EPA's Safer Choice Checklist for Formulations Containing Microorganisms

(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. 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/Safer Choice 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/Safer Choice 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, 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.

III. 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 (esp. 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, including 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.

IV. 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 recognition depends on the microorganism, its characteristics, product application, and comparison to what might be used in its stead.

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?

- 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.

Thank you for your help in understanding and improving the health and environmental profile of your formulations.