Chapter 4
Regulation of Genetically Engineered Microorganisms Under FIFRA, FFDCA and TSCA

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Abstract Since the dawn of civilization, humans have utilized microbial organisms of various sorts for food and agricultural production. More recently, microbes have been used for pesticidal, and environmental management purposes. With the advent of the development of recombinant DNA technology to genetically alter microbes, it became necessary for Federal regulators to assess the appropriate level, format, and application of their regulatory authorities. In 1986, the Office of Science and Technology Policy issued the Coordinated Framework for Regulation of Biotechnology. The Coordinated Framework constituted a comprehensive regulatory policy for biotechnology that, in essence, concluded that no new statutory authorities were necessary to effectuate a robust and efficient regulatory program for the products of biotechnology. The Framework articulated a division of regulatory responsibilities for the various agencies then involved with agricultural, food, and pesticidal products. Thus, in accordance with the Framework, USDA APHIS regulates microbes that are plant pests under the Plant Protection Act (PPA) and the National Environmental Policy Act (NEPA); the U.S. Environmental Protection Agency (U.S. EPA) regulates microorganisms and other genetically engineered constructs intended for pesticidal purposes and subject to the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA). The U.S. EPA also regulates certain genetically engineered microorganisms used as biofertilizers,

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bioremediation agents, and for the production of various industrial compounds including biofuels under the Toxic Substances Control Act (TSCA). The focus of this chapter is the regulatory process for approval of the use of genetically engineered microbes under the oversight of the U.S. EPA. We will also consider instances where organisms may be exempted from oversight and the outlook for the application of GE microbes in the future. This chapter does not seek to serve as a guidebook for navigating the details of the regulatory process, but rather as an overview of key considerations in risk assessment and risk management.

Keywords Algae • Bacteria • Baculovirus • Biofertilizer • Biofuel • Biopesticide • FFDCA • FIFRA • Fungi • Genetically engineered • Microorganism • MPCA • Plant pest • Plant protection act • Regulation • TSCA

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4.1 Introduction

4.1.1 Historical Regulatory Perspective

The regulation of products of biotechnology has a lengthy history in the United States. Prior to the development of a formal regulatory structure, many protracted discussions took place for well over a decade among scientists, government regulators, environmental activists, and representatives of industry (Berg and Singer 1995; Barinaga 2000). These discussions eventually resulted in the announcement by the Office of Science and Technology Policy (OSTP) of the Coordinated Framework for Regulation of Biotechnology **(**OSTP 1986).

In February 1975, the Asilomar Conference was convened with 140 scientists, lawyers, physicians, ethicists and other interested parties in Monterey, CA for a comprehensive discussion of the issues surrounding the release of genetically engineered organisms into the environment. A growing sense of concern was mounting among scientists regarding this new ability to reshuffle DNA between microbial agents and this was a major impetus for the conference. While a formal regulatory system would have to wait for further executive and legislative decisions, the Congress at Asilomar helped focus the publication of the National Institutes of Health (NIH) Recombinant DNA Guidelines in 1976 (NIH 1976), even though this project was already underway (Marchant 1988). The primary utility of the 'NIH Guidelines', as they came to be known, related to confined applications, e.g., laboratory

work for research purposes. Recognizing, however, that the NIH Guidelines did not provide genuine oversight for actual environmental releases of GE microbes, Federal regulatory agencies were considering appropriate means of adequately regulating such releases. (It should be noted that the NIH Guidelines are still in effect, with some modifications over the years, for their original intended purpose; NIH 2011).

The principal tenet of the Coordinated Framework was that existing statutes were sufficient to effectuate proper regulation of the products of agricultural biotechnology, i.e., that it was not necessary to legislatively create new statutory authorities specifically for the governance of products in the research pipeline and those that where then envisioned. Existing statutes were considered as a sound basis for oversight of biotechnology with modifications offered through promulgation of regulations via rulemaking.

Given the plethora of potential products to be derived from rDNA technology, the U.S. government was faced with the application of statutes already in use for regulation of pesticides (i.e., FIFRA), plant pests (i.e., Plant Pest Act) and pesticide residues on food and feed commodities (i.e., Federal Food Drug and Cosmetic Act) with implications for the associated agencies, EPA, USDA-APHIS, HHS-FDA, respectively. There were, of course, dissenting views as to whether relying on existing statutes was either sufficient or preferable with regard to necessary regulatory authorities applicable to these technologies and resulting products (Jones 1999).

Environmental releases of genetically modified organisms were proposed for the first time nearly simultaneously by Monsanto and Advanced Genetic Sciences, Inc. (AGS) (Watrud et al. 1985; Lindow 1985). AGS developed a product named FrostBan®, a *Pseudomonas syringae* engineered such that a gene coding for a protein necessary for ice-nucleation had been deleted, and conducted a field release on strawberry fields in University of California experimental plots under EPA and California Department of Food and Agriculture authority on April 24, 1987 (Smith 1997).

Initial approval granted by the NIH administrator (48FR9436; 48FR:24548) for this field test was overturned due to a May 16, 1984 decision (OTA 1988) that the environmental impacts under NEPA were not assessed, though the decision also affirmed that field testing could take place once an environmental effects assessment was performed (Pizzuli 1984). Through a series of events EPA was assigned the task of assessing environmental impacts, though the permit was withdrawn just prior to the 1987 field test when another test, this one an experimental rooftop injection of Frostban® into trees, was declared in violation of the issued permit resulting in a $20,000 fine – though AGS claimed the bacterium injected into trees was a contained use (New Scientist 1986). A field test of Frostban® on strawberry plants did occur at Conta Costa, CA following Federal and State approvals (Supkoff et al. 1988). Steve Lindow of the University of California at Berkeley also conducted frost prevention tests with his deletion mutant IceMinus *Ps. syringae* on potato plants at Tulelake, CA despite some vandalism by opponents of GE technology and a lengthy permitting process (Maugh 1987).

A subsequent genetically engineered construct involved transformation systems directing placement of *Bacillus thuringiensis* (B.t.) transgenic sequences into the bacterial chromosomes of *Clavibacter xyli* ssp. *cynodontis* and *Pseudomonas*

fluorescens, respectively. A system devised by Crop Genetics International (CGI) focused on delivery of the B.t. Cry1Ac δ-endotoxin in tissues of maize by introducing genetically modified *C. xyli* into maize xylem vessels (Turner et al. 1991). *C. xyli* is a natural endophyte of Bermuda grass, maize and several other plants, hence, its potential as a delivery agent of a biopesticidal protein was sought as a means of reducing feeding damage to corn earworm and European corn borers and as a way to reduce environmental exposure to non-target organisms (Lampel et al. 1994). Due in part to the overall concentration of the B.t. δ-endotoxin contained in the endophytic populations of *C. xyli* in maize, this construct ultimately failed to consistently deliver sufficient control during field trials. In addition, there were serious concerns about the possible uncontrolled spread of the genetically engineered microorganism to other plants. Yield was also affected in some maize varieties because of occlusion of xylem vessels with bacteria, particularly when drought stress was an issue (John Turner, personal communication 2011). Regulatory costs associated with field release permits (USDA-APHIS) and experimental use permits (US EPA) were a factor for CGI in that they were a relatively small company without a broad portfolio of products. In 1994, further research into this mechanism of delivery into maize was halted by CGI (Wrubel et al. 1997).

Monsanto's approach was to create an insecticidal, plant rhizosphere dwelling microbe by cloning the *Bacillus thuringiensis* subsp. *kurstaki* HD-1 crystalline protein gene into strains of *Pseudomonas fluorescens* (Obukowicz et al. 1986). Limited field releases of these live microorganisms occurred, though only with strain variants engineered with reporter genes (Kleupfel et al. 1991; Angle et al. 1995; Gagliardi et al. 2001). EPA questioned the safety of pseudomonads expressing B.t. endotoxins in aquatic environments, and this led to Monsanto's decision to cease work on use of engineered microbes as pesticides. Subsequent work in contained settings has shown that runoff from simulated agricultural plots containing *Pseudomonas chlororaphis* (*aureofaciens*) 3732 can be significant (Gillespie et al. 1995), and the general lack of available non-target aquatic invertebrate tests to evaluate such effects leaves regulatory certainty for this use in limbo.

Subsequently, between 1991 and 1996, four genetically engineered microbial preparations were registered under FIFRA as encapsulated *B. thuringiensis* δ-endotoxins in killed *Pseudomonas fluorescens*. Delivery of the B.t. δ-endotoxin in killed *Pseudomonas* had a distinct advantage over using live *B. thuringiensis* in that higher levels of toxins are produced by the pseudomonads during fermentations and some protection against UV light inactivation of the toxin was gained via encapsulation within the killed pseudomonad cell wall (OTA 1995; Mycogen 1998; Shand 1989). Additionally, the use of killed bacteria as the end product alleviates any concerns over spread and reproduction of the engineered pseudomonad; this was a consideration by both the company and EPA risk assessors (BLR 1988).

In addition to the transgeneric expression of B.t. δ-endotoxin genes in the heat-killed pseudomonads, creating a so called 'killed microbial' pesticide, several companies moved forward with engineering of *B. thuringiensis* strains directly, either modifying native *cry* gene sequences or adding to the resident *cry* genes with additional

cry genes in order to broaden the range of susceptible insect species (Baum et al. 1996; Sanahuja et al. 2011).

In addition to these pseudomonad constructs, six submissions were received by EPA for field testing of genetically modified baculoviruses from May 1995 through August of 1998. Four of these utilized the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) with additions of insect-specific toxin genes: three from two different scorpions (Summers 2006) and one from a mite (Tomalski et al. 1989). Two others are based upon modified *Helicoverpa zea* single-embedded nuclear polyhedrosis virus (HzSNPV) each using an insect-specific scorpion toxin from one of two scorpion species. Since the main issues are very similar between the various baculovirus constructs, only a few examples will be discussed in detail herein.

Work with engineered baculoviruses was quite active in the 1990s (Hughes et al. 1997), and even earlier in the UK (Bishop 1988) for control of insect pests on vegetables, ornamentals, and in forestry situations, and some of this work continues today (Tang et al. 2011). Much of the effort centered on addition of scorpion toxin genes to enhance the kill rate of AcMNPV and HzNPV without a consequent change in host range. Toxins from both *Leiurus quinquestriatus hebraeus* (Israeili yellow scorpion; LqhIT2) and *Androctonus australis hector* (Algerian scorpion; AaIT) were used by American Cyanamid and DuPont in an attempt to increase mortality in the target pest without altering the risk profile for non-target species that may feed on the infected insect pests (Bill Schneider, Personal Communication 2010; Gard et al. 2002; Heinz et al. 1995; American Cyanamid 1994, 1996; DuPont 1996; Kunimi et al. 1996).

These scorpion toxins act through either a depressant (LqhIT2) or stimulant (AaIT) capacity on neurons through sodium channel modulation, however, they do not have demonstrable vertebrate activity nor do they affect Crustacea (Hoover et al. 1996; Gard et al. 2002). EPA required testing of a range of surrogate species, including rats, Bobwhite quail, Mallard ducks, rainbow trout, and grass shrimp, which were fed infected *H. zea* larvae. Additional tests with NPV occlusion bodies (OBs) suspended in aqueous media indicated a lack of pathogenic or toxic effect on *Daphnia magna*, the water flea. Testing of human cell lines (liver, lung, intestine) was also performed with budding virus particles with no indication of alterations to cell morphology or timing of division. It is noteworthy that although guidance on assessing human health and environmental risks has adapted to newer technologies as they arose, many of the principals have been in place prior to the advent of biotechnology and rDNA methods (Engler 1974).

Additionally, the ecdysteroid UDP-glucosyl transferase gene (*egt*) had been found to alter ecdysoid hormone levels and influence killing rate, feeding period and molting of several insect species (O'Reilly and Miller 1989, 1991; Slavicek et al. 1999). Removal of the *egt* gene from the AcMNPV genome resulted in feeding cessation and wandering behavior of infected larvae, which succumbed to the viral infection prior to pupation. The combination of the AcMNPV/LqhIT2 toxin and deletion of *egt* resulted in a higher mortality rate during initial measurements soon after infection experiments comparing recombinant strains to wild type AcMNPV,

however, following extended incubation (e.g. a few days post infection to as many as 21 days depending on the virus:insect combination), mortality was equal between the two groups. The titer of occlusion bodies present in the AcMNPV/LqhIT2 strain was, however, significantly less than wild type infections (Tomalski and Miller 1991; Cory et al. 1994). Depending on the strain of virus and the intended host, reductions in yield of virus have varied from 30 to 50% and the rate of kill increased by as much as 95% (Cory 2000). The decreased viral load following infection and the limited host range of most baculoviruses fit prominently into the EPA's risk assessment for these modified biopesticides. The inability of these genetically engineered baculoviruses to persist in the environment and potentially exchange genes with wild type strains or related viruses reduced the uncertainty associated with field release of constructs previously evaluated in laboratory settings (OSTP 2001).

Another consideration of the risk assessment for AcMNPV/LqhIT2 and other recombinant baculoviruses was whether these novel strains could outcompete and eliminate wild type viruses over time. In addition to the noted decrease in viral load following host mortality, experiments and observations demonstrated that larvae infected with AcMNPV expressing insect-specific toxins were susceptible to 'knockoff' wherein they would drop from plant surfaces hours earlier than wild type infected larvae, thereby limiting spread of the OBs onto leaf surfaces where they may contact other larvae (Inceoglu et al. 2006). Further experiments with combinations of GE and wild-type NPVs also indicated that sequential passage to larval hosts resulted in the eventual elimination of the toxin expressing virus strains. In some instances, the GE baculoviruses were comparable in efficacy to conventional insecticides with a 30–40% increase in the speed of killing larvae as compared to non-GE baculoviruses (Hoover et al. 1996).

Shortly after the initial proposed field releases, non-pesticidal uses of genetically modified microorganisms began to be developed. By 1987 initial releases of *Ensifer* (*Rhizobium*) *meliloti* were under TSCA review and initial experimental releases took place by 1988 (EPA 1999). The Monsanto *Pseudomonas chlororaphis* (*aureofaciens*) strain containing reporter genes was also submitted for TSCA review in 1987 and went to the field that same year.

While this chapter considers the oversight of GE microorganisms by the US EPA, it should be noted that some of these organisms may also be regulated by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS). Both the Plant Protection and Quarantine (PPQ) and the Biotechnology Regulatory Services (BRS) divisions within USDA-APHIS may be involved in the importation, movement and field release of non-GE and GE microorganisms under the Plant Protection Act and National Environmental Policy Act (OSTP 2001). The Food and Drug Administration reviews all genetically engineered microorganisms that may cause an alteration in the nutritional state of a food, or otherwise contribute to a food safety issue. When in doubt as to which agencies may exercise regulatory authority over a particular microbe and its intended use, it is best to contact the agency directly for clarification.

4.2 FIFRA Risk Assessment of Genetically Engineered Microbial Pest Control Agents

Under FIFRA, microbial biopesticide products, as with all other pesticides, must be evaluated for their risks and benefits. Bacteria, viruses, protozoa, algae, and fungi intended for use as pesticides are regulated under FIFRA by the US EPA (40 CFR Part 158.2100). Additionally, the Agency evaluates the potential for effects upon threatened and endangered species under the Endangered Species Act, but this will not be discussed further in this section. There are three principal sections to the FIFRA risk assessment for genetically engineered microbial pest control agents (GE-MPCA): product analysis, human health, and environmental considerations (McLintock et al. 2000). The aim of this chapter is not to consider the data requirements associated with these sections in great detail, but, rather, to present an overview of key considerations. One important note: EPA evaluates an MPCA using the same data requirements, regardless of whether it is genetically engineered or naturally occurring (Baum 1998).

Under the product characterization section (40 CFR Part 158.2120) of the data requirements a summary of the taxonomy, natural history, target, and non-target host range is required. For any genetically modified MPCA, the product analysis portion of the data requirements seeks to provide the risk assessor with necessary information regarding the nature of the transformation event and includes DNA sequences of transgenes, associated vector sequences with restriction map, DNA source information and an indication of transgene stability over multiple generations or growth cycles (e.g., 5 batch analysis). Also critical to this section is the Confidential Statement of Formula, which details the active ingredient(s), inert ingredients, and concentration of the MPCA in its final product formulation. Any pesticide in use under a FIFRA Section 5 Experimental Use Permit, or Section 3 Registration, which is not in accord with the information present on the CSF is considered as 'Misbranded' and therefore illegal (FIFRA 2(q)).

Toxicology data requirements (40 CFR, Part 158.2140) explore the potential impact of the MPCA on humans in terms of toxicity, infectivity and pathogenicity. The MPCA is introduced via oral, pulmonary, and injection (intravenous or intraperitoneal) routes into rodent test animals functioning as surrogates. Animal body and organ weights, behavior, and mortality are all assessed as part of these studies, but most important is establishing clearance of the MPCA from the body over time. These high dose tests (at least 10^8 units of the MPCA per test animal) are intended to examine the outcomes following a single, significant contact with an MPCA by various exposure routes (mouth, nose, lungs, and dermal).

Non-target organism and environmental fate data requirements (40 CFR, Part 158.2150) evaluate the potential for the MPCA to impact organisms beyond the intended target pest(s). These studies require examination of pathogenicity on related (e.g., other insects) and unrelated (e.g., plants, birds, and mammals) organisms. The organisms chosen for study are functioning as surrogates, representative of broader groupings (e.g., Mallard duck for birds in general), and include wild mammals, birds, fish, beneficial insects, aquatic invertebrates, estuarine and marine organisms

(fish and invertebrates), plants, and honeybee testing. In accord with 40 CFR, Part 158.30, the Agency has flexibility in determining which of these data requirements must be in the form of generated data or related information, and which can be satisfied by waiver rationale.

The Environmental Fate data requirements focus on the fate of the organism in the area of application to determine the ability to persist and where the organism exists (e.g., in soil, associated with insects, etc.). The survivability and host range of an organism are key to understanding the ability of an MPCA to persist in the environment and potentially result in adverse effects (Hu and St. Leger 2002; 40 CFR 172.45(e)). For example, release of entomopathogens may require monitoring of resident arthropods to determine the ability to colonize and infect as a means of assessing persistence (St. Leger et al. 1996). Reproduction (e.g., sporulation) on cadavers of target hosts or lack thereof can be helpful in ascertaining the ability of the MPCA to persist following small scale release. Rhizospheric competence was also assessed with another set of constructs in *M. anisopliae* (now *M. robertsii*, J.F. Bisch., Rehner & Humber) as part of an investigation into survivorship in the environment (Hu and St. Leger 2002).

As with all pesticides applied to food or feed crops, a food tolerance or the exemption from the requirement of a food tolerance must be in place if any residues of the pesticide may be present on any food derived from the crop. In all cases to date, the MPCAs registered by the Agency have been granted an exemption from the requirement for a tolerance based upon a determination that there is a reasonable certainty that no harm will result from dietary exposure to the MPCA. In general, pesticides containing elements of any of the eight major allergens are not approved for use on most food or feed crops, which could also extend to any expressed proteins originating from peanuts, tree nuts, milk, soybeans, eggs, fish, Crustacea, and wheat (40CFR 180.950).

While the same set of data requirements are imposed upon naturally occurring and GE microbial agents, genetically modified MPCA and non-indigenous microbial species may be subject to additional data or information requirements on a case-by-case basis depending on the particular microbial agent and/or its parental strains, the proposed pesticide use pattern, and the manner and extent to which the organism has been genetically modified (FR 2007).

4.2.1 Biotechnology Notification Process for Microbial Pest Control Agents

At least 90 days prior to conducting any small scale test of a genetically modified microbial pesticide, other than those described at 40 CFR 172.45(d), a Notification must be submitted to the EPA in which the details of the genetic modification, proposed application methods and sites, and any potential toxicity or non-target organism effects are delineated. 40 CFR 172, subpart C. Measures must also be outlined in the Notification submission which indicate the methods of containment and monitoring used to ensure the GEO does not become established in the ecosystem. 40 CFR 172.48. The data required to support a request for a Notification are detailed in 40 CFR Part

172.48 (FR 59, 169, Sept. 1, 1994). If the proposed field test is to be greater than10 acres of treated land per pest evaluated or greater than1 acre, for aquatic uses, then an experimental use permit is necessary. 40 CFR 172.3.

Under FIFRA, a Biotechnology Notification Process (40 CFR, Part 172.43; BNP) for release of a GE-MPCA at any size test plot requires review and approval by the EPA prior to commencing experimentation. EPA requires notification prior to small scale field testing of genetically engineered and non-indigenous microorganisms not subject to USDA oversight to allow EPA to determine if an Experimental Use Permit is needed and to allow the applicant to gather data critical to the risk assessment process. Processing times for review and approval of BNP applications are considerably shorter than those encountered with Experimental Use Permits (EUPs) and Section 3 registrations, and they are intended for smaller (e.g., ≤1 A) field test plots than EUPs. It must be emphasized that with BNP approvals, any treated plants or materials are prohibited from entry into the food and feed supply unless a food tolerance or exemption from the requirement of a food tolerance under Section 408 of FFDCA is in place; these environmental releases are strictly for research purposes only. The treated produce of a BNP or EUP may be allowed for consumption by experimental animals, however, the products of those animals are not allowed for entry into the food or feed supply unless an appropriate food tolerance action is in place.

Several GE MPCA have been through the BNP successfully and field tested on a small scale (See Table 4.1). This includes the first approved field test of a GE microbe, strains of *Pseudomonas syringae* and *Erwinia herbicola* with an ice-minus phenotype applied to potatoes as a means of preventing frost and its associated plant damage (Lindow and Panopoulos 1988; Milewski 1987). Advanced Genetic Sciences (AGS) had engineered a *Ps. syringae* resulting in the absence of expression of a membrane protein responsible for ice nucleation, though the product currently marketed as 'Frostban®' is not genetically engineered and is a naturally occurring ice-minus strain. Another wildtype ice + strain of *Ps. syringae* is also marketed, as 'Snowmax' and is utilized in artificial snow-making operations, however, it is not regulated as an MPCA.

Other successful BNP environmental releases include two *Metarhizium anisopliae* strains modified to enhance virulence through addition of native protease genes (St. Leger et al. 1996) and, in a separate BNP, a gene derived from the scorpion *Androctonus australis* encoding a known neurotoxin active against tobacco hornworm (Wang and St. Leger 2007).

4.2.2 Experimental Use Permits for Microbial Pest Control Agents

When testing a MPCA at 10 acres or more (1 A or more for aquatic use), EPA requires an Experimental Use Permit before field testing naturally occurring or genetically engineered MPCA (40 CFR Part 158.2170; 40 CFR Part 172.3). EUPs for GE-MPCA typically involve larger acreages than those approved under a BNP; however, pesticide

Table 4.1 EPA/FIFRA regulation of genetically engineered organisms for environmental release

Year	Organism	Trait	Intended use/ref	Regulatory action	Registrant
1985, 1987	*Pseudomonas syringae* RGP36R2 and *Ps. fluorescens* GJP17BR2	Ice minus (absence of membrane protein inducing crystallization of water)	Frost prevention Smith (1997)	Biotech notification	Advanced Genetic Sciences
1987	*Pseudomonas syringae*	Ice minus (absence of membrane protein inducing crystallization of water)	Frost prevention Lindow and Panopoulos (1988)	Biotech notification	University of California – Berkeley
1988	*Clavibacter xyli* subsp. *cynodontis*	Insecticidal Cry toxin from *B. thuringiensis var. kurstaki*	Insecticide Turner et al. (1991)	Experimental use permit	Crop Genetics International
1987 1990	*Pseudomonas fluorescens*, M-Cap™ [b]	CryI proteins from *B. thuringiensis* var. *san diego*	Insecticide	Experimental use permit Registration	Mycogen
1990, 1991, 1994	*Pseudomonas fluorescens*, MVP®[b]	Chimeric Cry1Ac/ Cry1Cproteins from *B. thuringiensis* var. *kurstaki*	Insecticide Navon (2000)	Experimental use permit Registration	Mycogen
1991	*Pseudomonas fluorescens*, M-One Plus[b]	CryI proteins from *B. thuringiensis* var. *san diego*	Insecticide	Registration	Mycogen
1992	*Pseudomonas syringae* 742RS/*Ps. fluorescens* A506 and 1629RS	Ice minus (absence of membrane protein inducing crystallization of water)	Frost prevention	Registration	Frost Technology Corporation Plant Health Technologies
1993 1995	*Pseudomonas*[b] *fluorescens*, Mattch™	Cry IA(c)/CryIA(b) and CryIC/ CryIA(b) chimeric proteins from *B. thuringiensis* var. *kurstaki*	Insecticide Federal Register (1995)	Experimental use permit Registration	Mycogen
1993	*Bacillus thuringiensis*, ECX9399	Cry proteins from *B. thuringiensis* var. *kurstaki*	Insecticide All et al. (1994)	Experimental use permit	Ecogen
1994, 1996	Nuclear polyhedrosis virus of *Autographa californica* AcMNPV	Insecticidal toxin AaHTI from *Androctonus australis hector*	Insecticide	Biotechnology notification	American Cyanamid

1995, 1996, 1997, 1998	Nuclear[a] polyhedrosis virus of *Autographa californica* AcMNPV	Insecticidal toxin LqhIT2 from scorpion *Leiurus quinquestriatus hebraeus*	Insecticide Federal Register (1997)	Biotechnology notification	American Cyanamid
1995	*Bacillus thuringiensis* EG7673 Raven™ OF	Cry 3Bb and 3Aa proteins from *B. thuringiensis* var. *tenebrionis*	Insecticide Baum (1998)	Registration	Ecogen/Certis
1996	*Bacillus thuringiensis*, EG7841 Cry Max® WDG/WP	Cry 1C proteins from *B. thuringiensis* var. *aizawai*	Insecticide Baum (1998)	Registration	Ecogen/Certis
1996, 1997	*Helicoverpa zea* single-embedded nuclear polyhedrosis virus HzSNPV	Insect-specific toxin from the venom of the scorpion *Leiurus quinquestriatus hebraeus*	Insecticide Federal Register (1997)	Biotechnology notification	DuPont
1996	Nuclear polyhedrosis virus of *Autographa californica* AcMNPV	Insecticidal toxin (TxP-I) from *Pyemotes tritici*, straw itch mite	Insecticide Tomalski and Miller (1991)	Experimental use permit	American Cyanamid
1996	Nuclear polyhedrosis virus of *Autographa californica* AcMNPV	Insecticidal toxin (TxP-I) from *Pyemotes tritici*, straw itch mite	Insecticide Tomalski and Miller (1991)	Experimental use permit	DuPont
1996, 1997	Nuclear[a] polyhedrosis virus of *Autographa californica* AcMNPV	Insecticidal toxin from *Leiurus quinquestriatus hebraeus*	Insecticide Federal Register (1997)	Biotechnology notification	DuPont
1997	*Bacillus thuringiensis* EG7826 Lepinox™ WDG/G	Cry 1Ac/1 F[c] protein from *B. thuringiensis* var. *kurstaki/aizawai*	Insecticide Baum (1998)	Registration	Ecogen/Certis

(continued)

Table 4.1 (continued)

Year	Organism	Trait	Intended use/ref	Regulatory action	Registrant
1997	*Helicoverpa zea* single-embedded nuclear polyhedrosis virus HzSNPV	Insecticidal toxin from *Androctonus australis* AaH IT1 and prevent expression of the ecdysteroid UDP-glucosyltransferase gene (*egt*)	Insecticide	Biotechnology notification	American Cyanamid
1997	*Pseudomonas fluorescens*, M-Press®	Chimeric Cry1F/Cry1A(b) toxin from *B. thuringiensis* var. *aizawai*	Insecticide	Experimental use permit	Mycogen
1997	*Rhizobium* and *Sinorhizobium*	Expressing genes for trifolitoxin to outcompete soil bacteria and a hydrogenase for enhanced nitrogen fixation	Bactericide Federal Register (1997)	Biotech notification	Eric Triplett, University of Wisconsin-Madison
1998 2002	*Escherichia coli* K-12 derivative; heat-killed	Harpin protein from *Erwinia amylovora*	Plant disease prevention	Experimental use permit Registration	EDEN Biosciences Corp.
1999	*Agrobacterium radiobacter* K1026	Removal of DNA transfer function by deletion	Bactericide, Plant disease prevention	Registration	Bio-Care Technology Pty Limited
1999	*Metarhizium anisopliae*, ARSEF 1080	Enhanced protease production, Pr1 gene from *M. anisopliae*	Insecticide St. Leger et al. (1996)	Biotechnology notification	Ray St. Leger, Univ. of Maryland
2001, 2004	*Pichia pastoris*; heat killed	Insecticidal Trypsin Modulating Oostatic Factor	Larvacide for mosquitoes	Biotechnology notification, Registration (Technical Product only)	Insect Biotechnology, Inc.
2004	*Cryphonectria parasitica* ATCC 38755 ATCC 64671	Reduced virulence gene from mycovirus CHV1-Euro7	Fungicide/Protectant	Biotechnology notification	West Virginia University

2005	*Escherichia coli* K-12 derivative; heat-killed	Harpin αβ protein with components of *hrpN* and *hrpW* (*Erwinia amylovora), popA (Ralstonia solanacearum),hrpZ (Ps. syringae)*	Plant disease prevention, growth enhancement	Registration	EDEN Biosciences Corp.
2007	*Metarhizium anisopliae*, ARSEF 549	Scorpion toxin from *Androctonus australis* AaIT	Insecticide	Biotechnology notification	Ray St. Leger, Univ. of Maryland
2011	Citrus tristeza closterovirus	Anti-microbial peptides	Bactericide, Plant disease prevention	Biotechnology notification	Southern Gardens Citrus, University of Florida

Folimonov et al. (2007)

[a]See SIDEBAR No. II.A, BIOCONTROL USING A VIRUS (AcMNPV) following the Bt-maize case study. http://www.whitehouse.gov/files/documents/ostp/Issues/ceq_ostp_study3.pdf. Accessed 22 Nov 2010

[b]Cry proteins expressed in *Ps. fluorescens* cells, cells then killed prior to application

[c]Cry fusion protein

products used under an EUP also require an approved label for experimental use and interstate shipment (40 CFR Part 172.6); this is not the case for BNP testing. Several genetically modified biopesticides have been approved for use under EUPs; but, a number of these were never actually applied or in some cases only sparingly applied (Table 4.1). The reasons for this relate to issues of public perception (i.e., is the company or researcher willing to deal with public meetings and scrutiny?) and business decisions (e.g., is there sufficient market potential to warrant the development and regulatory costs?).

The data requirements for an EUP involving GE-MPCA are discussed at 40 CFR Part 174.3 and the specific tests, also germane to non-GE MPCA, are described in 158.2171–158.2174. In general, the data requirements for an EUP or Section 3 registration are similar, however, the limited exposure to the environment from the small scale field testing of an MPCA under an EUP does not require the same level of non-target organism testing as when full commercial registration is approved through registration procedures. This is due in large part to the limited scope of the environmental release at the EUP stage and the fact that much of the non-target effects information may be collected as part of the EUP overall plan.

4.2.3 Section 3 Registration of Microbial Pest Control Agents

Before any microbial pesticide registration is granted under FIFRA, EPA considers such issues as potential adverse effects to non-target organisms, environmental fate of the microorganism, and the potential toxicity, pathogenicity and infectivity of the microorganism to humans and other animals. These issues are the same as those considered for non-engineered microbial agents approved for pest management, and reflect the inherent similarities of the functional properties of the organism regardless of whether the traits of primary interest are derived from rDNA or not.

The data requirements for registration of a microbial biopesticide are delimited in 40 CFR 158.2120–158.2150. The data and information garnered from the fulfillment of these data requirements are used to inform the risk assessment process, just as with the EUP and BNP applications. All of the data requirements must be satisfied for a FIFRA Section 3 registration, however, in some instances rationale can be provided by the registrant to explain why the requirement is not applicable to the MPCA in question. For example, a psychrotropic bacterium which does not grow at temperatures greater than 20 °C is unlikely to result in mammalian pathogenicity given the body temperature of these animals, including man. Similarly, a microbial biopesticide labeled for use at residential sites only is unlikely to result in significant exposure to marine and estuarine environments. Explanation of factors affecting the applicability of a study outcome to a risk determination may be used to satisfy some data requirements. As always, it is important to discuss this with regulators prior to conducting any studies.

Relative to a BPN or an EUP, the number of studies requiring empirical data generation applied to the issuance of a Section 3 registration are typically greater as this regulatory action often coincides with commercial use on a larger scale than either of the two preceding regulatory actions. For both BPN and EUP actions, the

scope of the exposure of man and the environment to the novel pesticide is significantly reduced as compared to commercial use in most instances. Hence, the data and information required for an EUP or small scale field test under a BPN are more often limited to concerns of human health (e.g., infectivity) and environmental persistence than with longer term non-target effects, which will be addressed at the time of registration, with data obtained through earlier field tests in most cases.

It should be noted that all pesticide registrations are subject to periodic review and re-registration procedures as FIFRA is a licensing statute and statutory requirements exist in order to maintain that license or registration in good standing in order to enter the product into commerce.

The first genetically engineered MPCA registered under FIFRA was a pair of *Pseudomonas fluorescens* strains, each modified with a different type of δ-endotoxin from *B. thuringiensis*, for insect control. Mycogen chose to express their *kurstaki* and *san diego* type endotoxins in *Ps. fluorescens* to provide for adequate expression and accumulation of protein toxin, but also as a means of reducing inactivation of these proteins by ultraviolet light. These products were referred to as MVP and M-Trak, respectively, and did not contain any live organisms, so the risk assessment was not concerned with pathogenicity or infectivity issues.

4.3 Risk Assessment Considerations

4.3.1 FIFRA

As noted above, FIFRA's standard for registration decisions involves an assessment of risks and benefits of using a pesticide. This is to include a biological analysis of potential effects upon man and the environment as well as social and economic considerations resulting from a regulatory decision. The inclusion of an explicit risk-benefit calculation distinguishes FIFRA from most other U.S. environmental statutes.

One of the primary benefits of a biopesticide is the replacement of control measures that may pose greater risks, such as groundwater contamination, toxicity to non-target organisms, or dietary risks to infants and children. To date, decisions to approve nuclear polyhedrosis viruses (NPVs), plant viruses and bacteriophage have relied primarily on their lack of toxicity to all organisms except target pests with little or no animal testing conducted. EPA considers possible benefits that might result from use of viruses such as the NPV AcMNPV/LqhIT2 (OSTP 2001). Application of AcMNPV/LqhIT2 would likely reduce the use of other insecticides and thereby would avoid the types of impacts those less specific insecticides might have had, if applied to the same acreage as AcMNPV/LqhIT2.

Targeting an insect-specific toxin to the 'point of feeding' of pest insects should minimize the impact on non-target organisms and minimize ground water contamination, as may occur with use of more environmentally persistent chemical pesticides. Because many of the previously deployed insecticides were broad-spectrum in their activities, the potential for impacts on the beneficial insect populations was significant.

Populations of beneficial insects should increase over time as more MPCAs with host specificity are used and fewer broad-spectrum pesticides are applied. This has been shown in the context of B.t. corn crops, where increased abundance of arthropods was noted in B.t crop fields when compared to conventionally bred maize treated with insecticides (Marvier et al. 2007). Since some insecticides have effects on non-insect organisms (e.g. earthworms, nematodes), the reduction or elimination of these broad-spectrum pesticides will help to nurture these populations as long as cultural practices of soil management are adequate.

Additionally, the exposure of farm workers, pesticide applicators and the public at-large is often reduced when a biological pesticide takes the place of a chemical spray alternative. For example, residues on food are less of a concern with AcMNPV/LqhIT2, because the insect neurotoxin is known to be non-toxic to humans and other mammals. Spray drift is often problematic with chemical applications, but this is not a significant issue with target specific NPVs.

FIFRA also requires special consideration of public health pests, such as disease vectoring mosquitoes, cockroaches and rodents. Data detailing the ability of the MPCA to manage a pest situation are required for all registrations, however, these data must be submitted and reviewed for those involving public health pests prior to any such regulatory action being considered.

4.3.2 FFDCA

The Federal Food, Drug, and Cosmetic Act is largely the purview of the US Food and Drug Administration, except for residues of pesticides that may occur in food and feed (Section 408, FFDCA). Microbial biopesticides that pass Tier I testing without evidence of toxicity or pathogenicity will most often qualify for an exemption from the requirement of a numerical food tolerance (also referred to as a Maximum Residue Level in some countries). This regulatory action, determined following risk assessment and literature review, has afforded the determination that any level of the microbe present in food and feed resulting from use of the product as specified on the FIFRA label, will not result in harm by a variety of exposures. Among the exposure scenarios assessed for food safety are ingestion through food or water, inhalation, dermal and eye contact, and injection. While effects may be evident in some of these tests, the probability of exposure is also a consideration. Specific areas addressed under FFDCA (as applicable to microbial pesticides) are acute, subchronic and chronic dietary risks, occupational exposures, drinking water exposures, effects to the immune and endocrine systems, any dose response related information, exposures associated with day cares, residences and schools, exposure of sensitive populations, such as infants or children, aggregate effects for multiple exposures, and cumulative effects.

When assessing MPCA, there are the three endpoints of concern: infectivity, pathogenicity and toxicity. In some cases an analysis of potentially toxic metabolites is included in the food safety risk assessment and review of the primary literature. Some microbial species are known to produce metabolites or toxins which can have adverse effects upon man and livestock following consumption.

Note, if an organism is not completely identified or is closely related to a human pathogen, i.e., in the same genus, the literature review and subsequent risk assessment should be broad enough to cover the eventuality that the relevant pathogenicity factors and/or toxins are ruled out as not present in the test strains proposed for use as a pesticide.

4.4 Entomopathogenic Nematodes

Entomopathogenic nematodes have been applied to pest management of insects in diverse agricultural settings (de Doucet et al. 1998; Head et al. 2000; Martin 1997). While the number of nematode genera infecting insects and other arthropods is large and diverse, most of the research and development interest has been with the Steinernematid and Heterorhabditid groups targeting agricultural insect pests (Grewal and Peters 2005). Both of these genera rely on symbiotic (phoretic) bacteria to effect a lethal septicemia upon their hosts which results in degradation of internal tissues and organs, death of the insect host, and reproduction of the nematode and symbionts.

Members of the genera *Steinernema* and *Heterorhabditis* differ in their strategies of host location, host specificity, and survival mechanisms, they are both inherently susceptible to heat and desiccation in the soil environment. As a means of enhancing the heat tolerance of *Heterorhabditis bacteriophora*, an hsp70A gene from *Caenorhabditis elegans* was introduced to juvenile nematodes (Hashmi et al. 1995; Wilson et al. 1999). Although this effort was ultimately not successful at the field level in providing the necessary level of heat tolerance, it nonetheless raised some interesting regulatory issues (Gaugler et al. 1997).

The Code of Federal Regulations defines microorganisms considered as biopesticides to include viruses, bacteria, protozoa, algae and fungi (FR 2007). Absent from this list are nematodes and certain other microscopic, multicellular invertebrates. Nematodes may be included as biocontrol agents subject to oversight under the Plant Pest Act, yet this is less than apparent.

According to the Coordinated Framework for Biotechnology (OSTP 1986) when referring to EPA's oversight, "The Agency has determined that certain non-microbial organisms which fall within the definition of biological control agents are already addressed by other agencies, specifically USDA and the Department of the Interior. Examples of these biological control agents are vertebrates, insect predators, nematodes, and macroscopic parasites. Therefore, pursuant to section 25(b) of FIFRA and 40 CFR 162.5(c)(4), these nonmicrobial biological control agents have been exempted from regulation under FIFRA. However, if EPA, in cooperation with other agencies, determines that certain biological control agents exempted by § 162.5(c)(4) are not being adequately regulated, these organisms will be referred to the attention of the appropriate agency or added to the exceptions in § 162.5(c)(4) by amendment. In the latter case, those organisms would no longer be considered exempt from the provisions of FIFRA."

While entomopathogenic nematodes are included in this exemption, genetic engineering of either the nematode or the microbial symbiont could bring the new product back under FIFRA oversight as a pesticide.

Genetic engineering of the microbial symbionts (i.e., *Xenorhabdus* spp.; *Photorhabdus* spp.) would bring these organisms under the regulatory umbrella of the USDA-APHIS and EPA, however, modification of the nematode itself does not meet existing regulatory thresholds (FR 2007; Gaugler et al. 1997; Gaugler, personal communication). It should be noted that in the U.S., the importation and interstate movement of exotic entomopathogenic nematodes may be regulated by the USDA-APHIS' Plant Protection and Quarantine group (Rizvi et al. 1996; Selçuk et al. 2003) under the Plant Protection Act of 2000.

During laboratory and growth chamber experimentation with the *H. bacteriophora* hsp70A transformants, this issue was raised to the USDA-APHIS and EPA-BPPD for clarification (Randy Gaugler, personal communication; Chris Wozniak, personal communication). At the time, neither agency indicated jurisdictional oversight of these GE nematodes, but suggested that the Center for Disease Control be contacted as well. Communication with CDC (Wozniak, personal communication) likewise indicated that they did not claim oversight of the organisms for the intended purpose (i.e., pest control).

Faced with this lack of Federal oversight, yet concerned with public perception and local (i.e., State, University Institutional Biosafety Committees) considerations, the lead investigator, Dr. Randy Gaugler of Rutgers University, requested a review of the *H. bacteriophora hsp*70A, as applied to insect pest management, by the USDA-APHIS. This review resulted in a finding of no significant impact (FONSI) by the agency and a determination that environmental release would not result in injury to agricultural plants or their products as determined under the Plant Pest Act. Note that this finding does not preclude potential regulatory action by State or other local authorities, as is the case with all microorganisms, including pesticidal agents, intended for release into the environment.

While the lack of Federal regulation has obviously reduced costs and time necessary to bring an entomopathogenic nematode product to market, some have opined that this lack of oversight has resulted in some inferior products with exaggerated claims (Weinzierl et al. 2005). At least one of the authors (CAW) has had this unfortunate experience!

4.5 Considerations of Genetic Engineering and Gene Transfer

4.5.1 Public Perception of GE Microbials

During the early stages of the development of GE microorganisms, significant public debate occurred regarding the human health and environmental safety of these novel products of biotechnology (Marchant 1988; Barinaga 2000). As is often the case with public reaction to new technologies, the debate was not always centered on scientific facts or reasoned discussion, but was taken up by opponents of biotechnology as a

crusade against development of genetically engineered organisms regardless of intent or merit. Additionally, debate within the scientific community was needed to develop a regulatory system capable of responding to novel products and nuances to the technology as they developed. As evidenced by the early field experiments with ice-minus bacteria for frost prevention on strawberries and potatoes (Crawford 1986; Marchant 1988; Barinaga 2000), or the intentional degradation of an oil spill by hydrocarbon munching pseudomonads (Van 1989), public and, therefore, political considerations have influenced the field release and commercialization of GE microbes. Others have also expressed concerns (Dixon 2008).

Consideration of public perception and understanding of this novel technology led to business decisions that apparently did not necessarily reflect the actual science or potential risk associated with the proposed release of a particular GE microbial pest control agent. As is the case with GE plants, commercial considerations and the threat of lawsuits, with or without merit, persuaded individual concerns to halt research and development programs that may have lead to more environmentally benign alternative pest management measures (Phil Hutton Personal communication). Although regulatory requirements by EPA and USDA-APHIS may result in greater costs and longer lead times for commercialization of GE microbial products, we believe that, at least in some cases, companies were seeking regulatory approval as a means of indicating the safety of these products and did not perceive regulatory requirement as a deterrent to application of the products to market (Wrubel et al. 1997). Given the furor over the ice-minus and concurrent microbial field tests, regulatory oversight and approval may have enhanced public acceptance.

Many years later, as genetic engineering technology has progressed, significant numbers of GE microbial pest control agents exist on the market without the fanfare and protests characteristic of the early years of this technology. We believe that this bodes well for the potential of this technology to reduce the application of less environmentally benign technologies that ultimately have the potential for greater environmental effects.

4.5.2 Future for GE Microbials in Pest Management

The field of agricultural biotechnology has grown and developed so rapidly in the last 20 or so years that avenues to be taken, which we had not even anticipated 5 or 10 years ago, will continue to astound us in the future. The majority of this activity, at least in traditional agricultural terms, has been directly through engineering of plants for a variety of purposes, while the application of rDNA technology to microbial agents for pest and disease control has been slow in comparison. As can be evidenced by Table 4.1, the number of research efforts aimed at pest control through genetic engineering of MPCA have been numerous over the years. But, these efforts appear to have slowed, as recent actions are relatively few. There is, however, reason to expect that this may change in the future, at least in US and Canadian applications.

Despite the fact that some individuals are uncomfortable with microbes in general, based largely on a lack of understanding and encouraged by germ phobias, the instances where genetically engineered microbials have been utilized for nitrogen

fixation, soil amendments, biological control, and in bioremediation have not garnered the negative publicity to the degree that GE crop plants developed for agronomic, quality trait, and pest control purposes have. This was clearly not the case early on with the advent of biotechnology in agriculture – as was demonstrated by the furor over the early ice-minus field trials with pseudomonads in California or the first release of oil-degrading bacteria for cleanup of petroleum spills in marine environments.

The lack of attention to GE MPCA and other microbials may be in part due to the continued rancor over GE crops. There is also a common thread of mistrust among some of these groups toward large corporate interests (i.e., seed companies) such that the continued research and application of GE microbes flies largely under the radar of those who claim an innate aversion to this most promising of modern technologies. The majority of GE MPCAs are developed by small to mid-size companies without the visibility of those heavily involved in crop biotechnology.

One must also consider the use of GE microbes in food processing (e.g., chymosin, ascorbic acid production, flavor enhancers), even in countries where biotechnology is publically and officially shunned by many (e.g. the EU; GMO Compass 2010). These organisms and their products, when used as food processing aids, fail to trip the regulatory requirement for food labeling in stark contrast to those food and feed products derived from products of crop biotechnology. Perhaps this level of familiarity has garnered some trust with consumers or it simply has not made news enough to be noticed. Either way, it could bode well for GE microbial agents applied to agriculture and the environment.

4.6 TSCA Risk Assessment of Intergeneric Microorganisms

4.6.1 TSCA Regulation of Microorganisms

The United States Environmental Protection Agency is responsible for reviewing the risks associated with the commercial use or importation of chemical substances, including certain genetically modified microorganisms, under Section 5 of the Toxic Substances Control Act (TSCA). TSCA specifically excludes from review certain products that are subject to review by other federal agencies or under other statutes, including tobacco, nuclear materials, pharmaceuticals and cosmetics, and pesticides (but not pesticidal intermediates). TSCA's regulation of microorganisms is limited to those microorganisms that are "new", meaning that they are not listed on the TSCA Inventory of Chemical Substances. In this context, "new" microorganisms have been defined as those that are intergeneric, meaning that there has been the deliberate combination of genetic material originally isolated from organisms classified in different taxonomic genera. Also included in the definition of an intergeneric microorganism is a microorganism constructed with synthetic genes that are not identical to DNA that would be derived from the same genus as the recipient microorganism. Exclusions from TSCA review include naturally occurring microorganisms, as they

are considered to be implicitly listed on the TSCA Inventory, genetically engineered microorganisms other than intergeneric (e.g., intrageneric, physical or chemically mutagenized microorganisms), and intergeneric microorganisms resulting only from the addition of well-characterized, non-coding regulatory regions. TSCA section 5 only applies to microorganisms that are manufactured, imported, or processed for commercial purposes.

Intergeneric microorganisms subject to review under TSCA include a wide variety of biotechnological applications since TSCA is a gap-filling statute for biotechnology products not regulated under other statutes. Intergeneric microorganisms that may be subject to review under the Biotechnology Rule (40 CFR Parts 700,720, 721, 723, and 725 Microbial Products of Biotechnology: Final Regulation Under the Toxic Substances Control Act, FR Vol 62 No. 70 17909–17958, April 11, 1997) may be in applications including but not limited to biofuel production, biomass conversion, waste treatment, bioremediation, biomining, mineral leaching, oil recovery, desulfurization of fossil fuels, biofertilizers, biosensors, closed system fermentation for the production of enzymes and specialty chemicals, and pesticidal intermediates. Among these, biofertilizers (e.g., nitrogen fixers, mycorrhizae, phosphate solubilizers, etc.), algal biofuels, pesticidal intermediates, and perhaps, biosensors could have agricultural uses.

4.6.2 Categories of Premanufacturing Oversight

4.6.2.1 Microbial Commercial Activity Notice (MCAN)

Prior to manufacture or importation of an intergeneric microorganism, companies must make an appropriate submission to EPA's Office of Pollution Prevention and Toxics (OPPT). Subpart D of part 725 of the Biotechnology Rule establishes the reporting program for new microorganisms. New microorganisms that are to be manufactured or imported for distribution into commerce requires the submission of a Microbial Commercial Activity Notice (MCAN) 90 days prior to initiating manufacture or import, unless the activity is eligible for one of the specific exemptions.

The purpose of the MCAN is to supply EPA with information necessary to identify and list the new microorganism on the TSCA Inventory of Chemical Substances, and to determine whether the microorganism and the associated manufacture or importation may present an unreasonable risk of injury to human health or the environment. The MCAN information requirements closely parallel those developed for traditional chemical Premanufacturing Notices and differ only to the extent necessary to accommodate the specific characteristics of living microorganisms versus chemicals. All information on the microorganism identity and data on its actual and potential effects on human health and the environment that are available to the submitter, or are reasonably ascertainable are required in the MCAN. A detailed description of the genetic modifications to the recipient microorganism is necessary, along with data on the stability of inserted genetic material in the production strain

and the potential for transfer of this material to other organisms in the environment. In addition, a detailed complete description of the manufacturing process and design, production volumes, and containment and inactivation procedures are required. The requirements for information to be included in the MCAN are codified at § 725.155 and § 725.160.

4.6.2.2 Exemptions from Full Premanufacturing Notification

Research and Development Exemption

One exemption from MCAN reporting is the R&D Exemption. This is a complete exemption from TSCA § 5 reporting for certain R&D activities that are (1) conducted in contained structures, and (2) are subject to regulation by another Federal agency. As discussed in Subpart E of the Biotechnology Rule and codified at § 725.232, activities that meet these criteria are exempt from EPA review, reporting, and record keeping requirements for contained research conducted by researchers who are required to comply with the NIH Guidelines for Research Involving Recombinant DNA Molecules (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html).

Other manufacturers conducting contained TSCA research and development activities that are not subject to regulation by the NIH Guidelines may qualify for a more limited contained R&D exemption under § 725.234 and § 725.235. This exemption for R&D in contained structures specifies factors that a technically qualified individual (TQI) must consider in selecting the appropriate containment for this exemption. A structure is defined as a building or vessel which effectively surrounds and encloses the microorganism and includes features designed to restrict the microorganism from leaving. In proposing the Biotech Rule, EPA envisioned that this exemption would most likely apply to research performed in contained structures such as buildings, including laboratories, greenhouses, and pilot fermentation plants. etc., and in certain bioreactors used for waste treatment. However, other forms of structures could be used. EPA's approach relies on the experience and judgment of the TQI, recognizing that many different kinds of microorganisms displaying a wide range of characteristics could potentially be used in research. It also recognizes that appropriate types of controls (e.g., procedural. mechanical. and/or engineering) will vary with the microorganism and type of research. EPA expects that the TQI will be cognizant of these factors when selecting containment and inactivation controls appropriate to the microorganism(s) being utilized. The technically qualified individual is required to keep records to document both compliance with the containment requirements and compliance with the notification process for employees involved in the R&D process.

A major consideration of the R&D exemption in a contained structure is the structure itself. EPA may interpret the definition of a structure broadly given the intention of freely permitting research with contained microorganisms that meet the criteria of § 725.234. However, EPA encourages potential researchers who wish to perform their research in atypical contained structures to confer with EPA prior to initiating

their effort to confirm that the structure is considered “contained”. There may be instances in which a TSCA Environmental Release Application (TERA), which is a submission for field testing or intentional environmental release, may be required if the structure is not deemed “contained” (see below for TERA requirements).

Tier I and Tier II Exemptions

There are exemptions from MCAN reporting for certain industrial microorganisms used in closed systems so they likely have limited, if any, relevance to typical agricultural applications. As described in Subpart G, these Tier I and Tier II exemptions for closed systems are based on a three-pronged approach: use of a microorganism with a history of safe use, criteria that ensure the safety of the introduced DNA, and conditions for containment and inactivation of the microorganism to ensure low releases from the manufacturing/production facility. To qualify for the Tier I exemption, a manufacturer must use one of the ten recipient organisms listed at § 725.420 that have undergone categorical risk assessment, or any such microorganism subsequently listed after promulgation of the Biotechnology Rule through a petition process described in § 725.67. Currently, the eligible recipient microorganisms include the five bacteria *Acetobacter aceti, Bacillus licheniformis, Bacillus subtilis, Clostridium acetobutylicum, Escherichia coli* K-12, and the five fungi *Aspergillus niger, A. oryzae, Penicillium roqueforti, Sacharromyces cerevisiae,* and *S. uvarum.* In addition to the use of an approved recipient microorganism, there are four criteria for the genetic material introduced into these strains. There are also specific criteria for releases from the manufacturing facility and for inactivation of liquid and solid waste streams. For those manufacturers meeting Tier I requirements, only a brief notification to the Agency stating that fact is necessary. A manufacturer, who meets only the first two conditions of the Tier I exemption, but not the containment and inactivation criteria must submit a Tier II exemption notice to the Agency for a review of the process design and containment/inactivation conditions appropriate for the intergeneric microorganism.

Test Marketing Exemption (TME)

Another exemption from MCAN reporting requirements is the Test Marketing Exemption (TME) noted at § 725.300. Test marketing activities usually involve limited sale or distribution of a substance within a predetermined period of time to determine its competitive value when its market is uncertain. In general, EPA suggests that manufacturers who intend to test market a new microorganism file a MCAN rather than request a Test Marketing Exemption. However, there may be situations in which this exemption is appropriate, such as for microorganisms which were previously reviewed by EPA at the R&D stage. In addition to the general administrative requirements, certain technical information is required for each TME submission as noted in § 725.350 and § 725.355.

4.6.2.3 TSCA Experimental Release Application (TERA)

Another exemption from MCAN reporting requirements is available for R&D activities. The TSCA Experimental Release Application, described in Subpart E at § 725.238, is an exemption for R&D involving an intentional environmental release of an intergeneric microorganism. This exemption is likely to be a common one for many agricultural uses (e.g., biofertilizers, algae for biofuel production), as they generally involve field tests or may involve some release of subject microorganisms. Also, as previously mentioned, a TERA may be necessary for some contained R&D activities if such R&D is conducted in an atypical structure that does not meet the regulatory definition of a contained structure. The TERA is essentially an abbreviated MCAN for a field test or other intentional environmental introduction with a shortened review period of 60 days, although EPA may extend the review period for good cause. EPA must approve the TERA, with or without conditions, before the researcher may proceed, even if the 60-day period expires. EPA's approval is limited to the conditions outlined in the TERA notice and approval for the specific field test at the specified site(s).

A TERA must contain all available data in the possession or control of the submitter or reasonably ascertainable by the submitter on the microorganism(s) and the research and development activities that will allow EPA to make a reasoned evaluation of the planned test in the environment. The TERA must contain microorganism identity information and all available data concerning actual or potential effects on health or the environment of the new microorganism along with the phenotypic and ecological characteristics of the microorganism as they relate directly to the conditions of the proposed R&D activity. Persons applying for a TERA must also submit information about the proposed field testing activity including the objectives and significance of the activity with a rationale for testing in the environment, the numbers and frequency of microorganisms released by the proposed application method(s), the presence of target organisms, if applicable, and a full characterization of the test site(s) including location, geographical, physical, chemical, and biological features, and proximity to human habitation or activity. Also needed is a description of confinement procedures, mitigation and emergency procedures, and procedures for routine termination of the activity. The exact information requirements for a TERA are codified at § 725.255 and § 725.260.

Exemptions from a TERA for Eligible Microorganisms

There is an exemption from TERA reporting requirements for R&D field testing of two microorganisms with which EPA has had sufficient experience to determine that a submission is no longer needed. The exemption applies to two eligible microorganisms, *Bradyrhizobium japonicum* and *Sinorhizobium* (formerly *Rhizobium*) *meliloti*) providing certain conditions of the microorganisms and of the field testing are met. The introduced genetic material must comply with certain restrictions, the field testing must occur on no more than 10 terrestrial acres, and appropriate

containment measures must be selected to limit dissemination (see § 725.238 and § 725.239).

This TERA Exemption requires no upfront reporting to EPA, although a certification statement and recordkeeping are required. Guidance on how to submit a certification statement to EPA and on the recordkeeping requirements for field tests with these bacteria is provided at § 725.238.

4.6.3 Risk Assessment Process

Within the specified time period for each type of submission, EPA staff conduct a risk assessment on the intergeneric microorganism under the paradigm that Risk = Hazard × Exposure. There are a number of separate assessments made that are integrated into a final risk assessment. The components of the risk assessment include (1) a verification of the identification of the subject microorganism, (2) a human health hazard assessment, (3) an ecological effects hazard assessment, (4) a report that analyzes the construction of the microorganism and summarizes the pertinent chemical information and production volume known as the chemistry report, (5) an analysis of the genetic construct that evaluates any potential hazards associated with the genetic modifications and the potential for horizontal gene transfer, (6) an engineering report that assesses worker exposure and microbial releases to the environment through manufacturing or during field applications, and (7) an exposure assessment that evaluates the potential for survival, reproduction, and dissemination of the microorganism, and the exposure of the microorganism to environmental receptors and to the general population.

As noted below, there is no provision for a specified schedule of information elements under TSCA. Rather submitters must provide to EPA all relevant data and information in their possession or reasonably ascertainable. These data must be sufficient to enable EPA to complete a risk assessment. If a submission of any type contains insufficient information to proceed with a review, EPA may request an extension from the submitter to allow the submitter to provide the necessary information. EPA also has risk management options that may be employed to mitigate the effect of uncertainty due to data or information limitations as described below.

Since TSCA is a risk-benefit statute, the risks of using the microorganism determined in the risk assessment are weighed against the benefits to society (that are evaluated in an economics analysis) to arrive at the final risk management decision. Possible outcomes of the review process include a determination that there is (1) sufficient information to determine that the microorganism presents “no unreasonable risk of injury to human health or the environment” in which case the Agency takes no regulatory action and the company may commence manufacture after 90 days, (2) sufficient information to determine that the microorganism presents “an unreasonable risk of injury to human health or the environment” which means the Agency would take regulatory action to prohibit or restrict the production or use of the microorganism, and (3) insufficient information to determine effects, but the

possibility exists for unreasonable risk and/or substantial/significant exposure, in which case the Agency may negotiate a Section 5(e) Consent Order to restrict the use, and to specify the data needed to lift the Consent Order. The key element to the possible outcomes of EPA's review process is the amount of information that the Agency is supplied with or can obtain concerning the microorganism in order to make a determination of whether or not the use of the microorganism presents an unacceptable risk of injury to human health or the environment.

4.6.4 Data and Information Needs

Unlike many other statutes under which biotechnology products are reviewed, TSCA does not have specific initial data requirements. Rather, the submitter is required to provide relevant data and information that are available or reasonably ascertainable with the notification to EPA. In contrast, with microbial pest control agents (MPCAs) which are reviewed by EPA's Office of Pesticide Programs (OPP) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), there are a number of specific pathogenicity/toxicity/infectivity tests that must be conducted and submitted to the Agency. MPCAs, by their very nature, are designed to be either pathogenic or toxic to some pest, and consequently, their effects of pathogenicity/toxicity are fairly straightforward. The microorganisms that fall under TSCA review differ in that most are not likely pathogenic or toxic, but primarily are benign recipient microorganisms genetically engineered to synthesize a particular product or accomplish a particular task or transformation.

Obtaining sufficient information about the submission microorganism from the manufacturer or importer so that a scientifically credible risk assessment can be conducted by the Agency is critical to the review process. Information needs match the set of individual assessments (e.g., human health, ecological effects, etc.) that go into the comprehensive risk assessment described previously. Since combinations of microorganism and proposed use can vary widely, EPA prepared a guidance document, "Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms, June 2, 1997" (hereafter referred to as the Points to Consider document). This document is intended to assist manufacturers or importers in providing EPA's Office of Pollution Prevention and Toxics with both appropriate and sufficient information for EPA to conduct a robust risk assessment. It is intended that the Points to Consider document be a "living document" in that it will be updated periodically to reflect state-of-the-art biotechnological applications, risk assessment methodology, and current knowledge of microbial processes and characterization.

Although there are no data requirements that are applied routinely to each case, information that is both accurate and sufficient is necessary to evaluate the risks posed by the manufacture and use of genetically modified microorganisms. Each submitter must supply, as part of its notification requirements, all relevant data and information in its possession, or that is otherwise reasonably ascertainable. Information available in the literature or from sources other than the submitter is also used by the Agency in the evaluation of the hazards posed by the microorganism

and its ability to survive in the environment. The effects of genetic modifications of the recipient microorganism are then evaluated. For instance, if a recipient bacterium is known from the literature not to be a frank human pathogen, then it is unlikely that the introduction of one or several genes will create a pathogenic microorganism *de novo*. Likewise, if from the literature it is known that the recipient microorganism survives well in the environment, then the intergeneric microorganism also might be expected to survive well depending on whether the genetic modification altered any genes key to its survival characteristics. The Points to Consider document has been provided to guide submitters in selecting all the relevant information that the Agency may need for the review of all possible types of microorganisms and applications that may be subject to review under TSCA. All of the points or issues in the guidance document may not be appropriate for all cases. This document is not a schedule of data requirements but rather essentially a menu of data elements from which submitters are expected to choose the ones relevant to their particular microorganism and application. For example, information on substrate range and metabolic pathways may be applicable for a microorganism designed for bioremediation, but would be irrelevant for a microorganism designed for symbiotic nitrogen fixation. Identification of possible nontargets, i.e., potential legume hosts, may be important for symbiotic nitrogen-fixing rhizobia, but irrelevant to a microorganism used in a closed system for making an algal biofuel.

4.6.5 Applications of Genetically Engineered Microorganisms Reviewed to Date

4.6.5.1 Past Applications

A wide variety of intergeneric microorganisms have been reviewed under TSCA since the mid 1980s. Prior to the promulgation of the Biotechnology Rule in 1997, intergeneric microorganisms with TSCA uses were reviewed on a voluntary basis under the chemical Pre-Manufacturing Notification (PMN) system. Those intergeneric microorganisms and their genetic modifications with relevance to agriculture are listed in Table 4.2.

Following the promulgation of the Microbial Biotechnology Rule, various submissions types discussed above for intergeneric microorganisms have been received by the Agency. The majority of the submissions reviewed by EPA since publication of the Biotechnology Rule have been for closed system fermentation for enzyme production which were not relevant to agriculture, and thus, will not be elaborated on here. A complete list of all intergeneric microorganisms reviewed under TSCA to date can be obtained on the Biotechnology Program's website (http://www.epa.gov/oppt/biotech) under Notifications.

Table 4.3 presents those intergeneric microorganisms reviewed by EPA under TSCA since the promulgation of the Biotechnology Rule, having relevance to agriculture, all of which were TERA submissions.

Table 4.2 Agriculturally relevant genetically engineered microorganisms reviewed by EPA under TSCA under voluntary PMNs

Fiscal year	Company	Recipient microorganisms	Introduced genetic material	Purpose
1987	BioTechnica International, Inc.	Three strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa
1987	Monsanto Agricultural Company	*Pseudomonas aureofaciens* (currently *Pseudomonas chlororaphis*)	*lac* genes from *Escherichia coli*	"Marker" genes for monitoring the microorganism in the field
1988	BioTechnica, Agriculture, Inc.	Eight strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa
1988	BioTechnica, Agriculture, Inc.	Four strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa
1989	BioTechnica, Agriculture, Inc.	One strain of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa
1989	BioTechnica, Agriculture, Inc.	Two strains of *Bradyrhizobium japonicum*	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in soybeans
1990	BioTechnica, Agriculture, Inc.	One strain of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa
1991	Mycogen Corporation	Two strains of *Pseudomonas fluorescens*	Delta endotoxin genes from *Bacillus thuringiensis* var. *kurstaki* or var. *san diego*	TMEs of pesticidal intermediates of pesticides consisting of encapsulated killed cells for control of beetles and caterpillar pests
1992	Mycogen Corporation	Sixteen strains of *Pseudomonas fluorescens*	Delta endotoxin genes from *Bacillus thuringiensis* var. *kurstaki* or var. *san diego*	TMEs of pesticidal intermediates of pesticides consisting of encapsulated killed cells for control of beetles and caterpillar pests
1992	Research Seeds, Inc. (purchaser of BioTechnica Agriculture, Inc. strains)	Five strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in alfalfa

1993	Research Seeds, Inc.	Five strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Test marketing large scale field trials for one strain, RMBPC-2, symbiotic nitrogen fixation in alfalfa
1994	Research Seeds, Inc.	Four strains of *Bradyrhizobium japonicum*	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in soybeans
1994	Research Seeds, Inc.	Five strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Additional test marketing field trials for one strain, RMBPC-2, symbiotic nitrogen fixation in alfalfa
1995	Research Seeds, Inc.	Five strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Additional test marketing field trials for one strain, RMBPC-2, symbiotic nitrogen fixation in alfalfa
1995	Univ. of Wisconsin, USEPA Office of Research & Development	Two strains of *Rhizobium meliloti* (*Sinorhizobium meliloti*)	Nitrogen fixation genes and antibiotic resistance genes	Symbiotic nitrogen fixation in transgenic alfalfa
1995	Mycogen Corporation	One strain of *Pseudomonas fluorescens*	Delta endotoxin genes from *Bacillus thuringiensis*	TMEs of pesticidal intermediates of pesticides consisting of encapsulated killed cells for control of lepidopteran pests

Table 4.3 Agriculturally relevant genetically engineered microorganisms reviewed by EPA under TSCA under the biotechnology rule as TERAs

Fiscal year	Recipient microorganism	Introduced genetic material	Purpose
1998	Three strains of *Bradyrhizobium japonicum*	Nitrogen fixation and nodulation genes	Nitrogen fixation in soybeans
1999	Three strains of *Bradyrhizobium japonicum*	Nitrogen fixation and nodulation genes	Nitrogen fixation in soybeans
2003	*Alcaligenes xylosoxidans* subspecies *denitrificans* strain AL6.1	DsRed fluorescent protein marker gene	Detection in the environment – for eventual insertion of pesticidal gene for control of *Xylella fastidiosa* (Pierce's disease of grapes)
2004	*Alcaligenes xylosoxidans* subspecies *denitrificans* strain AL6.1	DsRed fluorescent protein marker gene	Detection in the environment – for eventual insertion of pesticidal gene for control of *Xylella fastidiosa* (Pierce's disease of grapes)
2005	*Alcaligenes xylosoxidans* subspecies *denitrificans* strain AL6.1	DsRed fluorescent protein marker gene	Detection in the environment – for eventual insertion of pesticidal gene for control of *Xylella fastidiosa* (Pierce's disease of grapes)

4.6.5.2 Potential Future Applications

Biofertilizers

As previously mentioned, there are many biotechnology applications of genetically engineered microorganisms that potentially may fall under the purview of TSCA including a number of uses that are relevant to agriculture. These include intergeneric microorganisms used as biofertilizers such as symbiotic nitrogen-fixers such as *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*. Field tests of numerous intergeneric rhizobia have gone through review under TSCA, and one particular strain of *S. meliloti*, RMBPC-2, was approved in 1997 for limited commercialization. In the future, there could be more submissions for more rhizobia for increased nitrogen-fixation ability, or perhaps, for enhanced nodulation efficiency. In addition, applications for other symbiotic nitrogen fixers, such as the actinomycete *Frankia* which is a Gram positive bacterium that forms symbiotic relationships with certain plants such as woody angiosperms referred to as actinorhizal plants, are a possibility. There may also be submissions for free-living nitrogen fixing microorganisms. In addition to nitrogen-fixing intergeneric microorganisms, other biofertilizer applications that would be reviewed under TSCA include phosphate-solubilizing microorganisms, mycorrhizal fungi, or other endophytic microorganisms that aid in nutrient absorption, plant hormone production, or other mechanisms that may increase plant productivity.

Biosensors

Microbial biosensors consist of the use of a microorganism that has some sort of reporter molecule that indicates the presence of a target molecule. The reporter genes used in recombinant DNA technology for microbial biosensors include those that can result in a signal that can be visible to the naked eye such as color production (e.g., blue color resulting from the breakdown of X-galactopyranoside by β-galactosidase), bioluminescence (e.g., *luc* or *lux* genes), or fluorescence (e.g. *gfp* or DsRed). One of the earliest genetically engineered microorganisms to be field tested was Monsanto's *Pseudomonas chlororaphis* (formerly *P. aureofaciens*) into which the β-galactosidase gene was inserted to enable detection of the microorganism in the environment. The *A. xylosoxidans* reviewed under TSCA that was eventually to be manipulated with pesticidal genes contained the DsRed protein for detection the microorganism in the environment as well. Other biosensors with reporter genes for detection of particular target molecules have been reviewed under TSCA as well. One such biosensor was a strain of *Pseudomonas fluorescens* Hk44 containing *lux* bioluminescence genes for detection of polycyclic aromatic hydrocarbons including naphthalene and methyl salicylate. Another reporter biosensor was a strain of *Pseudomonas putida* with genes for detection of unexploded ordinance, specifically trinitrotoluene (TNT). Another biosensor microorganism, a *P. putida* containing *lux* genes was reviewed that was developed for detection of trichloroethylene (TCE) and BTEX compounds (benzene, toluene, ethylbenzene, and xylene). Other genetically engineered microbial biosensors have been developed for *in situ* detection of metals such

as cadmium, nickel, cobalt, different forms of mercury, arsenite, and other heavy metals such as copper, zinc, and lead (as summarized in Shin 2010).

Potentially, there could be a number of biosensor applications developed that would be relevant to agriculture that would be subject to review under TSCA. Future developments could include the use of intergeneric microorganisms as biosensors for detection of bioterrorist agents, detection of other environmental pollutants, including pesticides, some of which may have relevance to agriculture. Other potential agricultural uses could be development of microbial biosensors for detection of pathogenic strains of *E. coli* or *Salmonella* in the environment, for instance, in irrigation water, in soils, in manures and other fertilizers that are used for food crop production. These types of biosensors may be particularly useful for produce often consumed raw such as lettuces, spinach, onions, etc. However, a biosensor such as this, if used to monitor contamination on the actual food product rather than the environment in which the crop is growing, would fall under the jurisdiction of the FDA rather than EPA. Other agriculturally relevant future biosensors could be for monitoring nutrient or water status of soils or contamination of water used in crop production or in aquaculture.

Pesticidal Intermediates

Pesticidal intermediates are an agricultural application reviewed under TSCA, and several of these were reviewed in the 1980s. A pesticidal intermediate is a live microorganism producing a pesticide that contains only inactivated microorganisms. The final pesticide product containing dead microorganisms is reviewed by EPA's Office of Pesticide Programs under FIFRA. However, the live microorganism used in the production of the pesticide is reviewed under TSCA as a pesticidal intermediate. Future submissions of pesticidal intermediates may also be expected.

Weather Modification

Some of the earliest biotechnology applications involving intergeneric microorganisms involved those in weather modification. There was the ice-minus *Pseudomonas syringae* for prevention of frost damage on strawberries. The commercial product called Snomax is a strain of *P. syringae* that increases the nucleation temperature of water, thereby increasing snow volume. Since strains of *P. syringae* are known plant pathogens, USDA had the lead in reviewing these two products in the 1980s under the Plant Protection Act. However, any such weather modification product produced in the future using an intergeneric microorganism that did not fall under review by another federal agency would be reviewed under TSCA.

Algal Biomass for Fuels and Other Uses Such as Animal Feeds, Aquaculture Feed, Etc

Currently there are extensive R&D activities on using algae as a biofuel feedstock. Characteristics of microalgae production that are advantageous include high biomass

yields per acre, a lack of competition for arable land and sometimes nutrients, the use of waste water, produced water, or saline water, the recycling of carbon through use of CO_2 from industrial flue gas or other sources, and because production is compatible with an integrated biorefinery concept. Other aspects of microalgal culture include rapid growth rate, high cell density, and high oil content. Algae may be able to produce several fuel types including gaseous compounds like hydrogen and methane, as well as a range of conventional liquid hydrocarbons. Most of the current focus with algal biofuels is on the development of liquid transportation fuels including gasoline, diesel, and jet fuel.

The U.S. Department of Energy biofuels roadmap (US DOE 2010) addressed many aspects of this rapidly developing industry, including the variety of algal types, methods to cultivate them, and processes to recover oil from them. Algae can be grown photosynthetically using natural daylight or with artificial lighting. Heterotrophic algae can be grown much like other industrial microorganisms via continuous culture in the dark although when grown this way, they require a fixed carbon source such as sugars. There are two primary cultivation approaches with many variations. Photobioreactors utilize closed cycle recirculation systems employing either ambient light or artificial illumination. Open pond production facilities are generally raceway ponds of a recirculating design using pumps and paddle wheels to circulate water, algae, and nutrients through shallow open ponds. Hybrid systems growing algae in the environment may also be used, however perhaps with enclosures such as plastic bags, to contain the algae rather than growing them in the open.

Commercial fuel production from algae is in its infancy, but the growth of algae for commercial production of high-value end products such as pharmaceuticals and "nutraceuticals" has existed for some time. Products such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, and biologically active molecules for use in human and animal health are produced by algae commercially (Oilgae 2010). Any intergeneric algae used for biofuel production would be reviewed under TSCA. Although these high-value end products other than fuels mentioned above would be reviewed by other federal agencies, TSCA would be involved if the algae were also producing biofuels.

4.7 Conclusions

Regulation of genetically engineered microbial agents, whether for pest management purposes or environmental bioremediation, has afforded the proper oversight of a novel technology as part of a larger attempt to reduce the uncertainty of the risk assessment process. With the advent of a new technology, uncertainties and lack of a proven track record necessitate thorough review of these microbes to ensure human health and environmental safety (Harrison and Bonning 2000). While the addition of a transgene to a familiar microbial genome may alter the phenotype of the microbe, these microbes are guided by the same biochemical and genetic processes as naturally occurring microbes (NRC 2000). Hence, they were assessed with that

fact in mind, albeit under an initial higher level of scrutiny and oversight. As indicated by Wrubel et al. (1997), decisions regarding further research and development of GE MPCA products may have been considerably influenced by unknowns in regulatory oversight, however, in the majority of cases an inability of the proposed product to live up to expectations was the driving force behind a products demise. One must not discount the perceived influence of public acceptance and its relationship to marketing of products, particularly when they involve food and feed.

Reports from the early field experiments with GE bacteria reveal how controversial and polarizing these first ventures were in the public arena (Griffin 1988; Berg and Singer 1995). Today this is largely not the case, although many have learned the value in public education and involvement in field testing novel technologies. It is still possible, however, to emote fear of the unknown without really intending to (Dixon 2008).

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