ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 725

[EPA–HQ–OPPT–2011–0740; FRL–9991–60]

RIN 2070–AJ65

Microorganisms; General Exemptions From Reporting Requirements; Revisions to Recipient Organisms Eligible for Tier I and Tier II Exemptions

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is issuing a final rule to add *Trichoderma reesei* (*T. reesei*) strain QM6a and its derivatives and *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) subspecies (subsp.) *amyloliquefaciens* to the list of recipient microorganisms that may be used to qualify for the Tier I and Tier II exemptions from full notification and reporting procedures under the Toxic Substances Control Act (TSCA) for new microorganisms that are being manufactured for introduction into commerce. EPA received petitions to add *T. reesei* and *B. amyloliquefaciens* to the list of microorganisms eligible for the exemption from full notification and reporting procedures under the TSCA for new microorganisms. Based on EPA's evaluation of these petitions, EPA has made the determination that certain strains of both microorganisms will not present an unreasonable risk of injury to health or the environment when used as a recipient microorganism provided that certain criteria for the introduced genetic material and the physical containment conditions are met.

DATES: This final rule is effective April 9, 2020.

ADDRESSES: The docket for this action, identified by docket identification (ID) number EPA–HQ–OPPT–2011–0740, is available at *http://www.regulations.gov* or at the Office of Pollution Prevention and Toxics Docket (OPPT Docket), Environmental Protection Agency Docket Center (EPA/DC), West William Jefferson Clinton Bldg., Rm. 3334, 1301 Constitution Ave. NW, Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the OPPT Docket is (202) 566–0280. Please review the visitor instructions and additional information about the docket available at *http://www.epa.gov/dockets.*

FOR FURTHER INFORMATION CONTACT:

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SUPPLEMENTARY INFORMATION:

I. Executive Summary

A. Does this action apply to me?

You may be potentially affected by this action if you produce, import, process, or use either intergeneric *T. reesei* or intergeneric *B. amyloliquefaciens* or any other eligible recipient microorganisms listed in 40 CFR 725.420. The following list of North American Industrial Classification System (NAICS) codes is not intended to be exhaustive, but rather provides a guide to help readers determine whether this document applies to them. Potentially affected entities may include:

- Basic Chemical Manufacturing (NAICS code 3251).
- Pesticide, Fertilizer and other Agricultural Chemical manufacturing (NAICS code 3253).
- Other Chemical Product and Preparation Manufacturing (NAICS code 3259).

If you have any questions regarding the applicability of this action to a particular entity, consult the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

B. What is the Agency's authority for taking this action?

This action is being taken under the authority of TSCA section 5(h)(4) (15 U.S.C. 2604(h)(4)). TSCA section 5(a)(1) requires that persons notify EPA at least 90 days before they manufacture (the term ''manufacture'' includes import under TSCA) for commercial purposes a ''new'' chemical substance, or manufacture (including import) or process a chemical substance for a ''significant new use'' (15 U.S.C. 2604(a)(1)(B)(i)). TSCA furthermore prohibits such manufacturing or processing from commencing until EPA has conducted a review of the notice, made an appropriate determination on the notice, and taken such actions as are required in association with that determination (15 U.S.C. 2604(a)(1)(B)(ii)). TSCA defines ''chemical substance'' broadly and in terms that cover intergeneric microorganisms as well as traditional chemical substances. Therefore, for the purposes of TSCA, a ''new microorganism'' is one that is not listed on the TSCA Chemical Substances Inventory (TSCA Inventory) compiled under TSCA section 8(b).

TSCA section 5(h)(4) authorizes EPA, upon application and by rule, to exempt the manufacturer of any new chemical substance from part or all of the provisions of TSCA section 5, if EPA determines that the manufacture, processing, distribution in commerce, use, or disposal of the new chemical substance will not present an unreasonable risk of injury to human health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified by the Administrator under the conditions of use.

C. What action is the Agency taking?

In 2012, EPA proposed to add *T. reesei* strain QM6a and its derivatives (hereafter, *T. reesei* QM6a) and *B. amyloliquefaciens* subspecies (subsp.) *amyloliquefaciens* to the list of recipient microorganisms in 40 CFR 725.420 that may be used to qualify for Tier I and Tier II exemptions from full notification and reporting procedures under TSCA for new microorganisms that are being manufactured into commerce. EPA is finalizing the proposal.

D. Why is the Agency taking this action?

EPA received petitions to add *T. reesei* and *B. amyloliquefaciens* to the list of microorganisms that may be used as recipient microorganisms in order to qualify for the exemption from full notification and reporting procedures under TSCA for new microorganisms that are being manufactured for introduction into commerce. EPA proposed to add certain strains of these two microorganisms to the list of recipient microorganisms based on EPA's preliminary determination. EPA is now issuing a final rule that incorporates certain changes in response to public comment.

E. What are the estimated incremental impacts of this final rule?

EPA has evaluated the potential costs of the addition of the two microorganisms to the list of recipient microorganisms eligible for Tier I and Tier II exemptions. The final rule is

expected to generate cost savings for organizations that, in the absence of the rule, would submit Microbial Commercial Activity Notices (MCANs) for new intergeneric *T. reesei* or *B. amyloliquefaciens* strains. The rule will result in costs savings for both the industry and the Agency. EPA estimates the annualized industry savings of the rule to be approximately $260,000 per year over a ten-year period, with a 3 percent discount rate, and $252,000 per year with a 7 percent discount rate. Annualized agency savings are approximately $178,000 per year with a 3 percent discount rate and $173,000 per year with a 7 percent discount rate over the ten-year period, for a total annualized savings to society of approximately $438,000 per year with a 3 percent discount rate and $424,000 per year with a 7 percent discount rate. The economic analysis is available in the docket and is summarized in Unit IX. of this final rule. Costs and benefits of adding *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* to 40 CFR 725.420 are also discussed in Unit VIII.C.2. through 4.

II. Background

EPA received petitions to add *T. reesei* and *B. amyloliquefaciens* to the list of recipient microorganisms at 40 CFR 725.420 that are eligible for the regulatory exemptions applicable to new microorganisms that are manufactured for introduction into commerce (Refs. 1–3). In the **Federal Register** of September 5, 2012 (77 FR 54499) (FRL–9348–1) (''2012 Proposed Rule'') (Ref. 4), the Agency proposed to add certain strains of these two microorganisms to the list of recipient microorganisms at 40 CFR 725.420 based on EPA's preliminary determination that both of the microorganisms, with certain limitations, meet the criteria for addition to the list—*i.e.,* they will not present an unreasonable risk of injury to health or the environment provided that the other conditions of the exemptions at 40 CFR part 725, subpart G, relating to the introduced genetic material, and the physical containment of the new microorganisms, have been met. EPA is now issuing a final rule that incorporates certain changes made in response to public comments received on the 2012 Proposed Rule. These changes are described in the following paragraphs.

In the 2012 Proposed Rule, EPA proposed to restrict the exemption for *T. reesei* to the *T. reesei* strain QM6a and its derivatives. In addition, EPA proposed to restrict the *T. reesei* QM6a exemption to use of the microorganism only under submerged standard industrial fermentation operations used for enzyme production; as described in this proposed rule, these conditions are typical throughout the fermentation industry and meet the existing physical containment and control requirements for the tiered exemptions under 40 CFR 725.422. Any subsequent deliberate fermentation of solid plant material or insoluble substrates with *T. reesei* QM6a and its derivatives as defined at 40 CFR 725.3 could only be initiated after inactivation of the viable *T. reesei* cells as delineated in 40 CFR 725.422(d), *i.e.,* by a procedure that has been demonstrated and documented to be effective in reducing the viable microbial population by at least 6 logs (*i.e.,* six orders of magnitude).

In addition, EPA proposed to limit the exemption for *B. amyloliquefaciens* to only strains of *B. amyloliquefaciens* that would fall under the subspecies *B. amyloliquefaciens amyloliquefaciens.*

In response to comments received on its original proposal, EPA has modified the regulatory text in 40 CFR 725.3 and 725.420 slightly to better clarify EPA's original intent. These revisions to the regulatory text in 40 CFR 725.3 and 725.420 merely represent a clarification of the original proposal.

Existing regulatory requirements and exemptions for intergeneric microorganisms are discussed in Unit III. of this proposed rule. EPA's response to public comments received on the 2012 Proposed Rule are provided in Unit IV. Unit V. provides EPA's evaluation of available information on *T. reesei* and *B. amyloliquefaciens* for the criteria delineated in 40 CFR 725.67. Physical containment and control technologies as well as release and exposure assessments for the two microorganisms are discussed in Unit VI. EPA's risk assessments for *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* are summarized in Unit VII., and EPA's rationale for adding the two microorganisms to the list of recipients eligible for exemption is discussed in Unit VIII. EPA's Risk Assessment documents (Refs. 5 and 6), available in the public docket, provide more detailed information, and supporting references, for EPA's evaluation of the available information and the potential risks to health and the environment.

III. Existing EPA Regulatory Requirements and Exemption Standard

Manufacturers are required to report certain information to EPA 90 days before commencing the manufacture of intergeneric microorganisms that are not listed on the TSCA Inventory. EPA regulations at 40 CFR part 725 establish the mechanisms for reporting this information. TSCA prohibits such manufacturing or processing from commencing until EPA has conducted a review of the notice, made an appropriate determination on the notice, and taken such actions as are required in association with that determination (15 U.S.C. 2604(a)(1)(B)(ii)).

Any manufacturer of a living intergeneric microorganism who is required to report under TSCA section 5 must file a MCAN with EPA, unless the activity is eligible for one of the specific exemptions. Section 5(h)(4) authorizes EPA, by rule and upon request, to exempt manufacturers from these requirements if the Administrator determines that the manufacture, processing, distribution in commerce, use or disposal of the chemical substance ''will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified by the Administrator under the conditions of use.'' TSCA section 3(4) defines ''conditions of use'' to mean ''the circumstances, as determined by the Administrator, under which a chemical substance is intended, known or reasonably foreseen to be manufactured, processed, distributed in commerce, used, or disposed of.'' TSCA section 3(12) defines ''potentially exposed or susceptible subpopulation'' to mean ''a group of individuals within the general population. . .who, due to either greater susceptibility or greater exposure, may be at greater risk than the general population of adverse health effects from exposure to a chemical substance or mixture, such as infants, children, pregnant women, workers, or the elderly.'' 15 U.S.C. 2601 *et seq.* The general procedures for filing MCANs are described in 40 CFR part 725, subpart B.

EPA regulations establish two exemptions for new microorganisms, after the research and development stage, which are being manufactured for introduction into commerce: Tier I and Tier II exemptions.

Under the Tier I exemption, if certain criteria are met, manufacturers are required to notify EPA 10 days prior to manufacturing a new microorganism that qualifies for this exemption, and to keep certain records. 40 CFR 725.400. To qualify for the Tier I exemption, a manufacturer must use one of the recipient organisms listed in 40 CFR 725.420, and must implement specific physical containment and control technologies listed in 40 CFR 725.422. In addition, the genetic material

introduced into the recipient microorganism must be well-characterized, limited in size, poorly mobilizable, and free of certain sequences. 40 CFR 725.421.

A manufacturer who meets the conditions of the Tier I exemption may modify the specified containment restrictions or level of inactivation but must submit a Tier II exemption notification 40 CFR 725.428. The Tier II exemption requires manufacturers to submit an abbreviated notification describing the modified containment and provides for a 45-day period during which EPA would review the proposed containment. 40 CFR 725.450 and 725.470. The manufacturer may not proceed under this exemption until EPA approves the exemption. 40 CFR 725.470.

EPA established a petition process at 40 CFR 725.67 for the public to propose additional microorganisms for the tiered exemptions. EPA's regulations at 40 CFR 725.67 direct petitioners to submit information to demonstrate that the activities affected by the requested exemption meet the requirements of TSCA section 5(h)(4), *i.e.* ''will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified by the Administrator under the conditions of use.'' 15 U.S.C. 2604(h)(4). In addition, a petitioner is responsible for providing supporting information for this determination in four general categories:

1. The effects of the new microorganism on health and the environment.
2. The magnitude of exposure of human beings and the environment to the new microorganism.
3. The benefits of the new microorganism for various uses and the availability of substitutes for such uses.
4. The reasonably ascertainable economic consequences of granting or denying the petition, including effects on the national economy, small business, and technological innovation.

The regulations at 40 CFR 725.67 specify that when applying to list a recipient microorganism for the tiered exemption under 40 CFR 725.420, petitioners should include information addressing six specified criteria, which EPA will use to evaluate the microorganism for listing. 40 CFR 725.67(a)(3)(iii). The six criteria are:

1. Identification and classification of the microorganism using available genotypic and phenotypic information.
2. Information to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on health or the environment.
3. A history of safe commercial use for the microorganism.
4. Commercial uses indicating that the microorganism products might be subject to TSCA.
5. Studies which indicate the potential for the microorganism to cause adverse effects to health or the environment.
6. Studies which indicate the survival characteristics of the microorganism in the environment.

IV. Response to Public Comments on the 2012 Proposed Rule

The Agency received three comments on the 2012 Proposed Rule (Ref. 4). One comment, from an anonymous submitter (Ref. 7), concerned mold problems in rental housing and thus was not relevant to the proposed rule. A second comment, from an individual (Ref. 8), supported the proposed rule.

The third comment was a joint set of comments from the Biotechnology Industry Organization (BIO) and the Enzyme Technical Association (ETA) (Ref. 9). While generally supportive of the proposed rule, BIO/ETA raised three important issues with respect to EPA's proposed rule.

First, BIO/ETA expressed concern that the proposed wording in section 725.420(k), that reads ''*Trichoderma reesei* strain QM6a used only in . . .'' does not accurately reflect the range of *T. reesei* strain QM6a microorganisms currently being used in standard industrial fermentations. BIO/ETA requested that the phrase be reworded as ''*Trichoderma reesei* strain QM6a and its derivatives used only in . . .'' EPA agrees that the commenter's suggested language more accurately reflects the Agency's original intent. EPA did not originally intend to restrict the exemption to the naturally occurring QM6a isolate. Most of the strains of *T. reesei* currently used in industrial production are not the naturally occurring QM6a isolate, but are strains derived from QM6a that have been modified by physical or chemical mutagenesis to obtain microorganisms with improved enzyme-producing abilities. Accordingly, EPA has adopted the commenter's suggested revision to clarify that the exemption applies not only to the naturally occurring strain, but also to any strain derived from the naturally occurring QM6a.

Second, BIO/ETA expressed concern that the proposed regulation was too broadly worded and as drafted would not clearly distinguish between standard industrial fermentation operations used to produce enzymes, and fermentation operations conducted for other purposes. Specifically, the commenter raised concern that the inclusion of an unqualified restriction in proposed 40 CFR 725.420(k) that ''no solid plant material or insoluble substrate is present in the fermentation broth'' would prohibit the use of *T. reesei* in submerged standard industrial fermentation operations used for enzyme production. Enzyme production is the first phase of some industrial applications such as cellulosic ethanol production where the first fermentation is to grow the microorganism to produce enzymes, followed by another fermentation of pretreated plant biomass for conversion of the cellulose and hemicellulose to simple sugars (*i.e.,* saccharification), followed by a third fermentation of the sugars to ethanol by yeast or another ethanologen. As part of the process of growing the microbes for enzyme production by *T. reesei* QM6a and its derivatives, nutrients need to be available, including those from plant materials such as soy or corn, which may contain insoluble components. The second fermentation operation of saccharification of plant biomass may occur only after the *T. reesei* microorganism has been inactivated. The use of nutrients supplied by plant material (*e.g.,* soy meal, corn steep liquor) in the first fermentation for enzyme production has a long history of safe use.

To address this issue, the commenter suggested revising the regulatory text to ensure that the typical industry practice of supplying nutrients in the form of solid plant materials during the initial enzyme fermentation would fall within the scope of the proposed exemption. EPA agrees and is therefore changing the regulatory text to allow the use of solid plant material in the enzyme fermentation step. Under the final regulatory text, the use of the conventional fermentation ingredients from solid plant material—for example, soy or corn meal and other insoluble fermentation ingredients from corn or soy which contain insoluble components—is allowed when used specifically to provide nutrients for growth of the microorganism during standard enzyme fermentation as described in part 1 of the definition at 40 CFR 725.3.

The commenter further suggested adding text to clarify that the requirement to inactivate the organism applies prior to ''subsequent fermentation operations, and not to the initial enzyme production stage.'' EPA agrees that the commenter has identified a reasonable basis for concern with respect to the proposed regulatory text. EPA acknowledges that nutrients for microbial growth in submerged standard industrial fermentation during the initial enzyme production phase of the

fermentation operation may be supplied by soybean meal, corn steep liquor, or other plant-derived materials that may contain insoluble substrates. The use of such plant materials as nutrient sources for microbial growth in submerged standard industrial fermentation operations used for enzyme production is a standard industry practice with a long history of safe use, and it does not result in the production of secondary toxic metabolites such as paracelsin because the fermentations involve the logarithmic growth of the cells in the presence of optimal concentrations of carbon and nitrogen and other nutrients (see Unit V. for more detail on paracelsin). EPA did not originally intend to preclude such operations and agrees that revision to the regulatory text is warranted to clarify that solid plant material can be used to provide nutrients for growth of the microorganism during submerged standard enzyme fermentation operations.

However, EPA continues to have concern about the potential for the production of paracelsin during the second fermentation phase of cellulosic ethanol production, *i.e.,* the saccharification of the pretreated plant biomass, because of the presence of solid surfaces and an excess of carbon substrate with live *T. reesei* QM6a (and its derivatives) cells. Therefore, EPA is retaining the requirement that fermentation operations subsequent to the enzyme production fermentation phase may only be initiated after inactivation of the viable *T. reesei* cells as delineated in 40 CFR 725.422(d) (*i.e.,* by a procedure that has been demonstrated and documented to be effective in reducing the viable microbial population by at least 6 logs). Inactivation of *T. reesei* QM6a prior to a subsequent or secondary fermentation that may contain solid plant material or insoluble substrates (as defined at 40 CFR 725.3) avoids the potential for production of paracelsin.

BIO/ETA also commented that paracelsin may be produced under non-standard conditions of fermentation, such as ''surface fermentation media with large concentrations of biomass,'' and requested that EPA revise the rule to reflect this. EPA interprets this comment to mean ''surface fermentation media with large concentrations of biomass'' is the only condition under which paracelsin can be produced and that BIO/ETA is requesting that the rule be amended accordingly. EPA agrees that paracelsin may be produced under non-standard conditions of fermentation, such as surface fermentation with large concentrations of biomass. However, available scientific literature indicates that paracelsin may also be produced under certain other fermentation conditions. Scientific literature suggests that surface fermentation is synonymous with solid-state fermentation where microorganisms are grown on the surface of a solid support that is not submerged. While it is likely that the potential for paracelsin production is greater with solid-state/surface fermentation, the production of peptaibols (of which paracelsin is one) by *Trichoderma* species has been shown to occur even in liquid broth culture in the presence of plant material or insoluble substrates in laboratory studies. Thus, paracelsin production potentially may be produced in fermentation broth amended with plant material providing excess carbon. Therefore, EPA is not amending the rule to indicate that the only conditions in which paracelsin potentially may be produced are with surface fermentations with large concentrations of biomass.

V. EPA's Evaluation of Available Information on *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* for the Criteria Delineated

Pursuant to 40 CFR 725.67, Genencor International, Inc., (subsequently supported by the Enzyme Technical Association (ETA)) and Novozymes North America, Inc., submitted Letters of Application to EPA requesting that *T. reesei* and *B. amyloliquefaciens* (Refs. 1 and 2) be added to 40 CFR 725.420 as candidate recipient microorganisms for the tiered exemptions. The letters of application provided information that the submitters believed demonstrate that activities affected by the requested exemptions would not present an unreasonable risk of injury to health or the environment. Information regarding the criteria specified in 40 CFR 725.67(a)(2) and 725.67(a)(3)(iii) were addressed in these letters of application to list *T. reesei* and *B. amyloliquefaciens* as recipient microorganisms under 40 CFR 725.420.

EPA has made the determination based on the information provided in the Letters of Application (Refs. 1 and 2), supplemental information provided by ETA (Refs. 10 and 11), and other information available to EPA that *T. reesei* QM6a, with certain restrictions, and *B. amyloliquefaciens* subsp. *amyloliquefaciens* will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation, when used as recipient microorganisms provided that: (a) The existing criteria for the introduced genetic material listed in 40 CFR 725.422 are met, and (b) the physical containment and control technologies criteria listed at 40 CFR 725.422 are met. In making this determination, EPA identified workers as a potentially exposed or susceptible subpopulation to the substances under the conditions of use and concluded that, with the limitations described above, the substances will not present an unreasonable risk of injury to health or the environment. EPA's Risk Assessments for these two microorganisms (Refs. 5 and 6) are available in the docket.

This unit presents a summary of EPA's evaluation of the available information pertinent to the six criteria delineated in 40 CFR 725.67(a)(3)(iii) for both microorganisms.

A. Evaluation of Available Information Relevant to the Criteria for T. reesei QM6a as a Recipient Microorganism with Specified Conditions of Growth

1. Identification and classification of the microorganism using available genotypic and phenotypic information.

T. reesei is a hypercellulolytic fungus originally isolated in the Solomon Islands in 1944. *T. reesei* was found on deteriorating military fabrics such as tents and clothing. This isolate, designated as QM6a, was initially named *Trichoderma viride.* Approximately 20 years later, QM6a was re-classified as *Trichoderma reesei.*

T. reesei is the species name given to the anamorphic form (this form reproduces asexually) of the fungus whose teleomorphic form (this form reproduces sexually) is now understood to be *Hypocrea jecorina.*

Recent taxonomic studies have shown that the species *T. reesei* consists only of this single isolate QM6a and its derivatives. Many other strains called *T. reesei* isolated elsewhere have now been proposed as belonging to a newly named species, *T. parareesei,* based on differences in habitat, sporulation, and metabolic versatility. *T. reesei* has been shown to belong to a single species now referred to as *H. jecorina/T. reesei* (QM6a) which reflects its relationship to its teleomorph *H. jecorina.* The only anamorphic strains within the species *H. jecorina/T. reesei* are those of QM6a and its derivatives. The petition to add *T. reesei* to the list of microorganisms at 40 CFR 725.420 requested that EPA include all strains of *T. reesei.* However, given these recent taxonomic publications, all fungal strains correctly named *T. reesei* are, by definition, QM6a or a derivative.

Adequate genotypic and phenotypic information is available for classification of *T. reesei* QM6a and its derivatives. The American Type Culture Collection (ATCC) designation for this original strain of *T. reesei* QM6a is ATCC 13631.

2. *Information to evaluate the relationship of the microorganism to any other closely related microorganisms that have a potential for adverse effects on health or the environment.*

Closely related members of section *Longibrachiatum* do not have a potential for adverse effects; other less closely related *Trichoderma* species have a potential to cause adverse effects as pathogens of commercially produced mushrooms. These less closely related species include various species of the Harzianum clade, *T. aggressivum, T. pleuotrophilum,* and *T. fulvidum* that are responsible for significant loss of the mushroom crops of *Agaricus bisporus* and *Pleurotus ostreatus.*

T. reesei/H. jecorina can be distinguished from other *Trichoderma* species by a comprehensive approach employing criteria of the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept, which commonly requires the use of genealogies of three or four genes, not just the sequences of spacer regions as previously utilized for identification. Use of the GCPSR protocol would separate *T. reesei* (sensu lato) from the opportunistic pathogens within the section *Longibrachiatum,* including *T. longibrachiatum* and *T. citrinoviride/H. schweinitzii,* as well as the mold disease pathogens of mushrooms.

3. *A history of safe commercial use for the microorganism.*

T. reesei QM6a has a long history of safe use producing a variety of commercial enzymes. *T. reesei* QM6a cellulases, beta-glucanases, and xylanases are used by the animal feed, baking, beverages, textile processing, detergent, pulp and paper, industrial chemicals, and biofuels industries.

For industrial enzyme production, *T. reesei* is generally grown in a closed, submerged standard industrial fermentation system. In submerged standard industrial fermentation operations used for enzyme production, growth of the microorganism occurs beneath the surface of the liquid growth medium. As described in this unit, this type of fermentation system appears to be typical throughout the industry, based on EPA's review of MCAN submissions over the years.

Under this type of fermentation system, the fermentation broth is a defined mixture of carbon and nitrogen sources, some of which may be supplied from plant material or soluble substrates (*e.g.,* soy meal, corn steep liquor), minerals, salts, and other nutrients, is maintained at optimal pH and temperature, and is typically aerated and mixed. These conditions support the active growth and productivity of the organisms for enzyme production. Submerged standard industrial fermentation operations used for enzyme production systems reduce the potential for exposure of workers to the production organism and fermentation broth aerosols, reduce the potential for contamination of the culture and make the collection of extracellular enzymes simpler and less costly. The fermentation process is terminated before the *T. reesei* QM6a organisms go into the stationary growth phase (*i.e.,* before secondary metabolism begins). At the end of the fermentation process, the production organisms are separated from the fermentation broth and inactivated.

Several enzymes produced by *T. reesei* QM6a have Generally Recognized as Safe (GRAS) status with the Food and Drug Administration (FDA) or FDA had no questions about the GRAS conclusions about them contained in GRAS submissions to FDA. This supports the Agency's conclusion that commercial use of *T. reesei* QM6a and its derivatives as a recipient microorganism for commercial enzyme production will not present an unreasonable risk of injury to health or the environment. *T. reesei* QM6a enzymes used in foods that have been granted GRAS status or for whose claimed GRAS status FDA had no questions include cellulase, hemicellulase, transglucosidase, pectin lyase, acid fungal protease, and a chymosin enzyme preparation. Data supporting the GRAS notices included the results of pathogenicity tests for the *T. reesei* QM6a production organisms and toxicity tests for the enzyme products. The data showed that the production strains are not pathogenic and did not produce toxins during enzyme fermentation.

4. *Commercial uses indicating that the microorganism products might be subject to TSCA.*

EPA has reviewed 48 MCANs involving intergeneric *T. reesei* production organisms used to manufacture a number of industrial enzymes, including amylases, glucosidases, proteases, phytase, laccase, and numerous cellulolytic enzyme preparations. Amylases and glucosidases are used for the breakdown of starch into sugars and have been used in laundry detergents and in textile processing. More recently, industrial enzymes produced by *T. reesei* have been produced for corn and cellulosic ethanol production. *T. reesei* produces numerous cellulases and hemicellulases that are efficient in degrading plant biomass. Intergeneric *T. reesei* strains could also be used to manufacture industrial chemicals other than enzymes such as surfactants or specialty chemicals. More detailed information on MCANs submitted to EPA can be viewed on EPA's TSCA Biotechnology Program web page: *https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/overview-biotechnology-under-tsca.*

5. *Studies which indicate the potential for the microorganism to cause adverse effects to health or the environment.*

a. *Human health hazards* — i. *Pathogenicity. T. reesei* QM6a is not pathogenic to humans. Due to its long history of use for production of enzymes used in food applications, the potential for the fungus and its products to be pathogenic or toxic to humans has been evaluated numerous times. Various studies have been conducted assessing *T. reesei* QM6a's pathogenic potential in healthy and immunocompromised laboratory animals. With the exception of one study where a high inoculum of intravenous (iv) and intraperitoneal (ip) injection of spores in immunocompromised mice resulted in pathogenic effects, studies have demonstrated a lack of pathogenicity of *T. reesei* QM6a. Numerous pathogenicity studies have been conducted as part of GRAS notices to FDA for several different enzymes used in the food industry. Studies using injection of *T. reesei* QM6a in rats, using both healthy and immunosuppressed rats, and using ip injection of viable and heat-killed cells of *T. reesei* QM6a in rats have all demonstrated a lack of potential pathogenicity to humans.

T. reesei QM6A is not known to possess any virulence factors associated with colonization or disease such as adherence factors, penetration factors, necrotic factors, toxins, or the ability to grow at human body temperature, 37 °C. There are no reports of harmful effects associated with the use of or exposure to *T. reesei* QM6A strains, even after decades of commercial use for enzyme production. The body of evidence indicates that *T. reesei* QM6A does not pose concerns regarding human pathogenicity.

ii. *Toxicity.* Available data indicate that *T. reesei* QM6a strains used in submerged standard industrial fermentation operations used for enzyme production do not present

human toxicity concerns. A number of studies have been conducted assessing the potential for *T. reesei* QM6a to produce toxins during submerged standard industrial fermentation operations used for enzyme production for food, pharmaceutical, or industrial uses. A cellulase enzyme known as celluclast produced by *T. reesei* QM6a has been tested for general oral toxicity and inhalation toxicity. Acute oral toxicity studies conducted in mice, rats, and dogs showed that *T. reesei* QM6a cellulase was not toxic to any of the test animals. Subchronic toxicity studies showed no evidence of systemic effects in dogs or rats. Additional toxicity studies have been conducted on other enzymes produced by *T. reesei* QM6a, the results of which have been presented in various GRAS petitions. Acute oral toxicity tests on two endoglucanases and a glucoamylase showed a lack of toxins. Subchronic feeding studies conducted on a cellulase, two xylanases, two endoglucanases, a protease, and a glucoamylase also showed a lack of toxicity in rats.

Under typical industry practice, industrial fermentations of *T. reesei* QM6a for enzymes to be used in food are routinely checked by the enzyme producers to confirm the absence of antibiotic activity and toxins (Ref. 12). Relying on the data that show *T. reesei* QM6a has a long history of safe use in the production of food enzymes, EPA has concluded that strains used industrially would not be expected to produce these toxins under the conditions of submerged standard industrial fermentation used for enzyme production.

iii. *Mycotoxins and other secondary metabolites.* The only health concern associated with *T. reesei* QM6a is its ability to produce a peptaibol secondary metabolite called paracelsin. Peptaibols are small linear peptides of 1,000–2,000 daltons characterized by a high content of the non-proteinogenic amino acid α-amino-isobutyric acid (Aib), with an *N*-terminus that is typically acetylated, and a *C*-terminus that is linked to an amino alcohol, which is usually phenylalaninol, or sometimes valinol, leucinol, isoleucinol, or tryptophanol. Peptaibols are associated with a wide variety of biological activities and have antifungal, antibacterial, sometimes antiviral, antiparasitic, and neurotoxic activity. Paracelsin has been shown to damage mammalian cells such as human erythrocytes with an *in vitro* hemolytic activity of $C_{50} = 3.7 \times 10^{5}$ mole/liter (mol/L) (Ref. 5).

Paracelsin has not been detected in the use of *T. reesei* QM6a under the submerged standard industrial fermentation operations used for enzyme production, and numerous toxicity studies on enzyme products of *T. reesei* QM6a have demonstrated a lack of toxicity to laboratory animals. EPA therefore expects that paracelsin production would be of insignificant concern, provided the microorganisms are produced with submerged standard industrial fermentation operations used for enzyme production as described at 40 CFR $_{725.3.}$

Under other conditions of fermentation, for example with the deliberate fermentation of cellulosic biomass for saccharification of plant material or extended fermentation, paracelsin may be produced (Ref. 5). Neither the information submitted with the petition, nor the information that is otherwise available is sufficient to allow EPA to determine the extent of paracelsin formation under these non-standard conditions. Consequently, EPA is unable to determine whether the use of the microbe under conditions other than submerged standard industrial fermentation operations used for enzyme production (*i.e.,* specific conditions under which paracelsin is not expected to be formed) will not pose an unreasonable risk to human health and/or the environment (Ref. 5).

b. *Environmental hazards*—i. *Hazards to animals. T. reesei* QM6a is not pathogenic to domesticated animals or wildlife. However, the secondary metabolite paracelsin has been shown to exhibit toxicity to aquatic species. A 24-hr exposure of paracelsin to *Artemia salina* (brine shrimp) resulted in a lethal concentration of 50% (LC_{50}) of 21.26 micromoles (μM) (40.84 micrograms per milliliter (μg/ml)) which decreased to 9.66 μM (18.56 μg/ml) with a 36-hr exposure. With *Daphnia magna,* paracelsin was found to be moderately toxic, with an LC_{50} of 7.70 μM (14.79 μg/ml) with a 24-hr exposure, and 5.60 μM (10.76 μg/ml) with a 36-hr exposure.

ii. *Hazards to plants. T. reesei* QM6a is not a pathogen of plants. Although it is capable of degrading cellulose and hemicellulose due to the copious quantities of the enzymes it can produce, it cannot be a primary colonizer on plant tissue. Genetic studies have shown that *T. reesei* QM6a does not contain any genes for ligninases, required for initial breakdown of plant material. This species is known as a wood rot fungus, but it apparently attacks only decaying plant material, not live plants.

iii. *Effects on other organisms.* Peptaibols are toxic to Gram-positive bacteria and various fungi. The inhibitory action of peptaibols on various fungi is the reason that many species of *Trichoderma* are used as biocontrol agents of plant pathogenic fungi. The peptaibol produced by *T. reesei,* QM6a paracelsin, has been shown to be inhibitory to one particular fungus, *Phoma destructiva.*

Some species of *Trichoderma,* specifically *T. aggressivum, T. pleuotrophilum,* and *T. fulvidum* are pathogens of mushrooms. However, *T. reesei* QM6a is not a pathogen of mushrooms.

6. *Studies which indicate the survival characteristics of the microorganism in the environment.* The species *T. reesei* is known only from the single original isolate QM6a from the Solomon Islands. Therefore, there is little information on its prevalence or behavior in the environment. Microcosm studies have been conducted that suggest it would survive in the environment in the plant rhizosphere and in bulk soils if inadvertently released.

Although *T. reesei* was originally isolated from a tropical climatic region, it would be expected to persist in soils for extended periods of time, even after cold temperatures.

B. Evaluation of Available Information Relevant to the Criteria for B. amyloliquefaciens as a Recipient Microorganism

1. *Identification and classification of the microorganism using available genotypic and phenotypic information.*

B. amyloliquefaciens was initially proposed as a unique species in 1943. The name *B. amyloliquefaciens* lost standing when it was not included on the Approved List of Bacterial Names with Standing in Nomenclature in 1980. Since classical phenotypic tests could not differentiate it as a unique species from *Bacillus subtilis,* it was regarded as a subspecies of *B. subtilis* for several decades. However, molecular evidence from subsequent studies led to the conclusion that *B. amyloliquefaciens* did indeed deserve independent status. The DNA homology between *B. subtilis* and *B. amyloliquefaciens* is only about 15%. In addition, there were several phenotypic properties that differed between the two species. Chemotaxonomic studies revealed additional capability of separating strains of *B. amyloliquefaciens* from the other related species, *Bacillus subtilis, Bacillus licheniformis,* and *Bacillus pumilus.* The species has remained within the genus *Bacillus sensu stricto* since it was last established as a separate species.

Recently, it has been proposed that there are two subspecies within the species *B. amyloliquefaciens, B.*

amyloliquefaciens subsp. *amyloliquefaciens* and *B. amyloliquefaciens* subsp. *plantarum.* The former subspecies includes the type strain and likely most, if not all, of the industrial strains of *B. amyloliquefaciens* used for enzyme production. The latter subspecies consists of plant-associated strains used as biocontrol agents due to the production of several antifungal lipopeptide and antibacterial polyketide toxins. This exemption is restricted to the subspecies *B. amyloliquefaciens* subsp. *amyloliquefaciens* which contains the industrial strains used for enzyme production. Adequate genotypic and phenotypic information is available to accurately identify *B. amyloliquefaciens* subsp. *amyloliquefaciens.*

2. *Information to evaluate the relationship of the microorganism to any other closely related microorganisms which have a potential for adverse effects on health or the environment.*

There are several species in the genus *Bacillus* that are known pathogens. These include *Bacillus anthracis,* which is pathogenic to humans and other animals, and *Bacillus cereus,* which is a common cause of food poisoning. *Bacillus thuringiensis, Bacillus larvae, Bacillus lentimorbus, Bacillus popilliae,* and some strains of *Bacillus sphaericus* are pathogenic or toxigenic to certain insects. The new subspecies *Bacillus amyloliquefaciens* subsp. *plantarum* has been shown to exhibit toxicity mainly to plant pathogenic fungi but can also be cytotoxic to mammalian cells. It is possible, using polyphasic approaches, to differentiate between *B. amyloliquefaciens* subsp. *amyloliquefaciens* and these other species and subspecies that have the potential to adversely affect humans or other organisms. *B. amyloliquefaciens* can be distinguished from the very similar *Bacillus subtilis* by a few phenotypic traits and DNA dissimilarity.

3. *A history of safe commercial use for the microorganism.*

B. amyloliquefaciens subsp. *amyloliquefaciens* has been used to produce commercial enzymes for more than 50 years. It produces carbohydrases, proteases, nucleases, xylanases, and phosphatases that have applications in the food, brewing, distilling, and textile industries.

For commercial enzyme production, *B. amyloliquefaciens* subsp. *amyloliquefaciens* is grown in a closed submerged fermentation. In submerged fermentation, growth of the microorganism occurs beneath the surface of the liquid growth medium. The fermentation broth is a defined mixture of carbon and nitrogen sources, minerals, salts, and other nutrients that is maintained at optimal pH and temperature. These conditions support the active growth and productivity of the organisms. Submerged fermentation systems reduce the potential for exposure of workers to the production organism and fermentation broth aerosols, reduce the potential for contamination of the culture, and make the collection of extracellular enzyme simpler and less costly. The fermentation process is terminated before the *B. amyloliquefaciens* subsp. *amyloliquefaciens* organisms enter the stationary growth phase, and the production organisms are separated from the fermentation broth and inactivated. The enzyme preparation may also be subjected to other purification processes.

B. amyloliquefaciens subsp. *amyloliquefaciens* has a long history of safe use for enzyme production in food and industrial applications with no incidences associated with human pathogenicity. In response to a petition from the ETA, FDA affirmed that carbohydrase enzyme preparations and protease enzyme preparations derived from either *Bacillus subtilis* or *Bacillus amyloliquefaciens* are GRAS for use as direct food ingredients. The European Food Safety Authority (EFSA) has put *B. amyloliquefaciens* on their list of bacteria that have a ''qualified presumption of safety'' because of a long history of apparent safe use in food and feed production. However, it was put on the list with a qualifier that only strains of *B. amyloliquefaciens* that do not have toxigenic potential be used.

One strain of *B. amyloliquefaciens* has been used as a biopesticide. A naturally occurring strain of *B. amyloliquefaciens* subsp. *plantarum* was registered in 2000 as a biopesticide active ingredient under the Federal Insecticide, Fungicide, and Rodenticide Act. It can only be used on certain ornamental, non-food plants in greenhouses and other closed structures.

4. *Commercial uses indicating that the microorganism products might be subject to TSCA.*

It is expected that intergeneric strains of *B. amyloliquefaciens* subsp. *amyloliquefaciens* would be used to produce enzymes and to manufacture other industrial chemicals subject to TSCA. Many enzymes produced by *B. amyloliquefaciens,* particularly α-amylase, are used in laundry detergents and in textile processing. *B. amyloliquefaciens* also makes a surfactant known as surfactin which functions as an antibiotic.

5. *Studies which indicate the potential for the microorganism to cause adverse effects to health or the environment.*

a. *Human health hazards*— i. *Pathogenicity. B. amyloliquefaciens* is not pathogenic to humans. There are no reports in the literature associating *B. amyloliquefaciens* with infection or disease in humans. *B. amyloliquefaciens* has been categorized as a Biosafety Level 1(BSL1) microorganism by the Centers for Disease Control and Prevention (CDC). BSL1 microorganisms are well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and which present minimal potential hazard to laboratory personnel and the environment. Animal toxicity studies were performed with *B. amyloliquefaciens* strain FZB24 to support its registration as a biopesticide. Tests for acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, and acute injection toxicity/pathogenicity showed little to no adverse effects, which indicated low mammalian toxicity and a lack of pathogenicity/infectivity.

ii. *Toxins and other secondary metabolites.* Although another species in the genus *Bacillus, B. cereus,* has the potential to produce food poisoning toxins which cause both emetic and diarrheal syndromes, and a variety of local and systemic infections, the risk of food-borne disease caused by bacilli other than *B. cereus* is generally considered to be negligible because usually only *B. cereus* has the genes that encode food poisoning toxins. Industrial strains of *Bacillus* species belonging to the *Bacillus subtilis* group, which includes *B. amyloliquefaciens,* do not express *B. cereus* toxins. In addition, there are no reported cases of food poisoning associated with *B. amyloliquefaciens.*

Some strains of *B. amyloliquefaciens* have been shown to produce bioactive cyclic lipopeptide metabolites such as iturin, surfactin, fengycin, and bacillomycin D. These are cyclical lipoprotein biosurfactants produced by non-ribosomal peptide synthesis. They have a low mammalian toxicity as demonstrated by a lethal dose of 50% (LD_{50}) of >2,500 milligram/kilogram (mg/kg) in an acute toxicity test of surfactin C, and a no observed adverse effect level of 500 mg/kg-day in a repeat dose oral gavage study. Some strains of *B. amyloliquefaciens* may also produce the polyketide toxins macrolactin, bacillanene, and difficidin. *B. amyloliquefaciens* also produces the protein toxin barnase and the antifungal protein baciamin.

There are reports of the isolation of *B. amyloliquefaciens* from water-damaged buildings in which occupants were suffering ill health symptoms. Extracts from biomass of isolated strains of *Bacillus* exhibiting antifungal properties were assessed for toxicity endpoints. All of the isolated *B. cereus* and *B. amyloliquefaciens* strains studied showed cytotoxicity as evidenced by inhibition of boar spermatozoa motility; however, the *B. amyloliquefaciens* strains affected boar spermatozoa differently from the indoor *B. cereus* isolates and the reference food-poisoning strain.

The isolation of cytotoxic strains of *B. amyloliquefaciens* from water-damaged buildings is of little concern in relation to the exemption of *B. amyloliquefaciens* subsp. *amyloliquefaciens.* It is important to note that the *Bacillus amyloliquefaciens* strains studied in water-damaged buildings were specifically selected for further study because the isolates exhibited antifungal activity. Some of the secondary metabolites produced by these strains of *B. amyloliquefaciens* also exhibited cytotoxicity to mammalian cells (*i.e.,* boar spermatozoa). However, industrial strains of *B. amyloliquefaciens* that are classified as *B. amyloliquefaciens* subsp. *amyloliquefaciens* have been shown not to produce most, if not all, of the antifungal and antibacterial lipopeptides and polyketides produced by the biocontrol-type strains. The genome of the type strain of *B. amyloliquefaciens* DSM 7^{T} (now *B. amyloliquefaciens* subsp. *amyloliquefaciens*) is very similar to the genome of the biocontrol strain FZB42 (*B. amyloliquefaciens* subsp. *plantarum*). However, the latter subspecies had genomic islands carrying prophage sequences, transposases, integrases, and recombinases that the DSM 7^{T} type strain did not have. The DSM 7^{T} type strain was shown to have a diminished capacity to non-ribosomally synthesize secondary metabolites with antifungal and antibacterial activities. The DSM 7^{T} type strain could not produce the polyketides difficidin or macrolantin, and could not produce lipopeptide such as iturin, macrolantin, and other compounds except for the compound surfactin.

Although there are isolated reports of toxin production in several antifungal, environmental isolates of *B. amyloliquefaciens,* the larger body of studies available on the safety and toxicity of *B. amyloliquefaciens* strains used industrially for enzyme production (Ref. 6) indicate that these strains are safe and non-toxic. For example, the industrial strains of *B. amyloliquefaciens, Bacillus subtilis,* and *Bacillus licheniformis* used for large-scale enzyme production did not exhibit any cytotoxicity in Chinese hamster ovary tests. In Europe, the toxicity of two strains of *B. amyloliquefaciens* used in the production of α-amylase and bacillolysin was assessed by EFSA's Scientific Panel on Additives and Products or Substances used in Animal Feed. The panel concluded that the *B. amyloliquefaciens* production strains DSM9553 and DSM9554, when used as a source of extracellular enzyme, do not present a toxigenic risk. Given its widespread distribution in the environment, its long history of safe use in industrial fermentation, the absence of reports on pathogenicity to humans, and the limited reports of cytotoxicity, EPA concludes that the use of *B. amyloliquefaciens* in fermentation facilities for production of enzymes or specialty chemicals does not present a human health concern.

b. *Environmental hazards*— i. *Hazards to animals.* There are no reports suggesting that *B. amyloliquefaciens* is pathogenic to domesticated animals or wildlife. The cytotoxicity of antifungal secondary metabolites to mammalian cells by biocontrol strains of *B. amyloliquefaciens* subsp. *plantarum* is discussed in this Unit.

ii. *Hazards to plants. B. amyloliquefaciens* is not pathogenic to plants. The plant-associated strains of *B. amyloliquefaciens* are beneficial to plants because they inhibit the growth of fungal plant pathogens. Antifungal and antibacterial secondary metabolites produced by strains of *B. amyloliquefaciens* such as iturins, surfactins, fengycin, bacillomycins, and azalomycin have been shown to inhibit the growth of *Rhizoctonia solani, Xanthomonas campestris* pv. *campestris, Alternaria brassicae, Botyris cinerea, Leptosphaeria maculans, Verticillium longisporum, Pythium ultimatum, Aspergillus* spp., *Fusarium* spp., *Bipolaris sorokiniana,* and *Fusarium oxysporum.*

In addition to producing antifungal and antibacterial compounds, *B. amyloliquefaciens* is known as a plant growth-promoting rhizobacterium, and some of the biological control strains of *B. amyloliquefaciens* were shown to produce the phytohormone indole-3-acetic acid.

6. *Studies which indicate the survival characteristics of the microorganism in the environment.*

Several studies assessing the survival of *B. amyloliquefaciens* are available in the public literature and are described in EPA's Risk Assessment of *B. amyloliquefaciens* (Ref. 6). Given that the natural habitat for *B. amyloliquefaciens* is typically in soil, on plant roots, or as an endophyte within the roots or stems of plants, the bacterium is likely to survive for a least some period of time if inadvertently released to the environment. However, like other bacilli, survival in soil may occur predominately as the resistant endospore state, whereas in the rhizosphere, it may exist as active vegetative cells.

VI. Physical Containment and Control Technologies for *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens*

A. Release and Exposure Assessment in Support of the TSCA Section 5(h)(4) Exemption for T. reesei QM6a

The estimated releases of the microorganism from an enzyme manufacturing facility and exposures of workers, the general population, and the environment to the microorganisms are based on a generic scenario developed by EPA in 1997 for large-scale closed system enzyme fermentation. The generic scenario assumes the facility operates 350 days/year, produces 100 batches/year, the maximal cell concentration in the fermentation broth is 1 x 10^{7} colony-forming units (cfu)/ml, and the volume of the fermentation broth is 70,000 L. The process consists of the main steps of laboratory propagation, fermentation, inactivation, and recovery where filtration operations separate out the microbial biomass from the concentrated desired product. The operations, sources of exposure and release are described in more detail in EPA's Release and Exposure Assessment (Ref. 13).

Exposures of workers to the microorganisms in during processing operations using submerged standard industrial fermentation do not pose concerns. The release of microbial cells in aerosols or in liquid and solid waste streams in submerged standard industrial fermentation operations with the containment and inactivation conditions of the Tier I exemption, are considered low. Thus, potential exposures to the general human population to the microorganism through inhalation or drinking water ingestion and to the environment are also low.

B. Release and Exposure Assessment in Support of the TSCA Section 5(h)(4) Exemption for B. amyloliquefaciens subsp. amyloliquefaciens

The estimated releases of the microorganism from an enzyme manufacturing facility and exposures of the microorganisms to workers, the general population, and the environment are based on a generic scenario developed by EPA in 1997 for large-scale closed system enzyme fermentation. The generic scenario assumes the facility operates 350 days/year, produces 100 batches/year, the maximal cell concentration in the fermentation broth is 1×10^{11} cfu/ml and the volume of the fermentation broth is 70,000 L. The process consists of the main steps of laboratory propagation, fermentation and then recovery where filtration operations separate out the biomass from the concentrated desired product. The operations, sources of exposure and release are described in more detail in EPA's Release and Exposure Assessment (Ref. 14).

Exposures of workers to the microorganisms during processing operations using submerged standard industrial fermentation do not pose concerns. The release of microbial cells in aerosols or in liquid and solid waste streams in submerged standard industrial fermentaion operations with the containment and inactivation conditions of the Tier I exemption are considered low. Thus, potential exposures to the general human population to the microorganism through inhalation or drinking water ingestion and to the environment are also low.

VII. Risk Assessment Overview for *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens*

EPA's Risk Assessment documents (Refs. 5 and 6) provide more detailed information, and supporting references, for EPA's evaluation of the available information and the potential risks to health and the environment. EPA has determined that because of the low hazard potential and safe history of use of *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens,* the TSCA section 5(h)(4) exemption will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk of injury to a potentially exposed or susceptible subpopulation under the conditions of use, provided that the other conditions of the exemptions at 40 CFR part 725, subpart G, relating to the introduced genetic material, and the physical containment of the new microorganisms, have been met.

A. Risk Assessment for T. reesei QM6a

There is only one potential concern for human health and environmental hazards associated with *T. reesei* QM6a, and that is for paracelsin production. Paracelsin production is not expected to occur in submerged standard industrial fermentation operations conducted solely for growth of the microorganism to produce enzymes. There is no concern for potential pathogenicity of *T. reesei* QM6a to humans, plants, domesticated animals, or wildlife. The body of evidence of pathogenicity testing on various industrial strains indicates that *T. reesei* is not pathogenic to humans. Toxicity testing on a number of enzymes produced by *T. reesei* indicates that the fungus does not produce toxins when used in the submerged standard industrial fermentation operations used for enzyme production.

T. reesei has a long history of safe use and is expected to present low hazard to workers, the general public, and the environment. Although direct monitoring data are unavailable, estimates of potential exposures made by EPA in its assessment of potential risks (Ref. 5) do not indicate high levels of exposure of *T. reesei* to either workers or the public from submerged standard industrial fermentation operations used for enzyme production. Standard industrial hygiene management practices currently used in the fermentation industry reduce the potential for adverse health effects in the workplace. The use of engineering controls (closed fermentation systems), appropriate work practices, personal protective equipment, and personal hygiene reduce the potential for worker exposure. Thus, current practices reduce the potential for the dermal and respiratory exposures estimated by EPA.

Based on worst-case exposure scenarios and toxicity of the microorganism, EPA has made the determination that the potential risk to workers, the general public, and to the environment resulting from the use of *T. reesei* QM6a in submerged standard industrial fermentation operations used for enzyme production is low, provided the additional criteria of the tiered exemptions for the introduced genetic material and the physical containment conditions are met (Ref. 5).

B. Risk Assessment for B. amyloliquefaciens subsp. amyloliquefaciens

Industrial strains of *B. amyloliquefaciens* subsp. *amyloliquefaciens* are not pathogenic to humans, plants, domesticated animals, or wildlife, and do not produce many of the toxic secondary metabolites found in biological control strains of *B. amyloliquefaciens* subsp. *plantarum.* The long history of safe use of enzymes produced by industrial strains of *B. amyloliquefaciens* in food is evidence that the bacterium does not produce toxins under standard conditions used for enzyme production.

Current practices in the fermentation industry reduce the potential for adverse health effects in the workplace. The use of engineering controls (closed fermentation systems), appropriate work practices, personal protective equipment, and personal hygiene reduce the potential for worker exposure and reduce the potential for the dermal and respiratory exposures.

Industrial strains of *B. amyloliquefaciens* have a long history of safe use and are expected to present low hazard to workers, the general public, and the environment. Although direct monitoring data are unavailable, exposure estimates do not suggest high levels of exposure of *B. amyloliquefaciens* subsp. *amyloliquefaciens* to either workers or the public resulting from the industrial fermentation procedures that are standard throughout the industry.

Based on worst-case exposure scenarios and toxicity of the microorganism, EPA has made the determination that the potential risk to workers, the general public, and the environment associated with the use of industrial strains of *B. amyloliquefaciens* subsp. *amyloliquefaciens* in submerged standard industrial fermentation is low provided the additional criteria of the tiered exemptions for the introduced genetic material and the physical containment conditions are met (Ref. 6).

VIII. Rationale for Adding *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* to the List of Recipient Microorganisms at 40 CFR 725.420

A. Statutory Background

On June 22, 2016, the ''Frank R. Lautenberg Chemical Safety for the 21st Century Act,'' amended TSCA (15 U.S.C. 2601 *et seq.*) (Ref. 15). Pursuant to TSCA section 5(h)(4), EPA is authorized, upon request and by rule, to exempt the manufacturer of any new

chemical substance from all or part of the requirements of TSCA section 5 if EPA determines that the manufacture, processing, distribution in commerce, use, or disposal of the chemical substance, or any combination of such activities, will not present an unreasonable risk of injury to human health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified by the Administrator under the conditions of use. The amended language of the statute with regard to section 5(h)(4) did not alter EPA's approach to balancing the considerations of the costs and benefits of issuing an exemption rule.

B. EPA's Approach for Assessing ''Unreasonable Risk'' for T. reesei QM6a and B. amyloliquefaciens subsp. amyloliquefaciens

In determining whether *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* will not present an unreasonable risk of injury to human health or the environment, the Agency considered more than just the inherent risks presented by the two microorganisms. The Agency also considered the full range of societal benefits associated with the exemption; for example, as discussed in more detail below, EPA considered not only the cost savings to the users of the microorganism, but also the societal benefits that flow from promotion of the use of low-risk recipient microorganisms, while allowing the Agency to direct its resources toward reviewing higher risk microorganisms.

It is important that EPA is revising one aspect of the existing tiered exemptions at 40 CFR 725.420 by expanding the exemption to apply to two specific microorganisms. The narrow scope of this action affected the scope of EPA's cost-benefit analysis in which EPA compared the risks and benefits of the two microorganisms being considered for an exemption with the risks that would have resulted if those same two microorganisms remained subject to full MCAN submission requirements and 90-day EPA review. EPA did not compare the risks and benefits that would result from use of these two microorganisms in the absence of any regulation.

It is also significant that the standard applicable to this rule is that the microorganisms ''will not present unreasonable risk,'' rather than ''no risk.'' It is not possible to eliminate all risks associated with the manufacture, processing, distribution in commerce, use, and disposal of any new microorganism.

C. Application of No Unreasonable Risk Factors for T. reesei QM6a and B. amyloliquefaciens subsp. amyloliquefaciens

The following is an explanation of the factors and their analyses relevant to the no unreasonable risk finding.

1. *Risks associated with these two microorganisms.* EPA's evaluation of the available information concerning *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* against these criteria is presented in detail in Unit V., and is summarized again here for the readers' convenience.

The Agency developed specific criteria in 40 CFR 725.67 that the Agency uses in determining the extent of a potential recipient microorganism's risks, and its eligibility for listing at 40 CFR 725.420. These criteria were explained in detail in the proposed ''biotech'' rule (Ref. 16), the final ''biotech'' rule (Ref. 17), and are discussed in Unit V. EPA's conclusions for these two microorganisms are based on the available data and EPA's experience under 40 CFR part 725. *T. reesei* QM6a is not pathogenic to humans, plants, domesticated animals, or wildlife and the fungus does not produce toxins under submerged standard industrial fermentation operations used for enzyme production. *T. reesei* QM6a has a long history of safe use and is generally expected to present low risk to workers, the general public, and the environment resulting from submerged standard industrial fermentation operations used for enzyme production that are standard throughout the industry.

Under non-standard conditions of fermentation, such as with the deliberate fermentation of cellulosic biomass for saccharification of plant material or extended fermentation, paracelsin may be produced. The risks associated with the production of paracelsin may be significant due to its toxicity to mammalian cells, aquatic species, Gram-positive bacteria, and various fungi. However, the potential risk associated with paracelsin production is expected to be significantly reduced by this rule, which limits the exemption to fermentation operations using submerged standard industrial fermentation operations used for enzyme production.

Industrial strains of *B. amyloliquefaciens* subspecies *amyloliquefaciens* are not pathogenic to humans, plants, domesticated animals, or wildlife, and do not produce toxins under standard conditions used for enzyme production. Industrial strains of *B. amyloliquefaciens* subsp. *amyloliquefaciens* used for the production of enzymes have a long history of safe use and are expected to present low hazards to human health and the environment.

Only strains of *B. amyloliquefaciens* that fall into the subspecies *B. amyloliquefaciens amyloliquefaciens* were considered as the eligible recipient microorganism at 40 CFR 725.420. In this rule, EPA is excluding other strains/subspecies of these two species for which:

- The Agency has insufficient data and review experience to find that they will not present an unreasonable risk of injury or
- The Agency has found that, under certain conditions, based on data on the species in question, a strain or subspecies may present an unreasonable risk, thereby requiring a closer examination of the conditions of manufacturing, processing, distribution in commerce, use, and disposal during a 90-day MCAN review. Consequently, additional information would be necessary to make an appropriate determination about the organisms' potential risks.

The Agency believes that the requirement for submission of a MCAN followed by a 90-day review period for new intergeneric microorganisms that use *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* as recipient microorganisms is not necessary to address the risks associated with these microorganisms and would not result in any additional protection than would be achieved by this rule. This conclusion is based, in part, on EPA's findings regarding the intrinsically low level of hazard that these two organisms pose to human health and the environment. The requirements of the Tier I and Tier II exemptions and the restrictions in this rule on fermentation conditions place sufficient constraints to significantly limit the potential risks of injury to human health or the environment, including potential risks to potentially exposed or susceptible subpopulations under the conditions of use. In making this determination, EPA identified workers as a potentially exposed or susceptible subpopulation to the substance under the conditions of use and concluded that, with the limitations described above, the substances will not present an unreasonable risk of injury to health or the environment.

The Agency concludes that the criteria set forth in this rule are sufficient to mitigate the identified risks associated with these microorganisms. Because of the low hazard potential and safe history of use of *T. reesei* QM6a and

B. amyloliquefaciens subsp. *amyloliquefaciens,* EPA concludes that the TSCA section 5(h)(4) exemption will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk of injury to a potentially exposed or susceptible subpopulation under the conditions of use, provided that the other conditions of the exemptions at 40 CFR part 725, subpart G, relating to the introduced genetic material, and the physical containment of the new microorganisms, have been met.

2. *Costs.* As discussed in Unit X., this rule is anticipated to reduce costs to currently regulated entities in the long run. Expanding the list of recipient microorganisms eligible for exemption does not otherwise impose any additional cost or other burden on currently regulated entities, or existing fermentation processes.

Limiting the use of this exemption to the identified fermentation conditions is also estimated to impose no burden on affected entities. The restriction merely codifies existing industrial fermentation procedures for manufacturing operations that currently seek to use tiered exemptions. Consequently, EPA expects that most, if not all, manufacturers using these microbes would already have the measures in place to qualify for the exemption. Equally important, this limitation would add no burden to any existing fermentation processes. Currently, fermentation operations with either of these microbes are not eligible for the tiered exemption, and thus a MCAN must be submitted. Any company that chooses to use a different fermentation process could continue to operate under the status quo and simply submit a MCAN. This rule simply offers an additional, less costly option, to facilities that choose to use the fermentation operations discussed in this rule.

3. *Benefits.* The following discussion describes the benefits of expanding the list of recipient microorganisms eligible for exemption in a qualitative manner; for a more quantitative approach, see the economic analysis prepared for this rule (Ref. 18). A summary of that economic analysis is also provided in Unit IX.

The benefits analyzed encompass more than the direct benefits associated with submitting a Tier I or Tier II exemption for a new intergeneric microorganism rather than a MCAN. EPA's benefit analysis included a consideration of the broader benefits to society. EPA's unreasonable risk determination is based on broader benefits to society as well as those benefits attributable to a reduction in the burden associated with submission of Tier I and Tier II exemptions rather than MCANs.

EPA has concluded that manufacturers of new intergeneric microorganisms based on these low-risk microorganisms currently bear an unnecessary regulatory burden. By adding *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* to the list of eligible recipient microorganisms in 40 CFR 725.420, the Agency removes unnecessary regulatory impediments to the design, manufacture, and commercialization of these low risk new intergeneric microorganisms, and of the chemical substances that can be produced by these safer microorganisms. This action will also reduce the costs associated with industry's reporting burden, including the costs associated with the preparation of the submission, and with the delay in the commercial market introduction of the new intergeneric microorganism. Some of the cost-savings benefits may accrue to small businesses, either as developers of the exempt microorganisms, as producers of fermentation chemicals using the live microorganisms, or as customers for enzymes or other products made using the microorganisms.

There will also be a reduction in the Agency review resources currently allocated to reviews of MCANs for these two microorganisms. These Agency resources will be shifted to the review of new intergeneric microorganisms or chemical substances of greater concern.

The addition of the two microorganisms to the list of microorganisms eligible for exemption is expected to encourage innovation in the industry. It is reasonable to assume that a new intergeneric microorganism would either possess a new function or serve an existing function more efficiently or at a lower cost. The reduction in delay for that new intergeneric microorganism to be introduced into commerce is expected to be a benefit to both manufacturers and the general public who will have access to the substance more quickly. The expected benefits to innovation have not been quantified but include: Reduced time to develop and commercialize organisms; decreased cost of some downstream industrial products, such as fuel ethanol; improved consumer appeal of some products, such as certain textiles; and reduced costs of some consumer products, such as detergent and leather goods.

4. *Risk/benefit balance.* Determining the presence or absence of an unreasonable risk for purposes of issuing an exemption pursuant to TSCA section 5(h)(4) requires balancing of the benefits and risks posed by a regulatory action. EPA has determined that the risks are generally low based on the inherent properties and intended uses of *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* and will be adequately managed by the restrictions in the rule, combined with the existing requirements of the Tier I and Tier II exemptions.

EPA anticipates that expanding the list of microorganisms eligible for exemption will impose no costs and will reduce costs to currently regulated entities that use those recipients. The limitation to certain fermentation conditions is not a cost that will be imposed by this rule but rather a limitation on the amount of regulatory relief it will provide. The limitations on fermentation conditions reflect industrial fermentation procedures that are currently common practices for the affected industry.

EPA has also concluded that the benefits of the addition of *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* as recipient microorganisms to the list of recipient microorganisms at 40 CFR 725.420 are quite significant. This addition reduces the overall regulatory burden for affected entities by reducing the reporting requirements and by eliminating the delay of these products into commerce. The rule benefits both regulated entities and the general public by promoting the expedited manufacture and use of the chemical substances produced using these low-risk organisms and manufacturing processes. There is also the added benefit of concentrating limited EPA resources on regulation of chemical substances which have a greater potential to present significant risks, rather than on these two microorganisms. While this is difficult to quantify, it is considered substantial.

In sum, the criteria set forth in this exemption are sufficient to mitigate the low level of potential risks presented by these organisms, particularly when compared to the benefits, *in toto,* of this exemption, to levels that are consistent with the statutory standard for an exemption. Consequently, EPA has determined that adding *T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens* as recipient microorganisms to the list of recipient microorganisms at 40 CFR 725.420 is appropriate. The two microorganisms

will not present an unreasonable risk of injury to human health or the environment when manufactured under the conditions of this exemption.

IX. Economic Impacts

EPA's economic analysis (Ref. 18) evaluates the potential for significant economic impacts as a result of the addition of two microorganisms (*T. reesei* QM6a and *B. amyloliquefaciens* subsp. *amyloliquefaciens*) to 40 CFR 725.420, which lists recipient microorganisms eligible for Tier I and Tier II exemptions. Over the course of the first 10 years after the effective date of the final rule, EPA estimates that the addition of the two microorganisms to the list will generate a total cost savings to society of approximately $4.5 million. Industry is estimated save approximately $2.7 million and the Agency approximately $1.8 million. The equivalent, annualized cost savings to industry are expected to be $260,000 per year and $252,000 per year at a 3% and 7% discount rate, respectively. EPA estimates that there will be a net decrease in burden to industry of 27,864 hours over this 10-year period.

X. Scientific Standards, Evidence, and Available Information

EPA has used scientific information, technical procedures, measures, methods, protocols, methodologies, and models consistent with the best available science, as applicable. These sources supply information relevant to a determination that the microorganisms subject to this rule will not present an unreasonable risk of injury to health or the environment, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified by the Administrator under the conditions of use. The clarity and completeness of the data, assumptions, methods, quality assurance, and analyses employed are documented, as applicable and to the extent necessary for purposes of this rule, in Units V. through VIII. and in the references. The extent to which the various information, procedures, measures, methods, protocols, methodologies or models used in EPA's decision have been subject to independent verification or peer review is adequate to justify their use, collectively, in the record for this rule.

XI. References

The following is a listing of the documents that are specifically referenced in this document. The docket includes these documents and other information considered by EPA, including documents that are referenced within the documents that are in the docket, even if the referenced document is not physically located in the docket. For assistance in locating these other documents, please consult the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

1. Genencor International, Inc. Letter of Application to list *Trichoderma reesei* as exempt under subpart G of 40 CFR part 725—Reporting Requirements and Review Processes for Microorganisms. March 17, 2005.

2. Novo Nordisk BioChem North America, Inc. Letter of Application to list *Bacillus amyloliquefaciens* as exempt under subpart G of 40 CFR part 725—Reporting Requirements and Review Processes for Microorganisms. November 7, 1997.

3. EPA, OPPT. Email confirming Novo Nordisk BioChem North America, Inc.'s letter of application to list *Bacillus amyloliquefaciens* as exempt under subpart G of 40 CFR part 725—Reporting Requirements and Review Processes for Microorganisms. August 3, 2009.

4. US EPA. Microorganisms; General Exemptions from Reporting Requirements; Revisions to Recipient Organisms Eligible for Tier I and Tier II Exemptions; Proposed Rule. RIN 2070–AJ65; FRL–9348–1. 77 FR 54499, September 5, 2012. (''2012 Proposed Rule'').

5. EPA, OPPT. Risk Assessment of *Trichoderma reesei* for Consideration of Addition to the List of Eligible Recipient Microorganisms for the 5(h)(4) Exemptions from MCAN Reporting Requirements. October 2011.

6. EPA, OPPT. Risk Assessment of *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* for Consideration of Addition to the List of Eligible Recipient Microorganisms for the 5(h)(4) Exemptions from MCAN Reporting Requirements. July 2015.

7. Anonymous Public Comment, Document ID: EPA–HQ–OPPT–2011–0740–0015; October 23, 2012.

8. Richard Fitti, West Chester University of PA Comment, Document ID: EPA–HQ–OPPT–2011–0740–0017; November 5, 2012.

9. Anthony T. Pavel, General Counsel & Secretary, Enzyme Technical Association (ETA) and Rina Singh, Director of Policy, Science & Renewable Chemicals, Industrial and Environmental Section, Biotechnology Industry Organization (BIO) Comment, *Document ID EPA–HQ–OPPT–2011–0740–0016;* November 2012.

10. ETA. Supplemental information on *Trichoderma reesei.* January 29, 2010.

11. ETA. Supplemental information on *Trichoderma reesei.* June 16, 2011.

12. Nevalainen, H., P. Suominen, K. Tasimisto. 1994. On the safety of *Trichoderma reesei.* J. Biotechol. 37:193–200.

13. EPA, OPPT. Release and Exposure Assessment in Support of the TSCA Section 5(h)(4) Exemption for *Trichoderma reesei.* June 2011.

14. EPA, OPPT. Release and Exposure Assessment in Support of the TSCA Section 5(h)(4) Exemption for *Bacillus amyloliquefaciens.* June 2011.

15. Legislative History of the Toxic Substances Control Act, pp. 409–423. House Report 1341, 94th Congress, 2nd Session. 1976.

16. EPA. Microbial Products of Biotechnology; Proposed Regulation under the Toxic Substances Control Act. **Federal Register** (59 FR 45526; September 1, 1994) (FRL–4774–4).

17. EPA. Microbial Products of Biotechnology; Final Regulation under the Toxic Substances Control Act. **Federal Register** (62 FR 17910; April 11, 1997) (FRL–5577–2).

18. EPA, OPPT. Economic Analysis for the Final Biotechnology Exemptions Rule for *Trichoderma reesei* and *Bacillus amyloliquefaciens.* October 2019.

XII. Statutory and Executive Order Reviews

Additional information about these statutes and Executive Orders can be found at *http://www2.epa.gov/laws-regulations/laws-and-executive-orders.*

A. Executive Order 12866: Regulatory Planning and Review and Executive Order 13563: Improving Regulations and Regulatory Review

This action is not a significant regulatory action and was therefore not submitted to the Office of Management and Budget (OMB) for review under Executive Orders 12866 (58 FR 51735, October 4, 1993) and 13563 (76 FR 3821, January 21, 2011).

B. Executive Order 13771: Reducing Regulations and Controlling Regulatory Costs

This is considered a deregulatory action under Executive Order 13771 (82 FR 9339, February 3, 2017) because this rule is expected to provide meaningful burden reduction by adding *T. reesei* and *B. amyloliquefaciens* subspecies a*myloliquefaciens* to the list of recipient microorganisms that may be used to qualify for the Tier I and Tier II exemptions from full notification and reporting under TSCA for new microorganisms that are being manufactured for introduction into commerce. The rule is expected to generate cost savings for organizations that, in the absence of the rule, would submit MCANs for new intergeneric *T. reesei* or *B. amyloliquefaciens* strains. EPA estimates that the rule will result in cost savings for both industry and the Agency.

C. Paperwork Reduction Act (PRA)

This action does not impose any new information collection requirements or related burden that would require additional review or approval by OMB under the PRA, 44 U.S.C. 3501 *et seq.* The information collection activities associated with the submission of Tier 1 and Tier 2 notices under TSCA have already been approved by OMB

pursuant to the PRA and are covered by the following existing Information Collection Requests (ICRs): OMB control numbers 2070–0012 (EPA ICR No. 0574.15) and 2070–0038 (EPA ICR No. 1188.11). In granting these exemptions, this rule does not impose any new information collection requirements and is expected to reduce the amount of required reporting by allowing firms to submit less information for qualifying microorganisms. Over the ten-year period, industry is expected to subtract a total of 27,864 hours at an average of 2,786 hours per year.

D. Regulatory Flexibility Act (RFA)

Pursuant to section 605(b) of the RFA, 5 U.S.C. 601 *et seq.,* I certify that this final rule will not have a significant economic impact on a substantial number of small entities. In making this determination, EPA believes that the impact of concern is any adverse economic impact on small entities, and that EPA may certify that a rule will not have a significant economic impact on a substantial number of small entities if the rule relieves regulatory burden, has no net burden or otherwise has a positive economic effect on the small entities subject to the rule. This action establishes exemptions from existing requirements that apply regardless of the size of the entity. The factual basis for this certification is presented in the small entity impact analysis that was prepared as part of the Economic Analysis for this rule (Ref. 18) and is briefly summarized in Unit VIII.

E. Unfunded Mandates Reform Act (UMRA)

This action does not contain an unfunded mandate as described in UMRA, 2 U.S.C. 1531–1538, and does not significantly or uniquely affect small governments. This action is not expected to impose enforceable duty on any state, local or tribal governments, and the requirements imposed on the private sector are not expected to result in annual expenditures of $100 million or more for the private sector. As such, EPA has determined that the requirements of UMRA sections 202, 203, 204, or 205 do not apply to this action.

F. Executive Order 13132: Federalism

This action does not have federalism implications as specified in Executive Order 13132 (64 FR 43255, August 10, 1999). It will not have substantial direct effects on the states, on the relationship between the national government and the states, or on the distribution of power and responsibilities among the various levels of government. EPA has no information to indicate that any state or local government commercially manufactures or processes the microorganisms covered by this action. Thus, Executive Order 13132 does not apply to this action.

G. Executive Order 13175: Consultation and Coordination With Indian Tribal Governments

This action does not have tribal implications as specified in Executive Order 13175 (65 FR 67249, November 9, 2000). It will not have substantial direct effects on tribal governments, on the relationship between the Federal government and the Indian tribes, or on the distribution of power and responsibilities between the Federal government and Indian tribes. EPA has no information to indicate that any tribal government commercially manufactures or processes the microorganisms covered by this action. Thus, E.O. 13175 does not apply to this action.

H. Executive Order 13045: Protection of Children From Environmental Health Risks and Safety Risks

EPA interprets Executive Order 13045 (62 FR 19885, April 23, 1997), as applying only to those regulatory actions that concern health or safety risks, such that the analysis required under section 5–501 of Executive Order 13045 has the potential to influence the regulation. This action is not subject to Executive Order 13045 because it does not establish an environmental standard intended to mitigate health or safety risks.

I. Executive Order 13211: Actions That Significantly Affect Energy Supply, Distribution, or Use

This action is not a ''significant energy action'' as defined in Executive Order 13211 (66 FR 28355, May 22, 2001), because it is not a significant regulatory action under Executive Order 12866, and is not likely to have a significant adverse effect on energy supply, distribution, or use.

J. National Technology Transfer and Advancement Act (NTTAA)

Since this action does not involve any technical standards, NTTAA section 12(d), 15 U.S.C. 272 note, does not apply to this action.

K. Executive Order 12898: Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations

This action does not entail special considerations of environmental justice related issues as delineated by Executive Order 12898 (59 FR 7629, February 16, 1994), because it does not establish an environmental health or safety standard.

VII. Congressional Review Act (CRA)

This action is subject to the CRA, 5 U.S.C. 801 *et seq.,* and EPA will submit a rule report to each House of the Congress and to the Comptroller General of the United States. This action is not a ''major rule'' as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 725

Environmental protection, Administrative practice and procedure, Biotechnology, Chemicals, Hazardous substances, Imports, Labeling, Microorganisms, Occupational safety and health, Reporting and recordkeeping requirements.

Dated: March 4, 2020.

Alexandra Dapolito Dunn,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

Therefore, 40 CFR chapter I is amended as follows:

PART 725—[AMENDED]

■ 1. The authority citation for part 725 continues to read as follows:

Authority: 15 U.S.C. 2604, 2607, 2613, and 2625.

■ 2. In § 725.3, add in alphabetical order a definition for ''Submerged standard industrial fermentation'' to read as follows:

§ 725.3 Definitions.

* * * * *

Submerged standard industrial fermentation means a fermentation system that meets all of the following conditions:

(1) Enzyme production is conducted under conditions of submerged fermentation (*i.e.,* growth of the microorganism occurs beneath the surface of the liquid growth medium).

(2) Any fermentation of solid plant material or insoluble substrates, to which *T. reesei* fermentation broth is added after the submerged standard industrial fermentation operations used for enzyme production is completed, may be initiated only after the inactivation of the microorganism as delineated in 40 CFR 725.422(d).

* * * * *

■ 3. In § 725.420, add paragraphs (k) and (l) to read as follows:

§ 725.420 Recipient microorganisms.

* * * * *

(k) *Trichoderma reesei* strain QM6a and its derivatives used only in

submerged standard industrial fermentation operations as defined at 40 CFR 725.3.

(l) *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens.*

[FR Doc. 2020–04746 Filed 3–9–20; 8:45 am]

BILLING CODE 6560–50–P

FEDERAL COMMUNICATIONS COMMISSION

47 CFR Part 54

[WC Docket Nos.19–126, 10–90; FCC 20–5; FRS 16498]

Rural Digital Opportunity Fund, Connect America Fund

AGENCY: Federal Communications Commission.

ACTION: Final rule.

SUMMARY: In this document, the Federal Communications Commission (Commission) adopts the framework for the Rural Digital Opportunity Fund. The Rural Digital Opportunity Fund builds on the Connect America Fund (CAF) Phase II auction, which allocated funds to deploy networks serving more than 700,000 unserved rural homes and businesses across 45 states. The Rural Digital Opportunity Fund represents the Commission's single biggest step to close the digital divide and connect millions more rural homes and small businesses to high-speed broadband networks.

DATES: Effective April 9, 2020, except of §§ 54.313(e), 54.316(a)(8), (b)(5), (c)(1), 54.804 (a) through (c), and 54.806. The Commission will publish a document in the **Federal Register** announcing the effective date of those rules.

FOR FURTHER INFORMATION CONTACT: Alexander Minard, Wireline Competition Bureau, (202) 418–7400 or TTY: (202) 418–0484.

SUPPLEMENTARY INFORMATION: This is a summary of the Commission's Report and Order (Order) in WC Docket Nos. 19–126, 10–90; FCC 20–5, adopted on January 30, 2020 and released on February 7, 2020. The full text of this document is available for public inspection during regular business hours in the FCC Reference Center, Room CY–A257, 445 12th Street SW, Washington, DC 20554 or at the following internet address: *https://www.fcc.gov/document/fcc-launches-20-billion-rural-digital-opportunity-fund-0.*

I. Introduction

1. Bringing digital opportunity to Americans living on the wrong side of the digital divide continues to be the Federal Communication Commission's top priority. It is imperative that the Commission take prompt and expeditious action to deliver on its goal of connecting all Americans, no matter where they live and work. Without access to broadband, rural communities cannot connect to the digital economy and the opportunities for better education, employment, healthcare, and civic and social engagement it provides.

2. In recent years, the Commission has made tremendous strides toward its goal of making broadband available to all Americans. But while the digital divide is closing, more work remains to be done. Therefore, in the Order, the Commission adopts the framework for the Rural Digital Opportunity Fund. It builds on the successful model from 2018's CAF Phase II auction, which allocated $1.488 billion to deploy networks serving more than 700,000 unserved rural homes and businesses across 45 states. The Rural Digital Opportunity Fund represents the Commission's single biggest step to close the digital divide by providing up to $20.4 billion to connect millions more rural homes and small businesses to high-speed broadband networks. It will ensure that networks stand the test of time by prioritizing higher network speeds and lower latency, so that those benefitting from these networks will be able to use tomorrow's internet applications as well as today's.

II. Discussion

3. To ensure continued and rapid deployment of broadband networks to unserved Americans, the Commission establishes the Rural Digital Opportunity Fund, which will commit up to $20.4 billion over the next decade to support up to gigabit speed broadband networks in rural America. The Commission opts to allocate this funding through a multi-round, reverse, descending clock auction that favors faster services with lower latency and encourages intermodal competition in order to ensure that the greatest possible number of Americans will be connected to the best possible networks, all at a competitive cost. In light of the need to bring service both to consumers in areas wholly unserved by 25/3 Mbps, as well as those living in areas partially served, the Commission will assign funding in two phases: Phase I will target those areas that current data confirm are wholly unserved; and, Phase II will target unserved locations within areas that data demonstrates are only partially served, as well as any areas not won in Phase I. By relying on a two-phase process, the Commission can move expeditiously to commence an auction in 2020 for those areas it already knows with certainty are currently unserved, while also ensuring that other areas are not left behind by holding a second auction once the Commission has identified any additional unserved locations through improvements to its broadband deployment data collection.

4. The Rural Digital Opportunity Fund Phase I auction will make use of many of the rules that made the CAF Phase II auction a success, with some exceptions to account for the passage of time and other changed circumstances. Most importantly, in addition to the weighting of performance tiers and latency, the Commission will assign support in the auction's clearing round to the bidder with the lowest weight. After the auction, the Commission will require Phase I support recipients to offer the required voice and broadband service to all eligible homes and small businesses within the awarded areas, without regard to the number of locations identified by the Connect America Cost Model (CAM), and instead as determined subsequently by the Wireline Competition Bureau (the Bureau). This approach differs from that used in the CAF Phase II auction, which tied the deployment and service obligations to a specific number of locations within awarded areas but allowed the recipients to demonstrate that their obligations should be reduced (along with a corresponding reduction in support) where there were fewer locations than the CAM specified. As discussed in the following, the Commission will use its cost model and current data to establish initial service milestones and to monitor interim progress, but the Commission emphasizes that Phase I bidders will be competing for support amounts to offer service to *all* locations ultimately identified in an area, not just to the specific number of locations in that area identified prior to the auction, without adjusting awarded support amounts.

5. The Commission adopts a term of support of 10 years for the Rural Digital Opportunity Fund. The Commission believes that the stability of a 10-year term of support was partially responsible for the robust participation that occurred in the CAF Phase II auction. The Commission expects that the same principles regarding encouraging long-term investments and auction participation will also apply to the Rural Digital Opportunity Fund. Most commenters addressing this issue agree that a 10-year term of support will provide the certainty and stability needed to encourage broadband deployment in unserved and