



United States Department of Agriculture

**USDA Review of High Oleic/Low
Linolenic Soybean Developed using
TALEN[®] Technology
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USDA History of Soybean with High Oleic and Low Linoleic Acid

- In 1997, APHIS deregulated the first modified high oleic acid soybean.
 - DuPont's soybean sublines G94-1, G94-19 and G-168 (USDA-APHIS, 1997).
 - This event involved a soybean with an inserted sense orientation soybean microsomal omega-6 fatty acid desaturase gene (GmFad2-1 gene).
- In 2010, APHIS deregulated Pioneer's soybean with high (oleic) fatty acid, low polyunsaturated fatty acids (linoleic and linolenic) and lower palmitic acid.
 - Pioneer event DP-3Ø5423-1
 - Inserted the GmFad2-1 gene

Retired Am I Regulated (AIR) Process

- The AIR process was used to determine whether-or-not an organism was a Regulated Article.
- Did not involve risk assessments.
- Regulated Article: Any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in §340.2 (legacy regulation) and meets the definition of plant pest.

Calyxt Soybean Reviewed Under AIR

- In March 2020, Calyxt submitted an AIR.
- This soybean contains a five-gene knockout (KO) achieved through expression of a specially designed TALEN[®] reagent.
- The soybean product is defined as a null-segregant of soybean lines developed by the expression of the construct.
- Rational for exemption:
 - Soybean is not itself a plant pest.
 - No plant pest sequences are contained in this soybean.

BRS Assessment of High Oleic Soybean via AIR

- Applicant described that a selectable marker was used to identify the soybean line with the TALEN[®] expression cassette and the selectable marker.
- Expression of the TALEN[®] reagent resulted in the targeted knockout of five genes in soybean.
- Polymerase chain reaction (PCR) was used to screen regenerated soybean plants to identify the specific transgenic plants that contained the targeted site disruption in the targeted genes.

BRS Evaluation of High Oleic Soybean via AIR

- Self-pollination of the soybean line resulted in the high oleic/low linolenic KO soybean product.
 - in which inserted DNA was removed
 - only the targeted disruption of the genes remained
- While the TALEN[®] reagent and certain other genetic sequences used in the development of high oleic/low linolenic soybean were derived from plant pests, none of the inserted DNA remained in the final soybean product.
- The soybean line was subjected to whole genome sequencing which confirmed that the final soybean product contains only the genetic material present in the original soybean plant and deletions in the five targeted genes.

USDA/BRS AIR Response

Based on the representations the applicant submitted, including the description of the results of their confirmation methods,

- 1) The genome edited high oleic/low linolenic soybean is not a plant pest.
- 2) The plant contains only deletions produced as a result of the plant's DNA repair mechanism.
- 3) USDA did not consider this soybean to be regulated pursuant to 7 CFR part 340.
 - Consistent with previous responses to similar letters of inquiry.



Revised Biotechnology Regulations

- How would USDA regulate the high oleic/low linoleic soybean under the revised 7 CFR part 340 Regulations?

Confirmation of Exemptions

- A plant is exempt from regulation if it contains a single targeted genetic modification that could be achieved through conventional breeding.
- Such a plant is not expected to pose any greater plant pest risk than a plant developed through conventional breeding.
- Developers can seek confirmation that a product is exempt from the revised 7 CFR part 340 regulations.
- Confirmation Request (CR) is a new process in the revised regulations.



Would this Soybean Qualify Under CR?

The specific soybean line reviewed under the AIR remains exempt.

The following discussion applies to a new soybean line that would contain the same five-gene knock-out.

“§ 340.1(b) The regulations in this part do not apply to plants that have been modified such that they contain either *a single modification* of a type listed in paragraphs (b)(1) through (3), or additional modifications as determined by the administrator (b4)”.

Would this Soybean Qualify Under CR?

Exemption B1 – Cellular repair without template

- § 340.1(b)(1) – The genetic modification is a change resulting from cellular repair of a targeted DNA break in the absence of an externally provided repair template.
 - TALEN reagents are nucleases that are used to cut DNA in specific locations. The plant then repairs the breaks.
 - This is a five (5) gene knock-out and not a single modification therefore it would not meet this exemption.

Would this Soybean Qualify Under CR?

Exemption B2 – Substitution

- § 340.1(b)(2) – The genetic modification is a targeted single base pair substitution.
 - This soybean does not meet this requirement.

Would it Qualify Under CR?

Exemption B3 – Insertion from the gene pool or generating modifications mimicking the ones that already exist in the gene pool

- § 340.1(b)(3) – The genetic modification introduces a gene known to occur in the plant's gene pool or makes changes in a targeted sequence to correspond to a known allele of such a gene or to a known structural variation present in the gene pool.
 - The five (5) gene knock-out would not qualify under this exemption.



How could this soybean be submitted under the revised regulations?

Since this soybean does not qualify for exemption, a Regulatory Status Review (RSR) could be submitted.

How would this soybean be evaluated under the RSR process?

RSR evaluates plant pest risk based on

- The biological properties of the plant;
- The trait (or new characteristic); and
- The mechanism of action (how the genetic modification causes the new trait to occur).

Regulatory Status Review (RSR)

- APHIS evaluates whether the modified plant requires oversight based on the characteristics of the plant itself rather than on the use of a plant pest in its development.
- If a plant developed using genetic engineering is found to be unlikely to pose a plant pest risk, APHIS will not require regulation under 7 CFR part 340.
- If APHIS is unable to reach such a finding, it will regulate the plant and it would be allowed to move only under permit.
- Once APHIS determines that a modified plant is not regulated, subsequent modified plants using the same plant-trait-mechanism of action combination would not be regulated.

Important Aspects of the RSR

- The RSR involves an **Initial Review** to determine if there is **any plausible pathway** to increased plant pest risk.
 - If a plausible pathway is not identified the plant would not be regulated.
 - If a plausible pathway is identified, then a PPRA is conducted to evaluate likelihood and consequence.
- **Feedback to applicants** regarding any identified plausible pathways. The data package is narrowed to only areas of concern.
- **Considers sexually compatible relatives** by putting SCR's of concern that are present in the U.S. through the analysis.





Questions?

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