Risk Assessment of Genetically Engineered and Synthetic Biology Microorganisms by EPA under the Toxic Substances Control Act

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Risk evaluation is an important societal aspect of synthetic biology, as the public expects that new technologies, and the products of those technologies, will be safety introduced into society. Under the Toxic Substances Control Act (TSCA) implementation of *the Coordinated Framework for Regulation of Biotechnology*, the U.S. Environmental Protection Agency (EPA) is responsible for assessing the risk of certain genetically engineered microorganisms (GEMs) that are "intergeneric," meaning that they are formed by the deliberate introduction of genetic material from a genus different from that of the recipient microorganism. The term *intergeneric* can include those GEMs created through the use of synthetic biology techniques if the introduced genetic material is not identical to that which could be found in the genus of the recipient. GEMs falling under TSCA's jurisdiction may be used for a variety of purposes including, but not limited to: fuel production, biomass conversion, waste treatment, biofertilizers, biomining, bioremediation, biosensors, microbially enhanced oil recovery, desulfurization of fossil fuels, and closed system fermentation for the production of enzymes and commodity or specialty chemicals.

EPA evaluates the risk posed by a use of a GEM under the paradigm of Risk = Hazard x Exposure. Because EPA under TSCA is responsible for premanufacturing review of GEMs, its analyses are necessarily predictive and need data and tools to perform those predictions. Because premanufacture notifications typically do not come with sufficient data to assess the substance, EPA must necessarily use indirect data, such as literature on the recipient and/or analog microorganisms, to predict how the prospective product organisms might behave under a wide range of circumstances.

In conducting its GEM risk assessments, EPA details the genetic modifications to the recipient organism and identifies the original donor organisms. The hazard assessment evaluates potential hazards posed by the introduced genetic material and includes an evaluation of the potential for horizontal gene transfer of this genetic material to other microorganisms in the environment. For human health, the assessment evaluates the potential pathogenicity/toxicity and allergenicity of the GEM. For ecosystems, it examines the potential for pathogenicity/toxicity to terrestrial and aquatic animals and plants. Potential hazards also include interactions of the GEM with other microorganisms in the environment and its effects on environmental processes such as biogeochemical cycling.

EPA's estimate of exposure considers production volumes and evaluates exposure of the GEM to workers, and releases of the GEM from production facilities or during field testing if the use anticipates an intentional environmental release. In addition, release estimates are used to estimate inhalation and drinking water exposures of the GEM to the general human population and exposures to the environment.

Synthetic biology may lead to novel microorganisms that make risk assessment challenging. Synthetic biology is usually thought of as a continuum from the use of just one or several chemically synthetized genes to extreme metabolic engineering to extreme novelty, such as the creation of organisms with alternate codon usage, or organisms with xenonucleic acids. If the

traits of a microorganism are radically changed from those of the recipient strain, comparison to the parental strain may no longer be appropriate.

In the absence of comparator microorganisms such as the wild-type, it may not be possible to predict the behavior of newly developed synthetic biology microorganisms in the environment and their interactions with other microorganisms. Likewise, there may be unknown effects on terrestrial and aquatic flora and fauna that cannot be predicted.

Completely synthesized genomes are another example of when there is no comparison to a wild-type organism. Even alterations in the cell wall or cell membrane of a microorganism for some useful purpose through synthetic biology techniques may make risk assessment difficult.

These issues create the need for additional research to support GEM risk assessment. They include the following:

- There is a need for extensive testing of some types of synthetic biology microorganisms before they are released into the environment. Research is needed to develop testing approaches that are inexpensive, reliable/reproducible, and generalizable over broad categories of GEMs.
- Microorganisms are typically considered to be sensitizing agents. Unfamiliar cell wall or membrane proteins may change their allergenic potential, and research is needed to develop methods for evaluating the allergenicity potential of GEMs.
- Comparison of orthogonal life to wild-type organisms cannot be made. Although the design of an organism by replacement of all copies of a particular codon with a different codon not found in naturally occurring microorganisms may be an intentional biocontainment strategy, research is needed to determine the stability of this strategy and whether reversion to naturally occurring codons can occur, particularly with DNA repair mechanisms inherent within the cell, or even uptake of naked DNA from the environment.
- The possibility for transfer and incorporation of xenonucleic acids into naturally occurring microorganisms and what effects may result in these microbial cells also needs investigation.
- Likewise, research is needed on the stability of use of xenonucleic acids as a biocontainment measure.