Challenges and Opportunities: *Managing US and International Regulation of Industrial Biotechnology Activities*

David J. Glass, Ph.D.
D. Glass Associates, Inc.

Overview

- International Biotechnology Regulation
  - Risk Assessment Issues
  - Regulatory Frameworks
- Case Studies
  - United States
  - Europe
  - Brazil
- Discussion (*time permitting*)
  - Is there a need for a common regulatory approach?
  - Is there a need for a common risk assessment framework?

Risk Assessment Principles

Risk assessment of industrial uses of microorganisms have been based on familiar principles.

- Does the organism have harmful or deleterious properties, e.g. toxicity, pathogenicity, enhanced competitiveness?
- Have these properties been altered by the genetic manipulations?
- If released to the environment, will the organism survive, multiply, compete and disseminate in the environment?
- Horizontal gene transfer: can genetic material be transferred to indigenous organisms?
- Will any of the above cause adverse ecological effects?

International Biotechnology Regulation

- Many national laws are based on the principles of the *Cartagena Protocol on Biosafety*, part of the Convention on Biological Diversity, which was adopted in January 2000.
- Under such laws, government approvals are generally needed for importation of Living Modified Organisms (LMOs) into countries, and for many industrial activities including “contained uses” or “environmental uses”.
- Such approvals may often require a risk assessment of the LMO and its proposed use.

Cartagena Protocol

- Transboundary shipment usually requires “Advanced Informed Agreements” (AIAs) with competent national authority.
- Article 3 defines “contained use”.

  "Contained use" means any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.
- Transboundary shipments of LMOs for contained use don’t require AIA if undertaken in compliance with applicable national law; labeling requirements may apply.

Cartagena Protocol: Implications for Biofuel Manufacture

- Ascertain “competent authority” in destination country.
- Shipment into country and subsequent contained use may require permits from competent authority.
- If country has no applicable biotech laws or regulations, ensure that competent authority is aware of shipment of LMO into country.
- May also need to provide a risk assessment (e.g. conducted by an agency of another government) and proof that the manufacturing process is “contained”.
- Risk assessment and other required information should be provided in accordance with Annexes I and III of the Protocol.

United States

Overview of U.S. Biotechnology Regulation

Environmental Protection Agency

- Microbial pesticides, plant pesticides.
- Engineered microorganisms used for other industrial purposes.

U.S. Department of Agriculture

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

Food and Drug Administration

- Foods, food additives, animal feed, feed additives.
- Pharmaceuticals.

EPA TSCA Biotechnology Regulations: *Overview*

- Regulations adopted in 1997 under the Toxic Substances Control Act (TSCA) cover commercial uses of new ("intergeneric") microorganisms not regulated by other agencies: primary rules covering industrial biotechnology.
- **R&D:** No oversight for contained activities; advance EPA approval needed for outdoor research (TSCA Experimental Release Application; TERA).
- **Commercial Uses:** Advance EPA review needed for most commercial applications through filing of Microbial Commercial Activity Notice (MCAN). Exemptions for well-understood industrial host species, e.g. *E. coli*, *S. cerevisiae* require strict adherence to containment provisions.

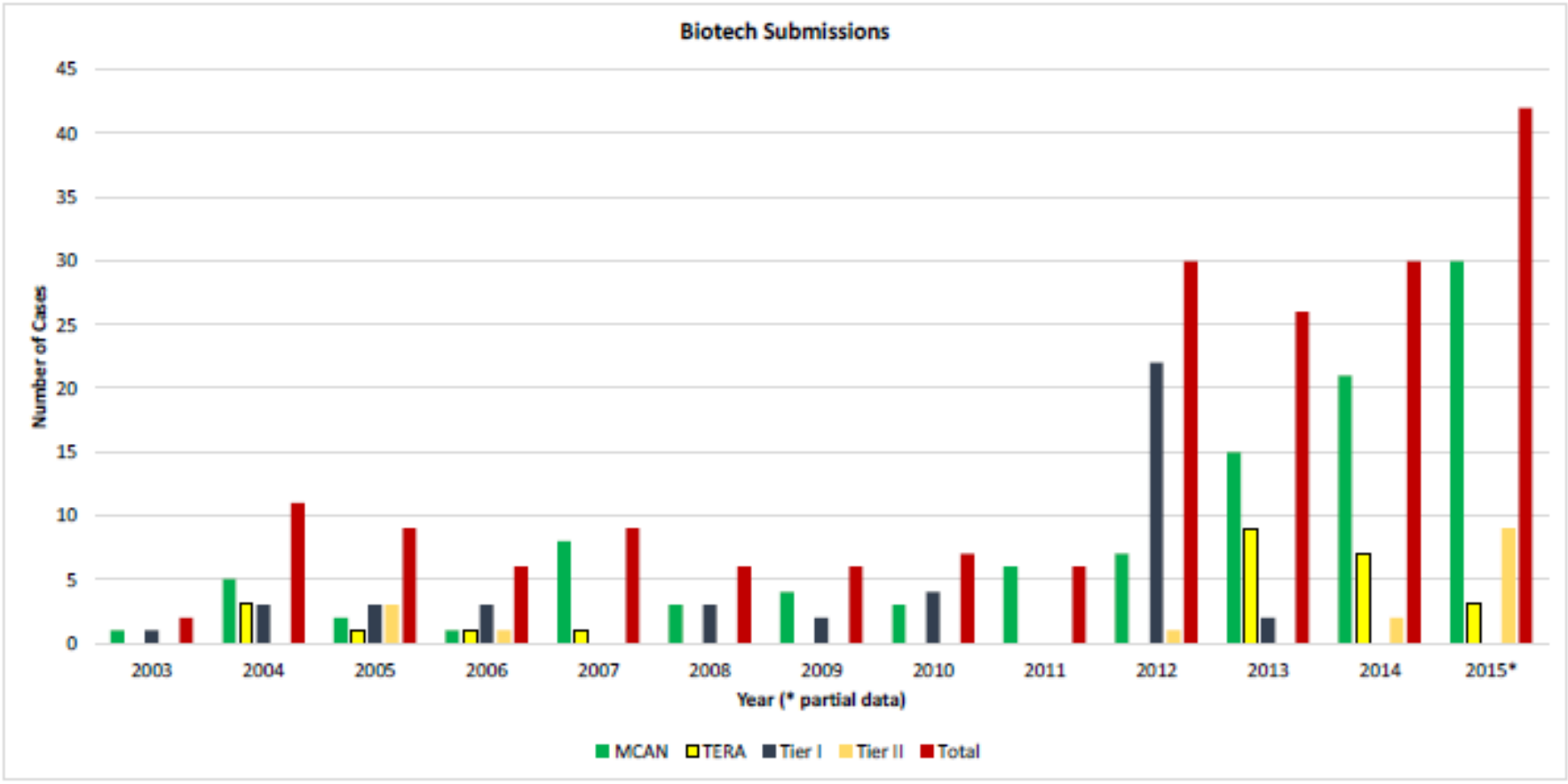
EPA TSCA Biotechnology Rule: *Microbial Commercial Activity Notifications (MCANs)*

- MCAN reporting required at least 90 days before commencing commercialization or importing a “new microorganism” for a TSCA purpose.
- MCAN requires submission of data to EPA.
  - Microorganism identity, construction and its properties.
  - Potential health and environmental impacts.
  - Information about the industrial process, control/containment measures, worker exposure, possible environmental release.
- EPA review, clearance of MCAN authorizes commercial use for any purpose. Most MCANs reviewed and cleared within 90 day review period.

EPA TSCA Biotechnology Rule: *Biofuel, Bio-Based Chemical MCANs*

- At least 85 MCANs filed since 1997. Number and frequency have increased in last 4-5 years.
- Most early MCANs covered GMOs for production of industrial enzymes.
- 18 MCANs for *S. cerevisiae*, 3 for *Zymomonas mobilis,* all for ethanol production.
- Four filed MCANs for cyanobacteria; 4 filed MCANs for modified microalgae.
- Complete list available at www.epa.gov/biotech_rule/pubs/submiss.htm.

Increase in TSCA Biotech Cases: 2003-Current



Case Study

Joule Unlimited MCAN for Ethanol-Producing Cyanobacteria

EPA Jurisdiction over Joule's production organisms

- Joule's modified biocatalysts for ethanol production are considered "new microorganisms" under EPA's TSCA biotechnology regulations (40 CFR Part 725):
  - The modified organisms include coding sequences from outside the *Synechococcus* genus and are considered "intergeneric".
  - The intended commercial use is for a purpose not regulated by any other federal agency.
- First biocatalyst: modified strain of the cyanobacterium *Synechococcus* for ethanol production.
- Laboratory, pilot and some demo plant activities qualify for the "contained structure" (R&D) exemption.
- Commercial use requires filing a Microbial Commercial Activity Notice (MCAN) at least 90 days before commercial use.

Joule Regulatory Strategy and Timeline

- Plan was to file first MCAN well in advance of anticipated start of commercial use.
- Early presubmission meeting with EPA staff (2011).
- First MCAN for commercial ethanol production strain (MCAN Number J12-0006) filed July 2012.
- Short-term goal was to gain approval to use this strain commercially at Joule's Demonstration Plant in Hobbs, New Mexico.
- EPA completed its review Fall 2012, began drafting Consent Order that would allow use of strain at Hobbs under specified conditions.
- Consent Order signed July 2013.

Joule MCAN and Consent Order: Issues Considered by EPA

- Minimal concerns for adverse human health effects, and minimal concerns for ecological effects from use in ethanol production.
- Introduced genes unlikely to pose potential hazards.
- Potential for horizontal gene transfer is expected to be low.
- Survival of the MCAN strain in Hobbs soil is expected to be low in the event of breach of containment.
- EPA was unwilling to extend approval to locations other than Hobbs, pending additional data on MCAN strain survival in other environments; so EPA required Joule to enter into a Consent Order limiting approved uses to Hobbs.
- Certain testing and data are required to allow an assessment of commercial use at sites other than Hobbs.

European Union

European Union

- Oversight under national laws adopted by EU member states based on binding EU Directives.
- ***Contained manufacturing***: Contained uses of LMO microorganisms require national government notification under EU "Contained Use" **Directive 2009/41/EC**.
- ***Open Ponds and Transgenic Plants***: Uses of LMOs in the open environment would be covered by EU **Directive 2001/18/EC** on "Environmental Release".

EU Contained Uses Directive

- "Contained Use" defined:

  "contained use" means any activity in which microorganisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment

- User has obligation to carry out risk assessment and choose a level of containment appropriate for the risks of the organism.
- User must notify competent national authority, and provide data and information specified in directive and under the applicable national law.

Contained Use in EU Member States

- Approval for contained uses of GMOs would come from applicable EU member state.
- The national laws and regulations of individual EU member states may have stricter requirements; permits or approvals may be needed for industrial uses.
- Applicants should identify national authority in countries of interest, obtain copies of laws and regulations.
- Early consultation with regulatory agencies in individual countries is recommended.

Case Study

ThyssenKrupp Industrial Solutions AG
Process Technologies, Germany

Applying Myriant`s E.coli KJ122 for Succinic Acid Production

German GenTG Jurisdiction over Myriant's production organism

- Myriant's modified *E.coli* KJ122 for succinic acid production is primarily considered a genetically modified microorganism under German GenTG biotechnology regulations (§5 Abs.1 and Appendix 1 GenTSV):
  - The modified organism has numerous deletions that have been artificially created and are thus a consequence of genetic engineering

- TKIS PT has applied for S1 approval of their Leuna Demonstration scale facility following the Risk- and Safety Assessment for genetic work as of §§4-7 GenTSV.

- Laboratory, pilot and all demo plant activities qualify for containment and regulated inactivation procedures.

TKIS PT Regulatory Strategy and Timeline

- Federal States treat S1 Regulations for Production Facilities differently strict in Germany – Not too much experience yet
- Early involvement of the Federal State Administration Bureau was key to approval
- One can announce S1 work in Germany and declare production, if you are sure you keep all regulations (containment, documentation, inactivation) but risk a stop in case they visit the site and find problems
- Alternatively you can go through the administration jointly (TKIS did), and it takes 4-6months for approval

TKIS PT Regulatory Strategy to mitigate S1 disposal cost

- Following S1 Approval, TKIS PT approached Federal State Administration to remove S1 categorization of *E. coli* KJ122 in August 2014

- No waste code for S1-*E.coli*-Biomass exists in Germany = leads to "specialty waste" label, which is significantly more expensive to dispose of (about 250€/mt)
  - each Waste Batch in Leuna = >15,000€ disposal
  - 4 mt Biomass Sludge / Batch = 1,000 € disposal / Batch
  - About 2-3 batches per week
  - Start-Up scenarios with frequent Waste Batches

TKIS PT Regulatory Strategy to mitigate S1 disposal cost

- Encouraged by successful determination that inactivated E.coli KJ122 could be disposed of as normal biogenic waste under EPA regulations in the USA (driven by Myriant Corporation and supported by D. Glass Associates Inc.), TKIS approached German authorities with similar argumentation / documents

- Lesson: Have all the documents you need; Be prepared to argue with specialists, and bureaucrats – no short-cuts. Federal State consulted National Center for Biological Safety in Berlin for Co-Approval.

- In November 2014, approval to exclude *E.coli* KJ122 from any regulations dictated by the German GenTG has been given. Biomass is now considered non-GMO biogenic waste. (70 €/mt)

Brazil

Brazil

- Signatory to Cartagena Protocol.
- National Biosafety Law adopted in 2005; regulations under the law also adopted in 2005.
- Created national framework administered by two interagency committees: CTNBio and CNBS.
- Applications would need to be submitted to government for approval for importation of GMO, use in a laboratory or facility, and use in manufacturing.
- There are now 6 approvals for contained industrial manufacturing using GMOs.

Brazil: Approvals for Contained Manufacturing with GM Microorganisms

The following commercial approvals of GM microorganisms are listed on the CTNBio website

- **Amyris**, *Saccharomyces cerevisiae* CEPA Y1979, February 2010; *S. cerevisiae* CEPA Y5056, May 2012, both expressing *Artemisia annua* farnesene synthase for production of farnesene.
- **Solazyme**, *Prototheca moriformis* for production of triglycerides, October 2013; September 2014.
- **Bio Celere Agroindustrial Ltda.**, *Saccharomyces cerevisiae* RN 1016 (Royal Nedalco strain, *Piromyces* xylA xylose isomerase) for production of ethanol, December 2013; November 2014.

Case Study

Comments on Amyris Experience in Brazil

Risk Assessments of GM Microorganisms

Risk Assessment Principles

Risk assessment of industrial uses of microorganisms have been based on familiar principles.

- Does the organism have harmful or deleterious properties, e.g. toxicity, pathogenicity, enhanced competitiveness?
- Have these properties been altered by the genetic manipulations?
- If released to the environment, will the organism survive, multiply, compete and disseminate in the environment?
- Horizontal gene transfer: can genetic material be transferred to indigenous organisms?
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Cartagena Protocol
Risk Assessment Guidance

(a) Recipient organism or parental organisms. The biological characteristics of the recipient organism or parental organisms.

(b) Donor organism or organisms. Taxonomic status and relevant biological characteristics of the donor organisms.

(c) Vector. Characteristics of the vector, including its source or origin, and its host range.

(d) Inserts and/or characteristics of modification. Genetic characteristics of the inserted nucleic acid and the function it specifies.

(e) Living modified organism. Identity of the LMO, and the differences from the recipient organism or parental organisms.

(f) Detection and identification of the living modified organism. Suggested detection and identification methods.

(g) Information relating to the intended use.

(h) Receiving environment. location, geographical, climatic and ecological characteristics of the likely potential receiving environment.

Containment Principles: Industrial Microorganisms

Containment principles for industrial manufacturing with modified microorganisms are well known.

Use well-established principles of Good Laboratory Practice, Good Large-Scale Practice, commensurate with Risk Group of the host organism. Practices to include:

- Controlled access to facility.
- Inactivation of liquid and solid wastes.
- Minimize release from air vents (e.g. HEPA filters), other potential release points.
- Institute spill control procedures and other emergency protocols.
- Worker training in proper microbiological techniques and emergency procedures.

Commentary

Pathways to Obtain Regulatory Approvals for the Use of Genetically Modified Algae in Biofuel or Biobased Chemical Production

David J. Glass

D. Glass Associates, Inc., Needham, MA

Recent years have seen an increased interest in developing genetically modified microalgae and cyanobacteria for use in biofuel and biobased chemical production, but this comes at a time when there is uncertainty within the industry and the academic community about how such uses will be regulated by governments in the US and elsewhere in the world. However, a reasonable road map is emerging of a regulatory regime that can allow pilot, demonstration, and commercial stage uses of modified algae without jurisdictional conflicts. In the US, regulations of the US Environmental Protection Agency (EPA) would govern the industrial use of algae or cyanobacteria in contained photobioreactors and open ponds, but regulations of the US Department of Agriculture (USDA) could in rare cases also apply. Although these regulations require assessments of potential environmental risks, recent government approvals show that the process can be successfully managed with proper preparation, and that approvals can also be achieved both for contained manufacturing as well as for proposed outdoor, open-pond testing of modified algae.

Introduction

Genetically modified microalgae, cyanobacteria, and other microorganisms are increasingly a focus of development for the production of renewable fuels and biobased chemicals. Innovations enabling biological methods of manufacturing commodity products currently made from petrochemical feedstocks promise to make an important contribution to the reduction of global carbon emissions and the movement to more sustainable industrial activities. The proposed use of genetically modified organisms offers potentially significant advantages over traditional industrial uses of microorganisms, such as improved productivity, decreased operational costs, the ability to use a more diverse range of feedstocks, and possibly more favorable carbon footprints. Recent interest in using modified microalgae and cyanobacteria for this purpose derives largely from the hope of being able to capitalize on the ability of photosynthetic organisms to synthesize useful compounds from sunlight, while also enabling capture and beneficial use of carbon dioxide from sources such as industrial waste streams.

However, in the US and most other countries around the world, manufacturing processes involving genetically modified algae and other microorganisms (GMMs) would likely trigger additional regulatory scrutiny before manufacturing could begin and products could be sold. This article reviews the regulations most applicable to fuel and chemical production using genetically modified microalgae and cyanobacteria, focusing mainly on the situation in the US, but including limited discussion of regulatory regimes elsewhere in the world. The article also discusses the scientific concerns that have led to the imposition of these regulations, and the issues underlying the risk assessments associated with such government oversight. With proper planning and management, approvals for research and development (R&D) or commercial use of genetically modified algae in industrial biotechnology should be relatively straightforward to obtain.

Potential Commercial Uses and Environmental Impacts of Genetically Modified Algae

STRATEGIES FOR GENETIC MODIFICATION OF ALGAE

Much of today's commercial activity using advanced biotechnology for biofuel or biobased chemical production focuses on the creation, selection or improvement of strains of desired microorganisms or algae having enhanced properties for functions important for the production process. Historically, production methods have made use of naturally occurring or classically selected microorganisms, but advanced biotechnologies are now being investigated or used to develop enhanced strains.

Although most industrial activity to date has focused on the use of heterotrophic microorganisms, photosynthetic organisms such as microalgae and cyanobacteria have also been used for commercial purposes.[1] Historically, species such as *Chlamydomonas, Chlorella, Haematococcus, Nannochloropsis, Dunaliela, Botryococcus, Scenedesmus,* and others have been used for the production of industrially useful compounds.[1–4] Genetic modifications are being considered for industrially useful strains of microalgae, to enable their use to produce commodity fuels and chemicals. Possible approaches to engineering microalgae are described in several recently published review articles and are summarized in *Table 1*.[2,3,5–11] These strategies range from traditional genetic engineering approaches to overexpress or knock out targeted functions, to the use of synthetic biology and other advanced techniques to modify metabolic pathways or to create entirely new pathways for synthesis of desired compounds.

GROWTH OF GENETICALLY MODIFIED ALGAE AT INDUSTRIAL SCALE

Growth of genetically modified microorganisms at industrial scales will usually involve the same hardware and processes

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Additional information available at our blog, ***Advanced Biotechnology for Biofuels*** (dglassassociates.wordpress.com)

Thank you very much

David J. Glass, Ph.D.
D. Glass Associates, Inc.
124 Bird Street
Needham, MA 02492
Phone 617-653-9945
dglass@dglassassociates.com
www.dglassassociates.com

Appendix/Additional Slides

EPA TSCA Biotechnology Regulations (1)

- Regulations adopted in 1997 under the Toxic Substances Control Act (TSCA) cover commercial uses of "new microorganisms".
- Regulations cover only those industrial uses not regulated by other agencies as foods, drugs, cosmetics, pesticides.
- Among covered activities: industrial enzyme production, bioremediation, biotreatment, manufacture of fuels, chemicals.
- New microorganisms are defined as "intergeneric": containing deliberate combinations of coding nucleic acids from more than one taxonomic genus.

EPA TSCA Biotechnology Regulations (2)

- Commercial use or importation of intergeneric organism requires 90 day advance notification to EPA, through submission of a ***Microbial Commercial Activity Notice (MCAN)***.
- Most research and pilot projects would not require EPA review if conducted in suitably “contained” facilities, with procedures for controlled access, inactivation of wastes, emission controls, worker notification.
- R&D with intergeneric organisms under non-contained conditions, such as open-pond algae reactors, would require EPA review through submission of a ***TSCA Experimental Release Application (TERA)*** 60 days in advance of proposed activity.

EPA TSCA Biotechnology Rule: *TSCA Experimental Release Applications*

- TERAs submitted to EPA 60 days in advance, describing the organism, the proposed research, and the proposed controls and monitoring procedures.
- EPA can approve or deny TERAs, or approve testing with limitations or required monitoring.
- To date, 30 TERAs submitted, most for agricultural or bioremediation microorganisms. All but three of these have been approved.
- The 5 TERAs most recently filed and approved were from Sapphire Energy, Inc., for open-pond research with modified algae.

Data Included in MCAN

In the MCAN, Joule provided all available information to enable a risk assessment for the MCAN biocatalyst strain, including:

- Description of strain construction.
- Biological characteristics of the MCAN strain.
- Genomic analysis and literature review to establish lack of evidence that the Joule host strain has any toxic, infectious, or pathogenic properties.
- Review of literature data on natural habitats and environmental incidence of the host strain.
- Discussion of ecology, geology of Hobbs site as they relate to environmental impacts: e.g. local wildlife and flora, depth of aquifer.
- Data on survival/persistence in Hobbs soil.
- Description of Joule's bioreactors, production process and containment features.

Summary: Consent Order Requirements

- **Commercial Use of MCAN Strain at Hobbs is allowed,** subject to terms of the Consent Order.
- **Soil Survival Testing.** Conduct additional studies of the survivability of the MCAN strain in Hobbs soil, using an EPA-approved protocol within one year of commencing commercial use of the MCAN strain at Hobbs.
- **Validation of Waste Inactivation.** During first year of use of the MCAN strain, monitor the efficacy of the waste inactivation system, using EPA-approved protocol, to show 6-log reduction.
- **Monitoring of Capsule Failures.** Required to keep appropriate records of capsule breaches and accidental spills, and to keep records documenting how these releases were cleaned up. Records to be available for EPA review upon request.

Pathway for approval:
Contained Use of Modified Microorganisms