Regulation of Genetically Engineered Microorganisms under the Toxic Substances Control Act

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Toxic Substances Control Act (TSCA)

- Enacted in 1976 – amended by the Frank R. Lautenburg Chemical Safety for the 21st Century Act (June 22, 2016)
- Under TSCA, EPA has authority to regulate the manufacture, use, distribution in commerce, and disposal of chemical substances and mixtures used for commercial purposes
- Covers chemical substances (industrial, environmental, or consumer products) not specifically excluded

Exclusions from TSCA

- Food, food additives
- Drugs
- Cosmetics
- Medical devices
- Pesticides (but not pesticide intermediates)
- Tobacco
- Nuclear material
- Firearms

History of Genetically Engineered Microorganisms (GEMs) and TSCA

- EPA issued 1984 Policy Statement and final Policy Statement in 1986 as part of the Federal "Coordinated Framework for the Regulation of Biotechnology" (Office Science Technology & Policy)
- 1986 "Coordinated Framework" (CF) specified lead agencies (FDA, USDA, EPA, OSHA, NIH) for regulation of various biotechnology products – Updated in 2017
- Proposed Microbial Biotechnology Rule 1994
- Final Rule: Microbial Products of Biotechnology 1997

TSCA Biotechnology Rule 1997

- Rule established procedures for premanufacture review of "new" microorganisms
- Rule retained EPA's interpretation of "new" (intergeneric) microorganisms as stated in the 1986 Coordinated Framework Policy Statement
- Applies to "new" microorganisms that are manufactured, imported, or processed for commercial activities, including R&D activities
- All requirements and procedures consolidated into one part of the CFR (40 CFR 725)

TSCA Inventory of Chemical Substances

Microorganisms subject to TSCA are treated as chemical substances. The rules for determining whether a substance is "new" or "existing" are:

- **An "existing" chemical substance is one that is listed on the TSCA Inventory**
- **A "new" chemical substance is one that does not appear on the TSCA Inventory**

"New" Microorganisms Subject to TSCA Section 5

New Microorganism = "intergeneric"

- **Microorganism formed by the deliberate combination of genetic material from organisms classified in different taxonomic genera**
- **Microorganism constructed with synthetic genes that are not identical to DNA that would be derived from the same genus as the recipient**
- **Not on the TSCA Inventory**
- **Used in TSCA applications**

Microorganisms Excluded from TSCA Reporting Requirements

- Naturally occurring microorganisms – implicitly listed on the TSCA inventory
- Intrageneric – those formed by the introduction of genetic material from organisms within the same genus
- Those containing only well-characterized, non-coding regulatory sequences

Definition of Microorganism

- Defined according to the 5-kingdom system of Whittaker (1969)
- Monera (or Procaryotae), Protista, Fungi, Chlorophyta and Rhodophyta of the Plantae, and viruses and virus-like particles
- Includes, but is not limited to, bacteria, protozoa, fungi, mycoplasmas, mycoplasma-like organisms, spiroplasmas, microphytoplanktons, green and red algae, viruses, and virus-like particles

TSCA Applications/Uses

- closed system fermentation – enzymes, specialty, and commodity chemicals
- fuel production
- biomass conversion
- waste treatment
- biofertilizers
- bioremediation
- biomining
- biosensors
- oil recovery

TSCA is a catch-all statute for biotechnology products not regulated by other Agencies

Submissions – Reporting Mechanisms

Microbial Commercial Activity Notice (MCAN)

- Any manufacturer, importer, or processor must file a Microbial Commercial Activity Notice at least 90 days prior to initiating manufacture/import (unless eligible for an exemption)

TSCA Experimental Release Application (TERA)

- Persons who wish to introduce a new microorganism into the environment, including those at the R&D stage if deemed commercial R&D, must submit a TERA 60 days prior to initiation of the field test

Exemptions from TSCA Section 5 Reporting Requirements (§ 725.110)

(a) Research & Development (Subpart E)

- § 725.232 – Activities subject to the jurisdiction of another Federal Agency (e.g., subject to the NIH Guidelines)
- § 725.234 – Activities conducted inside a structure
- § 725.238 – Activities conducted outside a structure using certain microorganisms specified at § 725.239 (*Bradyrhizobium japonicum* and *Rhizobium meliloti* with specific traits)

(b) Test Marketing Exemption (TME) § 725.300

(c) Tier I or Tier II Exemption § 725.420

Exemption from MCAN Reporting Requirements for Closed System Fermentation

Tier I and Tier II Exemptions (§ 725.420)

- Use of certain eligible recipient microorganisms
- Meet four criteria for the introduced genetic material
- Meet specific containment & inactivation criteria

Eligible Recipient Microorganisms for the Tiered Exemptions

Industry "workhorse" microorganisms with a long history of safe use

- *Acetobacter aceti*
- *Bacillus licheniformis*
- *Bacillus subtilis*
- *Clostridium acetobutylicum*
- *Escherichia coli* K-12
- *Aspergillus niger*
- *Aspergillus oryzae*
- *Penicillium roqueforti*
- *Saccharomyces cerevisiae*
- *Saccharomyces uvarum*

Additions to the list of eligible recipients in the 2020 Rule

- *Trichoderma reesei* (QM6a and derivatives)
- *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens*

Tier I and Tier II Exemptions Introduced Genetic Material

- Limited in size (structural genes of interest, regulatory sequences to control their expression, & associated sequences for movement or transfer)
- Well-characterized (function of all gene products, regulatory sequences, and associated sequences known)
- Poorly mobilizable (frequency of transfer of less than 10^{-8} transfer events per recipient)
- Free of certain sequences (must not contain a functional portion of any of the toxin-encoding sequences listed)

Tier I and Tier II Exemptions
Containment & Inactivation Criteria

Tier I

- Minimize aerosol releases
- 6-log reduction of viable cells in liquid and solid waste streams

'Postcard' submission – Minimal information notification to EPA that complying with the criteria of the Tier I Exemption

Tier II Exemption Containment & Inactivation Criteria

Tier II Exemption – does not meet the containment and inactivation requirements of the Tier I Exemption (for an individual facility only) – so transport to various facilities is not within the realm of the Tier I Exemption

- **Requires a submission to EPA for an evaluation of whether the proposed containment & inactivation conditions are appropriate for the microorganism(s)**
- **45-day review**

Commercial R&D

TSCA Section 5 - applies to microorganisms that are manufactured, imported, or processed for commercial purposes. EPA has defined manufacture or process for commercial purposes as "manufacture or process for purposes of obtaining an immediate or eventual commercial advantage" as determined by indicia of commercial intent.

Commercial R&D – means that the activities are conducted with the purpose of obtaining an immediate or eventual commercial advantage

- **R&D funded directly by a commercial entity regardless of who is actually conducting the research**
- **R&D not funded directly by a commercial entity, but the researcher intends to obtain an immediate or eventual commercial advantage**

Research and Development Exemption in a Contained Structure (§ 725.234)

A person who manufactures, imports, or processes a microorganism is not subject to reporting requirements if:

- **The microorganism is solely for research and development activities**
- **The microorganism is used by, or directly under the supervision of, a technically qualified individual (TQI) defined in § 725.3. The TQI must maintain documentation of the procedures selected to ensure compliance.**
- **There is no intentional testing of a microorganism outside of a structure**
- **There are containment and/or inactivation controls**

Structure - means a building or vessel which effectively surrounds and encloses the microorganism and includes features designed to restrict the microorganism from leaving

Risk Assessment

Risk = Hazard x Exposure

- **Taxonomic Identification Report**
- **Genetic Construction Report** - genetic modifications
- **Human Health Hazard Assessment** - including potentially exposed and susceptible subpopulations
- **Ecological Hazard Assessment** - pathogenicity to plants & animals, microbial interactions
- **Construct Hazard Analysis** - inserted genes, horizontal gene transfer (HGT)
- **Engineering Report** - use, worker exposure, releases to the environment
- **Exposure Assessment** - consumer, general population, and environmental exposures

OPPT Biotechnology Guidance Documents

Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms

- https://www.epa.gov/sites/default/files/2015-08/documents/biotech_points_to_consider.pdf

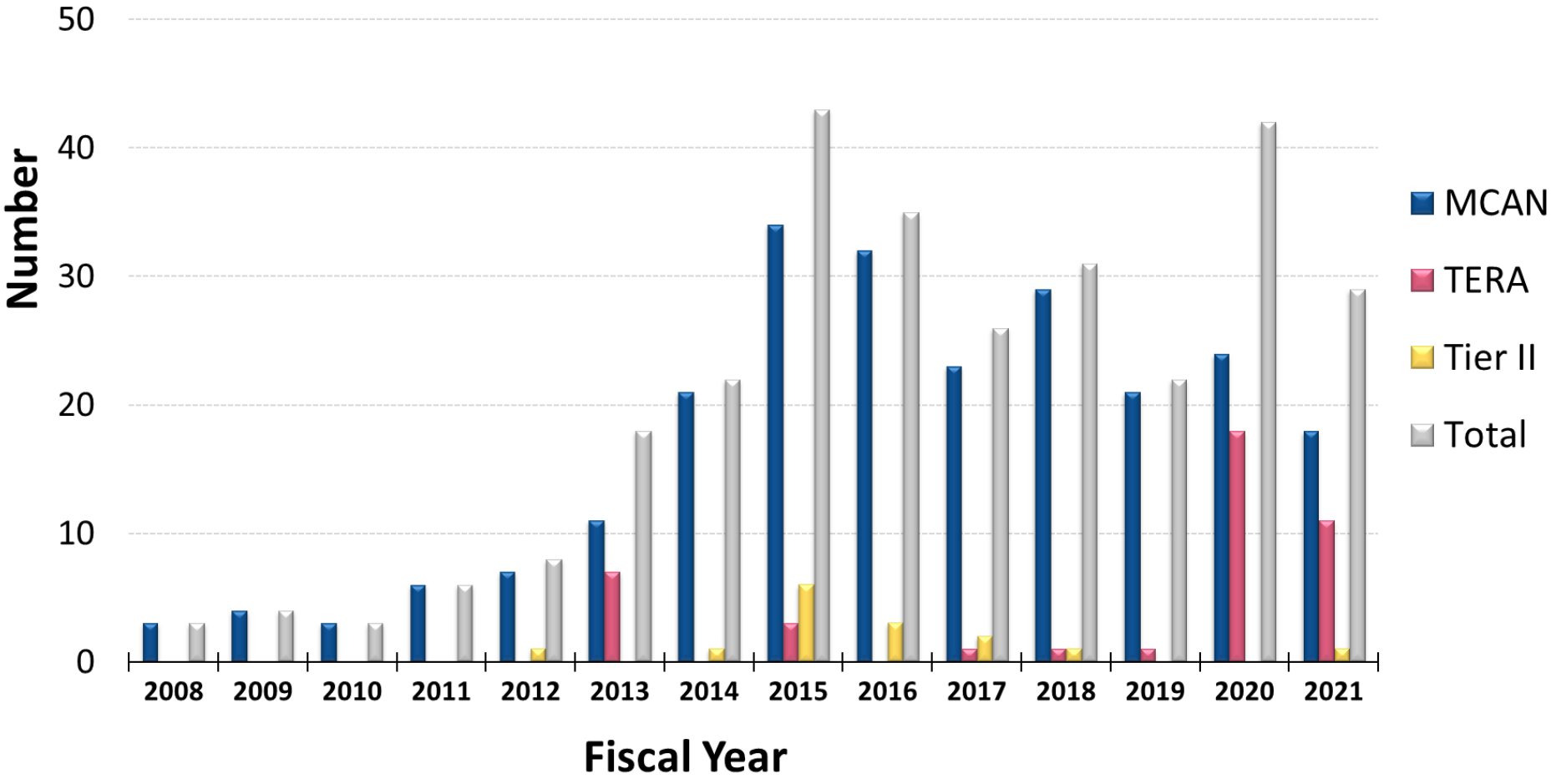
Algae Supplement to the Points to Consider

- https://www.epa.gov/sites/default/files/2020-10/documents/algae_supplement_091420.pdf

§ 725.155 Information to be included in the MCAN

§ 725.255 Information to be included in the TERA

Intergeneric Strains Reviewed



Synthetic Biology

- **Single Gene Modifications**
- **Chemically synthesized sequence to alter codon usage to fit preference of recipient microorganism (even a native gene) – considered "new" because not identical to that in the recipient**
- **Metabolic Engineering (ME) - Replace parts of, or entire pathways**

Biological Containment

- Engineered auxotrophy
- Kill switches

Orthogonal life

- Xenonucleic acids (XNA)
- Orthogonal protein translation
  - Synthetic codons
  - Synthetic tRNAs
  - Synthetic ribosomes
- Noncanonical amino acids

Minimal Genomes and Synthetic Genomes

Chemically synthesized genomes

- Recoded Organisms - e.g., *E. coli* - Multiplex Automated Genome Engineering/Conjugative Assembly Genome Engineering (MAGE/CAGE)
- The Venter Institute *Mycoplasma mycoides* synthesized genome
- Re-engineering whole genomes
    - The *S. cerevisiae* Sc 2.0 Build-a-genome (BAG) project

Use of biological parts (e.g., BioBricks)

Genome Editing

- TALENS (transcription activator-like effector nucleases)
- ZFNs (zinc finger nucleases)
- CRISPR-Cas (clustered regularly interspaced short palindromic repeats – Cas nucleases)

A microbial biotechnology product created using these genome editing techniques would not be regulated under Section 5 of TSCA unless intergeneric genetic material was introduced ("knock-in")

Office of Pollution Prevention and Toxics Biotechnology Program

Website: https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/overview-biotechnology-under-tsca

For questions regarding TSCA reporting requirements or pre-notice consultations:

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