



Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions

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Purpose of the Draft Algae Guidance

- To assist EPA's Office of Pollution Prevention and Toxics in updating its 1997 guidance document **"Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms"** for genetically engineered (GE) algae (cyanobacteria and microalgae)
- To identify issues that warrant consideration in the evaluation of the potential hazards and exposures with commercial-scale production of GE algae, and to identify uncertainties



Information Needs for Risk Assessment

- A. Recipient Microorganism Characterization**
- B. GE Alga Characterization**
- C. Genetic Modifications**
- D. Potential Human Health Effects of the GE Alga**
- E. Potential Ecological Effects of the GE Alga**
- F. Fate of the GE Alga**
- G. Information Applicable to Small-Scale Field Tests**
- H. Manufacturing Process Description and Production Volume**
- I. Exposures of the GE Alga**
- J. Monitoring of the GE Alga**
- K. Termination and Emergency Containment Procedures**



A. Recipient Microorganism Characterization

- **Taxonomy**
 - Sources/databases for cyanobacteria/algae classification
- **General Description & Characterization**
 - Growth forms (e.g., unicellular, filamentous, colonies)
 - Isolation source
 - Prevalence and distribution in the environment
 - Salinity tolerance & optima
 - Nitrogen fixation ability
 - Naturally transformable (cyanobacteria)
 - Dormancy structures
 - Importance in aquatic food webs/trophic level interactions



B. GE Algae Characterization

- **Taxonomy of GE Algae**
 - **If different from recipient microorganism**
- **Taxonomy of Donor Organisms**
- **General Description & Characterization**
 - **Introduced traits**
 - **Growth rate**
 - **Illumination conditions for optimal growth (e.g., intensity, photoperiod)**
 - **Photosynthetic ability**
 - **Nitrogen fixation ability**
 - **Salinity tolerance**
 - **Algal composition (e.g., protein, carbohydrates, oil content, lipid yields, specific fatty acids produced, ash-free dry wt.)**



C. Genetic Modifications

- **Construction of the GE Algae**
 - Gene sequences, isolation vs. chemical synthesis, codon optimization, vectors, methods of introduction
 - Selection marker genes (e.g., antibiotic resistance genes)
 - Site of insertion – chromosome or mobile genetic elements
 - Stability of gene integrations
 - Use of biological containment (e.g., auxotrophy, suicide genes, induced lethality)
- **Potential for Horizontal Gene Transfer (HGT)**
 - Mechanisms and frequency of HGT in eukaryotic algae - poorly understood compared to that of cyanobacteria
 - Naturally transformable cyanobacteria



D. Potential Human Health Effects

- Pathogenicity
- Toxin Production – cyanotoxins and phycotoxins under the conditions of use – and the presence in the genome of genes that encode toxins
- Secondary Metabolite Production
- Immunological Effects (e.g., allergenicity)
- Presence of Microbial Pathogens as Contaminants in Ponds or Bioreactors
- Prevention of Contaminants



E. Potential Ecological Effects

- Pathogenicity to Animals or Plants
- Toxicity to Animals
- Propensity for Bloom Formation – and the presence in the genome of gas vesicle (*gvp*) genes
- Effects on Primary Productivity
- Effects on Other Biogeochemical Cycles
- Effects on Aquatic Food Webs/Trophic Level Changes
- Effects on Ecologically Important Relationships
- Potential Effects on the Surrounding Environment
- Bioaccumulation of Metals in the GE Algae (e.g., from flue gas)



F. Fate of the GE Alga

- **Survival in Potential Aquatic and Terrestrial Receiving Environments**
 - Survival relative to the recipient microorganism
 - Ability to overwinter
 - Desiccation tolerance features
 - Known pathogens or grazers
- **Competition with Indigenous Populations**
 - Ability to outcompete/displace indigenous species
 - Selective advantages imparted to the GE algae
 - Effects on microbial community structure
- **Dissemination from the Site**



G. Information for Small-Scale Field Tests

- **Objectives**
- **Characteristics of the Site** (e.g., size, slope, proximity to water bodies, prevailing winds)
- **Field Test Design**
- **Application Methods**
- **Monitoring Endpoints**
- **Sampling Procedures**
- **Measurement Methodologies and QA/QC**
- **On-Site Containment Practices**
- **Termination and Mitigation Procedures**
- **Record Keeping and Reporting of Test Results**



H. Manufacturing Process Description

- **Heterotrophic Fermentation**
- **Photobioreactors (PBRs) – size, design, & construction materials**
- **Open Ponds – size, design, & construction materials**
- **Production Volume**
- **By-products**
- **Proposed Use Conditions**



Heterotrophic Fermentation

- **Number and Location of Sites**
- **Process Description**
- **Number of Batches per Year**
- **Fermentor Volume (or number of colony-forming units [CFUs] for continuous processes)**
- **Inactivation Methods**
- **Concentration of CFUs in Each Process Stream**
- **Cleaning of Fermentor**
- **Disposal/Use of Spent Biomass**
- **By-products**



Photobioreactors (PBRs)

- **Number/Volume of PBRs, Arrangement at the Site**
- **Size/Volume/Cell Density**
- **Number of Harvests/Batches per Year**
- **Harvesting Technologies**
- **PBR Construction Materials**
- **Integrity/Weatherability – Longevity/Replacement Time**



(PBRs), continued

- Junctions for Inlet and Outlet Tubing
- Amount and Source of CO₂, Nutrients, & Water
- Inactivation Methods
- Disposal/Use of Spent Biomass
- Releases of Wastewater
- Cleaning of PBRs for Re-use or Disposal of PBRs



flat plate PBRs

<http://www.nanovoltaiacs.com/portfolio/photobioreactors>. Used with permission from NanoVoltaics, Inc.



Open Ponds

- **Number/Dimensions/Surface Area**
- **Volume/Cell Density**
- **Number of Harvests/Batches per Year**
- **Harvesting Technologies**
- **Pond Construction Materials**
- **Use of Liners, Berms**



Open Ponds, continued

- **Circulation System and Potential for Bioaerosols**
- **Amount and Source of CO₂, Nutrients, & Water**
- **Inactivation Methods**
- **Disposal/Use of Spent Biomass**
- **Releases of Wastewater**
- **Cleaning of Ponds**



Photo courtesy of Arizona Center for Algae Technology and Innovation (AzCATI). Photo by Mark Segal, EPA, 2014.



I. Exposures of the GE Alga

- **Occupational Exposure**
- **Environmental and General Population Exposure**
- **Consumer Exposure**



Occupational Exposures

- **Processes Influencing Worker Exposure**
 - **Worker Activity**
 - **Number of Workers/Shifts**
 - **Exposure Days per Year**
 - **Exposure Duration**
 - **Personal Protective Equipment (PPE) Used**
- **Inhalation Exposure (CFUs)**
- **Dermal Exposure (CFUs)**



Environmental & General Population Exposures

- **Environmental Releases to Air, Water, and Land**
- **Inactivation Methods and Pollution Control Technologies**
- **Environmental Exposures**
- **General Population Exposures (including potentially exposed and susceptible subpopulations)**
 - **Inhalation Exposure**
 - **Drinking Water Exposure**
 - **Proximity to Human Populations, Urban Centers, Aquaculture Farms, etc.**



J. Monitoring

- **Monitoring Endpoints and Procedures**
 - Endpoints to be evaluated
 - Techniques used to detect the microorganism in test samples
 - Sensitivity and reliability of methods & limit of detection
 - Frequency and type of observations to be made
- **Sampling Procedures**
 - When, where, and how samples will be taken
 - Procedures for preserving, processing, and analyzing samples
 - Methods of measurement, accuracy and repeatability
 - Statistical analysis of data



K. Termination and Emergency Containment Procedures

- **Type of Unexpected Effects that would Necessitate Emergency Termination**
- **Emergency Termination Procedures to be Followed if Adverse Environmental Effects are Observed**
- **Handling of Leaks and Spills**



Summary

- EPA needs the information presented here for scientifically credible risk assessments of algae used in commercial-scale production.
- EPA requests your comments on whether these information/data needs are appropriate and sufficient elements for updating our Points to Consider guidance document for algae.

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