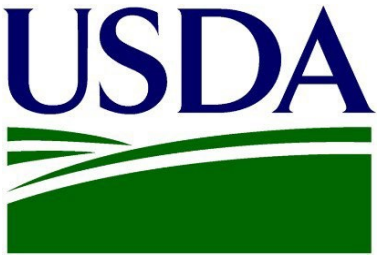
United States Department of Agriculture

Issuing Agency/Office:	Animal and Plant Health Inspection Service / Biotechnology Regulatory Services
Title of Document:	Guide for Requesting a Confirmation of Exemption from Regulations under 7 CFR part 340
Document ID:	BRS-GD-2020-0001
Date of Issuance:	August 31, 2022
Replaces:	Replaces BRS-GD-2020-0001 issued June 18, 2020
Summary:	This document provides recommendations on preparing requests for confirmation of exemption from regulations 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 guide 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

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

v. 08/31/2022

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 a guide. Except where noted, persons may choose to follow APHIS recommendations or follow different procedures, practices, or protocols that meet applicable statutes and regulations.

Language implying that mandatory action (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 recommendations contained in this document should not be construed as a guarantee of compliance with applicable statutes and regulations.

Guide 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 organisms that are modified or produced through genetic engineering that could pose a risk to plant health. The regulations specify certain plants that are exempt from the regulations. A person may, but is not required to, request confirmation from APHIS that a plant is exempt from 7 CFR part 340, based on the provisions in § 340.1 of the regulations. 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 guide 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]

[1] Phenotype. A set of observable characteristics of an organism resulting from the interaction of its genotype with the environment. A genetic locus controlling a trait within a species can have two or more alleles producing different observable characteristics producing different phenotypes. For example, flower color is a trait, and red and white flower colors are two different phenotypes for the flower color trait. When referring to an organism's observable characteristics, trait and phenotype are sometimes used interchangeably, but because a phenotype is more specific, it is often more helpful.

Exemptions

There are two categories of exemptions defined in § 340.1: § 340.1(b) and (c). The first category (b) covers modified plants that could otherwise have been developed through conventional breeding techniques (§ 340.1(b)(1-3)). Specifically, this category covers 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 second exemption category (§ 340.1(c)) covers plants with: 1) a plant-trait-MOA combination (PTMOA) that is the same as that in another plant of the same species APHIS previously reviewed in response to a Regulatory Status Review (RSR) submission in accordance with § 340.4 and determined not to be regulated under 7 CFR part 340, or 2) a PTMOA combination that is the same as that in a plant of the same species APHIS determined to be not regulated 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.

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 PTMOA combination. In general, multiple modifications made simultaneously in the same plant developed using genetic engineering 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 some or all PTMOA combinations in a plant have previously been combined in a single plant that was determined to be not regulated, the plant would not be regulated under § 340.1.

Exemptions for Plants with Modifications Achievable by Conventional Breeding

The exemption in § 340.1(b)(1) applies to plants in which repair of a targeted DNA break results in the same or different molecular alterations on one pair of homologous chromosomes, as long as each alteration results in the same functional change. In cases where there are functional differences between the two corresponding alleles, the exemption does not apply. Additionally, a sequence that is highly similar to the intended target sequence, such as sequences that may be found in multigene families, cannot be modified along with the intended target sequence (for more information, please see the section entitled "Unintended Modifications verses Off-Target Modifications" below). For modified plants that qualify for exemption, any exemption confirmed in one variety will be applicable to other varieties of the same crop, provided the modification is the same in the subsequent varieties, or it is in the same gene

[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. Transgenes from previously reviewed plant-trait-MOA combinations that have been determined not to be regulated are not considered part of the plant's gene pool.

and results in the same functional difference from the unmodified plant.

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 an RSR; 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 PTMOAs that are eligible for exemption under § 340.1(c).

Exemption for Previously Reviewed PTMOA

As indicated above, the exemptions in § 340.1(c) apply to a single PTMOA combination (or set of combinations) previously reviewed and found by APHIS not to be regulated. When determining whether an 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 5-enolpyruvylshikimate 3-phosphate synthase (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 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 PTMOA 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.

A separate consideration when determining whether an MOA is the same as that in another plant previously found by APHIS not to be regulated is the concentration of the gene product across time and tissues. Previous plant pest risk analyses may have evaluated MOA considering certain tissue types and concentration levels. Please refer to the PTMOA table to determine whether and in what way variation in expression was 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 PTMOA combination. In those instances, APHIS will specify when the

subspecies or variety is considered as part of the PTMOA for purposes of applying the exemption in § 340.1(c). Otherwise, once APHIS has determined that a PTMOA is not regulated, that determination will apply to all subspecies and varieties of the plant.

Unintended Modifications versus Off-Target Modifications

APHIS considers the unintended retention of exogenous DNA inserted as part of the modification process to be an unintended modification (e.g., DNA encoding genome modification machinery such as the Cas9 protein). Additionally, APHIS considers modifications to DNA sequences that are highly similar to the target sequence as unintended modifications (e.g., sequences found in multigene families that have the same or highly similar sequences as the intended target, pseudogenes, or other conserved sequences), as those sequences would likely be modified at frequencies exceeding low-similarity promiscuous binding. 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, and scientific methodology should include the design or verification steps taken to anticipate, reduce, and monitor unintended modifications of highly similar 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.

Off-target modifications occur at locations in the genome other than the intended target site and are indistinguishable from background mutations. APHIS will not review off-target mutations that occur during development of an exempt plant because (1) the rate of off-target modification 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 modifications are likely to be lost unless they are closely linked genetically to the targeted modification. Instead, APHIS' review will focus on the targeted modification.

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

Letters requesting confirmation of exemption from regulations under 7 CFR part 340 are limited to a single trait in a single species. Your letter must include the following information:

- Requestor's name and contact information, including email address.
- The plant's common name, 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.[5]
- 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 optional 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.

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, as detailed in the Guide for Submitting Confidential Business Information and in accordance with 7 CFR 340.7.

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

Ms. Cynthia A. Eck
301-851-3892
cynthia.a.eck@usda.gov

[5] 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 -PTMOA combination is the same as, or a subset of, 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.