Number: J-18-0041
TSCA Section 5(a)(3) Determination: Microorganism not likely to present an unreasonable risk (5(a)(3)(C))

Chemical Name:
Generic: *E. coli* K-12 C003: Arsenic detecting strain of *E. coli* with extra-chromosomal elements, including an intergeneric screening marker

Conditions of Use (intended, known, or reasonably foreseen)[1]:
Intended use(s) (specific): Imported in a cartridge containing 0.6 mL liquid suspension for use in a detection device for measuring arsenic in small water samples.
Known conditions of use: Applying such factors as described in footnote 1, EPA evaluated whether there are reasonably foreseen conditions of use and found none.
Reasonably foreseen conditions of use(s): Applying such factors as described in footnote 1, EPA evaluated whether there are reasonably foreseen conditions of use and found none.

Summary: The new microorganism is not likely to present an unreasonable risk of injury to health or the environment, without consideration of costs or other nonrisk factors, including an unreasonable risk to a potentially exposed or susceptible subpopulation identified as relevant by the Administrator under the conditions of use, based on the risk assessment presented below. The recipient strain is not pathogenic to humans or animals, and the introduced genetic modifications include an antibiotic resistance gene, $bla_{\text{TEM-1,}}$ which confers resistance to antibiotics of clinical importance. EPA concludes that the new microorganism is not likely to present an unreasonable risk under the conditions of use

Human Health Hazard[2]: 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

[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 chemical substance 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] 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.

the hazard (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 for the recipient parental strain as well as the genetic modifications. The recipient strain, derived from *E. coli* K-12, is well characterized and is not known to be a human pathogen. *E. coli* K-12 does not have the well-recognized pathogenic mechanisms found in the strains of *E. coli* which cause enteric infections, and it does not possess the O-antigen, a major virulence factor. The potential for the new microorganism to cause disease in humans is expected to be similar to the potential for the recipient strain to cause disease. The introduced genetic material does not increase the potential for pathogenicity or toxicity to humans over that of the recipient strain, but it does include an antibiotic resistance marker, $bla_{\text{TEM-1}}$, which confers resistance to β-lactam antibiotics including ampicillin, benzylpenicillin (penicillin G), and carbenicillin, which are critically important antibiotics according to the World Health Organization[3]. The use of this antibiotic resistance gene in the new microorganism could contribute to the loss of therapeutic value of these antibiotics if the resistance gene were to be horizontally transferred to a pathogen in the environment and if infections caused by those pathogens are treated with these antibiotics. The introduced plasmid which contains the antibiotic resistance marker does not encode mobilization or transfer factors related to plasmid mobility. The new microorganism maintains the plasmid in a stable manner, which was confirmed experimentally with data submitted with the MCAN which showed that the microorganism maintains the plasmid for at least sixteen generations without selection. The new microorganism lacks the fertility factor (F) which allows for genes to be transferred from it to other bacteria, but it could receive the F factor and subsequently transfer its genetic material, including the antibiotic resistance marker. Unless the microorganism is released in very large quantities, EPA expects that it will not contribute to the loss of therapeutic value of critically important antibiotics.

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 (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. EPA concludes that there is low environmental hazard for the microorganism. The recipient microorganism is not expected to be pathogenic to animals or plants, and the introduced genetic material does not result in increased survival, pathogenicity, or toxicity of the new microorganism, though it does include an antibiotic resistance marker as described above. Additionally, the new microorganism is not expected to survive if there were an inadvertent release to the environment.

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

[3] World Health Organization, Critically important antimicrobials for human medicine, 5th revision. 2017. Available at https://www.who.int/foodsafety/publications/antimicrobials-fifth/en.

[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.

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 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 the occupational exposures and environmental releases of the new microorganism using the 1997 Biotechnology Generic Scenario (https://www.epa.gov/tsca-screening-tools/using-predictive-methods-assess-exposure-and-fate-under-tsca) and information submitted in the MCAN. Under the conditions of use, EPA estimated negligible exposures to workers via all routes, as the new microorganism is imported in enclosed cartridges, and the microorganism is inactivated before the cartridges are disposed.

Data submitted in the MCAN indicated that the inactivation protocol will kill 100% of the new microorganisms, but EPA assessed a standard 6-log reduction (99.9999% inactivation efficiency) in assessing disposal via landfill. Under these conditions, environmental releases are negligible, so general population and environmental exposures are not expected and were not assessed. EPA considered worst-case scenarios, including cartridge breakage, and concluded that even under such conditions, dermal exposure would be very low (each cartridge contains only 0.6 mL media containing the new microorganism), inhalation exposure would be negligible, and environmental releases would be very small. Consumer exposures were not assessed because consumer uses were not identified as conditions of use.

The only identified hazard is that the new microorganism could contribute to the loss of therapeutic value of certain antibiotics if the resistance gene were to be horizontally transferred to a pathogen in the environment and if infections caused by those pathogens are treated with these antibiotics. Risks for this hazard were not identified, as releases and exposures are expected to be negligible under the conditions of use.

Given the low exposure to the new microorganism, EPA finds that it is not likely to present unreasonable risk to human health or the environment.

11/7/18
Date:

/s/
Jeffery T. Morris, Director
Office of Pollution Prevention and Toxics