RECEIVED
By kldiggs for BRS Document Control Officer at 10:39 am, Aug 19, 2022

Request for
Regulatory Status Review under 7 CFR part 340
For Safflower Plants Modified to Produce Gamma-Linolenic Acid (GLA)

(APHIS Number 22-145-01rsr with revisions as requested by APHIS-BRS)

To:

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services
RSRrequests@usda.gov

Requestor:

David Heron
Regulatory Affairs Manager
Moolec Science Limited
david@moolecscience.com

Confidential Business Information (CBI) Statement:
This document does not contain Confidential Business Information (CBI).

Consistent with the provisions in the APHIS regulation found at 7 CFR part 340, Moolec Science Limited is requesting a Regulatory Status Review (§ 340.4) for safflower plants which have been modified by genetic engineering to produce gamma-linolenic acid (GLA) in the safflower seeds.

Section 340.4 (b)(2) of the APHIS regulation states the following:

> *"If APHIS does not identify a plausible pathway by which the GE plant or its sexually compatible relatives would pose an increased plant pest risk relative to the comparator(s) in the initial review, the GE plant is not subject to the regulations in this part."*

Before we address the information requirements specified when requesting a Regulatory Status Review, we want to share our conclusions reached after careful study of the relevant definitions in 7 CFR part 340, as well as the implications for the APHIS analysis described in Section 340.4. We have concluded that introducing or disseminating safflower cannot pose a "plant pest risk", because safflower is not a "plant pest".

Definitions of "plant pest" and "plant pest risk"
Whether a plant can pose an increased plant pest risk depends upon the definitions in the regulation and the regulation's enabling statute, the Plant Protection Act of 2000. Under the definitions prescribed in Section 340.1 for "plant pest risk" and "plant pest", safflower cannot pose a plant pest risk. In order to pose a plant pest risk, as defined, an organism must first meet the criteria under the definition of "plant pest".

The definition of "plant pest" in Section 340.1 is verbatim with the definition of plant pest in the Plant Protection Act of 2000. This definition of plant pests specifically states that for a plant to be considered as a plant pest it must first be a "parasitic plant". Safflower is not a parasitic plant, and therefore does not meet the definition of plant pest in the regulation. The genetically modified safflower is likewise not

a parasitic plant, so it likewise does not meet the definition of plant pest. APHIS has published online its list of parasitic plant genera, and safflower (genus *Carthamus*) is not listed as a parasitic plant (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits/plant-pests/SA_Noxious_Weeds/parasitic-plant-genera-list).

Section 340.1 of the regulation states that "plant pest risk" is "the potential for direct or indirect injury to, damage to, or disease in any plant or plant product **resulting from introducing or disseminating a plant pest**, or the potential for exacerbating the impact of a plant pest." (Bold added for clarity)

The Plant Protection Act defines "plant pest risk" as "the potential for direct or indirect injury to, damage to, or disease in any plant or plant product **resulting from introducing or disseminating a plant pest**." (Bold added for clarity)

It is clear from the definition of plant pest risk in the Plant Protection Act that in order for the introduction or dissemination of an organism to pose a plant pest risk, the organism must be a plant pest as defined in the statute. We have searched the Congressional legislative records for the Plant Protection Act of 2000 and its predecessor statutes, and we have found nothing to contradict the conclusion that Congress intended that plant pest risks are posed by plant pests, not organisms that do not meet the statute's definition of plant pest.

Therefore, it is clear that introducing or disseminating safflower cannot pose a plant pest risk, because safflower is not a plant pest.

Request for Regulatory Status Review as described in Section 340.4
The section below provides the relevant information to meet the requirements for persons requesting a Regulatory Status Review for plants that have been modified by genetic engineering. In this case, the plant is safflower which has been modified by genetic engineering to produce gamma-linolenic acid in the seed.

Moolec Science Limited is requesting a Regulatory Status Review consistent with the provisions of §340.4 of the regulation (italicized text copied below for context).

> ***§ 340.4 Regulatory status review.*** *(https://www.ecfr.gov/current/title-7/subtitle-B/chapter-III/part-340)*
>
> *(a) (4) Information submitted in support of a request for a regulatory status review or re-review must meet the requirements listed in paragraphs (a)(4)(i) through (iii) of this section.*
>
> > *(i) A description of the comparator plant(s), to include genus, species, and any relevant subspecies information;*
> >
> > *(ii) The genotype of the modified plant, including a detailed description of the differences in genotype between the modified and unmodified plant; and*
> >
> > *(iii) A detailed description of the new trait(s) of the modified plant.*

1. Description of Comparator Plant:
The comparator plant is safflower, *Carthamus tinctorius*, L., cultivar Centennial. Safflower is an annual plant grown commercially mainly for edible oil that is extracted from the seeds. There are two types of safflower varieties: the type that produces oil high in monounsaturated fatty acids (oleic acid), and the type that produces oil with high concentrations of polyunsaturated fatty acids (linoleic acid).

Safflower has been the subject of extensive monographs that describe its biology, genetics, and agronomic production practices as an oilseed crop. The Organisation for Economic Cooperation and Development published a 61-page consensus document in 2020, and it can be found online (https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282020%2914&doclanguage=en).

In October 2019, The Australian Office of Gene Technology Regulator published version 1.2 of its 58-page document entitled "The Biology of Carthamus tinctorius L. (safflower)" (https://www.ogtr.gov.au/sites/default/files/files/2021-07/the_biology_of_safflower_-_v1.2_-_october_2019.pdf).

In addition, USDA-APHIS-BRS prepared an Environmental Assessment in conjunction with a 2006 request for a permit to conduct a confined environmental release of genetically engineered safflower. This document contains sections on safflower biology and sexually compatible relatives (https://www.aphis.usda.gov/brs/aphisdocs/06_36303r_ea.pdf). The APHIS analysis did not cite any evidence in the scientific literature that gene flow from any cultivated safflower to sexually compatible relatives increased the weediness of the offspring of such crosses.

Safflower is not a parasitic plant (https://web.archive.org/web/20161005194620/http://www.apsnet.org/edcenter/intropp/pathogengroups/pages/parasiticplants.aspx).

Because safflower is not a parasitic plant, it does not meet the definition of plant pest in APHIS regulation 7 CFR part 340 (see 7 CFR part 340.3 for definitions). The only plants that fall under this definition of "plant pest" are parasitic plants.

There is no plausible pathway by which safflower plants can be modified by genetic engineering to make it into a parasitic plant.

Safflower is not on the list of Federal Noxious Weeds (https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf).

2. Genotype of the Modified Plant
The subject genetically engineered (GE) safflower (GLA safflower) was generated by using an Agrobacterium-mediated transformation protocol to insert into the nuclear genome of *Carthamus tinctorius* L. cv. Centennial a genetic construct that enables the plants to produce elevated levels of the fatty acid gamma-linolenic acid (GLA) in their seeds (see Table 1 below). The inserted genetic construct consists enables expression of delta-6-desaturase for GLA production and phosphinothricin-N-acetyltransferase (PAT) as a selectable marker for transformation and regeneration of transformed plants.

The plasmid used for transformation, pSBS4119, contained cDNA encoding *Saprolegnia diclina* Humphrey delta-6-desaturase controlled by the *Arabidopsis thaliana* oleosin promoter (araP; GeneBank Accession X62353) and terminator (araT; GenBank Accession NM_118647). The plasmid pSBS4119 also contains cDNA encoding *Streptomyces viridochromogenes* phosphinothricin-N-acetyltransferase (PAT; GenBank Accession M22827) controlled by the *Petroselinum crispum* ubiquitin promoter (ubiT; GenBank Accession X64345) and the *P. crispum* ubiquitin terminator (ubiT) located between the right border and left border regions of the transfer DNA (T-DNA) region. Agrobacterium sequences were obtained from the *A. tumefaciens* plasmid pTiC58 T-DNA (GeneBank Accession J237588) (Figure 1).

Delta-6-desaturase converts linoleic acid to gamma-linolenic acid in the seeds of the GLA safflower plants.

PAT serves as a selectable marker used for the glufosinate-mediated selection of the genetically modified two-day-old cotyledons that had undergone genetic recombination following the transformation of *C. tinctorius* with pSBS4119.

The right and left border regions are recognized by endonucleases that mediate the transfer of DNA from pSBS4119 to the genome of *C. tinctorius* during *Agrobacterium tumefaciens*-mediated transformation.

The FASTA Format Information on genetic elements has been added August 19, 2022, per APHIS-BRS request. APHIS RSR number 22-145-01rsr)

RB (SBS)
>TTGATCCCGAGGGGAACCCTGTGGTTGGCTTGCACATACAAATGGACGAACGGATAAACCTTTTCACGCCCTTTT
AAATATCCGATTATTCTAATAAACGCTCTTTTCTCTTAGGTTTACCCGCCAATATATCCTGTCAAACACTGATAGTTT
AAACTGAAGGCGGGAAACGACAATCTGATCC

araP
>CTGCAGGAATTCGATCTCTATTGATTCAAATTACGATCTGATACTGATAACGTCTAGATTTTTAGGGTTAAAGCAA
TCAATCACCTGACGATTCAAGGTGGTTGGATCATGACGATTCCAGAAAACATCAAGCAAGCTCTCAAAGCTACACT
CTTTGGGATCATACTGAACTCTAACAACCTCGTTATGTCCCGTAGTGCCAGTACAGACATCCTCGTAACTCGGATTG
TGCACGATGCCATGACTATACCCAACCTCGGTCTTGGTCACACCAGGAACTCTCTGGTAAGCTAGCTCCACTCCCCA
GAAACAACCGGCGCCAAATTGCGCGAATTGCTGACCTGAAGACGGAACATCATCGTCGGGTCCTTGGGCGATTGC
GGCGGAAGATGGGTCAGCTTGGGCTTGAGGACGAGACCCGAATCCGAGTCTGTTGAAAAGGTTGTTCATTGGGG
ATTTGTATACGGAGATTGGTCGTCGAGAGGTTTGAGGGAAAGGACAAATGGGTTTGGCTCTGGAGAAAGAGAGT
GCGGCTTTAGAGAGAGAATTGAGAGGTTTAGAGAGAGATGCGGCGGCGATGAGCGGAGGAGAGACGACGAGG
ACCTGCATTATCAAAGCAGTGACGTGGTGAAATTTGGAACTTTTAAGAGGCAGATAGATTTATTATTTGTATCCATT
TTCTTCATTGTTCTAGAATGTCGCGGAACAAATTTTAAAACTAAATCCTAAATTTTTCTAATTTTGTTGCCAATAGTG
GATATGTGGGCCGTATAGAAGGAATCTATTGAAGGCCCAAACCCATACTGACGAGCCCAAAGGTTCGTTTTGCGT
TTTATGTTTCGGTTCGATGCCAACGCCACATTCTGAGCTAGGCAAAAAACAAACGTGTCTTTGAATAGACTCCTCTC
GTTAACACATGCAGCGGCTGCATGGTGACGCCATTAACACGTGGCCTACAATTGCATGATGTCTCCATTGACACGT
GACTTCTCGTCTCCTTTCTTAATATATCTAACAAACACTCCTACCTCTTCCAAAATATATACACATCTTTTTGATCAAT
CTCTCATTCAAAATCTCATTCTCTCTAGTAAACAAGAACAAAAAA

S diclina delta 6 desaturase

>ATGGTCCAGGGGCAAAAGGCCGAGAAGATCTCGTGGGCGACCATCCGTGAGCACAACCGCCAAGACAACGCGT
GGATCGTGATCCACCACAAGGTGTACGACATCTCGGCCTTTGAGGACCACCCGGGCGGCGTCGTCATGTTCACGC
AGGCCGGCGAAGACGCGACCGATGCGTTCGCTGTCTTCCACCCGAGCTCGGCGCTCAAGCTCCTCGAGCAGTACT
ACGTCGGCGACGTCGACCAGTCGACGGCGGCCGTCGACACGTCGATCTCGGACGAGGTCAAGAAGAGCCAGTCG
GACTTCATTGCGTCGTACCGCAAGCTGCGCCTTGAAGTCAAGCGCCTCGGCTTGTACGACTCGAGCAAGCTCTACT
ACCTCTACAAGTGCGCCTCGACGCTGAGCATTGCGCTTGTGTCGGCGGCCATTTGCCTCCACTTTGACTCGACGGC
CATGTACATGGTCGCGGCTGTCATCCTTGGCCTCTTTTACCAGCAGTGCGGCTGGCTCGCCCATGACTTTCTGCACC
ACCAAGTGTTTGAGAACCACTTGTTTGGCGACCTCGTCGGCGTCATGGTCGGCAACCTCTGGCAGGGCTTCTCGGT
GCAGTGGTGGAAGAACAAGCACAACACGCACCATGCGATCCCCAACCTCCACGCGACGCCCGAGATCGCCTTCCA
CGGCGACCCGGACATTGACACGATGCCGATTCTCGCGTGGTCGCTCAAGATGGCGCAGCACGCGGTCGACTCGCC
CGTCGGGCTCTTCTTCATGCGCTACCAAGCGTACCTGTACTTTCCCATCTTGCTCTTTGCGCGTATCTCGTGGGTGAT
CCAGTCGGCCATGTACGCCTTCTACAACGTTGGGCCCGGCGGCACCTTTGACAAGGTCCAGTACCCGCTGCTCGAG
CGCGCCGGCCTCCTCCTCTACTACGGCTGGAACCTCGGCCTTGTGTACGCAGCCAACATGTCGCTGCTCCAAGCGG
CTGCGTTCCTCTTTGTGAGCCAGGCGTCGTGCGGCCTCTTCCTCGCGATGGTCTTTAGCGTCGGCCACAACGGCAT
GGAGGTCTTTGACAAGGACAGCAAGCCCGATTTTTGGAAGCTGCAAGTGCTCTCGACGCGCAACGTGACGTCGTC
GCTCTGGATCGACTGGTTCATGGGCGGCCTCAACTACCAGATCGACCACCACTTGTTCCCGATGGTGCCCCGGCAC
AACCTCCCGGCGCTCAACGTGCTCGTCAAGTCGCTCTGCAAGCAGTACGACATCCCATACCACGAGACGGGCTTCA
TCGCGGGCATGGCCGAGGTCGTCGTGCACCTCGAGCGCATCTCGATCGAGTTCTTCAAGGAGTTTCCCGCCATGTA
A

araT

>GTTACCCCACTGATGTCATCGTCATAGTCCAATAACTCCAATGTCGGGGAGTTAGTTTATGAGGAATAAAGTGTTT
AGAATTTGATCAGGGGGAGATAATAAAAGCCGAGTTTGAATCTTTTTGTTATAAGTAATGTTTATGTGTGTTTCTAT
ATGTTGTCAAATGGTCCCATGTTTTTCTTCCTCTCTTTTTGTAACTTGCAAGTGTTGTGTTGTACTTTATTTGGCTTCT
TTGTAAGTTGGTAACGGTGGTCTATATATGGAAAAGGTCTTGTTTTGTTAAACTTATGTTAGTTAACTGGATTCGTC
TTTAACCACAAAAAGTTTTCAATAAGCTACAAATTTAGACACGCAAGCCGATGCAGTCATTAGTACATATATTTATT
GCAAGTGATTACATGGCAACCCAAACTTCAAAAACAGTAGGTTGCTCCATTTAGTAACCTGAATTGCCTCCTGATTC
TAGTTGATCCCGGTACC

ubiP

>GAATCCAAAAATTACGGATATGAATATAGGCATATCCGTATCCGAATTATCCGTTTGACAGCTAGCAACGATTGT
ACAATTGCTTCTTTAAAAAAGGAAGAAAGAAAGAAAGAAAAGAATCAACATCAGCGTTAACAAACGGCCCCGTTA
CGGCCCAAACGGTCATATAGAGTAACGGCGTTAAGCGTTGAAAGACTCCTATCGAAATACGTAACCGCAAACGTG
TCATAGTCAGATCCCCTCTTCCTTCACCGCCTCAAACACAAAAATAATCTTCTACAGCCTATATATACAACCCCCCCT
TCTATCTCTCCTTTCTCACAATTCATCATCTTTCTTTCTCTACCCCCAATTTTAAGAAATCCTCTCTTCTCCTCTTCATTT
TCAAGGTAAATCTCTCTCTCTCTCTCTCTCTCTGTTATTCCTTGTTTTAATTAGGTATGTATTATTGCTAGTTTGTTAAT
CTGCTTATCTTATGTATGCCTTATGTGAATATCTTTATCTTGTTCATCTCATCCGTTTAGAAGCTATAAATTTGTTGAT
TTGACTGTGTATCTACACGTGGTTATGTTTATATCTAATCAGATATGAATTTCTTCATATTGTTGCGTTTGTGTGTAC
CAATCCGAAATCGTTGATTTTTTTCATTTAATCGTGTAGCTAATTGTACGTATACATATGGATCTACGTATCAATTGT
TCATCTGTTTGTGTTTGTATGTATACAGATCTGAAAACATCACTTCTCTCATCTGATTGTGTTGTTACATACATAGAT
ATAGATCTGTTATATCATTTTTTTATTAATTGTGTATATATATATGTGCATAGATCTGGATTACATGATTGTGATTAT
TTACATGATTTTGTTATTTACGTATGTATATATGTAGATCTGGACTTTTTGGAGTTGTTGACTTGATTGTATTTGTGT
GTGTATATGTGTGTTCTGATCTTGATATGTTATGTATGTGCAGCCAAGGCTACGGGCGATCCACC

PAT

>ATGTCTCCGGAGAGGAGACCAGTTGAGATTAGGCCAGCTACAGCAGCTGATATGGCCGCGGTTTGTGATATCGT
TAACCATTACATTGAGACGTCTACAGTGAACTTTAGGACAGAGCCACAAACACCACAAGAGTGGATTGATGATCTA
GAGAGGTTGCAAGATAGATACCCTTGGTTGGTTGCTGAGGTTGAGGGTGTTGTGGCTGGTATTGCTTACGCTGGG
CCCTGGAAGGCTAGGAACGCTTACGATTGGACAGTTGAGAGTACTGTTTACGTGTCACATAGGCATCAAAGGTTG
GGCCTAGGTTCCACATTGTACACACATTTGCTTAAGTCTATGGAGGCGCAAGGTTTTAAGTCTGTGGTTGCTGTTAT
AGGCCTTCCAAACGATCCATCTGTTAGGTTGCATGAGGCTTTGGGATACACAGCCCGGGGTACATTGCGCGCAGC
TGGATACAAGCATGGTGGATGGCATGATGTTGGTTTTTGGCAAAGGGATTTTGAGTTGCCAGCTCCTCCAAGGCC
AGTTAGGCCAGTTACCCAGATCTGA

ubiT

>GTCGACCGAATGAGTTCCAAGATGGTTTGTGACGAAGTTAGTTGGTTGTTTTTATGGAACTTTGTTTAAGCTAGCT
TGTAATGTGGAAAGAACGTGTGGCTTTGTGGTTTTTAAATGTTGGTGAATAAAGATGTTTCCTTTGGATTAACTAG
TATTTTTCCTATTGGTTTCATGGTTTTAGCACACAACATTTTAAATATGCTGTTAGATGATATGCTGCCTGCTTTATTA
TTTACTTACCCCTCACCTTCAGTTTCAAAGTTGTTGCAATGACTCTGTGTAGTTTAAGATCGAGTGAAAGTAGATTTT
GTCTATATTTATTAGGGGTATTTGATATGCTAATGGTAAACATGGTTTATGACAGCGTACTTTTTTGGTTATGGTGT
TGACGTTTCCTTTTAAACATTATAGTAGCGTCCTTGGTCTGTGTTCATTGGTTGAACAAAGGCACACTCACTTGGAG
ATGCCGTCTCCACTGATATTTGAACAA

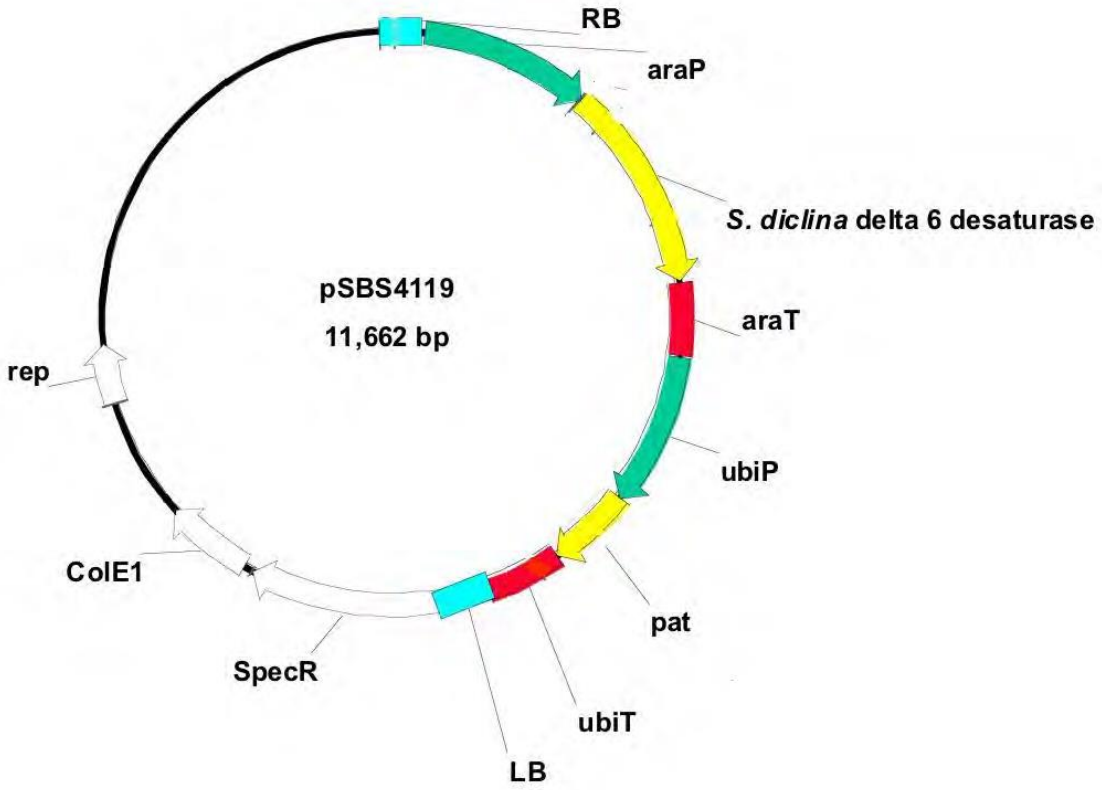
LB (SBS)

>CAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCTGC
CACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAAAATCACCACTCGATACAGGCAGCCCATCAGTCC
GGGACGGCGTCAGCGGGAGAGCCGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGGAAGAACG
GCAACTAAGCTGCCGGGTTTGAAACACGGATGATCTCGCGGAGGGTAGCATGTTGATTGTAACGATGACAGAGC
GTTGCTGCCTGT

Table 1 (revised). The genetically engineered (GE) safflower plant (GLA safflower) was generated by using an Agrobacterium-mediated transformation protocol to insert the following genetic construct elements in order to enable the plants to produce elevated levels of the fatty acid gamma-linolenic acid (GLA) in their seeds. **(On August 19, 2022, we revised the table content per APHIS request to add nucleotide position and sequences between elements, which are noted here as 'linker')**

Genetic element	Donor organism	Function	Nucleotide position	Sequence
Right Border (RB)	*Agrobacterium tumefaciens*	LB and RB involved in transfer and integration of intervening genetic elements of the construct	1 – 184	184 bp
araP	*Arabidopsis thaliana*	Promoter for oleosin	185 – 1293	1108 bp
linker	synthetic	linker	1294 – 1295	>CC
cDNA encoding delta-6-desaturase	*Saprolegnia diclina*	GLA production	1296 – 2658	1362 bp
linker	synthetic	linker	2659 – 2662	>GCTT
araT	*Arabidopsis thaliana*	Terminator for oleosin	2663 – 3143	480 bp
linker	synthetic	linker	3144 – 3149	>GAATTC
ubiP	*Petroselinum crispum*	Promoter for ubiquitin	3150 – 4148	998 bp
pat	*Streptomyces viridochromogenes*	Phosphinothricin acetyl transferase is a selectable marker used for the glufosinate-mediated selection of the genetically modified two-day-old cotyledons	4149 – 4701	552 bp
ubiT	*Petroselinum crispum*	Terminator for ubiquitin	4702 – 5192	490 bp
linker	synthetic	linker	5193 – 5198	>AGAATT
Leftt Border (LB)	*Agrobacterium tumefaciens*	LB and RB involved in transfer and integration of intervening genetic elements of the construct	5199 – 5510	311 bp

Figure 1.
Plasmid map of pSBS4119 used in Agrobacterium-mediated transformation of two-day-old cotyledons of safflower cv. Centennial



3. Detailed description of New Trait of the Modified Plant

Intended trait. The safflower plants have been modified with a gene which encodes a delta-6-desaturase enzyme which allows the conversion of linoleic acid to gamma linolenic acid (GLA), a fatty acid that otherwise is undetectable in the fatty acid profile of safflower. GLA is found naturally in oils obtained from other plants, including evening primrose oil, borage oil and black currant oil.

The safflower plants also have been modified to express the PAT enzyme which serves as a selectable marker in the transformed safflower plants, because it inactivates the glufosinate-ammonium herbicides used in transformation and regeneration.

The modified GLA-producing safflower plants do not encode a product intended for pharmaceutical or industrial use.

Intended phenotype. The modified safflower plants have an altered fatty acid profile in the oil produced in their seeds with the result that gamma-linolenic acid (GLA)-containing safflower oil (GLASO) is approximately 40% GLA. The mechanism-of-action (MOA) for producing GLA in the seeds of these plants is conferred by insertion of a delta-6-desaturase gene with under control of a seed-selective promoter and terminator sequences derived from the oleosin gene of *Arabidopsis thaliana*.

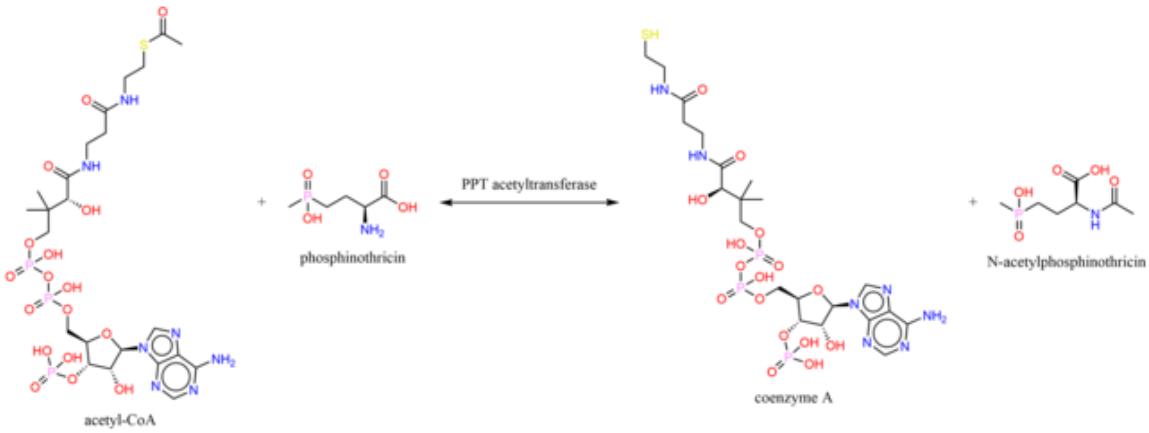
The GLASO produced by these GE safflower plants is intended for food use. These food uses have already been successfully reviewed under regulations and procedures implemented by the United States Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetics Act.

A more detailed description of the fatty acid profile of GLASO can be found in the documents in the FDA GRAS inventory online, in particular the FDA response letter and the documentation submitted to FDA as part of the GRAS notification (GRN-652).

- https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=652
- https://www.fda.gov/food/gras-notice-inventory/agency-response-letter-gras-notice-no-grn-000652
- https://wayback.archive-it.org/7993/20190208035755/https:/www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm510909.pdf

The GLA-producing plants also express phosphinothricin acetyltransferase (PAT) (EC 2.3.1.183), an enzyme, which serves as a selectable marker in transformation and regeneration, but is not used in the agronomic production conditions used for these GLA-expressing safflower plants. The MOA of PAT to confer tolerance to glufosinate-ammonium herbicides is well described in the literature, including an OECD consensus document (https://www.oecd.org/env/ehs/biotrack/46815628.pdf) frequently cited by APHIS in biotechnology assessments. PAT catalyzes the chemical reaction shown in Figure 2.

Figure 2: Inactivation of phosphinothricin via PAT



Key supplemental information to aid in the APHIS analysis for Regulatory Status Review:
In addition to supplying the information above, as stipulated in 340.4 for persons requesting a Regulatory Status Review under 7 CFR part 340, we provide below a summary of our consideration of the issues are described in 340.4 (1) (i-iii) that prescribe the factors upon which APHIS will conduct its initial review to determine whether there is a plausible pathway for the GE plant (or its sexually compatible relatives) to pose an increased plant pest risk. We offer a summary of our analysis as a supplement that we hope will facilitate the APHIS role in considering section 340.4 (1) (i-iii) of the regulation.

Prior to requesting this Regulatory Status Review under the provisions in 7 CFR part 340, we have done our own analysis of the regulation to reach the conclusion that these GLA-producing safflower plants are not subject to this regulation.

We have summarized below rationale relevant information used to support the conclusion that there is no plausible pathway by which the subject GE safflower plants or their sexually compatible relatives would pose an increased plant pest risk relative to the comparator non-GE safflower cv. Centennial, and are therefore not subject to the regulations found at 7 CFR part 340.

Section 340.4 (b)(2) of the APHIS regulation states the following in describing the basis for reaching a conclusion that a GE plant is not subject to this regulation:

> *"If APHIS does not identify a plausible pathway by which the GE plant or its sexually compatible relatives would pose an increased plant pest risk relative to the comparator(s) in the initial review, the GE plant is not subject to the regulations in this part."*

Our analysis relied on this regulation's definitions of "plant pest" and "plant pest risk", since these are central to the basis for APHIS reaching a conclusion that a GE plant is not subject to the regulation as part of its Regulatory Status Review. We have concluded that (1) GE and non-GE safflower cannot pose a "plant pest risk" as defined, and (2) GE and non-GE safflower plants do not meet the definition of "plant pest".

> Section 340.3 Definitions https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/biotech-rule-revision/secure-rule/secure-text/sr-text
>
> *"Plant pest risk. The potential for direct or indirect injury to, damage to, or disease in any plant or plant product resulting from introducing or disseminating a plant pest, or the potential for exacerbating the impact of a plant pest."*
>
> *"Plant pest. Any living stage of a protozoan, nonhuman animal, parasitic plant, bacterium, fungus, virus or viroid, infectious agent or other pathogen, or any article similar to or allied with any of the foregoing, that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product."*

1. The regulation found at 7 CFR part 340 defines "plant pest risk" as "the potential for direct or indirect injury to, damage to, or disease in any plant or plant product resulting from introducing or disseminating a plant pest, or the potential for exacerbating the impact of a plant pest." We have underlined the part of the definition that is the same as the definition of plant pest risk used in the Plant Protection Act of 2000 (PPA), which is the enabling statute for the APHIS regulation 7 CFR part 340. If an organism does not meet the definition of plant pest, introducing or disseminating that organism cannot pose a plant pest risk. The additional clause "or the potential for exacerbating the impact of a plant pest" added by APHIS when amending the regulation in 2020 is vague, and it appears to go beyond the letter and intent of the authority granted by Congress when it amended the PPA in 2000 to consolidate the prior statutes of the Federal Plant Pest Act, the Plant Quarantine Act, and the Noxious Weed Act. There is nothing in the legislative record of the PPA which supports the APHIS choice to add such language to the definition of "plant pest risk" in 7 CFR part 340. If APHIS wants to regulate non-parasitic plants (whether GE or non-GE), these can be regulated under the existing APHIS noxious weed regulations. APHIS has made

many communications to the public prior to and after publication in 2020 of the final amended rule for 7 CFR part 340 that this regulation would use the plant pest provisions of the PPA.

2. Safflower is not a plant pest as defined in Section 340.3, so introducing or disseminating safflower cannot pose a plant pest risk. Note that in the definition of "plant pests", only parasitic plants are included among the taxa of plant pests. Safflower is not a parasitic plant. APHIS publishes lists of plant genera that are parasitic plants, and safflower is not among these listed parasitic plants. Using genetic engineering techniques to add two genes to encode delta-6-desturase and PAT does not make the safflower into a parasitic plant, so the GE safflower does not meet the definition of "plant pest" found in the regulation found at 7 CFR part 340. Safflower, whether GE or non-GE, cannot meet this definition of "plant pest", because they are not parasitic plants.

As part of the procedure for a Regulatory Status review, Section 340.4 of the regulation states that APHIS will consider the following:

i) The biology of the comparator plant(s) and its sexually compatible relatives;
(ii) The trait and mechanism-of-action of the modification(s); and
(iii) The effect of the trait and mechanism-of-action on:
(A) The distribution, density, or development of the plant and its sexually compatible relatives;
(B) The production, creation, or enhancement of a plant pest or a reservoir for a plant pest;
(C) Harm to non-target organisms beneficial to agriculture; and
(D) The weedy impacts of the plant and its sexually compatible relatives.

The biology of the comparator plant(s) and its sexually compatible relatives.
The comparator plant for the subject GE safflower is safflower (*Carthamus tinctorus*, cv. Centennial). The OECD has recently published an extensive consensus document on the biology of safflower (https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)14&doclanguage=en).

We agree with the APHIS statement in its previous environmental assessment that "Safflower is not weedy and will not establish itself readily without human intervention." See https://www.aphis.usda.gov/brs/aphisdocs/06_36303r_ea.pdf and references therein.

Regarding sexually compatible relatives of safflower, the above cited APHIS document states the following:

"Outcrossing between safflower plants has been reported to be anywhere from 0 to 100% (Claassen, 1950); (Knowles, 1980) with an average between 15 and 20% (based on dominant flower-color markers). Wild relatives of cultivated safflower, *Carthamus creticus* and *C. oxyacanthus*, have been reported to occur sporadically in several U.S. states (Kartesz, 2004) and are listed as noxious weeds. *C. creticus* is not sexually compatible with cultivated safflower due to its chromosome number (2n=20 or 4n=44 compared to that of cultivated safflower with a chromosome number of n=12). Although, *C. oxyacanthus* has been reported in California (specifically in Monterey County), this sexually compatible species is rare and has not been detected in Washington (Kartesz, 2004); (Kiel and Turner, 1993). Noxious weeds are carefully monitored, quarantined, and subject to eradication efforts thereby minimizing the possibility

these species will establish. Because they are listed as noxious weeds, there are detailed records of their distribution."

APHIS Plant Protection and Quarantine's Noxious Weed Program has posted online the current Federal Noxious Weed List and the sole species of Carthamus on the list is wild safflower, *C. oxyacantha.* (https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf).

There are no records that support a conclusion that the offspring of cultivated safflower with wild safflower has increased the weediness of the offspring of such crosses. Modifying safflower varieties for seed oil profiles is not considered to provide a likely mechanism for increasing the weediness of safflower or sexually compatible relatives. It is also important to note that glufosinate-ammonium herbicides are not used in the cultivation of these GLA-producing safflower plants or any safflower.

The trait and mechanism-of-action of the modification(s)
The subject GLA-producing safflower plants have an altered seed oil profile, with approximately 40% GLA content. There is no indication in the scientific literature that altering the seed oil profile of safflower to contain GLA or expression of the PAT gene poses a plant pest risk any different from safflower plants that do not have this trait.

The effect of the trait and mechanism-of-action on:

(A) The distribution, density, or development of the plant and its sexually compatible relatives;
The subject GLA-producing safflower plants grow the same as its comparator safflower cv. Centennial, and there are no special cultural conditions for the GLA safflower that differ from the cultural practices for safflower. Glufosinate-ammonium herbicides are not used for the field grown GLA safflower plants. The GLA safflower plants are unlikely to interact with their sexually compatible relatives in a manner that differs from non-GE Centennial safflower plants. It is important to note that the sexually compatible relatives of safflower are not parasitic plants. According to the definition of "plant pest" in Section 340.3 the only plants that are considered plant pests are parasitic plants. Neither safflower nor its sexually compatible relatives meet this regulation's definition of "plant pest", so they cannot pose a "plant pest risk" as defined in this regulation.

(B) The production, creation, or enhancement of a plant pest or a reservoir for a plant pest;
The subject GLA-producing safflower plants have not been modified to differ in their interactions with plant pests or pathogens, so they are not likely to differ from non-GE Centennial safflower plants with regard to them serving as a reservoir for plant pests.

(C) Harm to non-target organisms beneficial to agriculture; and
The subject GLA-producing safflower plants do not have target or non-target organisms, a term typically used for plants modified to resist pathogens or pests of plants. The subject GLA-producing safflower plants are expected to interact with agriculturally beneficial organisms in a manner that is indistinguishable from their comparator, non-GE safflower cv. Centennial. The GLA safflower plants will produce an altered seed oil profile that is rich in GLA, but this is unlikely to pose harm to organisms beneficial to agriculture.

The GLA safflower plants will produce the PAT protein, but this protein is not toxic or harmful (see OECD consensus document cited above and numerous environmental assessments done by the APHIS biotechnology regulatory program over the past 30 years). Glufosinte-ammonium herbicides are not registered by the Environmental Protection Agency for use on the subject GLA safflower plants, and glufosinate-ammonium herbicides are not used in the field cultivation of these plants. The resistance to glufosinate-ammonium herbicides was only used as a selective marker trait in the transformation and regeneration laboratory stages of development for the GLA safflower plants.

Intensive breeding in the United States for safflower oil content and quality has been ongoing for a century, and in other parts of the world even longer. To date, there are no scientifically substantiated reports of safflower cultivation causing harm to non-target organisms beneficial to agriculture.

(D) The weedy impacts of the plant and its sexually compatible relatives.
We can find no reports of safflower affecting the weedy impacts of its sexually compatible relatives. Safflower has been modified for its oil seed content over many decades, and there are no reports of this affecting "weedy impacts" of safflower (noting that safflower is not considered a weed) or "weedy impacts" of the sexually compatible relatives of safflower.

The U.S. Environmental Protection Agency regulates the use of glufosinate-ammoniuim herbicides, and these are not registered for use on safflower (https://www3.epa.gov/pesticides/chem_search/ppls/088685-00003-20130226.pdf). Even if the *pat* gene were to be transferred from the subject GLA-producing safflower plants to a sexually compatible relative, the resulting offspring would pose no greater risk as weeds than those without the *pat* gene, because all of the other current weed control strategies for the sexually compatible relative would still be effective. Expression of the PAT enzyme only confers resistance to glufosinate-ammonium herbicides, and it does not protect the plant from other herbicides (e.g., glyphosate). Glufosinate-ammonium herbicides are not used typically to control wild safflower, because other registered herbicides are more cost effective.

We can find no reports of safflower affecting the weedy impacts of its sexually compatible relatives. Safflower has been modified for its oil seed content over many decades, and there are no reports of this affecting "weedy impacts" of safflower (noting that safflower is not considered a weed) or "weedy impacts" of the sexually compatible relatives of safflower.

Under the Plant Protection Act of 2000, problematic non-parasitic plants are regulated under the Noxious Weed Provisions, and APHIS has separate Noxious Weed Regulations for regulating plants that meet the statutory definition of "noxious weed". APHIS Plant Protection and Quarantine administers the APHIS Noxious Weed Program and implements the Noxious Weed Regulations (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/sa_weeds/sa_noxious_weeds_program/noxious-weeds-regs).

Summary of the Requestor's Analysis

Based on our careful review of the APHIS regulation found at 7 CFR part 340 and its enabling statute, the Plant Protection Act of 2000, we have concluded that these GE GLA-producing safflower plants should not be considered to be subject to the regulations in this part, because there is no scientifically plausible pathway by which these GLA-producing safflower plants or their sexually compatible relatives would pose an increased plant pest risk relative to the comparator(s).