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October 19, 2023

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By kldiggs for BRS Document Control Officer at 2:42 pm, Oct 20, 2023

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services: **Revised Resubmission of 23-096-03rsr**

Contains Confidential Business Information

Re: Request for Regulatory Status Review for Group 10 (glufosinate) and Group 2 (sulfonylurea and imidazolinone) Herbicide Tolerant *Camelina sativa* derived from construct [ ] **CBI-deleted**

Yield10 Bioscience is requesting a regulatory status review for its glufosinate and group 2 herbicide tolerant *Camelina sativa* lines based on the provisions in 7 CFR § 340.4.

1. Requestors

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2. Confidential Business Information (CBI) Statement

This RSR request contains CBI.

CBI Justification:
The Freedom of Information Act (FOIA) exempts federal agencies from releasing trade secrets and commercial or financial information that is privileged or confidential (5 U.S.C.552(b)(4)). Yield10 Bioscience considers certain information in this document as trade secret or commercial information that is privileged and confidential. Disclosure of this information would cause substantial competitive harm to Yield10 Bioscience by allowing other companies to unfairly compete with it.

First, Yield10 Bioscience must protect the nature of traits which it has selected to be important, that, if imparted to a new plant variety, would represent a competitive advantage in the marketplace. Disclosure of this information would reveal the company's marketing strategy, which identifies targets of potential commercial opportunity. Second, Yield10 Bioscience must keep some aspects of its research confidential: what it is doing, how it is doing it, and how far along it is. Disclosure of this information would enable competitors to duplicate Yield10 Bioscience research and development without incurring the investment of time and money expended by the company. This information would also provide competitors with commercially valuable knowledge about the specific products Yield10 Bioscience is interested in commercializing and the likely time for commercialization. Moreover, Yield10 Bioscience must protect its intellectual property. Yield10 Bioscience must keep research information strictly confidential because in some cases, patent applications have not been filed or patents are pending and have not been published.

Specifically, Yield10 Bioscience designates the following as Confidential Business Information:

Genetic elements used to create trait. Yield10 Bioscience biotechnology traits consist of vectors transferred into plants, which comprise genes for the expression of traits and regulatory sequences such as promoters, enhancers, signaling peptides and terminators. Disclosure of genetic elements comprising the genotype of the resulting plants would provide the company's competitors with the knowledge of what genetic sequences the company is using. Disclosure of this information may also reveal the specific modifications the company made in assembling the DNA constructs and enhance their usefulness. It is in Yield10 Bioscience's commercial interest that these trade secrets are not publicly disclosed.

Construct Identity. Yield10 Bioscience technology traits are encoded by genetic constructs that the company identifies with unique nomenclature. Disclosure of the construct identity could enable competitors to determine the status of the company's proprietary research and development programs, if construct identity is linked to the genetic elements contained in these constructs.

Phenotype Descriptions. Yield10 Bioscience seeks to improve certain characteristics of plants that are economically important to its customers. Disclosure of the phenotypes that the company is pursuing would enable competitors to determine the company's R&D objectives and the status of its R&D programs.

Accordingly, Yield10 Bioscience requests all FOIA protections for the CBI submitted herein.

This information is both customarily and actually treated as private information by Yield10 Bioscience and was provided to the government under an assurance of privacy. We consider this information as confidential within the meaning of 5 U.S.C §552(b)(4), the Freedom of Information Act's Exemption 4.

3. Description of Comparator Plant

Scientific Name: *Camelina sativa*

Common Name: Camelina, False Flax, Gold of Pleasure

Cultivar and/or Breeding Line: [ ]. These breeding lines will be used to produce the transgenic herbicide tolerant plants. CBI-deleted

There are two types of Camelina, spring and winter, named for their respective growth habit. Both are promising crops that produce high levels of seed oil (Vollmann and Eynck, 2015; Berti et al. 2016.; Malik et al. 2018). The spring growth habit is dominant in Camelina. Winter lines are better adapted as cover crops in colder, temperate climates. Winter lines require vernalization, or exposure to cold, with temperatures below 8°C for 2-3 weeks at seedling to rosette stage to induce bolting and flowering, whereas spring lines do not (Anderson et. al. 2018). Camelina plants are highly sensitive to herbicides including glufosinate, glyphosate, sulfonylureas (SUs), and imidazolinones (IMIs). Yield10 Bioscience is engineering glufosinate and group 2 tolerance [ ]. CBI-deleted CBI-deleted

4. Genotype of the Modified Plant

Transformation construct [ ] has an expression cassette for the *bar* gene, encoding phosphinothricin acetyltransferase, providing engineered plants with spray tolerance to glufosinate. It also has an expression cassette for the AHAS gene, encoding acetohydroxyacid synthase, with mutations engineered to provide tolerance to sulfonylureas and imidazolinones (Group 2 herbicides). CBI-deleted

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a. Sequence of the Insertion

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b. Annotation of the Inserted Genetic Material

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c. Information about insertion site. For transformation construct **[ ]**, plants containing single copy inserts of the T-DNA have been isolated after *Agrobacterium*-mediated transformation of Camelina lines **[ ]**. After identification of the best events for commercial advancement in field trials, whole genome sequencing will be carried out to ensure the presence of the desired expression cassettes for *bar* and AHAS and the absence of unintended DNA fragments derived from the vector (plasmid) backbone. Sequence analysis of flanking genomic DNA regions will also be used to determine the genomic location of the T-DNA insert and its junctions with the plant genome to confirm insert integrity and location. **CBI-deleted** **CBI-deleted**

5. Description of New Trait

a. Intended trait. Herbicide tolerance. The inserted genetic material imparts Camelina plants with tolerance to glufosinate, a broad spectrum post-emergent broadleaf herbicide, and Group 2 herbicides (sulfonylureas and imidazolinones).

b. Intended phenotype. Tolerance to spray applications of commercial herbicides containing the active ingredient glufosinate. Tolerance to Group 2 herbicides, such as sulfonylureas and imidazolinones.

c. Description of the Mechanism of Action (MOA).

The mechanism of action of the phosphinothricin N-acetyltransferase (PAT) protein, providing tolerance to the herbicide glufosinate, has been well studied (for review see Takano and Dayan, 2020). Glufosinate (or L-phosphinothricin) inhibits glutamine synthase (FIG. 1.A) when applied to plants such that high levels of ammonia accumulate and inhibition of the photorespiratory pathway and photosynthesis occur, killing the plant (Takano and Dayan, 2020). Expression of the *bar* gene from **[ ]** in transgenic plants results in the production of PAT, a protein which catalyzes the conversion of L-phosphinothricin (L-PPT) to the non-phytotoxic form, N-acetyl-L- phosphinothricin, by acetylation (FIG. 1B). Plants engineered with a genetic construct for expression of the *bar* gene are thus tolerant to post-emergent applications of glufosinate containing herbicides (Thompson et al., 1987; De Block et al., 1987; Wehrmann et al., 1996). **[ ]** RSR 22-174-01rsr has previously been submitted to USDA-APHIS by Yield10 Bioscience for expression of the *bar* gene in Camelina. **[ ]**. **CBI-deleted** **CBI-deleted** **CBI-deleted** **CBI-deleted** **CBI-deleted** **CBI-deleted** **CBI-deleted**

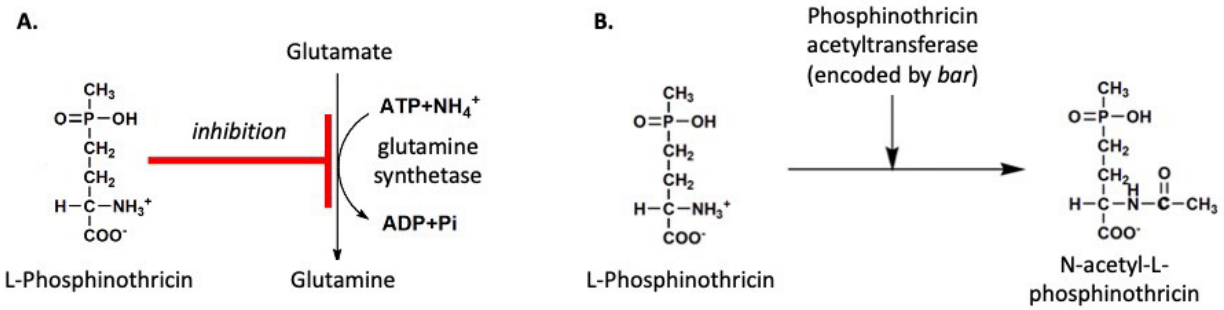

Figure 1. Glufosinate detoxification by expression of the *bar* gene encoding phosphinothricin acetyltransferase. **A.** Glufosinate (also known as L-phosphinothricin) exhibits its herbicidal action through inhibition of glutamine synthetase, resulting in the accumulation of ammonia and inhibition of photosynthesis. **B.** Expression of the *bar* gene in transgenic plants, encoding phosphinothricin acetyltransferase, converts L-phosphinothricin to N-acetyl-L-phosphinothricin such that the transgenic plant is tolerant to glufosinate.

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Group 2 herbicides include imidazolinones, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinones, sulfonylureas, and triazolopyrimidines. Their mode of action is inhibition of acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS),

a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine (WSSA, 2011) (**Fig. 2.A**). Crop tolerance to Group 2 herbicides has been achieved through mutations in the coding sequence of the *AHAS* gene that result in specific amino acid substitutions in the AHAS protein (**Fig. 2.B**). The amino acid substitutions in AHAS prevent the herbicide from binding to the protein and confer herbicide tolerance. [CBI-deleted
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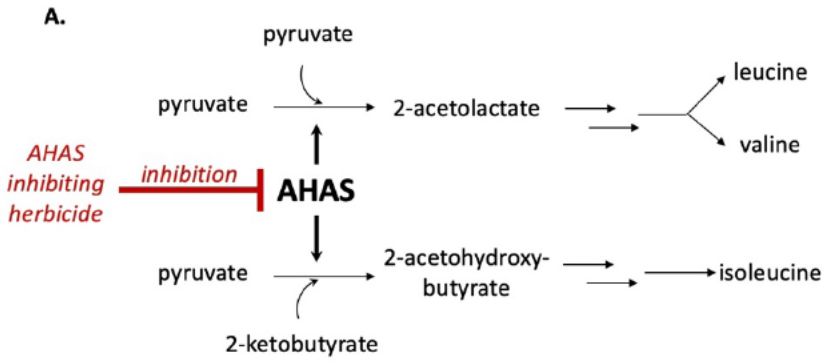

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Figure 2. Group 2 herbicides inhibit acetolactate synthase (AHAS). A. Inhibition of AHAS by Group 2 herbicides affects production of branched chain amino acids. [CBI-deleted
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We have provided the following elements which support our RSR request:

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3. The mechanism of action of genetically engineered glufosinate tolerance in plants is well studied (Figure 1).
4. **[** **CBI-deleted**
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5. The mechanism of action of genetically engineered tolerance to Group 2 herbicides such as sulfonylureas and imidazolinones is well studied (Figure 2).

Please let us know if you have any further questions or need additional information as you review our request.

Sincerely,

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Karen Bohmert-Tatarev
Senior Director of Regulatory Affairs
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References

1. Anderson, J. V., Horvath, D. P., Doğramaci, M., Dorn, K. M., Chao, W. S., Watkin, E. E., Hernandez, A. G., Marks, M. D. and Gesch, R. 2018. Expression of FLOWERING LOCUS C and a frameshift mutation of this gene on chromosome 20 differentiate a summer and winter annual biotype of Camelina sativa. Plant Direct 2, e00060.
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3. **[CBI-deleted CBI-deleted] CBI-deleted**
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