999; OECD, 2002). Expression of the PAT protein in KWS20-1 sugar beet results in the ability to convert L-phosphinothricin to the non-herbicidal N-acetyl- L-phosphinothricin, thus conferring glufosinate tolerance to the crop.

Phosphinothricin N-acetyltransferase (PAT) proteins have been isolated from two separate species of *Streptomyces*, *S. hygroscopicus* (Thompson et al., 1987) and *S. viridochromogenes* (Wohlleben et al., 1988). The PAT protein isolated from *S. hygroscopicus* is encoded by the *bar* gene, whereas the PAT protein isolated from *S. viridochromogenes* is encoded by the *pat* gene. These PAT proteins are made up of 183 amino acids with 85% identity to each other at the amino acid level (Wohlleben et al., 1988). Based on previous studies (Wehrmann et al., 1996) that have extensively characterized PAT proteins produced from *bar* and *pat* genes, it has been noted that both proteins are so similar as to be functionally equivalent (OECD, 1999). Expression of the *pat* gene in KWS20-1 sugar beet results in a single polypeptide of 182 amino

acids with an apparent molecular weight of ~22.3 kDa. Data from N-terminal sequencing analysis of the KWS20-1 sugar beet-produced PAT protein indicate that it is identical to the wild type PAT protein encoded by *S. viridochromogenes* and to the PAT proteins produced in several glufosinate-tolerant crops previously deregulated by USDA-APHIS (e.g., USDA-APHIS Petitions #94-357-01p, #00-136-01p, #03-353-01p and #15-113-01p corn; #98-278-01p and #01-206-01p canola; #96-068-01p, #98-014-1p, #12-215-01p, #11-234-01p and #09-349-01p soybean; #97-336-01p sugarbeet, #98-329-01p rice; and #12-185-01p, #13-262-01p and #08-340-01p cotton) (Hérouet et al., 2005; ILSI-CERA, 2011), except for the first methionine that is removed due to cotranslational processing in KWS20-1 sugar beet. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinnel and Giglione, 2008).

PAT proteins have been extensively assessed by regulatory agencies in at least 15 different countries for more than 30 biotechnology-derived events in several different crop species (e.g., corn, soybean, cotton, canola and sugar beet). Prior assessments of the PAT proteins expressed in these other biotechnology-derived crops are directly applicable to the KWS20-1 sugar beet PAT protein because the amino acid sequence of the KWS20-1 sugar beet PAT protein is identical to the PAT proteins in these biotechnology-derived crops that are derived from the *pat* gene.

The PAT protein expressed in KWS20-1 sugar beet is highly specific for glufosinate. Enzyme assays indicated that the PAT protein does not acetylate other common L-amino acids that are structurally similar to L-phosphinothricin, and substrate competition assays showed no inhibition of glufosinate acetylation in the presence of high concentrations of L-amino acids that are structurally similar to L-phosphinothricin (including the glufosinate analog L-glutamate) (Wehrmann et al., 1996). Recent metabolic profiling reported some non-specific PAT (*bar*)-mediated acetylation of two amino acids (aminoadipate and tryptophan) in senescent leaf extracts from *A. thaliana* and also in PAT (*pat*)-expressing soybean (Christ et al., 2017). However, the activity level for these two amino acids was very low relative to the activity for L-phosphinothricin, indicating that PAT (*pat*) has a very high level of specificity for the herbicidal molecule.

The data and information summarized in this section confirm that the molecular mechanism-of-action of the KWS20-1 sugar beet PAT protein that confers glufosinate tolerance is well understood, that the KWS20-1 sugar beet PAT protein is identical to the PAT proteins present in several biotechnology-derived crops that have been deregulated by USDA-APHIS, and is highly specific for glufosinate. Aside from glufosinate tolerance, no changes to metabolism, physiology or development of the KWS20-1 sugar beet plant are expected. Thus, prior evaluations for the PAT protein are directly applicable to the KWS20-1 sugar beet PAT protein.

CP4 EPSPS Protein

KWS20-1 sugar beet contains a codon optimized coding sequence of the *aroA* gene from the soil bacterium *Agrobacterium* sp. strain CP4 that expresses the CP4 EPSPS protein (Padgette et al., 1996; Barry et al., 2001). 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme of the shikimate pathway of aromatic amino acid biosynthesis, and is present in plants, bacteria and fungi. The molecular mechanism of the CP4 EPSPS protein is well understood, where

EPSPS catalyzes a reversible reaction that produces EPSP and inorganic phosphate (Pi) from phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Levin and Sprinson, 1964), known to specifically react with these substrates (Gruys et al., 1992). In most plants, the endogenous EPSPS is inhibited by the herbicide glyphosate that causes cell death (Franz et al., 1997). The CP4 EPSPS protein is structurally similar and functionally equivalent to endogenous plant EPSPS enzymes, but has a much-reduced affinity for glyphosate relative to endogenous plant EPSPS (Barry et al., 2001; Padgette et al., 1996). The presence of this protein renders the plant tolerant to glyphosate. Expression of the CP4 EPSPS protein in KWS20-1 sugar beet is targeted to the chloroplast by a CTP, where the plant EPSPS resides and is the site of aromatic amino acid biosynthesis (Klee et al., 1987; Kishore et al., 1988).

The *cp4 epsps* expression cassette contains the *cp4 epsps* gene encoding a precursor protein of 531 amino acids (i.e., 455 amino acids encoded by the *cp4 epsps* gene and 76 amino acids encoded by the *CTP2* gene for targeting the CP4 EPSPS protein into chloroplasts). KWS20-1 sugar beet expresses an ~43.5 kDa CP4 EPSPS protein, consisting of a single polypeptide of 455 amino acids starting at the methionine position 77 (Padgette et al., 1996) after a complete cleavage of the chloroplast transit peptide (CTP2).

The CP4 EPSPS protein present in KWS20-1 sugar beet is similar to EPSPS proteins consumed in a variety of food and feed sources. CP4 EPSPS protein is homologous to EPSPS proteins naturally present in plants, including food and feed crops (e.g., corn and soybean) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe consumption (Harrison et al., 1996; Padgette et al., 1996). The CP4 EPSPS protein in KWS20-1 sugar beet is also produced in several glyphosate-tolerant crops previously deregulated by USDA-APHIS (e.g., USDA-APHIS Petitions #00-011-01p, #04-125-01p, #10-281-01p, #13-290-01p and #19-316-01p corn; #98-089-1p and #11-188-01p canola; #93-258-01p, #06-178-01p and #09-201-01p soybean; #03-323-01p sugar beet; #04-110-01P alfalfa; and #95-045-01p and #04-086-01p cotton).

Additionally, the CP4 EPSPS protein has been extensively assessed by regulatory agencies in at least 20 different countries for more than 15 biotechnology-derived events in several different crop species (e.g., corn, soybean, cotton, canola, alfalfa and sugar beet). Prior assessments of the CP4 EPSPS protein expressed in these other biotechnology-derived crops are directly applicable to the KWS20-1 sugar beet CP4 EPSPS protein because the amino acid sequence of the KWS20-1 sugar beet CP4 EPSPS protein is identical to the CP4 EPSPS protein in these biotechnology-derived crops that are derived from the *cp4 epsps* gene.

The data and information summarized in this section confirm that the molecular mechanism-of-action of the KWS20-1 sugar beet CP4 EPSPS protein that confers glyphosate tolerance is well understood, that the KWS20-1 sugar beet CP4 EPSPS protein is identical to the CP4 EPSPS proteins present in several biotechnology-derived crops that have been deregulated by USDA-APHIS, and is highly specific for glyphosate. Aside from the intended glyphosate tolerance, no changes to metabolism, physiology or development of the KWS20-1 sugar beet plant are expected. Thus, prior evaluations for the CP4 EPSPS protein are directly applicable to the KWS20-1 sugar beet CP4 EPSPS protein.

V.D Conclusion

This request for Regulatory Status Review (RSR) of KWS20-1 sugar beet, provides details on a next generation herbicide-tolerant GE sugar beet product following the guidance outlined in the Agency's document titled "Guidance for Requesting a Regulatory Status Review (RSR) under 7 CFR part 340", Document ID BRS-GD-2020-003. Molecular characteristics, as well as the mechanism-of-action for the expressed proteins, are provided herein and support the conlcusion that KWS20-1 sugar beet does not include any expected changes in metabolism, physiology or the development of the sugar beet plant due to the trait/genetic modification.

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APPENDICES

Appendix A: Sequence of the Insertion for KWS20-1 Sugar Beet

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stephen.lamitola@bayer.com
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Signer Events	Signature	Timestamp
Ronald Schneider ronald.schneider@bayer.com Bayer PROD Default (Useranlage) Security Level: Email, Account Authentication (Required)	Ronald Schneider Signature Adoption: Pre-selected Style Signature ID: EE81EB78-8197-4199-BDEB-EECB0E2774AF Using IP Address: 161.69.116.11 With Signing Authentication via DocuSign password With Signing Reasons (on each tab): I am the author of this document	Sent: 8/30/2022 10:33:23 AM Viewed: 8/30/2022 10:35:53 AM Signed: 8/30/2022 10:37:06 AM
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In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
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Signing Complete	Security Checked	8/30/2022 10:37:06 AM
Completed	Security Checked	8/30/2022 10:37:06 AM

Payment Events	Status	Timestamps

Electronic Record and Signature Disclosure

Electronic Signatures – Consent and Privacy statement

Herewith, signers agree that the electronic signatures are intended to be the legally binding equivalent of traditional handwritten signatures according to CFR – Code of Federal Regulations Title 21, Part 11 Electronic Records; Electronic Signatures, Subpart C – Electronic Signatures.

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The personal data, which we process, are **identifying information** like your name, e-mail address and contact details, and **document management and signature related information** like date of signature, history of document-related actions (e.g. review, sign and IP address). Furthermore, the electronically signed documents may contain **personal data of any type** that is not directly related to the electronic signature process.

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Your personal data may be transferred to and processed in a country outside the European Economic Area for which the European Commission has not decided that it ensures an adequate level of data protection. In such cases, we will set up appropriate protection measures as required by GDPR. For example, we generally will apply the so-called "standard

data protection clauses" adopted by the European Commission. You can obtain a copy from us on request. Alternative protection measures may be implemented as well.

Your personal data will be **retained and archived** according to legally required retention and archiving periods applicable to business related files. Your personal data will then be deleted if there are no other legal reasons to further processing.

You have the right to request information from us about your personal data, access to and rectification or erasure of personal data or restriction of processing concerning your personal data or to object to processing as well as the right to data portability. You also have the right to lodge a complaint with the data protection supervisory authority.

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