EPA's Safer Choice Criteria for Microorganism-based Products

November 2024

Safer Choice's Microorganism Review Checklist (Appendix A) describes the basic information needed to assess the potential hazards of a microorganism, including methods for proper species identification, sources for a thorough human health and ecological effects literature search, and possible exposure patterns based on product use. Assessment of the risk, which is a function of both potential hazards and exposure, evaluates whether the microorganism is a potential pathogen to any of a broad spectrum of organisms (including humans, other mammals, avian species, aquatic vertebrates and invertebrates, plants, and others), whether there are any other adverse effects, and the likelihood of those effects that may result from exposure to this microorganism in the specific use of the product.

- Primary considerations for partnership: The risk assessment concludes that the microorganism is not pathogenic to any species with which it will come into contact and will not cause any other adverse human health or ecological effects (e.g., producing metabolites that are more toxic than the parent) in the specific use of the product. All non-microorganism ingredients must have an acceptable health and environmental profile (as per the Safer Choice and Design for the Environment [DfE] Standard).

Please note that Safer Choice typically partners with formulators of end-use products. For microbiological-based products, partnership preference is given to companies who manufacture the microorganism and formulate products for end use and thereby maintain maximum control over product formulation. Safer Choice may also partner with companies that incorporate a third-party's microorganism into their end-use product, provided that they are able to fully address all partnership elements.

Safer Choice will consider the following additional elements as part of its decision to offer partnership to a manufacturer of a microorganism-based product. These elements may be adapted or modified to fit the specific circumstances of the product review (e.g., microorganism type, intended use, and method of application) with special attention to the potential for human or environmental exposures. (Note: All elements not specifically addressed in the Partnership Agreement will be incorporated by reference.)

I. Consistency in Use of the Strain.

The manufacturer must commit to formulating with only those microorganism strains that were the subject of the risk assessment and Safer Choice review and agreed to in the Partnership Agreement. These strains must be identified through a rigorous taxonomic review (including but not limited to 16S rDNA or rRNA sequencing), which can be provided by a recognized full-service culture collection, whether or not the strain is part of the collection, or by other appropriate means. Such collections may be commercial or governmental (US or foreign) but should be listed with the World Federation for Culture Collections and must offer comprehensive identification services as one of its products. Alternatively, the strains may be identified by an established expert in the systematics of the organism used. The strain must not change without prior Safer Choice notification and review. The manufacturer may not substitute a strain of different species without first securing a third-party risk assessment and Safer Choice review and approval. The manufacturer may substitute another strain of the same species (e.g., a related wild-type or a more productive strain) following a careful evaluation of the taxonomic designation and a determination that a new risk assessment is not needed. Consistency in use of strain helps ensure reproducible, consistent formulations, reliable product performance, and a positive health and environmental profile.

II. Product Purity and Quality Assurance.

(A). Key Elements. Related to consistency of strain is product purity (i.e., measures taken to ensure that the product does not become contaminated with other microorganisms during the manufacturing or formulating process). The manufacturer must have quality assurance/control provisions to ensure product purity both during manufacture and any subsequent processing. An example of useful principles of quality assurance/control measures can be found in the test guidelines for microbial pesticides that the U.S. EPA Office of Pesticide Programs has issued (U.S. EPA Microbial Pesticide Test Guidelines OPPTS 885.1200 and 885.1300). While these guidelines are prescriptive and directed toward the specific needs of regulating microbial pesticides, the elements are informative of the kinds of considerations that can be employed in a quality assurance program for microorganism production. The pesticide guidelines include the following:

- A description of the basic manufacturing process, the starting and intermediate materials, and the steps taken to limit extraneous contamination, both chemical and biological;
- A theoretical discussion on the formation of unintentional components, including microbial contaminants, with a list of procedures to ensure the purity of unformulated products; and
- A demonstration that human or other animal pathogens are not present in the final product.

(B). Testing. The purity testing should occur on a periodic basis; at a minimum, the testing should occur at the time of product formulation and at a time that approximates the end of shelf life. Records of test results should be available to Safer Choice upon

request. Product purity is key to both safety and reliable product performance.

(C). Modifications to Formula. The product must not be modified in any way without providing prior notice to Safer Choice (as specified in the Partnership Agreement, Section 12). Water may be added by a licensed processor according to the manufacturer's purity specifications, incorporated by reference in the Partnership Agreement. The manufacturer must document its legal relationship with the processor.

(D). Product Containers. Manufacturers, and any downstream processors, must have quality assurance/control provisions to ensure that containers do not contaminate the formulation. Containers must meet the primary packaging criteria in Sections 4.2.5.1 through 4.2.5.3 of the Safer Choice and DfE Standard.

III. Functionality and Product Performance.

(E). Utility of Product Ingredients. The manufacturer must demonstrate that each ingredient contributes to product performance (with evidence of the efficacy of that performance) and would not compromise product purity in any way. Use of certain added fillers or carriers might contaminate the approved microbiological blend (i.e., add foreign bacteria) or otherwise interfere with performance (e.g., impede digestion of organic waste constituents, cause increased clogging of drainfield soils, pass through the system to the receiving environment, etc.).

(F). Product Performance. The manufacturer must provide performance testing that demonstrates performance that meets its users' needs. In its review criteria of chemical-based products, Safer Choice outlines several ways to demonstrate performance: by comparison testing with a market leading product, by using a standard test method (such as ASTM), or using a non-standard test protocol approved by Safer Choice in cases where standard methods are not available or not applicable.

Given the lack of standardized testing for biological-based products, a manufacturer must provide a literature reference that describes the functionally appropriate use of the relevant microorganism strains (e.g., certain pseudomonads degrade chlorinated solvents); alternatively, if there is not a literature reference, a manufacturer may use a non-standard method. An example of the latter might involve the lab scale application of microorganisms to media or substrates (e.g., sewage sludge), while simulating real-world conditions (temperature, time, oxygen levels).[1] A small-scale test offers several

[1] Performance Testing. A Safer Choice product should perform on a par with industry leading products. Assays should be designed to compare the certification candidate to a currently certified product or industry leader and to replicate use directions and, to the extent possible, real-world application conditions. The number and type of substrates tested will be left to the manufacturer's discretion, but test results should support any product performance claims. Degradation of the substrate (e.g., fats, oils, grease [FOG]) should be measured through an appropriate method, for example, respirometry that indicates oxygen consumption or evolved carbon dioxide. Representative photographs would provide helpful documentation to support quantified measurements at various stages of degradation, but are not required. In all cases, protocols should be submitted to and approved by Safer Choice before any testing

advantages: reproducibility, comparability among microorganisms, and affordability. Manufacturers of other products for a similar purpose would be held to a comparable product performance level.

(G). Shelf life. Shelf life should not exceed the period during which microorganisms are efficacious. The manufacturer or formulator must provide evidence that demonstrates product efficacy during the period of potential sale.

IV. Limitations on Product Eligibility

(H). Products for Use in Indoor Environments. Safer Choice is presently reviewing the appropriateness of indoor use of microorganism-based products. Until this review is complete, Safer Choice will not review or consider for partnership microorganism-based products intended for use on carpets, hard surfaces, or other indoor environments.[2]

(I). Septic System and Drain Line Applications, including any application where effluent may be released to a septic system or directly to the environment (e.g., holding ponds or lagoons; products for bioremediation would be an exception). Microorganism-based products for septic system, drain line, holding pond, or similar applications must contain only live or dormant (i.e., capable of germinating) microorganisms and water (limited use of Safer Choice-acceptable nutrients, stabilizers, additives, and colorants would be allowed) and no added emulsifiers (e.g., surfactants or added enzymes) or other ingredients that might interfere with microbial digestion of wastes and the proper functioning of the drainage system. Surfactants are poorly degradable in a tank's anaerobic environment. Many municipalities have prohibited the use of added emulsifiers in septic or drain line maintenance products for industrial or institutional applications (e.g., Corpus Christi, TX and Davidson County, TN).

V. The Partnership Agreement

(J). To obtain Safer Choice certification for a microorganism-based product, the manufacturer must comply with the above-listed information elements and enter into a Partnership Agreement with Safer Choice. The partnership agreement governs the relationship between EPA and its partner, the product manufacturer. It contains, among other elements, provisions covering the following: full ingredient disclosure;

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is performed.

Note on grease traps: Evidence should be provided that the degradation products would improve drain operations, for example, are of a more fluid consistency (i.e., less sticky) than the subject substrate.

[2] Safer Choice is exploring whether the use of microorganism-based products in these applications raises a concern for hypersensitivity pneumonitis (HP), a group of immunologically mediated lung diseases in which repeated exposures to finely dispersed antigens evoke a hypersensitive reaction resulting in granulomatous inflammations in the distal bronchioles and alveoli. (Note: HP has typically been considered an adult disease because of its association with occupational exposures, but it has been shown to occur in children from exposure to antigens in the home.) Repeated exposure to vegetative *Bacillus subtilis* cells and spores may result in hypersensitivity pneumonitis.

notification of changes in formula and the need for prior Safer Choice approval; the manufacturer's commitment to continuous environmental improvement; limitations; audit requirements; responsibilities regarding use of the Safer Choice certification and label; and partnership termination and opportunity for renewal. A sample Partnership Agreement is available in Annex A of the Safer Choice and DfE Standard.

(K). As a condition for certification and a provision of the Partnership Agreement, a manufacturer must agree to include on product labels and literature, the following statement: "Product contains live microorganisms."

(L). Based on the increasing incidence of microbial resistance to antibiotics, the manufacturer must test the microorganism(s) in its certified product(s) for resistance to a representative set of antibiotics, as specified by Safer Choice.

Appendix A. EPA's Microorganism Review Checklist

Note: This document is principally derived from "Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms," US EPA/OPPT, 6/2/97, available on the web at http://www.epa.gov/biotech_rule/pubs/pdf/ptcbio.pdf. The document also includes points from Environment Canada's "Guidelines for the Notification and Testing of New Substances," Sec. 4: Technical Information Requirements.

The following information will help Safer Choice assess the human health and environmental profile of your product. For certain microorganisms and uses (i.e., those that have been well characterized and present minimal potential for exposure), Safer Choice may only need information from Parts I, II, and IV.

I. Manufacture and Use

_____ Company Status

- Are you a manufacturer or blender (or both) of microbiological products?
- If you are a blender, who is your microorganism supplier?

As with chemical toxicological assessments, potential for harm from microorganisms is a function of both inherent characteristics and dose (i.e., number of cells or colony forming units [CFUs]) to which another living thing is exposed. Information in this section helps define potential exposure pathways and the magnitude of exposure.

_____ Uses

- Describe the intended use(s) of the microorganism (e.g., drain maintenance; fats, oils, grease [FOG] degradation; hydrocarbon remediation; etc.) or products the microorganism is intended to produce (e.g., enzymes for detergents). Provide commercial product name for each use, if available. Also, list past and potential future uses for the microorganism or microbial product.
- Provide a general description of the locations of the application (e.g., hazardous waste sites, grease traps, industrial wastewaters, etc).
- Describe the method of application or use and quantity, frequency, and duration of application (e.g., product label instructions); potential for human contact or unintended release (include, if possible, the number of persons that may be exposed and the degree of exposure).
- Describe the mechanisms of dispersal of the microorganism and modes of interaction with any dispersal agents.

- Describe the known mode of action in relation to the intended use(s).

- Describe the enzymes produced by the microorganism(s) for the intended uses, and whether the enzymes are produced intracellularly or extracellular by the microorganism(s).

II. Microorganism Identity

All formulators using microbiological ingredients should know with as much certainty as possible the identity of the microorganism(s) in their products.

Step one in assessing the potential hazard of a microorganism is to verify its identity. Since there are no universally applicable methods for identifying microorganisms, expert judgment and experience must inform this process and guide the pairing of microorganism with test method, as necessary, to increase identification confidence levels.

The taxonomy of microorganisms is undergoing rapid change, and this makes even more difficult the complex task of properly identifying microorganisms. EPA experience has revealed that attempts at shortcuts to microbial identification often yield equivocal results. Recognizing the difficulties likely to be encountered and considering the central role of product identification in permitting a meaningful review, EPA will work with applicants to ensure that appropriate identification information is obtained and provided to the Agency.

The following is general guidance for this process. The applicant may need to expect that this will be an iterative process, with EPA supplying suggestions, where available, for specific methods or approaches tailored to the specific organisms proposed by the applicant.

_____ Taxonomic designation, including strain (if possible)

- If the microorganism is a strain identified by a national service culture collection, provide appropriate documentation (e.g., collection number and product information sheet; purchase order; any testing used in identification; etc).

- If the microorganism is not obtained from a national service culture collection, verify the taxonomic designation by providing one of the following: 1) a letter from a source culture collection that describes how the identification was performed, including available test data and literature references, or 2) results of tests to determine characteristics (e.g., commercial methods such as Biolog/Microlog, API 20NE Rapid NFT; research methods such as GC-FAME, 16S rDNA sequencing; or specific tests unique to the supplier of the identification) and a statement addressing who performed the tests (i.e., the submitter, a commercial

service, a consultant, etc.). Also, for species that are difficult to identify, a specific description of the methods used to distinguish and detect the microorganism.

- Synonyms, common names, and superseded names.

- Identification of any patent or application for a patent.

Depending on the species/strain type, the Agency may recommend particular methods or approaches that have been found to provide species identification with better degrees of confidence. All test reports should include confidence levels, name of lab, date of testing, etc.

Additional Characterization

- Life cycle

- Growth characteristics: Generation time, growth temperature (optimum and range), pH, oxygen requirements, and preferred energy and carbon sources.

- Factors affecting growth, survival, or reproduction (e.g., sporulation, encystment, other non-vegetative growth stages, ability to exist in the viable but non-culturable [VNBC] state, auxotrophy, etc.).

- Resistance to antibiotics and tolerance to metals and pesticides.

II. Potential Human Health and Environmental Effects

Microorganisms, like individual chemicals, vary greatly in the degree to which they have been characterized toxicologically. Depending on microorganism type and product application, the following information may be pertinent in developing a human health and environmental profile and assessing the hazards the microorganism may pose.

Note that in most cases the information listed below (especially on human health effects) will be available in microbiology texts or from literature/database sources (see for example: Current Contents, Medline, Biosis, Science Citation Index, Agricola, Enviroline, TOXNET, Biotechnology Citation Index, Current Biotechnology Abstracts, Current Research Information System [CRIS]).

The search should provide information for a thorough overview of the requested information. If most of this information is available in recent reports, a search of the literature dating back a number of years may not be necessary. Where recent reports are unavailable, inconclusive or contradictory, a more extensive search over a longer time period may be needed. The literature search report should indicate the time period of the search, the information sources, title of published papers, and search strategy, including search terms.

Whenever possible, the information should be provided for the specific organism in your formulation. Where there is little information available on this organism, information on a surrogate organism may be substituted (please consult with Safer Choice on the choice of a suitable surrogate). When there is no relevant information from the scientific literature or unpublished studies for items pertaining to human health effects or ecological hazards, laboratory tests may be required.

_____Human Health

- Nature and degree of pathogenicity (including the capacity to act as an opportunistic pathogen), virulence, or infectivity in humans.
- Nature and degree of toxigenicity and toxicity (host tissue damage) to humans.
- Nature and degree of allergenic or immunological responses in humans after exposure via ingestion, inhalation, or dermal contact.
- Potential host range of the microorganism(s), infective dose, routes of transmission, and normal reservoir for the microorganism(s).
- Ability to colonize humans or grow at body temperature.
- Susceptibility to control measures, e.g., antibiotics or disinfectants.

_____Ecological

- Nature and degree of pathogenicity, virulence, or infectivity to mammals, fish, insects, other invertebrates, and to plants, including host range. Include test data that supports potential effects.
- Toxicity of microbially produced toxins and toxigenicity to mammals, fish, insects, other invertebrates, and to plants.
- Identification of plant and animal species likely to be exposed and, where infectivity, pathogenicity, toxicity, or toxigenicity to non-human organisms has been identified, the identification of the receptor species likely to be exposed.
- Potential for gene transfer to other microorganisms of traits for pathogenicity, infectivity, toxicity, toxigenicity to non-human species or of antibiotic resistance.
- Potential for causing adverse effects on mammals, fish, insects, other invertebrates, and plants, indirectly through means such as, but not limited to, changes in the availability of nutrients, changes in the solubility or oxidation states of metals, creation of anoxic conditions in surface waters, etc. Involvement in or effects on biogeochemical processes (e.g., effects on nutrient cycling,

particularly C, N, P, and S; effects on primary production [CO_2 fixation]; utilization of complex substrates, such as cellulose and lignin degradation; effects on nitrogen fixation and nitrification).

- Known or predicted effects on other organisms, including microorganisms in the environment and effects on competitors, prey, hosts, symbionts, predators, parasites, pathogens, community structure, and species diversity.

- Known or expected substrate range of degradative gene protein products, including both contaminant compounds to be bioremediated and environmental substrates (e.g., lignin) on which gene protein products may also act.

- Known or expected metabolic pathways of both target xenobiotic compounds and other contaminants present during degradation.

- Nature and degree of toxicity of metabolites (dead-end or intermediate products resulting from degradation of the target compound) to mammals, fish, insects, other invertebrates, and to plants.

Survival and Environmental Fate

- Natural habitats and prevalence of the microorganism(s) in the environment, including a description of habitats where the microorganism may persist or proliferate.

- Habitat at the potential locations of introduction, and the nature of selection pressure that may operate on the microorganism at these locations.

- Survival/persistence in in the environmental media (e.g., water, soil, air) into which the microorganism(s) is to be introduced, including an estimate of the quantities of the microorganism in those media at the points of introduction and the estimated population trends.

- Survival/persistence in environments other than the intended introduction site (surface water, ground water, other soils) into which the microorganisms may disseminate.

- Known and predicted environmental conditions that may affect survival, multiplication, etc.

- Method of detection and detection limits.
- Prevalence of natural gene exchange among the microorganism(s) and natural microbial populations.

III. Measuring the Health/Environmental Benefits

In determining whether a microorganism has a more positive health and environmental profile or contributes to a formulation with these characteristics, it is important to understand the formulatory context, in other words, what were the choices, among microorganisms or chemical ingredients, in formulating a particular product. Whether a product receives Safer Choice certification depends on the microorganism, its characteristics, product application, and comparison to what might be used in instead.

Once a more positive profile is established, it is important to ensure that a product maintains that profile (i.e., that its microorganism make-up remains consistent and free of harmful contaminants).

Comparing Ingredients

- What other microorganisms might be substituted for your microorganism for each of the uses listed in Part III B (page 3 of this document)?

- Does your product replace a microorganism- or chemical-based product? If so, what ingredients does the microorganism- or chemical-based product contain?

Quality Assurance/Quality Control

- For each production lot or batch, verification of absence of pathogenic microorganisms as contaminants. Species of concern include not only frank pathogens, but certain opportunistic microorganisms such as, but not limited to, *Pseudomonas aeruginosa* and *Burkholderia cepacia.*

- For each production lot or batch, verification of (certificate of analysis) consistency of total bacterial counts or cell density (CFU/ml); activity of microorganisms/efficacy of product; concentration of surfactants/emulsifiers; and concentration of free enzymes; concentration of other ingredients.

- Shelf-life of product.