Number: J-19-0001
TSCA Section 5(a)(3) Determination: The microorganism is not likely to present an unreasonable risk (5(a)(3)(C))

Chemical Name:
Specific: *Trichoderma reesei* 3CH-3

Conditions of Use (intended, known, or reasonably foreseen)[1]:
Intended use(s) (specific): Manufacture for use in the production of enzymes which will be used to saccharify agricultural waste to simple sugars, consistent with the manufacturing, processing, use, distribution, and disposal information described in the MCAN.
Known conditions of use: Applying such factors as described in footnote 1, EPA evaluated whether there are known conditions of use and found none.
Reasonably foreseen conditions of use: Applying such factors as described in footnote 1, EPA has identified manufacture, processing, or use of the new microorganism in a manner that could result in the production of paracelsin (e.g., proceeding to saccharification without first inactivating viable microorganisms in the fermentation broth) as reasonably foreseen based on the inclusion of such conditions in the initial MCAN submission; the submission was later amended to include an inactivation step before saccharification of agricultural waste.

Summary: The microorganism is not likely to present an unreasonable risk based on low human health hazard and low environmental hazard associated with the recipient microorganism and introduced genetic material under the intended conditions of use, as well as the terms of the proposed Significant New Use Rule (SNUR) signed by EPA.[2] The recipient microorganism,

[1] Under TSCA § 3(4), the term "conditions of use" means "the circumstances, as determined by the Administrator, under which a chemical substance (including an intergeneric microorganism) is intended, known, or reasonably foreseen to be manufactured, processed, distributed in commerce, used, or disposed of." In general, EPA considers the intended conditions of use of a new microorganism to be those identified in the section 5(a) notification. Known conditions of use include activities within the United States that result from manufacture that is exempt from MCAN submission requirements. Reasonably foreseen conditions of use are future circumstances, distinct from known or intended conditions of use, under which the Administrator expects the MCAN microorganism to be manufactured, processed, distributed, used, or disposed of. The identification of "reasonably foreseen" conditions of use will necessarily be a case-by-case determination and will be highly fact-specific. Reasonably foreseen conditions of use will not be based on hypotheticals or conjecture. Accordingly, EPA will apply its professional judgment, experience, and discretion when considering such factors as evidence of current use of the new microorganism outside the United States, evidence that the MCAN microorganism is sufficiently likely to be used for the same purposes as existing microorganisms that are similar, and conditions of use identified in an initial MCAN submission that the submitter omits in a revised MCAN. The sources EPA uses to identify reasonably foreseen conditions of use include searches of internal confidential EPA MCAN databases (containing use information on analogous microorganisms), other U.S. government public sources, and Internet searches.

[2] Reasonably foreseen conditions of use subject to a proposed SNUR are not likely to present an unreasonable risk of injury to health or the environment. Based on EPA's experience, it is the Agency's judgment that a new use would not commence during the pendency of a proposed SNUR because web posting of a proposed SNUR serves as the cut-off date for a significant new use. Therefore, manufacturers and processors would not commence a prohibited new use that would be legally required to cease upon the finalization of the SNUR. Once a SNUR is final and effective, no manufacturer or processor – including the MCAN submitter – may undertake the conditions of use identified as a significant new use of the new microorganism in the SNUR. EPA must first evaluate the new use in accordance with the requirements of TSCA Section 5 and (a) either conclude that the new use is not likely to present

Trichoderma reesei, is not pathogenic to humans or animals and has an extensive history of safe use. The introduced genetic modifications pose low concern for health and environmental hazard. EPA identified the production of paracelsin as a hazard. Paracelsin is a short polypeptide that is potentially toxic to humans and aquatic invertebrates. The MCAN describes conditions of use that mitigate the human health and environmental risks. Therefore, EPA concludes that the new microorganism is not likely to present unreasonable risk to human health or the environment under the intended conditions of use.

As set forth below, the information available to EPA is sufficient to permit the Agency to conduct a reasoned evaluation of the health and environmental effects of the new microorganism under the conditions of use that are not subject to the proposed SNUR, in order to determine that the new microorganism is not likely to present an unreasonable risk under those conditions of use. As such, EPA does not need to impose testing requirements to conduct this evaluation. Whether testing is needed to evaluate the effects of the intended, known, or reasonably foreseen conditions of use of a new microorganism subject to an MCAN is determined on a case-by-case basis. To the extent that testing may be necessary to conduct a reasoned evaluation of the health or environmental effects of the reasonably foreseen conditions of use that are subject to the proposed SNUR, EPA will make the appropriate determination if an MCAN is submitted for a significant new use following finalization of the SNUR.

EPA found no known conditions of use, assessed the intended conditions of use, and addressed reasonably foreseen conditions of use by proposing a SNUR. Therefore, EPA determines the new microorganism is not likely to present unreasonable risk to human health or the environment.

Human Health Hazard[3]: Human health hazard is relevant to whether a new microorganism is likely to present an unreasonable risk because the significance of the risk is dependent upon both the hazard (e.g., pathogenicity/toxicity) of the microorganism and the extent of exposure to the microorganism. EPA estimated the human health hazard of this microorganism based on data and information for the recipient strain as well as the genetic modifications. There is low concern for human health hazard for the microorganism based on the recipient strain not being a human pathogen and the introduced genetic material encoding a protein that is not expected to increase the potential for adverse human health effects. The only human health hazard EPA identified is the production of a peptide antibiotic (peptaibol) known as paracelsin. Peptaibols are associated with a wide variety of biological activities as they modify membranes, causing pore formation resulting in leakage from the cell. Peptaibols have been associated with cytotoxicity and neurotoxicity. Paracelsin is not known to be produced during standard submerged industrial

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an unreasonable risk under the conditions of use; or (b) take appropriate action under section 5(e) or 5(f). If EPA were not to finalize the proposed SNUR, then that decision would be based on information and data provided to the Agency during the comment period demonstrating that the reasonably foreseen conditions of use subject to the proposed SNUR are not likely to present an unreasonable risk. Under either scenario, the reasonably foreseen condition of use is not likely present an unreasonable risk.

[3] A microorganism is considered to have low human health hazard if it is not known to be a frank human pathogen that causes disease in healthy adults, and/or animal studies have demonstrated a lack of pathogenicity or toxicity; a microorganism is considered to have high human health hazard if there is evidence of adverse effects in humans or conclusive evidence of severe effects in animal studies. In the absence of animal data on a microorganism, EPA may use other data or information obtained through literature searches.

fermentation, but may be produced when *Trichoderma reesei* is grown outside of submerged media, or is introduced to solid plant material or insoluble substrates.

Environmental Hazard[4]: Environmental hazard is relevant to whether a new microorganism is likely to present unreasonable risks because the significance of the risk is dependent upon both the hazard (e.g., pathogenicity/toxicity) of the microorganism and the extent of exposure to the microorganism. EPA estimated the environmental hazard of this microorganism based on data for the recipient strain as well as information on the genetic modifications. There is low concern for environmental hazard for the microorganism based on the recipient strain not being an animal or plant pathogen and the introduced genetic material encoding a protein that is not expected to increase the potential for adverse effects on animals or plants. The only environmental hazards EPA identified are those associated with the production of paracelsin, which has been shown to interact with the cell membranes of aquatic invertebrates, resulting in cytotoxicity.

Exposure and Risk Characterization: The exposure to a new microorganism is potentially relevant to whether a new microorganism is likely to present unreasonable risks because the significance of the risk is dependent upon both the hazard (e.g., pathogenicity/toxicity) of the microorganism and the nature and extent of exposure to the substance.

EPA considers workers to be a potentially exposed or susceptible subpopulation (PESS) on the basis of greater exposure potential compared to the general population. EPA also considers PESS in conducting general population drinking water exposures by evaluating risks associated with water intake rates for multiple age groups, ranging from infants to adults. EPA considers consumers of specific products to be a potentially exposed or susceptible subpopulation on the basis of greater exposure potential compared to the general population who do not use specific products.

EPA estimated occupational and environmental exposures to the new microorganism. No consumer use was identified, so exposures to consumers were not assessed.

Under the intended conditions of use described in the amended MCAN, the new microorganism is not expected to produce paracelsin because paracelsin is not expected to be produced during the standard submerged industrial fermentation for enzyme production, and the viable microorganisms in the fermentation broth will be inactivated before the broth is used for saccharification. Because the only hazards identified by EPA were those resulting from the potential production of paracelsin, the new microorganism is not likely to present an unreasonable risk to human health or the environment under the intended conditions of use.

It is reasonably foreseen, based on information in the initial MCAN which was subsequently amended, that the new microorganism could be used for saccharification of agricultural waste without first inactivating the viable microorganisms in the fermentation broth. The SNUR that has been proposed for this microorganism defines certain conditions of use as significant new uses. The proposed significant new uses include any addition of fermentation broth containing

[4] A microorganism is considered to be of low ecological hazard if it is not known to be an animal or plant pathogen, and the genetic modifications do not impart pathogenic or toxigenic traits, and the introduced genetic material does not provide a selective growth advantage in outcompeting indigenous microbial communities in the environment.

viable *Trichoderma reesei* 3CH-3 cells to solid plant waste or other insoluble substrates prior to the completion of an inactivation step. Conditions of use that fall under the restrictions of the proposed SNUR are not likely to present unreasonable risk of injury to health or the environment because (1) those conditions of use are not likely to be commenced during the pendency of the proposed SNUR, and (2) upon finalization of the SNUR, those conditions of use would be prohibited unless and until EPA makes an affirmative determination that the significant new use is not likely to present an unreasonable risk or takes appropriate action under section 5(e) or 5(f).

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