U.S. and International Regulation of Microbial Inoculants and Soil Additives

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Overview of Presentation

- U.S. EPA regulation of modified microorganisms under the Toxic Substances Control Act (TSCA).
  - *Regulatory program that might govern testing and uses of certain modified soil microorganisms.*
- Overview of international regulations affecting uses of microorganisms in agriculture.
  - *Regulatory regimes exist in most countries under which approvals likely needed for field tests of modified soil microorganisms.*

U.S. REGULATION OF AGRICULTURAL BIOTECHNOLOGY

Overview of U.S. Biotechnology Regulation

Environmental Protection Agency

- Microbial pesticides, plant pesticides. (FIFRA)
- Engineered microorganisms used for other industrial purposes. (TSCA)

U.S. Department of Agriculture

- Transgenic plants, potential plant pests.
- Plant-produced industrial products.

Food and Drug Administration

- Foods, food additives, pharmaceuticals, cosmetics.
- Animal drugs, foods and food additives

History and Scope of TSCA Biotechnology Regulation

- **Toxic Substances Control Act of 1976 (TSCA)**: "Gap-filling" statute to cover chemicals not regulated elsewhere in the government.
- In the 1986 Biotechnology "Coordinated Framework", it was decided to use TSCA to regulate uses of modified microorganisms not regulated by other federal agencies.
- Interim policy took effect in 1986, but final regulations (40 CFR Part 725) not issued until 1997.

Industrial Applications Subject to TSCA Jurisdiction

TSCA Biotechnology Rule covers uses of “new microorganisms” used for the following purposes.

- Nonpesticidal agricultural microorganisms.
  - Biofertilizers: e.g. microorganisms for improved nitrogen fixation
  - Biostimulants (if not regulated as pesticides)
  - Possible overlap with USDA regulations, “*if plant-associated microorganism poses a plant-pest risk*”
- Production of pesticide intermediates.
- Bioremediation, biotreatment.
- Manufacture of industrial enzymes.
- Biofuel, bio-based chemical manufacture.

U.S. Regulation of Biostimulants

- Not explicitly defined in US regulation, may be distinct from pesticides or fertilizers.
- **2018 Farm Bill**: “plant biostimulant” is considered a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield.
- **EPA 2019 Draft Guidance**: “A naturally-occurring substance or microbe that is used either by itself or in combination with other naturally-occurring substances or microbes for the purpose of stimulating natural processes in plants or in the soil in order to, among other things, improve nutrient and/or water use efficiency by plants, help plants tolerate abiotic stress, or improve the physical, chemical, and/or biological characteristics of the soil as a medium for plant growth.”
- Biostimulants and biofertilizers may be regulated as ”fertilizers” under state laws in some U.S. states.

Definition of “New Organism” as “Intergeneric” Organism

- TSCA covers only “new chemical substances” used in commerce, so the Biotech Rule covers only “new microorganisms”.
- “New microorganisms” defined as “***intergeneric***”: i.e., containing ***deliberate combinations of coding nucleic acids from more than one taxonomic genus***.
- Organisms modified solely by classical mutagenesis, gene deletions, directed evolution or gene editing, with no intergeneric sequences, would be outside of TSCA scope.

Overview: TSCA Biotechnology Regulation

	R&D Use	Commercial Use
Contained Use (laboratories, pilot production facilities, greenhouses)	Exempt: self-certifying, no approval needed; largely independent of scale (may include pilot-plant uses)	**Microbial Commercial Activity Notice (MCAN):** File 90 days before commercial use or importation
Outdoor Use (open field, algae open ponds)	**TSCA Environmental Release Application (TERA):** File 60 days before beginning outdoor experimentation	**Microbial Commercial Activity Notice (MCAN):** File 90 days before commercial use or importation

TSCA ENVIRONMENTAL RELEASE APPLICATIONS (TERAs)

Outdoor R&D Use under TSCA

- Most outdoor R&D (”deliberate releases”) requires prior EPA approval under TSCA Environmental Release Applications (TERAs).
- However, the regulation provides limited exemptions for *S. meliloti* and *B. japonicum* (based on early field tests carried out under EPA’s interim biotechnology policy).

TERAs: Data Requirements

- Detailed description of the proposed research and development activity, including details of microorganism construction and objectives, design of the test.
- Information on monitoring, confinement, mitigation, and emergency termination procedures.
- All available data concerning actual or potential effects on health or the environment of the new microorganism that are in the possession or control of the submitter.

EPA Review of TERAs

- EPA Review period is 60 days following 30-day prescreening to determine completeness. Early approval is possible.
- EPA conducts risk assessment and will approve the TERA If it determines that the proposed R&D does not present an unreasonable risk to health or the environment; but may impose requirements and conditions in its approval.
- If EPA denies the TERA, it will provide reasons for the denial in writing.
- Early consultation with the agency is recommended.

EPA-Approved TERAs, 1998 to date

38 Approved TERAs, 5 withdrawn

- Improved nitrogen fixation: 7
- Pesticide research: 4
- Biofuel research (open-pond use of algae): 10 (*including several large-scale tests*)
- Hazardous waste detection (bioindicators): 13
- Bioremediation: 2
- Enzyme production: 2 (using B*. thuringiensis Israelensis*).

Approved TERAs for Agricultural Applications

- **Early research: Rhizobia for nitrogen fixation:**
  - Five TERAs from 1998-2000 for field tests of modified *B. japonicum*.
  - Earlier field tests of *B. japonicum* and *S. meliloti* took place under PMNs before issuance of 1997 rule.
  - One *S. meliloti* strain approved for commercial use (*discussed later*)
- **Recent research: Improved nitrogen fixation:**
  - Two TERAs, 2021-22, for "Bacilli strains engineered to affect nitrogen production," for "evaluation of the engineered strains' ability to effect nitrogen uptake in plants".

Approved TERAs for Agricultural Applications

- **Pesticide research:**
  - Three TERAs from 2003-05 from UC Riverside for modified strains of *Alcaligenes xylosoxidans,* to determine biology and behavior of the strain to develop a paratransgenesis system to control Pierce's disease of grapes.
- **Other:**
  - 2020 TERA for use of Alphaproteobacteria strain engineered to express a bioluminescent marker protein "for investigating microbial colonization of plants" (soybeans).

Microbial Commercial Activity Notifications (MCANs)

- Commercial use or importation of “new microorganisms” requires MCAN reporting at least 90 days before commencing commercialization or importing microorganism.
- Applies to contained manufacturing and commercial uses in the environment: requires submission of data package and other information to EPA.
- MCANs for commercial environmental use should be supported by data from field tests under TERAs.
- Only one MCAN for environmental use: a marker strain of *S. cerevisiae* as reference strain for cell counting, approved in 2018.

Commercial Approval of Modified Rhizobia

- In 1998, EPA issued a Significant New Use Rule (SNUR) that allowed limited commercial use of *S. meliloti* strain RMBPC-2.
- Granted at request of Research Seeds, Inc. (under EPA interim policy), codified at 40 CFR §721.9518.
- The company was limited to maximum production volume of 500,000 lbs. in any consecutive 12-month period.
- Other applicants would need to submit new TSCA notice (TERA or MCAN) for use of this strain.

INTERNATIONAL REGULATION

- Approvals likely needed in most countries for field tests of modified microorganisms
- Definitions of “modified organism” differ from country to country, but nature of risk assessment will largely be the same

Cartagena Protocol

- International treaty on biodiversity (Convention on Biological Diversity): Biosafety Protocol adopted January 2000.
- Most countries in the world are signatories; many have adopted Biosafety Laws modeled on the principles of the Protocol.
- Provides minimal set of regulations, particularly for transboundary shipment of Living Modified Organisms (LMOs), for which "Advanced Informed Agreements" (AIAs) with competent national authority would usually be required.
- Most environmental uses of LMOs would require government approval.

Biotechnology Regulation in the European Union

- The EU has adopted two directives on biotechnology: contained use and environmental release. The latter, Directive 2001/18/EC, would govern genetically modified microorganisms used in agriculture.
- EU directives must be implemented in each member state by appropriate national laws.
- National approval needed for GM field tests; all member states can review and comment on proposed commercial approvals.

Other EU Regulation

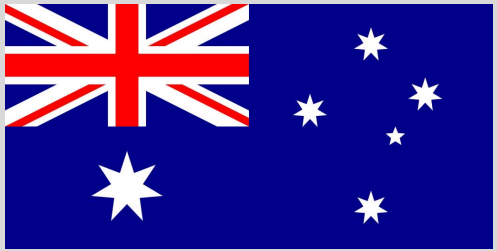
- Microbial biostimulants would be governed by the **Fertilizer Regulation**, Directive 2019/1009.
- Microbial plant control agents (biopesticides) would be governed by the **Plant Protection Regulation**, Directive 1107/2009.
- These regulations would apply to GM micro-organisms as well as nonmodified micro-organisms.

Biotechnology Regulation in Canada

- Canada regulates microorganisms under the **Canada Environmental Protection Act**, in the New Substance Notification (NSN) regulations.
- Notifications are needed in advance of importation or use of microorganisms in Canada (similar to US TSCA).
- The NSN regulations cover *any* microorganism that has not previously been used commercially in Canada: even nonmodified strains.
- Reduced requirements for experimental field studies (total area < 100 hectares).
- Organisms regulated under the Fertilizers Act or Pest Control Products Act are exempt from NSN reporting.

Biotechnology Regulation in Australia

- Use of GM microorganisms regulated under Gene Technology Act of 2000.
- Field tests and environmental uses are “Dealings Involving Release” and require government approval.
- Field tests not judged to pose unreasonable risks are approved within 150 working days: other releases have longer timelines.
- Pest control products regulated by federal government; fertilizers regulated by individual states.



Biotechnology Regulation in Japan

- Agricultural field tests of living modified organisms are “Type 1” uses under Japanese law, and require advance approval.
- Regulatory agency would be the Ministry of Agriculture, Forest and Fisheries (MAFF).

Summary

- U.S. regulation of modified microorganisms under the Toxic Substances Control Act (TSCA).
  - *Take-home lesson: it is possible to obtain approval from the U.S. EPA for field tests of genetically modified microorganisms.*
- Overview of international regulations affecting uses of microorganisms in agriculture.
  - *Take-home lessons: approvals likely needed in most countries for field tests of modified microorganisms; definitions of "modified organism" differ from country to country.*

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ADDITIONAL SLIDES

Rhizobia exemption 40 CFR 725.239

(a) Bradyrhizobium japonicum. To qualify for an exemption under this section, all of the following conditions must be met for a test involving Bradyrhizobium japonicum:

(1) Characteristics of recipient microorganism. The recipient microorganism is limited to strains of Bradyrhizobium japonicum.

(2) Modification of traits.

(i) The introduced genetic material must meet the criteria for poorly mobilizable listed in Sec. 725.421(c).

(ii) The introduced genetic material must consist only of the following components:

(A) The structural gene(s) of interest, which have the following limitations:

(1) For structural genes encoding marker sequences, the gene is limited to the aadH gene, which confers resistance to the antibiotics streptomycin and spectinomycin.

(2) For traits other than antibiotic resistance, the structural gene must be limited to the genera Bradyrhizobium and Rhizobium.

(B) The regulatory sequences permitting the expression of solely the gene(s) of interest.

(C) Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

(D) The vector nucleotide sequences needed for vector transfer.

(E) The vector nucleotide sequences needed for vector maintenance.

(3) Limitations on exposure.

(i) The test site area must be no more than 10 terrestrial acres.

(ii) The technically qualified individual must select appropriate methods to limit the dissemination of modified Bradyrhizobium japonicum.

Coordinated Framework 2017 Update

Product Area	Source Organism or Culture		
	Genetically Engineered Plant	***Genetically Engineered Animal***	***Genetically Engineered Microbe or Cultured Cell***
Biomass conversion for chemical production, microbial fuel cells, mining and resource extraction, building materials, waste remediation and pollution control, non-pesticidal agriculture applications like biofertilizers, weather and climate modification, various consumer products, and all other applications of intergeneric microbes not otherwise excluded under TSCA[92]	Not applicable to this product area	Not applicable to this product area	EPA/OPPT If microbe is intergeneric, and is manufactured or processed for commercial production purposes,[88] including R&D for commercial purposes,[89] for a use that is not excluded under TSCA,[90] and is not otherwise exempt from reporting.[91]
Other (non-food, non-chemical producing, non-drug producing, non-biologic producing, non-pesticidal organisms)[93]	USDA/APHIS For ornamental, silvicultural, or turfgrass crops, if plant poses a plant pest risk USDA/APHIS For ornamental, silvicultural, or turfgrass crops, if plant poses plant noxious weed risk	FDA/CVM USDA/APHIS If animal poses a plant pest risk USDA/APHIS/VS If animal poses health risk to livestock[65]	USDA/APHIS If plant-associated microorganism poses a plant pest risk EPA/OPPT If microbe is intergeneric, and is manufactured or processed for commercial production purposes,[88] including R&D for commercial purposes,[89] for a use that is not excluded under TSCA,[90] and is not otherwise exempt from reporting.[91]

Detailed Contents of TERAs: §725.255(e)

(1) Detailed description of the proposed research and development activity.

i. The objectives and significance of the activity; rationale for the test.
ii. Number of microorganisms released; method(s) of application or release.
iii. Characteristics of the test site(s), including location, geographical, biological and other features of the site.
iv. Target organisms (e.g., prey) of the modified microorganism (if any)
v. Planned start date and duration of each activity.
vi. Whether State and/or local authorities have been notified of the activity, evidence of notification.

Detailed Contents of TERAs : §725.255(e)

(2) Information on monitoring, confinement, mitigation, and emergency termination procedures.

i. Confinement procedures, access and security measures, procedures for routine termination.

ii. Mitigation and emergency procedures.

iii. Measures to detect and control potential adverse effects.

iv. Name of principal investigator and chief of site personnel responsible for emergency procedures.

v. Personal protective equipment, engineering controls, procedures to be followed to minimize dispersion.

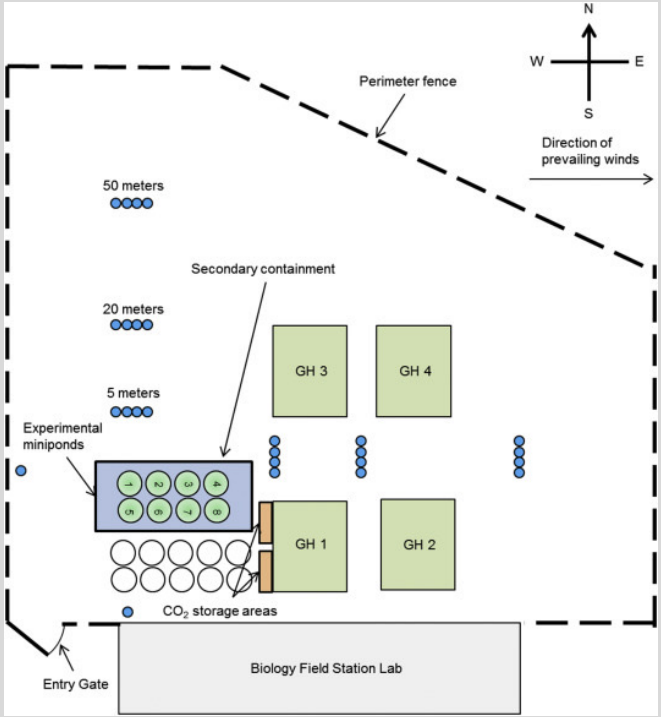
vi. Procedures for disposal of articles, waste, or equipment involved in the release, methods for inactivation of the microorganism(s), and for containment, disinfection, and disposal of contaminated items.

Additional Contents of TERAs: §725.260

Each TERA must contain all available data concerning actual or potential effects on health or the environment of the new microorganism that are in the possession or control of the submitter and a description of other data known to or reasonably ascertainable by the submitter that will permit a reasoned evaluation of the planned test in the environment.

Approved TERAs for Algae

- **R-13-0003 thru 0007.** Sapphire/UCSD, August 2013. Five genetically modified strains of *Scenedesmus dimorphus.*
- **R-17-0002.** Arizona State University, May 2016. Two modified strains of *Chlorella sorokiniana*.
- **R-18-0001.** Arizona State University, August 2017. Modified strain of *Chlorella sorokiniana*, expressing SNF related kinase from *Picochlorum soloecismus.*



Source: Szyjka, et al. (2017) Algal Research, Volume 24, Part A, pages 378-386.

Approved TERAs for Algae

Synthetic Genomics, Inc. (Viridos): 3 Approved TERAs.

- 2019: small-scale open-pond testing of a proprietary strain engineered with a marker gene.
- 2020, 2021: larger-scale open-pond testing of multiple green microalgal strains engineered for enhanced lipid productivity

CAAF is first commercial R&D facility for GE algae authorized by US EPA

	Prior TERAs	SGI 2019 TERA 1	SGI 2020 TERA 2	SGI 2021 TERA 3
# Genera (cumulative)	1	1	2	2
# Strains (cumulative)	5	1	16	19
Duration (months)	2	2	4	18
Pond area (m^2)	32	810	4900	9100

viridos

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Image courtesy of Viridos, Inc.: L. Brown "The path to scale at the California Advanced Algae Facility" 2021 Algal Biomass Summit (virtual).

Research Needs

- Baseline information on wild type microorganism.
- Persistence in soil, water.
- Potential for environmental dispersal.
- Adverse effects on other microflora.
- Adverse effects on plants, animals, humans?