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May 9, 2023

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Re: Request for a Regulatory Status Review of Dicamba Tolerant Canola MON 94100

Bernadette Juarez:

Bayer CropScience LP is submitting this request to USDA-APHIS for an initial Regulatory Status Review (RSR) of the enclosed information in regard to a determination of nonregulated status for the new genetically engineered (GE) canola product, MON 94100, any progeny derived from crosses between MON 94100 and conventional canola, and any progeny derived from crosses between MON 94100 and other GE canola not subject to 7 CFR Part 340 regulations.

Bayer has developed herbicide tolerant MON 94100 canola, which is tolerant to the herbicides dicamba (3,6-dichloro-2-methoxybenzoic acid). MON 94100 canola will be combined, through traditional breeding methods, with other deregulated canola events (e.g., glyphosate-tolerant trait) and will offer North American growers multiple choices for effective weed management including tough-to-control and herbicide-resistant broadleaf and grass weeds. MON 94100 canola combined with glyphosate-tolerant canola systems will provide additional weed management tools and flexibility to enhance weed management and maintain or improve canola yield and quality. This enhanced system will help meet the growing needs of food, feed, and industrial markets, provide broader grower choice, improve production efficiency, increase pest control durability, and promote sustainable agriculture practices.
The enclosed information is being submitted in accordance with the Guidance for Requesting a Regulatory Status Review under 7 CFR part 340 (USDA-APHIS Document ID BRS-GD-2020-003).

We would be pleased to meet with you and other USDA officials and scientists to respond to any questions you may have, or to provide you with additional information that you may request. Should you have any questions on this letter, the enclosed

information or wish to set up a meeting to further discuss MON 94100 please contact James Nyangulu, Federal Engagement Lead, at (202) 304-6594, or Aster Beyene at (314) 370-9565.

Yours sincerely,

DocuSigned by:
Aster Beyene
Signer Name: Aster Beyene
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Aster Beyene, Ph.D.
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Request for a Regulatory Status Review for Dicamba Tolerant Canola MON 94100

OECD Unique Identifier: MON-941ØØ-2

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

May 9, 2023

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Aster Beyene
Signer Name: Aster Beyene
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Aster Beyene, Ph.D.
Global Regulatory Affairs Manager

Bayer RSR Number: CA281-23U1

Submitted by:

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RELEASE OF INFORMATION

Bayer CropScience LP (hereafter Bayer) is submitting this request for a Regulatory Status Review (RSR) by the USDA. Bayer understands that the USDA complies with the provisions of the Freedom of Information Act (FOIA). In the event the USDA receives a FOIA request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of the information in this request, Bayer expects that, in advance of the release of the document(s), USDA will provide Bayer with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g., responsiveness, confidentiality, and/or competitive concerns. Bayer understands that a CBI-deleted copy of this information may be made available to the public in a reading room and made available via the internet as part of a public comment period. Bayer also understands that if the review proceeds to the plant pest risk assessment (PPRA) step and the RSR request has been deemed complete, a copy of the RSR request may be posted to the USDA-APHIS BRS website or other U.S. government websites (e.g., www.regulations.gov). Except in accordance with the foregoing and required under applicable law, Bayer does not authorize the release, publication or other distribution of this information without Bayer's prior notice and consent.

ABBREVIATIONS AND DEFINITIONS

APHIS	Animal and Plant Health Inspection Service
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic acid
DMO	Dicamba mono-oxygenase
dmo	Coding sequence for the DMO protein
DCSA	Dichlorosalicylic acid
GE	Genetically engineered
OECD	Organization for Economic Co-operation and Development
RSR	Regulatory Status Review
T-DNA	Transfer deoxyribonucleic acid
USDA	United States Department of Agriculture

I REQUESTOR

The submitters of this initial Regulatory Status Review request for canola MON 94100 is:

Bayer CropScience LP
700 Chesterfield Parkway West
Chesterfield, MO 63017

Communications with regard to this submission should be directed to Aster Beyene, Ph.D., Global Regulatory Affairs Manager, at the Bayer address listed above, or by email at aster.beyene@bayer.com

II RATIONALE FOR THE DEVELOPMENT OF MON 94100

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 is submitting this request for an initial RSR to APHIS for the agency to evaluate whether the genetically engineered (GE) product, MON 94100, any progeny derived from crosses between MON 94100 and conventional canola, and any progeny derived from crosses between MON 94100 and other GE canola not subject to 7 CFR Part 340 regulations should continue to be regulated by APHIS.

II.B Rationale for the Development of Herbicide-Tolerant Canola

Bayer CropScience LP has developed herbicide tolerant MON 94100 canola, which is tolerant to the herbicide dicamba. MON 94100 canola contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein that confers tolerance to the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid).

MON 94100 will provide canola growers with an additional herbicide option for effective and sustainable weed management, including control of glyphosate resistant weeds. The best management practices for minimizing the development of herbicide resistant weeds are built on the concepts of implementing diversified weed management programs, which includes using multiple herbicides with different sites-of-action, either in mixtures, in sequences or in rotation.

MON 94100 canola may be combined with other deregulated herbicide tolerance traits (*e.g.*, Roundup Ready) through traditional breeding methods to create commercial products with tolerance to multiple herbicides. These next generation combined-trait canola products will continue to offer broader grower choice in herbicide options and support continued weed control durability.

III DESCRIPTION OF COMPARATOR PLANT

Canola (*Brassica napus*) variety 65037 and the hybrid 55076+65037 were used as the conventional canola comparators to support the safety assessment of MON 94100 canola. MON 94100 canola was derived from a single plant transformant of canola variety 65037. MON 94100 and the conventional control 65037 variety or 55076+65037 hybrid have similar genetic backgrounds with the exception of the *dmo* expression cassette, thus, the effect of the *dmo* expression cassette and the expressed DMO protein can be assessed in an unbiased manner in comparative safety assessments. The 65037 variety was developed via conventional breeding and is adapted to Canada and U.S. canola growing regions. The 65037 variety was used as the conventional control in molecular characterization studies and the 55076+65037 hybrid was used as the conventional control in compositional analysis and in phenotypic, agronomic, and environmental interactions assessments.

IV GENOTYPE OF THE MODIFIED PLANT FOR MON 94100

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 MON 94100

MON 94100 was developed through an *Agrobacterium* -mediated transformation of canola tissue using the transfer DNA (T-DNA) transformation vector PV-BNHT508701 (~17248 kb) based on the method described by Radke et al. (1992). This plasmid vector contains two T-DNAs, which are delineated by Right and Left Border regions. T-DNA I contains the *dmo* expression cassette and T-DNA II contains the *splA* and *aadA* selectable marker expression cassettes. Although both *aadA* and *splA* selectable marker expression cassettes were present in PV-BNHT508701, selection for transformants was based on spectinomycin resistance conferred by *aadA* and *splA* was not used as a selectable marker. Subsequently, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *dmo* expression cassette and lack the *splA* and *aadA* cassettes. MON 94100 was selected as the lead event based on superior agronomic, phenotypic and molecular characteristics. The nucleotide sequence of the inserted genetic material in MON 94100 is provided in Appendix A and an annotation of the different genetic elements is provided in Table IV-1.

Table IV-1. Annotation of the Inserted Genetic Material in MON 94100

Genetic Element	Location in Sequence	Function (Reference)
B1[1]-Right Border Region[r1]	1-70	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)
Intervening Sequence	71-104	"Synthetic[2] sequence" used in DNA cloning
P[3]-*PClSV*	105-537	Promoter for the full length transcript (FLt) of peanut chlorotic streak caulimovirus (PClSV) that directs transcription in plant cells (Maiti and Shepperd, 1998)
Intervening Sequence	538-557	Sequence used in DNA cloning
L[4]-*TEV*	558-689	5' UTR leader sequence from the RNA of tobacco etch virus (TEV) that is involved in regulating gene expression (Niepel and Gallie, 1999)
Intervening Sequence (IS)	690	Sequence used in DNA cloning
TS[5]-*RbcS (Ps)*	691-933	Targeting sequence and the first 24 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)
Intervening Sequence	934-942	Sequence used in DNA cloning
CS[6]-*dmo*	943-965	Coding sequence for the dicamba monooxygenase (DMO) protein from *Stenotrophomonas maltophilia* (Wang et al., 1997, Herman et al., 2005)
Intervening Sequence	966-2034	Sequence used in DNA cloning
T[7]-*guf-Mt1*	2035-2534	3' UTR from an expressed gene of *Medicago truncatula* of unknown function (GenBank Accession MH931406) that directs polyadenylation of mRNA
Intervening Sequence	2535-2632	Sequence used in DNA cloning
B-Left Border Region[r1]	2633-2913	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983)
3' Flanking DNA	2914-2913	DNA sequence flanking the 3' end of the insert

[1] B, Border

[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 94100 was truncated compared to the sequences in PV-BNHT508701.

[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] P, Promoter

[4] L, Leader

[5] TS, Targeting Sequence

[6] CS, Coding Sequence

[7] T, Transcription Termination Sequence

V DESCRIPTION OF THE NEW TRAIT FOR MON 94100

This section describes the intended MON 94100 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 MON 94100 Trait

MON 94100 is intended to provide herbicide tolerance to dicamba.

V.B Intended Phenotype of MON 94100

Herbicide-tolerant canola MON 94100 is intended to provide tolerance to dicamba and will be combined with other herbicide tolerance traits to 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 MON 94100

DMO Protein

MON 94100 canola 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 MON 94100 is targeted to the chloroplast by a chloroplast transit peptide (CTP), that is post-translationally processed during chloroplast targeting into two forms of the DMO protein; referred to as DMO and DMO+27, contain 339 amino acids (DMO) and 367 amino acids (DMO+27) and have apparent molecular weight of ~38 and 39.4 kDa, respectively. MON 94100 DMO and DMO+27 are identical to the DMO and DMO+27 proteins expressed in MON 87708 dicamba-tolerant soybean for which the characterization and safety assessment of this protein was reviewed by USDA-APHIS (Petition #10-188-01p) and resulting in a determination of nonregulated status in 2015. In addition, MON 94100 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 (MON 88701 cotton, USDA-APHIS Petition #12-185-01p and MON 87419 maize, USDA-APHIS Petition #15-113-01p). Thus, prior evaluations of the DMO expressed in other biotechnology-derived crops are directly applicable to the DMO protein expressed in MON 94100.

The DMO protein is specific for the oxidative demethylation of dicamba, forming DCSA. This demethylation is very specific to dicamba, where both the carboxylate moiety and the chlorine atoms help position the substrate at the active site of the enzyme (D'Ordine et al., 2009, Dumitru et al., 2009). Crystallography studies of the substrate in the active site demonstrated that these chlorines function as steric “handles” that position the substrate in the proper orientation in the

binding pocket (Dumitru et al., 2009). Potential plant substrates (o-anisic acid, vanillic acid, syringic acid, ferulic acid and sinapic acid) that are structurally similar to dicamba, were not metabolized by an *E. coli*-produced DMO in laboratory tests indicating that the DMO enzyme is specific for dicamba. Given the limited amount of chlorinated metabolites with structures similar to dicamba in plants and other eukaryotes (Wishart et al., 2009, Wishart, 2010) it is unlikely that MON 94100 DMO will catalyze the conversion of other endogenous substrates. Therefore, the activity of the enzyme is specific for dicamba while it maintains many structural properties common to oxygenases that are ubiquitous to all organisms.

The data and information summarized in this section confirm that the molecular mechanism-of-action of the MON 94100 DMO protein that confers dicamba tolerance is well understood, that the MON 94100 DMO protein is specific for dicamba, and that aside from dicamba tolerance, no changes to metabolism, physiology or development of the MON 94100 plant are expected. Furthermore, the MON 94100 DMO protein is structurally and functionally homologous to the DMO proteins present in biotechnology-derived crops that have been previously deregulated by USDA-APHIS.

VI Conclusion

This request for Regulatory Status Review (RSR) of MON 94100, provides details on a next generation herbicide-tolerant GE canola 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 conclusion that MON 94100 canola does not include any expected changes in metabolism, physiology or the development of the canola plant due to the trait/genetic modification.

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APPENDICES

Appendix A: Sequence of the Insertion for MON 94100

CCAGTCAGCATCATCACACCAAAAGTTAGGCCCGAATAGTTTGAAATTAGAAAGCTCGCAATTGAGGTCTGTCGACC
CTGCAGGTACACTGGCGCGCCGGCCGCAGATCTTGAGCCAATCAAAGAGGAGTGATGTAGACCTAAAGCAATAATGG
AGCCATGACGTAAGGGCTTACGCCCATACGAAATAATTAAAGGCTGATGTGACCTGTCGGTCTCTCAGAACCTTTAC
TTTTTATGTTTGGCGTGTATTTTTAAATTTCCACGGCAATGACGATGTGACCCAACGAGATCTTGAGCCAATCAAAG
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