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Summary:	This document provides guidance on preparing requests for confirmation of exemption from regulation under 7 CFR part 340. APHIS protects and enhances U.S. agricultural and natural resources using a science-based and risk-based regulatory framework to ensure the safe movement – including importation, interstate movement, and confined environmental release – of organisms developed using genetic engineering. APHIS receives its regulatory authority from the Plant Protection Act of 2000, and oversees organisms developed using genetic engineering in accordance with its regulations under 7 CFR part 340 (*Movement of Organisms Modified or Produced Through Genetic Engineering*).
Disclaimer:	The contents of this guidance document do not have the force and effect of law and are not meant to bind the public in any way. This document is intended only to provide clarity to the public regarding existing requirements under the law or agency regulations.

USDA-APHIS Biotechnology Regulatory Services

Guidance for Requesting a Confirmation of Exemption from Regulation under 7 CFR part 340

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Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
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The information contained in this document is intended solely as guidance. Except where noted, persons may choose to follow APHIS guidance or follow different procedures, practices, or protocols that meet applicable statutes and regulations.

Language implying that guidance is mandatory (e.g., "shall," "must," "required," or "requirement") should not be construed as binding unless the terms are used to refer to a statutory or regulatory requirement.

Following the guidance contained in this document should not be construed as a guarantee of compliance with applicable statutes and regulations.

Guidance for Requesting a Confirmation of Exemption

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Introduction to the Confirmation of Exemption Process

APHIS regulations at 7 CFR part 340 govern the movement of certain organisms that are modified or produced through genetic engineering. The regulations specify certain plants that are exempt from the regulations. A person may request confirmation from APHIS that a plant is exempt from 7 CFR part 340, based on the provisions in § 340.1 of the regulations. Confirmation that a plant is exempt from the regulations is not mandatory. APHIS will provide a written response within 120 days of receiving a sufficiently detailed confirmation request, except in circumstances that could not reasonably have been anticipated. Upon completion, APHIS will post confirmation requests and responses on the APHIS-BRS website, typically within 1 – 2 business days of providing the response to the requestor, with any information claimed as Confidential Business Information (CBI) or personal identifying information redacted, as appropriate. APHIS is providing the following guidance to help with preparing a request for confirmation of exemption from regulations under 7 CFR part 340. We recommend discussing your request for an exemption with APHIS prior to your first submission.

Important Definitions

Gene pool: Germplasm within which sexual recombination is possible as a result of hybridization, including via methods such as embryo culture or bridging crosses (§ 340.3).

Mechanism of Action (MOA): The biochemical process(es) through which genetic material determines a trait (§ 340.3).

Trait: An observable (able to be seen or otherwise identified) characteristic of an organism (§ 340.3).[1]

Exemptions

There are four exemptions defined in § 340.1. The first three exemptions cover modified plants that could otherwise have been developed through conventional breeding techniques (§ 340.1(b)(1-3)). These exemptions cover plants modified to contain a single targeted genetic modification of one of the three types listed below:

(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 (§ 340.1(b)(1));[2] or
(2) The genetic modification is a targeted single base pair substitution (§ 340.1(b)(2));[3] or
(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 (§ 340.1(b)(3)).[4]

The fourth exemption (§ 340.1(c)) covers plants with: 1) a plant-trait-MOA combination that is the same as that in another plant of the same species previously reviewed by APHIS in accordance with § 340.4 and determined by APHIS not to be regulated under 7 CFR part 340, or 2) a plant-trait-MOA combination that is the same as that in a plant of the same species APHIS determined to be nonregulated in response to a petition submitted prior to October 1, 2021, pursuant to § 340.6 of the previous regulations found at 7 CFR part 340.

When determining whether a MOA is the same as that in another plant previously found by APHIS not to be regulated, it is important to recognize both that the same trait may be conferred by multiple distinct MOAs, and that the same MOA can be conferred by distinct genes. For example, one MOA for resistance in plants to the herbicide glyphosate relies on inactivation of glyphosate by the protein glyphosate acetyl transferase (GAT), while a second MOA for resistance relies on an inability of glyphosate molecules to bind and inactivate an enzyme called EPSPS, which is responsible for an essential step in a biochemical pathway for the synthesis of certain amino acids. GAT and EPSPS catalyze different biochemical reactions and are distinct MOAs. Therefore, a glyphosate resistant plant that uses a *gat* gene would not provide a basis for exempting a glyphosate resistant plant of the same species that uses an *epsps* gene. However, EPSPS-mediated glyphosate resistance has been developed using *epsps* genes from both corn (*mepsps*) and a strain of *Agrobacterium* (*CP4 epsps*). In both cases the added gene encodes an EPSPS protein that does not bind to glyphosate. Both proteins catalyze the same biochemical reaction and the MOAs are

[1]The trait is sometimes also referred to as the phenotype or the phenotypic trait. Because a trait's manifestation is the result of an underlying genotype and its interaction with the environment, a genetic locus controlling a trait within a species can have two or more alleles producing different observable characteristics termed as phenotypes. For example, flower color is a trait, and red and white flower colors are two different phenotypes for the flower color trait.

[2] Examples of such changes are insertions, deletions, changes that result in both insertion and deletion during break repair, and non-templated base pair substitutions.

[3]The single base pair substitution results from a templated repair.

[4] Such changes generally involve the use of an externally provided template for repair and may involve the insertion and/or replacement of genetic material.

equivalent even though the two genes share only a low level of sequence similarity. Therefore, a glyphosate resistant plant that uses an *epsps* gene encoding a glyphosate-insensitive EPSPS protein from one source would provide a basis for exempting a glyphosate resistant plant that uses an *epsps* gene encoding a glyphosate-insensitive EPSPS protein from another source. In general, when evaluating whether two plant-trait-MOA combinations are the same, APHIS considers, in addition to the plant and the trait, whether the introduced genetic sequences result in the same biochemical process. When a specific biochemical reaction is catalyzed by different enzymes, the MOA will be considered the same as long as the different enzymes do not catalyze any additional biochemical reactions that differ between them.

As another example, a coleopteran resistance trait can be conferred to a modified plant by expression of a Cry protein or by expression of a silencing complex targeting ribonucleic acids (RNA) in the coleopteran pest. These are different MOAs and a plant modified to confer one of these MOAs would not be exempt from regulation solely because APHIS had previously determined that a plant of the same species modified to confer the other MOA was not regulated. In addition, different Cry proteins may act in different ways or have different molecular specificities, and thus they may have different MOAs.

There may be instances where variation in gene expression could lead to differences in plant pest risk, and, thus, instances where APHIS determinations may be conditioned on where, when, or to what level the gene is expressed. In those cases, APHIS will specify[5] whether and in what way variation in expression is considered as part of the MOA for purposes of applying the exemption in § 340.1(c). Similarly, there may be rare instances where plant pest risk could differ among subspecies of plant varieties modified with the same trait-MOA combination. In those instances, APHIS will specify when the subspecies or variety is considered as part of the plant-trait-MOA for purposes of applying the exemption in § 340.1(c). Otherwise, once APHIS has determined that a plant-trait-MOA is not regulated, that determination will apply to all subspecies and varieties of the plant.

As indicated above, the exemptions in § 340.1(b)(1-3) apply to a single targeted modification, while the exemption in § 340.1(c) applies to a single plant-trait-MOA combination. In general, multiple modifications made simultaneously in the same plant will not qualify for an exemption under § 340.1. However, if single modifications that are exempt from regulation are subsequently combined through conventional breeding, the resulting offspring will not be subject to regulation under § 340.1. In addition, if a plant was modified to contain multiple plant-trait-MOA combinations and the same set of plant-trait-MOA combinations was previously determined to be not regulated, the plant would not be regulated under
§ 340.1.

The exemption in § 340.1(b)(1) applies to plants in which repair of a targeted DNA break results in different molecular alterations on two homologous chromosomes, as long as each alteration results in the complete loss of gene function. In cases where there are functional differences between the two corresponding alleles, the exemption does not apply. Similarly, any exemption confirmed in one variety will be applicable to other varieties of the same crop, provided that the modification is the same in the subsequent varieties, or that it is in the same gene and results in the same functional difference from the unmodified plant.

[5]APHIS is developing an online plant-trait-MOA table to list combinations that have been found nonregulated. This table will include the specifications discussed above. APHIS anticipates posting this table on its website in early August 2020, and will update this guidance to include a link to the table.

The exemptions in § 340.1(b)(1-3) apply to modifications made to any two homologous chromosomes regardless of whether the plant is diploid or polyploid. If a developer wants to make corresponding changes to additional homologous chromosomes or to homoeologous chromosomes, they can submit the plant for a regulatory status review; if APHIS determines that the plant is unlikely to pose an increased plant pest risk, the plant-trait-MOA combination will be added to the list of plant-trait-MOAs that are eligible for exemption under § 340.1(c).

Request for Confirmation of Exemption

If you are seeking confirmation that your plant is exempt from the regulations in 7 CFR part 340, you must electronically submit (ConfirmationRequests@usda.gov) your confirmation request as a letter containing the information described below to:

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services

Your letter requesting confirmation of exemption from regulations under 7 CFR part 340 must include the following information:

- Requestor's name and contact information, including email address.
- A description of the plant's genus, species, and, if relevant, subspecies or ecotypes.
- A clear statement of which regulatory exemption the requestor is claiming for the plant and why the plant qualifies for that exemption. Specifically include reference to either § 340.1(b)(1), § 340.1(b)(2), § 340.1(b)(3), or § 340.1(c).
- A description of the trait (it is helpful to also include a description of the intended or actual phenotype(s) of the plant).
- A description of the intended and/or actual genetic modification in the plant sufficient to enable APHIS to confirm the plant is eligible for the exemption, including:
    - For exemptions under §§ 340.1(b)(1-2), the type of genetic modification (e.g., insertion, deletion, single base pair substitution, as applicable), the targeted gene or genetic element, and the method used to produce the modification.
    - For exemption under § 340.1(b)(3), the type of genetic modification, the gene or genetic or structural element, the donor organism or the organism on which the modification is based and evidence that the modification exists in the gene pool, and the method used to produce the modification.
    - For exemptions under § 340.1(c), the trait(s) and associated MOA(s), including a molecular description of the inserted genetic material and method used to produce the modification.[6]

[6]The molecular description could be a list or table identifying the genetic elements introduced into the plant sufficient for APHIS to be able to confirm that the plant-trait-MOA combination is the same as a combination previously determined to be not regulated. The genetic elements need not be identical to those used in the previous combination (e.g., a different promoter or a different gene could be used), as long as the same MOA is conferred.

- Details about the scientific methodology used, or intended to be used, to verify the plant qualifies for the specified exemption, with sufficient information to enable APHIS to assess the efficacy of the methodology.

Your letter requesting confirmation of exemption from regulations under 7 CFR part 340 may also include the following additional information and data you deem necessary to substantiate your request:

- The function of the modified gene or genetic element
- Molecular characterization data (e.g., Southern blots)
- DNA sequence data
    - Sequence data should encompass the modification
    - Any sequencing strategy and methodology should be clearly presented

APHIS' reply to your request will vary slightly depending on whether the request is for a plant that has already been developed or is for a plant that has yet to be developed. APHIS' reply will also vary depending on the extent of supporting information and data you submit.

Off-Target Mutations

Off-target mutations are mutations that occur at locations in the genome other than the intended target site. APHIS will not review off-target mutations that occur during development of an exempt plant because (1) the rate of off-target mutation is low relative to the background mutation rate that occurs in conventional breeding without raising unique plant pest risk concerns, and (2) due to the nature of plant breeding, where populations are created and evaluated and individuals are selected and advanced for further breeding, deleterious off-target mutations are likely to be lost unless they are closely linked genetically to the targeted modification. Instead, APHIS' review will focus on the targeted modification.

APHIS does not consider the unintended retention of exogenous DNA that was inserted as part of the modification process to be an off-target mutation (e.g., DNA encoding genome modification machinery such as the Cas9 protein). To qualify for exemption under §§ 340.1(b)(1-2) there must not be any retention of DNA that was deliberately inserted as part of the modification process including vector sequences. For exemptions §340.1(b)(3) and § 340.1(c), APHIS will carefully review the information and any data provided regarding exogenous DNA retained in the plant to determine if the plant qualifies for the exemption.

Confidential Business Information

If your confirmation request, as well as any follow-up documentation you provide, does not contain Confidential Business Information (CBI), it must be marked "**No CBI.**"

If your confirmation request, as well as any documentation you provide, contains CBI, you must submit a CBI copy, a CBI-deleted copy, and a CBI justification.

CBI Justification

If the confirmation request contains CBI, the CBI claims must be justified in terms related to competitive harm due to its release. Information is not protected from disclosure simply because the requestor does not want the information to be made public. The requestor must include a statement justifying all claims of CBI. The statement must be detailed enough to demonstrate that each piece of information claimed as CBI meets the definitions of trade secret or commercial or financial information. Each piece of information claimed as CBI must be justified in the statement. For examples of the type of information that can be claimed as CBI and the definitions of commercial or financial harm, see: https://www.aphis.usda.gov/brs/pdf/CBI_Submission_Guidance.pdf.

APHIS will review each claim of CBI, and will discuss with the requestor any claims that do not meet the criteria for CBI.

Preparation of a Confirmation Request with CBI

If the confirmation request contains CBI, you must submit two copies of the document:

- Each page of a document containing CBI must have "CBI Copy" marked in the upper right corner of the page.
- Each page of a CBI-deleted document (i.e., the CBI text is removed) must have "CBI-deleted Copy" marked in the upper right corner.
- In a document containing CBI, mark with square brackets ("[ ]") only the specific words or phrases claimed as CBI, and in the right margin for each set of brackets write "CBI."
- In the CBI-deleted copy, replace with blank spaces the words or phrases marked in the CBI copy, mark the spaces with square brackets, and in the right margin for each set of brackets write "CBI-deleted."
- The CBI-deleted copy should be identical to the CBI copy, except 1) blank spaces surrounded by square brackets occurring in the text where the CBI text has been redacted and 2) "CBI-deleted Copy" should appear in the upper right corner of each page instead of "CBI Copy."
- The CBI-deleted copy must be paginated identically to the CBI copy. The CBI-deleted copy should be made directly from the same document which originally contained CBI.
- Do not insert additional text (transitions, paraphrasing, or generic substitutions, etc.) into the spaces of the CBI-deleted copy.
- All published references that appear in the CBI copy should be included in the reference list of the CBI-deleted copy.

Preparation of a Confirmation Request without CBI

If the confirmation request does not contain CBI, only submit one copy. This document should be clearly marked "No CBI" in the upper right corner of the page.

For additional questions about CBI and CBI formatting, please contact the BRS Document Control Officer:

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