Government Regulation of the Uses of Genetically Modified Algae and Other Microorganisms in Biofuel and Bio-based Chemical Production

David J. Glass

Abstract Recent years have seen an increased interest in developing genetically modified algae and other microorganisms for use in biofuel and bio-based chemical production. However, this comes at a time when there is uncertainty within the industry and the academic community about how such uses will be regulated by governments in the U.S. and elsewhere in the world, as well as concerns by some observers over the adequacy of existing regulations to cover organisms created using techniques known as synthetic biology. However, a reasonable road map is emerging of a regulatory regime that can allow pilot, demonstration and commercial stage uses of modified microorganisms. In the U.S., regulations of the U.S. Environmental Protection Agency and possibly of the U.S. Department of Agriculture might govern the industrial use of microorganisms in contained photobioreactors or algae in open ponds, and these regulations generally require conducting assessments of the potential environmental risks of such large-scale uses. The EPA regulations include a mechanism by which outdoor experimentation of modified microorganisms can take place in a stepwise approach, with risks assessed as the scale of experimentation increases, which provides an accessible path to exploration of the use of modified algae in open ponds. Such risk assessments will address legitimate questions of potential ecological impact, such as the potential survival and dissemination of the production organism, the potential for heterologous genes to horizontally transfer to indigenous microorganisms, and the chance for other unintended effects on nontarget species. Numerous companies have successfully navigated these regulations, including some recent project approvals in the U.S. and elsewhere in the world.

Keywords Genetic modification • Genetically modified organism • Government regulation • Environmental impact • Risk assessment • Biofuel • Bio-based chemical

D.J. Glass (✉)
D. Glass Associates, Inc., 124 Bird Street, Needham, MA 02492, USA
e-mail: dglass@dglassassociates.com

A. Prokop et al. (eds.), *Algal Biorefineries*, DOI 10.1007/978-3-319-20200-6_2

1 Introduction

Genetically modified microorganisms, including microalgae and cyanobacteria, are increasingly being developed for the production of renewable fuels or bio-based chemicals. The development of biological methods of manufacturing commodity products currently made from petrochemical feedstocks promises to make an important contribution to the reduction of global carbon emissions and the movement to more sustainable industrial activities. Microbiological methods have long been used for the production of ethanol or other industrial chemicals, but the proposed use of genetically modified organisms offers potentially significant advantages over traditional methods, such as improved productivity, decreased operational costs, the ability to use a more diverse range of feedstocks, and possibly more favorable carbon footprints.

In the U.S. and most other countries around the world, manufacturing processes involving genetically modified microorganisms (GMMs) would likely trigger additional regulatory scrutiny before manufacturing could begin and products could be sold. This chapter will review the regulations that are applicable to fuel and chemical production using genetically modified algae, cyanobacteria and other microorganisms in the United States and elsewhere in the world, and which would also apply to organisms created for these purposes using synthetic biology. The chapter will also discuss the scientific concerns that have led to the imposition of these regulations, and the issues underlying the risk assessments associated with such government oversight. With proper planning and management, it should be relatively straightforward for most applicants to obtain the approvals needed to proceed with R&D or commercial use of genetically modified microorganisms in industrial biotechnology, as will be demonstrated by discussions of several cases where regulatory approvals for uses of genetically modified microorganisms have been obtained in the U.S. and elsewhere.

2 Potential Commercial Uses and Environmental Impacts of Genetically Modified Microorganisms

2.1 Strategies for Genetic Modification of Microorganisms

Much of today's commercial activity using advanced biotechnology for biofuel or bio-based chemical production focuses on the creation, selection or improvement of strains of desired microorganisms having enhanced properties for functions important for the production process. Microbiological methods for producing ethanol, fuels or other chemicals generally rely on the use of one or more selected microbial strains to catalyze biosynthesis of the desired compound, generally through a traditional fermentation process. Historically, these methods have made use of naturally-occurring or classically selected microorganisms, but in recent years the power of the new biotechnologies to develop enhanced strains is being investigated or used by numerous companies.

Much of the industrial interest in the use of microbial processes has been for the production of commodity products used as automotive fuels, primarily ethanol and diesel substitutes such as biodiesel, with some processes using modified microorganisms already in commercial use. Processes are also under development for microbial manufacture of other fuels or fuel additives, including n-butanol, isobutanol, and mixtures of alkanes or lipids that can be drop-in replacements for diesel, gasoline, or jet fuel (USDOE 2013). Biological methods are also under development or in commercial use for a variety of chemical compounds of many different uses and industrial applications. Examples include succinic acid, butadiene and its downstream products, isobutene, propanediol, and the monomeric units for numerous bioplastics such as polylactic acid, polypropylene and others (Golden and Handfield 2014).

Most commercial activity today is focused on the use of heterotrophic microorganisms, both prokaryotic and eukaryotic, and including a number of species and strains that have been used for decades in industrial production, as well as other strains not previously utilized commercially. Historically, this has involved species such as *Saccharomyces cerevisiae* and other yeasts; fungal species such as *Aspergillus* and *Trichoderma* (especially for the production of industrial or food enzymes), and bacterial species such as *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens, Clostridium acetobutylicum*, species of *Corynebacterium* and *Lactobacillus*, and others (Adrio and Demain 2010). More recently, with the advent of genetic engineering, various strains of *Escherichia coli* and other commonly-used laboratory species have been considered for industrial use, and there has more recently been interest in utilizing less-common strains with interesting or valuable properties, such as various thermophilic microorganisms and the radiation-resistant *Deinococcus* species.

Recent years have seen an explosion in academic and industry activity in developing strategies for the use of advanced biotechnology techniques to improve such microorganisms for the production of fuels or chemicals. This is evidenced by the large and growing number of review articles that have appeared just in the last few years summarizing these strategies. For example, recent reviews of advanced biotechnology strategies for improved biofuel production include Colin et al. (2011), de Jong et al. (2012), Dellomonaco et al. (2010), Jang et al. (2012), Kung et al. (2012), Lennen and Pfleger (2012, 2013), Peralta-Yahya and Keasling (2010), and Zhang et al. (2011). Recent reviews of strategies focused on improving bio-based chemical production include He et al. (2014) (*Zymomonas* as production organism), Hong and Nielsen (2012) and Nielsen et al. (2013) (*S. cerevisiae* as production organism), Cao et al. (2011), Yu et al. (2011), and Chen et al. (2013) (*E. coli* as production organism), as well as Cao et al. (2013), summarizing methods for production of succinic acid, and more general reviews from Adrio and Demain (2010), Chen and Nielsen (2013) and Buschke et al. (2013).

The different strategies being pursued for the improvement of microorganisms for industrial purposes have been exhaustively summarized in these review articles. A brief summary is presented in Table 1. These include strategies to enhance the productivity of existing biosynthetic pathways, knocking out competing pathways

Table 1 Genetic engineering strategies for microorganisms

Overexpress key endogenous enzymes to increase yield of desired product
Modify the properties of key endogenous enzymes (e.g. directed evolution, codon optimization) to increase productivity of desired biosynthetic pathways
Introduce new enzymatic activities to enable use of different feedstocks or compounds as energy sources for the production organism
Introduce two or more genes encoding heterologous enzymes, to create entirely new biosynthetic pathways for desired products
Improve carbon flow into a desired pathway by knocking out genes encoding enzymes in competing pathways

to improve carbon flow into a desired pathway, and creation of entirely new pathways by introducing genes encoding multiple enzymes. The newer techniques of synthetic biology are often useful in implementing any of these strategies.

In spite of the commercial focus to date on heterotrophic microorganisms, photosynthetic organisms such as microalgae and cyanobacteria have also been used for commercial purposes (USDOE 2010). Species such as *Chlamydomonas, Chlorella, Haematococcus, Nannochloropsis, Dunaliela, Botryococcus, Scenedesmus* and others have been used for the production of industrially-useful compounds (Rosenberg et al. 2008; Larkum et al. 2012; USDOE 2010; Trentacoste et al. 2014). Because the processes required to grow algae, harvest the organisms and purify the product tend to be rather expensive, algal production has historically been mostly limited to specialty chemical or pharmaceutical products, characterized by low volumes and high profit margins, or products like nutritional supplements that don't require as much downstream processing as do specialty chemicals.

Similar genetic modifications are being considered for industrially-useful strains of microalgae to enable their use to produce commodity fuels and chemicals, especially to improve productivity or efficiency to overcome economic and other factors that have hindered development of the technology (USDOE 2010). Here too, progress in the field is attested by the significant number of recently-published review articles summarizing applicable advanced biotechnology strategies for strain improvement (Rosenberg et al. 2008; Radakovits et al. 2010; Jones and Mayfield 2012; Larkum et al. 2012; Rosgaard et al. 2012; Work et al. 2012; Nozzi et al. 2013; Henley et al. 2013; Enzing and Nooijen 2012). Some of the approaches being contemplated are shown in Table 2.

2.2 Potential Environmental Impacts of Genetically Modified Microorganisms

As the biotechnology industry grew, government regulatory frameworks developed in order to ensure the safe conduct of larger-scale industrial uses of genetically modified organisms. Early concerns about the technology focused on the potential public health impact of the creation of so-called novel life forms, but because almost

Table 2 Genetic engineering strategies for algae

Enhance photosynthesis; improve carbon fixation
Enhance pathway proteins like RuBisCO
Introduce new carbon fixation pathways
Enhance or alter lipid biosynthesis for improved diesel, jet fuel production
Enable secretion of lipids to improve harvesting and separation
Express, enhance transporter proteins
Alter cell wall composition for easier cell lysis
Metabolic engineering to enhance existing pathways
Maximize carbon flow to desired product(s)
Eliminate competing pathways
Remove toxic, harmful compounds
Introduce new pathways for desired products
Ethanol
Butanol
Improved production of hydrogen for fuel use

all industrial applications of modified microorganisms utilized well-studied species and strains known to be nonpathogenic, such concerns tended to be less prominent in the regulation of commercial activities. For larger-scale industrial uses (and agricultural applications) the emphasis of government regulation shifted to the need to assess potential environmental impacts, and to the development of appropriate risk assessment methods in support of such regulatory programs.

Microorganisms need to be grown in large scale in order to have industrial utility, especially for the production of commodity products like biofuels. For the most part, this will involve the hardware and processes that have usually been used for those native microbial species that have been exploited commercially, which might typically involve traditional industrial fermentations. These would be conducted under familiar conditions that have long been used commercially and which would be expected to afford some protection against exposure or accidental release of the microorganism. However, industrially-useful algae strains (and to some extent cyanobacteria) have traditionally been grown in open-pond reactors, where the algal cultures are exposed to the environment (USDOE 2010; Enzing and Nooijen 2012). The use of such reactors for genetically modified algae would pose much different issues for regulators conducting a risk assessment because of the inherent exposure of the production organism to the environment.

Consideration of the potential environmental risks of genetically engineered microorganisms began in earnest as the agricultural biotechnology industry was developing in the mid-1980s, with early publications such as Alexander (1985) setting forth the factors to be considered in environmental risk assessments. This concern led to more formal inquiries assessing environmental concerns in a general way, including a pioneering effort by a group of prominent ecologists and other scientists convened by the National Academy of Sciences (Tiedje et al. 1989), which generally concluded that modified microorganisms would behave in the environment

similarly to nonengineered strains introduced into new environments, and that such behavior could be predicted and monitored using appropriate risk assessment tools. These early scientific reviews provided the framework for some of the earliest regulatory risk assessments of proposed field tests and other uses of GMMs, under which a number of modified microorganisms and plants were used in field experimentation beginning in the mid to late 1980s. No evidence of environmental harm was seen in any of the field tests of modified microorganisms conducted during those years (see Glass 1995 for an early review of some of these results, and Viebahn et al. 2009 for a more recent review).

As shown in Table 3, there are legitimate scientific concerns about the potential environmental effects of microorganisms having novel traits. Among the issues identified as important for a risk assessment are (a) the toxicity, infectivity or other risks inherent to the GMM itself or that might have been introduced by the genetic modifications; (b) the ability of the GMM to persist or become established in the environment; (c) the ability of the GMM to compete with or displace natural microflora at the release site; (d) the possibility that the GMM could spread or be dispersed from the release site; and (e) the possibility that genes introduced into the GMM could themselves spread through horizontal gene transfer to be taken up by and expressed in different microbial species. Other potential risks are unique to algae or cyanobacteria, such as concerns over possible impacts on native algae populations or the potential to create or exacerbate harmful algal blooms. Some of these concerns are not unique to engineered organisms, and many observers would have similar concerns about large-scale industrial uses or releases of any novel organism, whether recombinant or not (for example, see Gressel et al. 2013). Although some observers within environmental groups and the general public fear that engineered microorganisms and plants inherently have potentially serious environmental risks (e.g. Glaser and Glick 2012; Ryan 2009), especially in the context of microorganisms improved by synthetic biology (Dana et al. 2012; Snow and Smith 2012), many scientists and industry officials feel that whatever risks may exist are easily assessable and manageable, and in any event do not differ in degree from the risks posed by similar uses of naturally-occurring organisms.

Although some of the public concerns over outdoor testing of GMMs and transgenic plants began to subside (with activists' attentions shifted to food uses of modified plants), the potential environmental impacts of GMMs continued to be the

Table 3 Key issues in risk assessments of large-scale industrial uses of microorganisms, including algae and cyanobacteria

Stability of vector and introduced genes.
Possible deleterious functions encoded by transgene(s) such as toxins.
Potential for horizontal gene transfer, crossing to native species.
Potential for engineered strain to be transported outside facility, survive and compete in environment.
Potential persistence in the environment: soil or water in vicinity of site of use.
Potential disruption of natural ecosystems, such as native algae populations.
Creation or enhancement of harmful algal blooms or ecologically disruptive algal blooms.

subject of academic study, often in the context of deliberate environmental uses of GMMs in agriculture or for bioremediation. (Sayler and Ripp 2000; Davison 2005; Viebahn et al. 2009; Urgun-Demirtas et al. 2006; Singh et al. 2011). Because so much of this research has involved microorganisms intended for uses in the open environment (e.g. in agriculture), not all of it may be directly applicable to industrial uses of GMMs that are either contained in traditional fermentation processes or conducted in controlled outdoor reactors; however what has been learned from such studies may present baseline information to help assess potential adverse effects should there ever be a large-scale accidental release of industrial GMMs from production vessels.

Viebahn et al. (2009) presents a fairly recent, quite comprehensive, review of literature relating to possible survival of GMMs in the environment, as well as potential ecosystem effects and impacts on non-target species. In summarizing a large number of studies involving microorganisms that might be used in agriculture or soil remediation, these authors concluded that in most cases, GMMs did not persist in the environment nor have adverse effects on indigenous microflora or other non-target organisms, but that such effects (such as population increases in the environment) were sometimes seen in some studies. Where non-target effects were seen, they were often "transient and small compared to natural variation". In many cases, the GMM behaved similarly to its nonmodified parent, but here too there were some studies showing the opposite. These authors did not draw any broad, generalized conclusions from the literature they reviewed, and recommended that each proposed use of a GMM be assessed on its own merits.

Urgun-Demirtas et al. (2006) also review experimental results (again, largely from the perspective of bioremediation) relating to the possible environmental impacts of introduced GMMs, including a comprehensive review of studies pertaining to possible horizontal gene transfer (HGT) between introduced species and native microflora. These authors summarize several studies in which evidence for HGT has been seen between native species and from GMMs to native species, but they note that lab or microcosm studies may overestimate the extent this occurs in nature. The authors conclude that, the possibility of HGT from use of GMMs is a "crucial [issue] regarding the potential impact of [GMM] release into the field", but that studies in contained systems "have generally indicated that this may not be an insurmountable problem".

Singh et al. (2011), while largely a review of genetic engineering approaches to improve microbial bioremediation, discusses the factors that might prevent modified strains from competing with native microflora in the environment, and proposes a 6-step decision tree for performing risk assessments of modified bacteria intended for deliberate release into the environment.

The potential environmental impacts of the use of GM algae and the types of risk assessments needed to evaluate such potential impacts are similar to those discussed above, with some added concerns due to the nature of algae and cyanobacteria. Consideration of these factors has been the subject of several recently-published papers (Henley et al. 2013; Snow and Smith 2012; Gressel et al. 2013; Gressel et al. 2014; Menetrez 2012), as well as a recent workshop (Enzing and Nooijen 2012), the conclusions of which coincide with many of the points raised in these recent papers. Henley et al. (2013) presents the most comprehensive review of the potential environmental impacts of the "commodity-scale" use of GM algae, discussing such

issues as the potential of a released strain to grow, persist and mutate in the environment, the possibility that GM algae could produce toxins or harmful algal blooms (HABs) or have other negative effects of aquatic ecosystems, and the possibility that introduced genes could spread by horizontal gene transfer and be expressed in indigenous microorganisms. In a shorter paper, Snow and Smith (2012) cover many of these same issues, particularly the need to assess environmental survival and persistence of an introduced strain and the potential for horizontal gene transfer. Both papers speculate on possible physical barriers or biological containment (e.g. so-called "suicide genes"; also discussed by Gressel et al. 2013) that might be effective in reducing environmental dispersal or survival of a released GM algae strain.

In two recent papers, Gressel, et al. (2013, 2014) assess the possible risks of large-scale industrial uses of both naturally-occurring and modified algal strains that have been domesticated for industrial use, and conclude that environmental risks should be assessed prior to large-scale use of either type of strain, particularly since accidental releases from production reactors are likely inevitable, even from contained photobioreactors. These authors propose mitigation strategies designed to limit the ability of production strains to survive and persist in the environment in the event of escape from production facilities. In particular, they advocate risk management strategies similar to the principles of Good Industrial Large Scale Practice, such as limiting large-scale uses to algae strains known to be nonpathogenic and to have a history of safe use.

These are the scientific issues that need to be addressed, at least at some level, before any proposed large-scale industrial use of modified organisms is to proceed. Regulatory frameworks around the world have been developed to carry out the needed risk assessments, and these should be applicable regardless of whether the organism was constructed by traditional recombinant DNA techniques or by newer techniques such as metabolic engineering or synthetic biology. However, one can have the expectation in most cases that microorganisms chosen for commercial uses at large-scale will be nonpathogenic and will not have other traits which might cause adverse environmental or health effects.

3 U.S. Framework for Regulation of Biotechnology

3.1 Overview

Regulatory frameworks have been developed in the United States and other countries to provide oversight over biotechnology and its commercial uses and to ensure that these potential environmental impacts are assessed. Because the earliest debates over biotechnology regulatory and public policy were often contentious or even confrontational, the perception developed within the industry that such government regulations were difficult to navigate, and that this, coupled with negative public opinion, placed significant barriers against the possible use of GMOs in industrial or agricultural applications, particularly those involving open environment use. Although this is not true, this misperception persists in many quarters to this day, and so it is useful to put today's regulatory frameworks into some historical perspective.

3.1.1 History

The following is a brief discussion of how the regulations that would affect uses of GMMs for fuel and chemical production evolved in the U.S. and elsewhere. More detailed summaries can be found elsewhere, including Glass (2003), Wozniak et al. (2012), Wrubel et al. (1997) and others. Biotechnology regulatory frameworks in most countries arose out of the health and safety issues that were initially raised by scientists and eventually debated by the public, shortly after recombinant DNA (rDNA) techniques were first developed in the early to mid 1970s (this early history is well documented by others, including Krimsky 1985; Glass 1991, and Glass 2003). The initial concerns were over the potential public health and safety threats that might be posed during laboratory research, if organisms having new traits were inadvertently released outside the laboratory, and this concern led to the adoption of research guidelines, which in some cases had limited applicability (e.g. the U.S. National Institutes of Health recombinant DNA guidelines, binding only on federally-funded research). As the biotechnology industry developed in the 1980s, the focus of regulatory concern shifted not only to the larger scale uses inherent in commercial application of this new technology, but also to deal with the intended use of engineered plants, animals and microorganisms for use outside the lab, in the open environment (e.g. in agriculture). In fact, the driving force for much of the regulatory action in the 1980s was concern over such "deliberate releases" to the environment, even though most governments instituted regulations that covered a wider range of commercial activities.

In the United States, the outcome of several years of public policy discussions was the adoption of a "Coordinated Framework" for biotechnology regulation in 1986 (OSTP 1986). Under this framework, it was decided that the commercial products of biotechnology would be regulated under existing laws and regulations and that it was not necessary to enact a specific law broadly covering all biotechnology activities.[1] Thus, the use of biotechnology to produce drugs, vaccines, diagnostic products, foods and food additives would be regulated by the Food and Drug Administration (FDA), using existing regulatory authority; biotechnology-derived pesticides would be governed by existing rules of the Environmental Protection Agency (EPA)[2]; and most other agricultural products would be regulated by the U.S. Department of Agriculture (USDA).

The effect of this decision was that the vast majority of biotechnology products, especially in the early years of the industry, were to be governed by the existing regulatory programs of the FDA and EPA with little or any regulatory revision; however it was also necessary to create new regulatory structures for some classes of commercial

[1] This is in contrast to most other countries in the world, which have generally created a single national biotechnology ("biosafety") law, often in compliance with the Cartagena Protocol on Biosafety (see below).

[2] EPA developed regulations under the U.S. pesticide law (the Federal Insecticide, Fungicide and Rodenticide act; FIFRA) to regulate proposed uses of modified and unmodified microorganisms as biopesticides. These regulations encompass risk assessments similar to those discussed in this chapter, but pesticides and other agricultural uses of microorganisms are outside this chapter's focus on fuels and chemicals. See references such as Glass (2003) or Wozniak et al. (2012) for more details on FIFRA biopesticide regulation.

product that could be anticipated to arise from biotechnology. Specifically, although it was decided that existing laws administered by the USDA could be used to regulate genetically engineered (transgenic) plants, new regulations under those laws would be needed. In addition, there were a number of potential uses for genetically modified microorganisms that could be regulated under EPA's existing statutory authority to regulate new chemicals (TSCA; discussed below), but here too a set of new rules would be needed to use this law to regulate microorganisms.

3.1.2 Applicability to Biofuels and Bio-based Chemicals

In fact, it is these EPA and USDA regulations that may govern the use of modified organisms for production of fuels or chemicals in the U.S. Many uses of modified microorganisms would be subject to regulations adopted in 1997 by the U.S. EPA under the Toxic Substances Control Act (TSCA) (Glass 2003; Bergeson et al. 2014; Wozniak et al. 2012). These regulations require notification to the agency before commercial use or importation of certain modified microorganisms, as well as agency review for proposed outdoor R&D activities of such modified organisms, e.g. open-pond growth of modified algae. The biotechnology regulations of the U.S. Department of Agriculture would, in many cases, cover uses of transgenic plants as biofuel or chemical feedstocks – these regulations will be briefly described below although they would apply to proposed uses of modified microorganisms only in rare cases.

Certain uses of modified microorganisms or algae could fall subject to FDA regulations as well. Naturally, microbial production of foods, pharmaceuticals, or other products within FDA's traditional jurisdiction would be subject to that agency's oversight, however, the nature and scientific basis for such regulation is outside the scope of this chapter and will not be discussed here. However, a common strategy for companies developing modified yeasts or other nonpathogenic microorganisms for ethanol, fuel or chemical production is to plan to use of the spent biomass that remains after the production process in animal feed. This has traditionally been done in the ethanol industry, through the production of dried distillers grains containing inactivated yeast for use in animal feed. Any proposed use of modified microorganisms in animal feed would likely require review by the animal feed division of the FDA (or equivalent bodies in other countries), although in the U.S., FDA shares some responsibility for oversight over animal feed ingredients with the Association of American Feed Control Officials (AAFCO; see below).

3.2 EPA Regulation of Industrial Uses of Modified Microorganisms

3.2.1 Overview

The use of certain genetically modified microorganisms in biofuel or bio-based chemical production may be subject to regulations promulgated by the U.S. Environmental Protection Agency under TSCA (Glass 2003; Wozniak et al. 2012; Bergeson et al.

2014). EPA uses TSCA to regulate commercial applications of genetically modified microorganisms that are not regulated by other federal agencies. TSCA (15 U.S. Code 2601) is a law requiring companies or individuals to notify EPA at least 90 days before commencing manufacture or importation of any "new" chemical, i.e., one that is not already in commerce in the United States, and which is intended to be used for a purpose not subject to federal regulation as a pesticide or under the food and drug laws. It is viewed as a "gap-filling" statute, that is meant to cover chemicals falling through the cracks of other regulatory authority, but it is also a "notification" statute, with government notice required for all new chemicals, regardless of risk, with the idea that the agency would review all the notifications and single out for further oversight those chemicals that appeared to pose potentially unacceptable risks to the environment or public health. The large majority of chemical notifications received by EPA under TSCA are cleared within the 90 day period after only brief agency review.

In the Coordinated Framework of June 1986 (OSTP 1986), EPA proposed to use TSCA in the same "gap-filling" way as it is used for chemicals, to capture those modified microorganisms to be used in commerce but that were not regulated by other federal agencies. The primary areas which were expected to became subject to the TSCA biotechnology regulations were (a) microorganisms used for production of non-food-additive industrial enzymes, other specialty chemicals, and in other bioprocesses; (b) microorganisms used as, or considered to be, pesticide intermediates; (c) microorganisms used for nonpesticidal agricultural purposes (e.g. nitrogen fixation); and (d) microorganisms used for other purposes in the environment, such as bioremediation. As the field of industrial biotechnology has developed, production of biofuels and bio-based chemicals have become the most prominent "bioprocessing" applications that might fall subject to TSCA.

Although EPA established an interim policy of TSCA regulation under the 1986 coordinated framework, because of political difficulties and interagency disputes (Glass 2003) the agency was not able to publish proposed biotechnology regulations until 1994 and was not able to finalize these regulations until 1997 (USEPA 1997b). These rules, when finally issued, amended the existing TSCA regulations by creating a new section of the Code of Federal Regulations (40 CFR Part 725), which specifies the procedures for EPA oversight over commercial use and research activities involving microorganisms subject to TSCA. The net result was to institute reporting requirements specific for microorganisms (but which paralleled the commercial notifications used for new chemicals), while also creating new requirements to provide suitable oversight over outdoor uses of those genetically modified microorganisms subject to TSCA jurisdiction.

3.2.2 Scope of the TSCA Biotechnology Regulation

The biotechnology rule requires premanufacture reporting for new organisms intended for commercial use, but it was a long-running challenge in the development of the regulations to adequately define "new organism". The final rule defines a "new organism" as an "intergeneric organism", which is defined to mean "a microorganism that is formed by the deliberate combination of genetic material

originally isolated from organisms of different taxonomic genera". This is the same definition originally proposed by EPA in the Coordinated Framework and used under the agency's interim policy. The rationale for this definition, which was admittedly somewhat arbitrary, was that microorganisms that are classified within the same genus were more likely to be able to exchange genetic information in nature than microorganisms found in different genera, so that an "intergeneric" combination of genes was judged to be less likely to have occurred naturally (without human intervention) than an "intrageneric" combination. Under this formulation, microorganisms that are not intergeneric are considered not to be new, and such organisms, including naturally occurring and classically mutated or selected microorganisms, as well as GMMs modified only through gene deletions or directed evolution approaches, are exempt from reporting requirements under TSCA. Note that EPA's need to limit the TSCA regulations to "new" microorganisms in this way gives the rule a narrower scope than other U.S. federal regulations as well as the laws and regulations of other countries, in potentially excluding certain categories of modified microorganisms from the rule.

Although there has been some uncertainty in the past, it now seems clear that genetically modified algae strains would fall under EPA jurisdiction under TSCA if intergeneric and if used for a TSCA-regulated purpose. This interpretation is supported by language in EPA's 1997 Federal Register notice instituting the biotechnology rule (USEPA 1997b) which included "green and red algae" among a list of types of organisms covered by the definition of the term "microorganism", and a discussion in its Regulatory Impact Analysis accompanying the rule (USEPA 1997d), which stated that "Language in the Act has been interpreted to include living microorganisms (i.e., microscopic living cells such as bacteria, fungi, protozoa, microscopic algae, and viruses)". As discussed below, in recent years proposed uses of algae and cyanobacteria have indeed begun to be regulated under TSCA.

3.2.3 Regulation of Commercial Uses of Modified Microorganisms

Commercial uses of "new microorganisms" used for a "TSCA purpose" (that is, not regulated elsewhere in the federal government) generally require notification to EPA at least 90 days in advance of commercial use or importation. This notification takes the form of Microbial Commercial Activity Notices (MCANs). An individual MCAN is needed for each modified strain intended to be commercialized, although EPA maintains procedures to facilitate submission and review of "consolidated" MCANs covering up to six related strains of similar genetic make-up.

The information and other data that applicants need to submit in the MCAN are listed in Section 725.155 of the regulations and summarized in Table 4. Much of the required information has to do with the biological characterization of the modified microorganism and a detailed description of how it was constructed, but information is also to be submitted on the proposed use of the microorganism, the proposed production process, the containment and control procedures to be used, the likelihood for worker exposure and the steps taken to control exposure, and an assess-

Table 4 MCAN data requirements

Microorganism identity, including taxonomic identification of the "recipient" organism and the "donor" organisms that are the source of the introduced genes.
Detailed information about the construction of the microorganism.
Biological characterization of the microorganism.
Potential health effects of the microorganism, such as pathogenicity or toxicity (can be addressed from testing or from the literature).
Potential environmental effects of the microorganism (can be addressed from testing or from the literature).
Detailed information about the industrial process, including the measures that will be taken to minimize release of the microorganism from the facility.
The extent to which workers might be exposed to the microorganism.
The extent to which the microorganism might be released into the environment as a result of the process.

ment of the potential health and environmental effects of the microorganism should it be released from the facility. It is important to note that, in MCANs or other submissions to EPA under the biotechnology rule, the applicant can claim much or all of the submitted information as "confidential business information", which the agency must keep confidential and which cannot be released to the public, but the applicant must provide EPA with the justification for the confidentiality claim (in fact, this justification must be included within the MCAN filing). EPA has published a detailed "Points to Consider" document (USEPA 1997c) summarizing the required data and the format for submission, which, together with guidance from the publicly-available versions of previously-filed MCANs (i.e. the parts of prior MCANs not claimed by the applicant as confidential), can be used to help applicants prepare MCAN submissions.

In most cases, EPA review of MCANs can be expected to be fairly straightforward, and would include consideration of the potential risks and benefits of the commercial use of the modified microorganism. Most of EPA's prior reviews of MCANs have taken place within the 90-day period specified in the regulations, although EPA has the power to unilaterally extend the review period by an additional 90 days, or to ask the applicant to voluntarily suspend the review period, if the Agency decides it needs more time to complete its review or needs to request more data. MCANs for the contained use of new microorganisms in bio-based manufacturing have generally not caused any concerns or significant issues in EPA's review, and most have been routinely cleared for commercial use without any delays or difficulties; however it is possible that MCANs for algae or cyanobacteria might take slightly longer for EPA review, due to initial unfamiliarity with the species and its proposed conditions for growth and manufacture.

MCANs (like chemical PMNs) are not "approved" per se, but if no issues emerge they are cleared for commercialization if EPA takes no action and drops the MCAN from review within 90 days. However, if issues are identified, EPA has the authority to require additional data from the MCAN submitter or to limit approved uses of the microorganism in a variety of ways, including controls on workplace and/or envi-

ronmental exposure and limitations on the amount of the organism that can be used commercially. Once an MCAN is dropped from review, an applicant must file a Notice of Commencement within 30 days of beginning commercial use or importation of the microorganism, a notice that requires submission only of minimal information, but which triggers recordkeeping and reporting requirements once commercialization begins. Even if EPA does not impose any restrictions on use prior to commercialization, the agency has the power to regulate microorganisms after commercialization, for example by imposing requirements for testing and submission of health and safety data, as well as taking other steps that the agency may decide is necessary to address unreasonable risks to health or the environment.

As of this writing, EPA has reviewed 63 or more MCANs (USEPA 2014; Bergeson et al. 2014), with most of the more recent ones covering microorganisms intended for use in biofuel or bio-based chemical production. These will be discussed in more detail below.

The biotechnology rule provides certain exemptions from MCAN reporting. These are the so-called "tiered exemptions" available for certain uses of modified strains of well-studied, common industrial microorganisms. First, the host, or recipient, organism must be one that is included on the list found in Section 725.420 of the regulations. This list includes many well-studied species including *E. coli* K12, *Saccharomyces cerevisiae, Bacillus subtilis,* and others, but does not include any algae or cyanobacteria species. The regulations also include a procedure whereby manufacturers may petition for inclusion of a new host on this list. In September 2012, in response to industry petitions, EPA published a notice proposing to add *Trichoderma reesei* strain QM6a and *Bacillus amyloliquefaciens* subsp. amyloliquefaciens (industrial strains) to the list of potentially exempt species, but as of this writing, the proposed rule has not yet been finalized (USEPA 2012).

Second, as specified in Section 725.421, the introduced genetic material in the microorganism must be well characterized (that is, the function of all introduced DNA is known); must be limited in size to the minimal genetic information needed; must be poorly "mobilizable", which is defined in the regulation to mean that the ability of the genetic material to be transferred and mobilized has been inactivated and that the frequency of transfer is less than 10^{-8} transfer events per recipient; and must be known to be free of harmful sequences. Given the precision possible in today's recombinant DNA techniques, these conditions should be easy to meet.

Third, in Section 725.422, the regulations specify specific containment and control procedures to minimize the possibility that the engineered microorganism might inadvertently be released from the facility. If an organism meets the first two sets of criteria (the "biological" criteria), and the applicant can certify that it will use the microorganism in strict compliance with the provisions of Section 422, the process is eligible for a "Tier I" exemption and the microorganism can be used commercially merely upon 10 days advance notice to EPA. Note, however, that in EPA's current interpretation of the Section 422 provisions, the Tier I exemption is not available if significant quantities of a live microorganism are to be transported from one facility to another (K. Moss, personal communication). For microorganisms meeting the biological criteria but which are intended for use under conditions less

strict than the Section 422 procedures, the applicant can submit a petition for a "Tier II" exemption 45 days before intended manufacture. EPA would approve the Tier II request if it felt that the proposed containment and control procedures, although not identical to the Section 422 procedures, were sufficient for the organism in question. Note that the Section 422 procedures are also recommended for use with microorganisms subject to MCAN reporting.

In addition to the tiered exemptions, the TSCA regulations also provide a procedure by which companies can apply for an exemption for test marketing purposes. This requires submitting certain information to EPA 45 days in advance of the proposed activity. According to Bergeson et al. (2014), from 1997 through 2013, EPA received and approved 118 Tier I and two Tier II exemption requests, as well as one request for a test marketing exemption.

The use of genetically modified microorganisms to produce chemicals for commercial use may trigger additional regulation under TSCA. If the chemical substance synthesized by the microorganism has never before been used in commerce in the U.S., the manufacturer may have to file a traditional premanufacture notice (PMN) for the new chemical (Bergeson et al. 2012). Examples might include novel enzymes (e.g., having new activity or a novel, artificially-designed amino acid sequence) or organic chemicals that have not previously had any commercial utility. The use of a novel (i.e. intergeneric) microorganism to produce known compounds or chemical substances presents a somewhat more complicated picture. If the substance being commercialized is a single chemical compound that can be represented by a definite chemical structural diagram, it is known under TSCA as a Class 1 substance, and in most cases, if a Class 1 substance has been used in commerce and is on the TSCA Inventory, producing that substance by a novel production process, such as by a novel microorganism, would not require filing a new PMN. However, TSCA also defines another class of substances, Class 2 substances, as those that are of "unknown or variable composition, complex reaction products, [or] biological materials" (known as UVCB substances), which cannot be easily represented by a structural diagram. As described in Bergeson et al. (2012), there are several types of Class 2 substances, which are generally listed on the TSCA inventory by a definition specifying the source from which the substance has been derived. In many cases, production of a Class 2 substance by a novel microbiological method will require filing a new PMN in addition to an MCAN, since the source of the substance will differ from the source defined on the Inventory listing. For example, a substance comprising a range of alkanes or alkyl molecules for use in diesel fuel would likely require a new PMN as a UVCB substance if produced by a novel microbial method. See Bergeson et al. (2012) for more detail on scenarios that might require PMN filing in addition to MCAN filing for the production of bio-based chemicals using modified microorganisms.

Because of the statutory limitations of TSCA, most chemicals requiring PMNs under TSCA would generally not be regulated elsewhere in the federal government, but there are exceptions. Notably, microbially-produced substances intended for use as automotive fuels or fuel additives are required under the Clean Air Act to obtain registration from EPA's Office of Transportation and Air Quality under 40 CFR Part

79. This requirement is to ensure that novel fuels will not harm engines or cause undue air pollution, and would apply not only to diesel or gasoline substitutes but also to additives such as ethanol or butanol. New aviation fuels or fuels intended for use in any of the branches of the U.S. military also must undergo a registration process, which often requires establishment of, and compliance with, standards and specifications adopted by ASTM International. Furthermore, there are additional compliance requirements for manufacturers of novel fuels wishing to take advantage of the economic credits (Renewable Identification Numbers) available under the U.S. Renewable Fuel Standard. Discussion of these regulatory programs is beyond the scope of this article, but see Slating and Kesan (2012) and Danish et al. (2014) for more information.

3.2.4 Regulation of Research Uses of Modified Microorganisms

TSCA is a commercial statute, and so its jurisdiction generally does not include R&D activities. Under the parts of TSCA regulations that cover new chemicals, there is an exemption for "small quantities" of new chemicals used solely for R&D. This exemption was largely carried over into the biotechnology rule, except that EPA made the somewhat arbitrary distinction that microorganisms, because they are self-replicating, could not be considered to ever be used solely in "small quantities" unless certain restrictions were placed on how they were used. Thus, new microorganisms used solely for R&D purposes could qualify for the exemption only if they were used under suitably contained conditions (i.e., in "contained structures"). Under this definition, it is likely that most laboratory research using GMMs in biofuels or bio-based chemicals would be exempt from commercial reporting. In addition, many uses of engineered microorganisms in biofuel or bio-based chemical pilot plants or demonstration plants could also qualify for this exemption. However, R&D use of intergeneric microorganisms in the open environment, or in vessels or facilities judged not to be suitably contained, requires notification to EPA at least 60 days before the proposed use, under an application known as a TSCA Experimental Release Application (TERA; described in more detail below).

The key issue in determining if an activity qualifies for the R&D exemption is whether or not it will take place in a "contained structure". The term "structure" is defined in the biotechnology rule at Section 725.3, and includes any "building or vessel which effectively surrounds and encloses the microorganism and includes features designed to *restrict* the microorganism from leaving" (emphasis added). The key point of this definition is that the structure *minimize* (rather than *prevent*) the potential for microorganisms to escape and become established in the environment.

Under the rule, activities in contained structures would qualify for the small quantities exemption if conducted "solely for research and development" and meeting other procedural requirements. For example, the R&D must be conducted under the supervision of a technically qualified individual, who must adopt specific con-

tainment procedures. In addition, appropriate records must be kept and workers must be adequately notified of any risks. R&D meeting these requirements can be conducted with no EPA oversight or prior notice (in fact, entities determine for themselves if they are in compliance).

The rule gives EPA staff and the regulated community broad leeway in determining which structures are suitably "contained". Although there was some initial uncertainty when the rule was first proposed, it has since become clear that EPA interprets the definition broadly, so that many laboratories and greenhouses, as well as most fermentation reactor vessels, would meet the definition. Fermenters need not be indoors to meet the definition, so that large outdoor vessels can qualify if certain procedures are followed and suitable controls for the process and the facility are maintained.

It should therefore be possible for most companies to take advantage of this exemption for traditional fermentation processes taking place at pilot or demonstration plants, as long as the microorganism were used solely for research and development and neither the organism or its product are used or sold commercially. It is also likely that many uses of algae or cyanobacteria in enclosed photobioreactors would qualify as contained structures, depending on the specifics of reactor design and operation, and if the procedural requirements for the exemption are also met. However, most open-pond algae reactors would not qualify as contained structures, and would likely require prior EPA review under the TERA process.

The TERA process provides an expedited review procedure for small-scale field tests and other outdoor R&D uses of new microorganisms. Applicants proposing such uses must file a TERA with the EPA at least 60 days in advance of the proposed activity. The data requirements for TERAs are outlined in Sections 725.255 and 725.260 of the regulations, and include information about the microorganism and how it was constructed, a description of the field experimentation proposed to be conducted, along with the proposed confinement conditions and steps to be taken to monitor the possible dissemination of the organism from the test site.

EPA is required to review the submitted information and decide whether or not to approve the proposed outdoor R&D activity within 60 days, although the agency could extend the review by an additional 60 days. If EPA determines that the proposed activity does not present an unreasonable risk of injury to health or the environment, it will notify the applicant in writing that the TERA has been approved. When a TERA is approved, the applicant must carry out the testing under the conditions and limitations described in the TERA application document, and also in accordance with any requirements or conditions included in EPA's written approval. In most cases, it is likely that EPA will require applicants to conduct some form of monitoring, to detect the possible spread or dispersal of the microorganism from the test site, or to detect any other potential adverse environmental effects. EPA may require collection and submission of other data as well. EPA's approval is legally binding on the applicant, and the Agency has the additional authority to modify or

revoke the approval upon receipt of evidence that raises significant questions about the potential risk of the activity.

The regulations provide some exemptions from TERA reporting for certain qualifying outdoor uses of modified microorganisms, but these exemptions are very limited, in covering only certain uses of the nitrogen-fixing bacteria *Bradyrhizobium japonicum* and *Rhizobium (*now *Sinorhizobium) meliloti*, species which were field tested in closely monitored experiments under EPA's interim TSCA regulatory policy. These exemptions would not be expected to apply to any potential use of modified algae or other microorganisms for fuel or chemical production.

As of this writing, there has only been limited experience with TERAs, with only 30 TERAs filed since the biotechnology rule was put into place in 1997 (USEPA 2014; Bergeson et al. 2014). These will be discussed in more detail below.

Many ecologists and public sector critics of use of GMMs in the environment have raised questions about how well the risks of such uses can be assessed. The answer to these concerns is not to prevent any outdoor uses until all risks are ruled out (e.g., as proponents of the precautionary principle would demand), but instead to allow risks to be addressed through the stepwise progression from small scale to larger scale, under a regulatory regime that not only provides oversight but also flexibility and accountability. The TERA process is well-suited for this purpose, to allow outdoor uses of GMMs to take place in a stepwise fashion under appropriate monitoring and agency oversight, to enable environmental risk assessment questions to be addressed with data from actual small-scale environmental use, thus facilitating subsequent risk assessments for larger-scale uses. Although there is no doubt that outdoor uses of genetically modified microorganisms will receive greater regulatory scrutiny than uses in contained manufacturing, EPA's TERA process should allow such uses to proceed through the normal phases of scaled testing in an orderly and responsible manner, under a level of regulatory scrutiny that is accessible to academic scientists as well as companies.

None of the projects covered by any of the previously-filed TERAs have progressed to commercial use, although EPA has approved commercial sale under TSCA of one live, modified agricultural microorganism. In September 1997, EPA approved limited commercialization of the intergeneric microorganism *Sinorhizobium meliloti* strain RMBPC-2, a modified strain with improved capacity to provide fixed nitrogen to alfalfa plants as a nutrient. Because this product was field tested under approvals granted by EPA under its pre-1997 interim biotechnology policy, EPA concluded that the commercial use of this inoculant did not pose significant environmental risks, provided it was subject to certain production limits (USEPA 1997a). Although this is the only live engineered microorganism approved for commercial use in the open environment under the EPA TSCA regulations, it does establish a precedent that EPA would be prepared to grant such approvals where warranted by the science and the data package accumulated by the applicant.

3.3 USDA Biotechnology Regulations

The U.S. Department of Agriculture (USDA) maintains regulations at 7 CFR Part 340, that have been the major U.S. government rules that have covered uses of transgenic plants in agriculture and more recently the increasing interest in using plants for other industrial purposes, such as production of pharmaceuticals, industrial products, and phytoremediation. A small number of modified agricultural microorganisms have also fallen under this regulation, and it is worth noting that there have been some in the algae community that have expressed a preference for the USDA to use this regulation to assert jurisdiction over industrial uses of modified algae, due to the commonalities between algalculture and agriculture and USDA's historical support for, and involvement with, the algae industry (Trentacoste et al. 2014; Henley et al. 2013). However, as explained below, this rule covers only outdoor uses or interstate movement of those organisms to which it applies, and so its potential applicability to contained manufacturing using GMMs is quite limited.

These rules were put into place in 1987 as an immediate outgrowth of the "Coordinated Framework" for biotechnology regulation. USDA proposed to use its existing statutory authority under a law then known as the Plant Pest Act to regulate interstate transport and field testing of genetically engineered plants intended for use in the open environment, to assess the potential environmental effects of such uses. The basis for this rule was the possibility (however remote) that such engineered plants might pose a plant pest risk, based on the presence of nucleic acid sequences arising from genera listed in the rule. These regulations were finalized in June 1987 (USDA 1987), and have been administered by a dedicated biotechnology office within USDA's Animal and Plant Health Inspection Service (APHIS).

The possible applicability of the rule to engineered microorganisms rests within its definitions. Under the rules, "regulated articles" are defined to include only "organisms that are or contain plant pests", which has been interpreted to cover only those plants (or microorganisms) engineered to contain nucleic acid sequences from certain specific microbial, plant and animal genera that contain species that were considered to be potential plant pests. The regulations included a fairly broad list of such genera, and this had the practical effect of causing most transgenic plants to be captured by the regulations: this was because the genus *Agrobacterium* was on the list, and in practice, DNA sequences from *Agrobacterium tumefaciens* were almost universally used in plant transformation procedures, and the presence of *A. tumefaciens* DNA in the resulting plant would often be enough to subject the transgenic plant to regulation under this rule. The list of known or potential plant pest species is contained in 7 CFR Part 340.2. Although the list includes several genera which might include industrially-useful species (e.g., *Pseudomonas*, *Streptomyces*), the rule would only apply if any microorganism containing nucleic acid sequences from these genera were intended to be deliberately used in the environment, which is not likely for production strains for industrial products. The rule could cover open-pond uses of algae, but the list in the regulations does not appear to include any of the genera of algae that have been suggested for industrial use. A modified algae might

fall under the rule if it contained nucleic acid sequences from *Agrobacterium* or another listed genus.

The regulations give APHIS the leeway to make a determination that an organism altered or produced through genetic engineering is a plant pest or that there is reason to believe the organism is a plant pest,[3] but generally speaking, if an engineered microorganism is not from one of the genera shown on the list in Part 340.2, or does not contain any nucleic acids from any such genera, it would not *a priori* be subject to regulation under the existing rules. So, it is unlikely that USDA would use its regulatory leeway to assert authority over a proposed industrial use of a modified microorganism unless it was a fairly large-scale open-pond commercial use (e.g. of a modified algae), and only if there were some clear link, such as a possible plant pest risk, to agriculture or to a particular region or sector of U.S. agriculture. However, in view of the discretion afforded to USDA under the regulations, companies considering the use of modified algal or microbial species not having long histories of industrial use should consider informally consulting with USDA before commercial use or interstate transport of the organism. It should also be noted that several states have regulations that may affect uses of modified organisms or require state participation in USDA biotechnology reviews, particularly for activities with modified organisms conducted outside of containment.

Although the USDA rule initially required submission of permit applications for all proposed outdoor uses of organisms covered under the regulation, the regulations were substantially relaxed on two occasions (USDA 1993, 1997), with the creation of a much simpler notification process for those plant species deemed to have low potential risks. Under the current version of the regulations, transgenic varieties of most common agricultural crops and other familiar plant species meeting criteria specified in the regulations can be used in research field tests simply upon 30 days advance notice to APHIS, and the submission of only minimal information about the modified plants and the proposed field use. Such field tests must be conducted in accordance with performance standards specified in the regulations. Only uses of less-familiar transgenic plants, and presumably any modified microorganisms falling under the regulations, would now be required to undergo the longer permitting process. If an open-pond use of a modified microorganism were judged to fall under these regulations, it is likely that permits would be needed for outdoor testing, even at small scale. Commercial use would require USDA approval through the provisions under the regulation requiring applicants to petition the agency for a determination that regulated articles are determined to qualify for "nonregulated status". Such decisions by USDA clear the organism for commercial use, but in recent years have required the agency first to prepare Environmental Assessments justifying such actions.

[3] USDA now has potentially broader regulatory ability. In 2000, the Plant Pest Act, the law on which the Part 340 regulations was based, was combined with other statutes to create a new law, the Agriculture Risk Protection Act, which includes language that could give USDA the ability to regulate modified organisms based on potential invasiveness or weediness. In 2008, USDA published some possible options to amend the regulations to accomplish this, but to date the Department has never proposed any specific regulations for this purpose.

A number of modified microorganisms have received permits or have otherwise been allowed to be field tested or imported into the United States under the USDA regulations, including species such as *Pseudomonas syringae*, *Xanthomonas campestris*, *Aspergillus flavus*, and various rhizobia (Glass 2003). It is believed that these have all been for agricultural purposes and have been intended to be used under non-contained conditions.

3.4 FDA Regulation of Modified Microorganisms Used in Animal Feed

The use of spent biomass in animal feed, or to produce a substance to be used in animal feed, would be regulated in the U.S. by the Food and Drug Administration (FDA), through its Center for Veterinary Medicine (CVM). FDA does not require premarket review of most human or animal "food" per se: whole food or feed products are presumed to be safe for consumption, but FDA has enforcement powers to be sure marketed products are not adulterated. So, FDA regulation is largely directed at new substances proposed for use as human food additives or as animal feed additives. Under the Federal Food, Drug and Cosmetic Act (FFDCA), most such new substances that are intended to be components of food or to affect components of food are considered to be "food additives" and must be approved through the submission of a Food Additive Petition or, in the case of products for animal consumption, "feed additives" requiring Feed Additive Petitions. However, some substances can be used without approval of a Food or Feed Additive Petition: The FFDCA provides that "substances that are generally recognized, among experts qualified by scientific training and experience to evaluate their safety as having been adequately shown … to be safe under the conditions of their intended use," are not considered as food additives. This created the category of substances known as GRAS: "generally recognized as safe", and many food or feed substances are used in food or feed on this basis.

Companies seeking to use spent microbial biomass in animal feed theoretically have several options to obtain clearance for such uses. One option is to file a Feed Additive Petition, which requires compilation of a significant amount of data and an often-lengthy FDA review process. The primary alternative would be to achieve GRAS status for the product, for which several routes are available. It is permissible under the law for a manufacturer to self-certify that a substance is GRAS for a specific use, if supported by appropriate publicly-available data or expert opinion, while another option would be to seek FDA's concurrence to a GRAS determination using the GRAS Notification procedure, a relatively new process instituted by FDA's Center for Veterinary Medicine in 2010 (following the successful use of a similar program within FDA's human food branch) (USFDA 2014).

However, a third option also exists. Although the law and regulations give FDA the ultimate authority to make decisions on food or feed additive petitions and

GRAS determinations, in practice CVM operates in cooperation with the Association of American Feed Control Officials (AAFCO), which is composed of state, federal, and international regulatory officials who are responsible for the enforcement of state laws regulating the safe production and labeling of animal feed. FDA CVM and AAFCO work together on animal feed regulation, particularly in the establishment of definitions to describe new feed ingredients. Each year AAFCO publishes its Official Publication which includes a model feed bill for states to adopt in regulating feed products and a list of accepted feed ingredients. Most states have adopted all or part of the model feed bill and allow feed ingredients listed in the publication to be used in their respective territories.

In many cases, it may be necessary to obtain an AAFCO ingredient definition for a new animal feed product, particularly to allow sale and use of the product in certain states within the U.S. New feed additives approved by FDA under the petition process are generally accepted as new ingredients by AAFCO, but this may not be true for products self-certified as GRAS. It is possible to work directly with AAFCO to obtain a new ingredient definition for a GRAS substance or other feed ingredient, an action to which FDA may later consent. One example of a company that has successfully obtained clearance both from FDA and AAFCO for the sale of distillers' grains containing genetically modified *S. cerevisiae* is Mascoma Corporation, which has to date obtained approvals for two such modified yeast strains (BusinessWire 2013).[4]

Regardless of the regulatory route chosen, the scientific criteria that would be considered in the regulatory risk assessments for feed use would be different from the environmental effects issues that would be considered for the programs described above, in part because of the different intended use, and in part because microorganisms used in animal feed have generally been inactivated before such uses. Therefore, these regulatory programs will not be discussed here in any additional detail.

4 International Biotechnology Regulations

Biotechnology regulations exist throughout the world, although they have developed differently than in the U.S. Many other industrialized countries or regions, particularly the European Union, Canada, Australia and Japan, implemented biotechnology laws and regulations in the early days of the growth of the industry (i.e., the 1980s and 1990s), in ways that were consistent with the regulatory approaches of these jurisdictions, resulting in some idiosyncrasies among these regulations, although there are some similarities, such as those between the U.S. and Canadian approaches. As described below, more recently, many other countries around the world have adopted biotechnology or "biosafety" laws and regulations based on the principles of an international convention adopted in 2000 – the Cartagena Protocol on Biosafety. Countries

[4] The author has consulted for Mascoma in the past, but at this writing has no financial interest in this company.

taking this route generally have a single biotechnology law that, in principle, is applicable to all research and industrial uses of genetically modified organisms, although much of the focus of the Cartagena Protocol is on agricultural applications of GMOs in the open environment, and cross-boundary movement of GMOs.

4.1 Cartagena Protocol

The Cartagena Protocol on Biosafety was adopted on January 29, 2000 as a supplementary agreement to the Convention on Biological Diversity, and took effect on September 11, 2003 (Eggers and Mackenzie 2000). Under national biosafety laws modeled on the Cartagena Protocol, government approvals are generally needed for importation of living modified organisms (LMOs), and for many industrial activities including "contained uses" or "environmental uses". Such approvals may often require a risk assessment of the LMO and its proposed use. The principles of the Protocol are often a useful guide to the biosafety policies or regulations of many governments, particularly in the developing world.

Under the Cartagena Protocol, "LMO" is defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology, with "modern biotechnology" defined to include in vitro nucleic acid techniques as well as "fusion of cells beyond the taxonomic family". Although definitions of "GMO" vary around the world, most countries have adopted a definition such as this, but it is notable that the definitions in the U.S. EPA TSCA regulations and the USDA regulations are narrower, with EPA's limited to "intergeneric" microorganisms and USDA's requiring the presence of nucleic acids from suspected plant pest species.

The Protocol is primarily intended to ensure that national authorities are notified of any proposal to introduce LMOs into their countries, particularly for the purpose of deliberate release into the environment or for use in food or feed, and further to ensure that information about uses of LMOs is provided to the public and to other countries and interested parties. A key provision of the Protocol is to require there to be "Advance Informed Agreements" (AIAs) when LMOs are shipped across national boundaries, to ensure that the recipient nation is notified of the proposed shipment, and to allow the recipient nation to conduct needed risk assessments.

In most countries, uses of microorganisms within contained manufacturing will differ from applications such as open-pond cultivation of algae, and in general will be subject to far less stringent oversight. "Contained Use" is defined in Article 3 of the Protocol as "any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment". However, Article 6(2) of the Protocol provides an exemption from the AIA procedures for shipments of LMOs intended solely for contained use. Unfortunately, the definition of "contained use" in the Protocol does not distinguish between research uses and commercial uses, an ambiguity which is also found in a number of national laws, sometimes making it unclear whether there might be

any permit requirements for commercial "contained uses" over and above the notification and labeling requirements under the Protocol. The Protocol can be viewed as establishing minimum requirements for applicants proposing to use LMOs in contained commercial manufacturing, such as the requirement to notify the competent national authority), with the understanding that national laws may impose additional requirements in certain countries.

In principle, under the Protocol, the required procedures to use an LMO in the open environment (e.g. in an open-pond algae reactor) would not be much different than for a proposed use in contained manufacturing, in that the recipient national government would need to be notified and would need to conduct a risk assessment. However, in the case of an intended "release" to the environment, an Advance Informed Agreement would absolutely be required (which is not the case for a proposed contained use) and the risk assessment would almost certainly be more rigorous. The Protocol provides specific guidance for the risk assessments to be conducted, with minimal information for the AIA found in Annex I and guidance for the risk assessment in Annex III. In many countries, a permit or some affirmative government permission would be needed before the LMO could be used in the open environment. Such proposals may also engender public or community interest and perhaps opposition.

4.2 European Union

The EU has adopted two directives to cover biotechnology – one covering contained uses of modified organisms, and the other covering uses of modified plants and other GMOs in the open environment (Enzing and Nooijen 2012). Each EU member state is obligated to adopt national laws corresponding to EU directives, and so all 28 EU members should have their own biotechnology laws or regulations that mirror the provisions of the two EU directives.

Uses of modified microorganisms in contained manufacturing would require national government notification, and in some cases possibly also approval, in accordance with the EU "Contained Use" Directive 2009/41/EC (European Union 2009). Article 2 of the directive defines "contained use" in a way that gives an applicant proposing to use a GMM in Europe a fair amount of leeway in determining that a system or process is "contained". Article 4 of the directive requires the user to carry out a risk assessment of the microorganism, using considerations set forth in Annex III of the directive. As a result of this assessment, the user would determine which of four containment levels is appropriate for the organism, and would be obligated to adopt appropriate containment measures in accordance with Annex IV of the directive. These requirements are similar to most other international biosafety guidelines, and most microorganisms used for fuel or chemical production would qualify to be included within the lowest level of containment. Article 6 of the directive requires users to notify the government agency designated in national legislation as having jurisdiction to enforce the contained use directive before a facility is to be used with GMMs for the first time. Annex V specifies the information required

to be submitted with such notifications, and for organisms in the lowest class of risk, the necessary information is fairly minimal. The laws of individual EU nations should conform to these provisions, and in most cases there would not be any need to seek government approval for contained uses, beyond the notifications described here, although it is likely that the laws of some EU nations may require government review and approval of such proposals.

Uses of modified algae or other microorganisms in open ponds would be covered by national laws corresponding to EU Directive 2001/18/EC on "Environmental Release" (European Union 2001). Generally speaking, any outdoor activities with LMOs in Europe, including small scale field testing, would require approval from the country in which the activity is to take place. Applications for commercial use are more complicated, in that all EU member states have some say in commercial approvals granted by individual countries. Although most if not all EU members have approved numerous field tests of transgenic plants over the past two decades (most of which have been for food-producing crops), commercial approvals for food crops have proven extremely problematic, and at times have effectively been barred in Europe.

4.3 Canada

Both contained and open-pond uses of modified microorganisms may require approval from Environment Canada under the New Substances Notification regulations under the Canadian Environmental Protection Act (Darch and Shahsavarani 2012). These regulations, which in many ways resemble the U.S. TSCA biotechnology regulations, cover the use of any microorganism that is new to commercial use in Canada, and potentially cover many modified microbial strains as well as unmodified microorganisms that have not previously been used in Canada. This represents one difference from the situation in the U.S., where unmodified microorganisms (as well as some modified microbes) are not covered by the TSCA regulations. The regulations potentially cover both contained and open-environment use of microorganisms, with a greater level of scrutiny dedicated for the latter, however, as of this writing the only microorganisms approved under the program have been for enzyme manufacture or for uses not related to biofuels.

4.4 Brazil

Under the National Biosafety Law, all proposed uses of living modified organisms would require approval from the Biosafety National Technical Committee (CTNBio), followed by authorization from the applicable Ministry. CTNBio is part of the Ministry of Science and Technology and it is a multidisciplinary committee composed of representatives from many different ministries and branches of the

government, which is responsible for the technical reviews of biotechnology applications. Responsibility for formulation and implementation of the National Biosecurity Policy falls to the National Biosecurity Council (CNBS), which reports to the Presidency of the Republic. Once CTNBio grants an approval for a project, the formal authorization is granted by a government ministry: either the Ministry of Agriculture for most agricultural activities; the Ministry of the Environment for nonagricultural activities taking place in the environment, or the Ministry of Health for "human and pharmaceutical uses". There have been recent approvals for contained uses of LMOs for biofuel or bio-based chemical production that have been issued by the Ministry of Health after CTNBio review (CTNBIO 2014), which are discussed below.

4.5 Japan

Japan is a signatory to the Cartagena Protocol, and it has adopted Law 97 of 2003, entitled "Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms." This law placed Japanese law in conformance with the Protocol, and forms the basis for Japan's biotechnology regulatory regime (Yamanouchi 2005). Among the defined terms of Law 97 are definitions of two categories of use of LMOs. "Type 1" uses correspond to what is typically called "deliberate releases", while "Type 2" uses are "contained uses". Under this scheme, proposed uses of modified microorganisms in contained manufacturing would be regulated as Type 2 uses, although the Ministry having jurisdiction may vary, and could be either the Agriculture Ministry or the Environment Ministry. Uses of microorganisms in open ponds would be regulated more stringently as Type 1 uses, requiring submission of a greater amount of data and triggering a more intensive risk assessment.

4.6 Australia

Australia has one of the more developed biotechnology regulatory frameworks in the world, through the Gene Technology Act of 2000, which has been implemented by the Gene Technology Regulations of 2011 (Tribe 2012). Under the Gene Technology Act and its regulations, both contained and non-contained uses of LMOs would require a license from the government, through the Office of the Gene Technology Regulator (OGTR).

The Australian law uses the terminology "dealings" to refer to any proposed use of a genetically modified organism. Contained uses of microorganisms would be considered as "dealings not involving release" (DNIR). Commercial and R&D DNIRs both require government review and approval, but in general, proposals for contained uses would face a shorter, easier approval process than would a proposal

for outdoor uses of GMOs. Open-pond uses would be regulated by OGTR as "dealings involving release" (DIR). These proposed uses would be subject to greater scrutiny and a more involved risk assessment than DNIR applications, but the Australian government has approved a significant number of these applications. It appears that all the approved licenses have been for transgenic crop plants or for genetically modified vaccines, and none appear to cover either modified energy crops or modified microorganisms.

4.7 China

Under China's Biosafety laws and regulations, open-pond use of modified microorganisms would likely require approval from the Agriculture Ministry. Jurisdiction over contained uses is less certain, although approval would be needed to import LMOs into China for any purpose. See Chen et al. (2006) and Gupta and Falkner (2006) for more information on the Chinese regulatory regime.

5 Successful Regulatory Applications for Industrial Uses of Genetically Modified Microorganisms

5.1 Approvals Under EPA MCANs

EPA has been receiving MCANs and other notifications of biotechnology products under its interim TSCA policy since 1987 and under the current rules since 1997, and these regulations have not proven to be a barrier to industrial biotechnology companies, including those developing biofuel products or processes. As of this writing, there are 63 MCANs listed on the EPA website (USEPA 2014) as having been filed from the 1997 inception of the regulations through December 2013. The number and frequency of these filings have increased substantially in the last 3 years, as can be seen in Fig. 1, due to a greater number of proposals for biofuel or bio-based chemical production (Bergeson et al. 2014). All but one of the MCANs listed on the EPA website were favorably reviewed by EPA, and the intended products of the microorganisms covered by these MCANs are summarized in Table 5. The breakdown of MCANs by genus is shown in Table 6.

The greatest number of MCANs cleared by EPA have been for uses of intergeneric microorganisms to manufacture industrial enzymes. Many of these, particularly in recent years, have been for enzymes intended for use in the production of cellulosic ethanol or other biofuels. In recent years, the number of MCANs for biofuel or bio-based chemical production organisms has dramatically increased, such that production of fuel ethanol has become the second largest category: notably, 16 of the 22 MCANs for this purpose have involved the use of modified strains

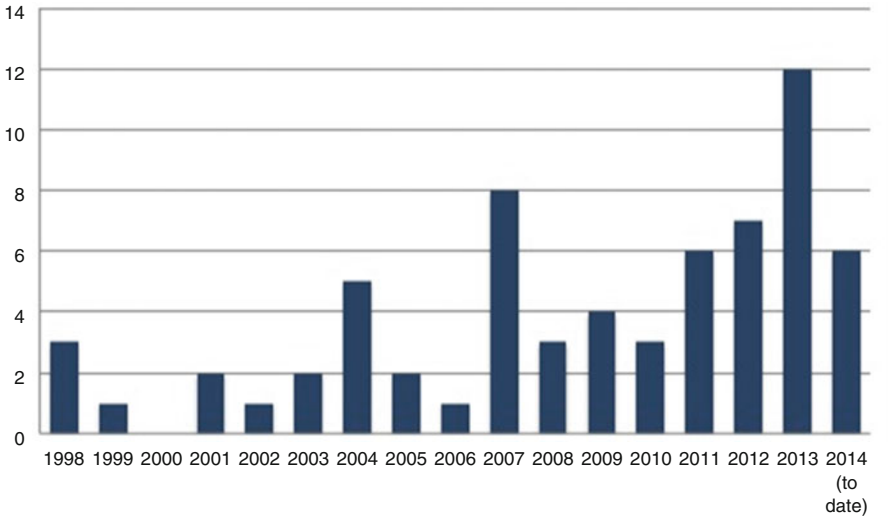

Fig. 1 MCANs Submitted to EPA, by U.S. Government Fiscal Year (through December 2013) (Source: http://www.epa.gov/biotech_rule/pubs/submiss.htm, accessed on October 23, 2014 (USEPA 2014). Data includes MCANs identified as filed through December 2013)

Table 5 Products produced by MCAN microorganisms

Industrial Enzymes[a]	27
Ethanol	22
Bio-Based and Other Specialty Chemicals	11
Research Enzymes	3
Total	**63**

Source: http://www.epa.gov/biotech_rule/pubs/submiss.htm, accessed on October 23, 2014 (USEPA 2014). Data includes MCANs identified as filed through December 2013

[a]Includes at least 6 MCANs for production of enzymes used in biofuel manufacture

of *Saccharomyces cerevisiae*. Although *S. cerevisiae* strains potentially qualify for the tiered exemptions described above, it has become common for developers of such strains to file MCANs to simplify the transfer and use of the strains to third parties, such as under partnership or licensing business models. This is largely due to EPA's policy that the Tier I exemption doesn't apply if significant quantities of live organisms are to be transferred between facilities, but also because once an MCAN is reviewed and approved by EPA, and the developer files a Notice of Commencement indicating that commercialization has begun, the strain is deemed to be placed on the TSCA Inventory and can therefore be used commercially by any

Table 6 Host organisms in MCANs filed through december 2013

Genus	Number
Trichoderma	18
Saccharomyces	16
Escherichia	5
Pichia/Komagataella	3
Zymomonas	2
Bacillus	2
Pseudomonas	2
Microalgae (unspecified species)	2
Klebsiella	1
Synechococcus	1
Unspecified (i.e. claimed as confidential)	11
Total	**63**

Source: http://www.epa.gov/biotech_rule/pubs/submiss.htm, accessed on October 23, 2014 (USEPA 2014). Data includes MCANs identified as filed through December 2013

party under any conditions. In contrast, the tiered exemptions are facility-specific, so that third party users of a modified strain for which one party has obtained an exemption would have to obtain their own approvals, through MCANs or tiered exemptions, before being allowed to use the strain.

There have been MCANs covering other species of microorganism for ethanol production, including *Zymomonas* and *E. coli*. As shown in Table 5, there have also been a considerable number of MCANs for various microbial production processes of bio-based or specialty chemicals, although the names of the applicant, the chemical and/or the production microorganism are often claimed as confidential in these filings. One series of MCANs identifies the product as an unspecified organic acid, although the identity of the submitter and the production organism have been claimed as confidential.

Among the most recent filings are two MCANs submitted by Solazyme, which are the first received and favorably reviewed by EPA under TSCA for the industrial use of modified eukaryotic algae. Although the identity of the microalgae species has been claimed as confidential in these MCANs, presumably one or both are for modified versions of the same algae species, *Prototheca moriformis*, that has been identified in online documents describing Solazyme's approvals for commercial use in Brazil (described below). Unlike many industrial uses of microalgae, Solazyme grows its algae strains in traditional contained fermentations, with the organisms growing heterotrophically, i.e. deriving their energy from chemical nutrients rather than via photosynthesis. These modified algae would be used to produce one or more chemicals, the identities of which have been claimed as confidential by the company.

MCANs have also been submitted for modified cyanobacteria. In 2012, Joule Unlimited Technologies filed the first MCAN for a modified strain of *Synechococcus*

for production of ethanol, and although at this writing not yet listed on EPA's website, it is known that Algenol has also filed an MCAN for a modified cyanobacteria strain for ethanol production (P. Ahlm, personal communication).

Joule's MCAN is unique among all previously-filed MCANs in that the organisms would be grown outdoors, in durable, contained transparent photobioreactors arrayed horizontally to gather sunlight, rather than in a traditional stainless-steel fermenter.[5] In its evaluation of Joule's MCAN, EPA had no health or safety objections to use of the modified strain at Joule's Hobbs, New Mexico facility. However, because of the innovative nature of Joule's photobioreactors, EPA was not prepared to simply drop the MCAN from review, thereby granting the company unlimited rights to use the MCAN strain under any conditions. Instead, EPA and Joule entered into a voluntary consent order, which allows Joule to use the strain commercially at the Hobbs facility, while also providing EPA with further data resulting from such use. According to the EPA website, as of this writing Joule's MCAN is the only one which EPA has regulated with a consent order.

5.2 Approvals Under EPA TERAs

There has only been limited experience with TERAs since the biotechnology rule was put into place in 1997. According to EPA's website, there have been 30 TERAs submitted for field use of engineered microorganisms, almost exclusively for agricultural microorganisms, or for microorganisms to be used for bioremediation or for detection of hazardous contaminants in soil (USEPA 2014). All of these have been to propose small-scale, early-stage R&D projects, and all but three of these were approved. Two recent TERAs from 2013 were submitted by the U.S. Army Engineer Research and Development Center and the US Army Corps of Engineers to propose the use of modified strains of *Gordonia terrae* and *Rhodococcus jostii* in a field demonstration of bioaugmentation to enhance the degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in contaminated groundwater (USEPA 2014). All the TERAs previously approved by EPA had been for use of the organisms in soils or encapsulated in devices for contaminant detection: these two TERAs appear to be the first in which release of GMMs into the groundwater was approved. EPA's approval included significant monitoring and reporting requirements.

More recently in the same year, EPA approved the first TERAs submitted for the experimental outdoor use of genetically modified algae. These are a series of applications submitted by Sapphire Energy, Inc., for open-pond testing of five intergeneric strains of the photosynthetic green algae *Scenedesmus dimorphus*. Sapphire submitted these TERAs on August 1, 2013, and EPA approved them on September 25, 2013, within the 60-day review period allotted under the regulations. The Sapphire TERAs proposed the testing of five different intergeneric strains of

[5] The author coordinated the preparation of Joule's MCAN and handled all interactions with EPA during its review of the filing, while employed by Joule Unlimited. The author also declares a financial interest in this company.

Scenedesmus dimorphus in open ponds, with the stated goals of evaluating the translatability of the genetically modified strains from the laboratory to an outdoor setting, and characterizing the potential ecological impact (dispersion and invasion) of the genetically-modified microalgae. The field trials were proposed to be conducted at the University of California San Diego Biology Field Station (BFS) in La Jolla, California, in collaboration with investigators from the university.

Sapphire's TERA included results of studies in both soil and water to show that the strains showed poor survival (i.e., zero or negative growth) in these environments, and also included a detailed description of the proposed outdoor experimentation and the procedures that will be followed to minimize and monitor the potential release of the organism from the test plots.

The studies under this TERA have been carried out. Among the main findings were that the modified algae were capable of dispersing and colonizing trap tanks up to 50 m distant from the test site, but that the rate of dispersal declines with distance; that both the modified and the wild-type algae were capable of growing in water from nearby lakes; and that the GM algae had no apparent effects on biomass, diversity or composition of native algae species found in the nearby lakes. In particular, the studies showed that the GM *Scenedesmus* is ecologically indistinguishable from the wild-type strains in its impact on native ecosystems (J. Shurin, personal communication).

5.3 Approvals Outside the United States

Although there have likely been other government approvals elsewhere in the world for use of modified microorganisms for fuel or chemical production, Internet searches for relevant information are difficult. While many countries and the Biosafety Clearinghouse that administers the Cartagena Protocol maintain detailed online records of approvals involving genetically modified crop plants, the same is generally not true for approvals for proposed uses of modified microorganisms under contained conditions; and where such online records exist, they often cover R&D as well as commercial applications, and in many cases would not distinguish industrial processes from pharmaceutical manufacture. Furthermore, within the European Union, approvals are granted by each individual EU member, meaning that there is no central location at which to search for approved industrial uses in Europe.

One exception is Brazil, where there has been considerable interest in commercialization of processes for manufacture of ethanol or bio-based chemicals, and where some records are available online. As of this writing, the advisory committee CTNBio (described above) has granted at least five approvals for industrial (i.e. non-pharmaceutical, non-food) uses of modified microorganisms in Brazil (CTNBIO 2014). These are: two approvals to Amyris Brazil S.A. for the use of modified strains of *S. cerevisiae* to produce farnesene (which Amyris uses to produce a jet fuel substitute and other products); one approval to Bio Celere Agroindustrial Ltda. for an *S. cerevisiae* strain modified to express a *Piromyces*

xylA gene encoding xylose isomerase, for ethanol production; and two approvals to Solazyme Renewable Oils and Bioproducts Brazil Ltda. for the proposed use of the genetically modified microorganism *Prototheca moriformis* for the commercial production of triglycerides and bioproducts. *Prototheca moriformis* is a single-celled non-chlorophyll-containing obligatory heterotroph which reproduces asexually and does not produce spores. The latter two are notable in that they are approvals for genetically modified algae strains, which may correspond to Solazyme's two U.S. MCANs described above.

In Canada, Environment Canada maintains a website listing its risk assessment decisions and approvals of proposed uses of microorganisms subject to its New Substances Regulations described above (Environment Canada 2014). At this writing, the site lists 18 decisions, dating back to 2002; however, due to the scope of these regulations, these decisions cover proposed applications in many fields, including human and animal healthcare, and only a few relate to industrial biotechnology projects. These have included proposals for the use of modified microorganisms to produce industrial enzymes in contained manufacturing, and some proposed research projects relating to bioremediation. As noted above, these regulations administered by Environment Canada would be ones that would cover uses of modified microorganism or algae to produce fuels or chemicals.

6 Research Needs

There must be a comprehensive research base to support risk assessment if there is to be effective, science-based regulation that does not pose arbitrary barriers to commercialization. There is already a good deal of starting data regarding the environmental impacts of modified microorganisms, particularly those intended for use in agriculture or bioremediation. The available record to date gives some comfort that large-scale uses of modified microorganisms are not likely to have significant negative environmental effects. Nevertheless, there is a clear need for additional research and data, as several authors have suggested (see, for example, Snow and Smith 2012; Dana et al. 2012), particularly on microbial and algal species most likely to have industrial applicability. It may be useful to foster partnerships among industry, academia and government to focus additional research on organisms created by synthetic biology to develop this additional data.

Much of the needed research can take place at the laboratory or bench-scale, or can be conducted without the need for actual introductions of novel strains into the environment. For example, basic research into the biology, ecology and natural history of commercially-relevant wild type algae, cyanobacteria or other microbial strains would be necessary to develop data on baseline environmental behavior of such species. Basic research could also be carried out to address the important regulatory concerns discussed above, such as gene transmissibility, survival, persistence in the environment, and the genetics of algal toxin production. Laboratory, microcosm and macrocosm studies can be used to address these questions and to model

the behavior of modified species in the environment. Such studies could have significant value, while recognizing the limitations of attempting to model environmental behavior in the laboratory (e.g., see Gressel et al. 2013, 2014 on the "plankton paradox").

Because of these limitations, for proposed uses of modified algae in open ponds, it will ultimately be necessary to assess environmental impacts through actual field experimentation, as has been common in agricultural research. It is appropriate to begin with small field studies designed with features to minimize potential dispersal of the test species in the environment (so-called "confinement" or even some measure of "containment"), along with soil, water and air monitoring as appropriate to detect possible dispersal or environmental persistence. If data derived from such small-scale studies provide no evidence suggesting any potential environmental harm, such studies could be followed up with larger-scale studies, in much the way new plant varieties and other new agricultural products are field tested in progressively larger field trials. In the case of genetically modified algae, even small-scale field trials would likely require some regulatory oversight, and the TERA process under EPA's TSCA regulations, discussed above, provides a very appropriate framework for doing so. Small-scale field trials of modified algae conducted under a TERA would be designed to include monitoring and other procedures to develop the data needed to support progressively larger field trials, and to ultimately support regulatory applications for approvals for commercial use in fuel or chemical production.

Biotechnology regulations around the world are structured so that conducting such studies in support of risk assessments would be the responsibility of the applicant. However, there is no reason that for-profit companies proposing larger-scale uses could not collaborate with academic or government scientists in carrying out such studies, in much the way Sapphire Energy collaborated with the University of California San Diego in carrying out the studies covered by the TERAs discussed above. Academic scientists wishing to propose and carry out their own studies of the environmental behavior of modified algae or cyanobacteria species could also take advantage of the TERA process: a number of the TERAs that EPA has reviewed and approved over the years originated from academic investigators, and the paperwork and other requirements to apply for TERA approval need not be burdensome. Finally, government agencies and their staff scientists could also become involved in carrying out such small-scale testing, as collaborators or principal investigators on such studies, or by offering field test sites at the national laboratories or other agency-controlled sites. This has often been done in the environmental remediation field, with many field tests of novel remediation technologies having been conducted at contaminated sites within facilities operated by the DOE or the military branches.

7 Conclusions

As more companies and research groups begin to contemplate or implement the use of genetically modified microorganisms, including organisms produced using synthetic biology, for biofuel or bio-based chemical production, greater attention will

be turned to the need for appropriate science-based regulation and risk assessment. The scientific basis for such risk assessments is well-understood and is itself the subject of ongoing research, and regulatory frameworks exist around the world to ensure that such assessments take place. Review and approval for use of microorganisms in contained reactors (e.g. photobioreactors) should be fairly straightforward, as any potential risks would largely be mitigated by the choice of the production organism and design features of the reactor (e.g. in accordance with Good Industrial Large Scale Practice). Risk assessments of proposed open-pond uses might need to more rigorously address the key issues, but regulatory procedures like the TERA process of the U.S. EPA can ensure that risks are assessed in a stepwise manner, as field experimentation moves from small-scale to large-scale under conditions designed to minimize the potential spread of the organism from the test plot, and with appropriate monitoring and data-collection to support later experimentation or use at larger scale. Although critics may argue to the contrary, there is no reason to think that these regulations and risk assessments could not apply equally to organisms created through synthetic biology as they do for strains created by more established means of genetic manipulation. Collaborations between industry, academia and government can ensure that the technology moves forward in a responsible manner, to support the development of new processes that can address critical worldwide needs of developing novel sources of energy, while reducing carbon emissions and avoiding other detrimental environmental impacts.

List of Acronyms

EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
LMO	Living Modified Organism
MCAN	Microbial Commercial Activity Notice
PMN	Premanufacture Notice
TERA	TSCA Experimental Release Application
TSCA	Toxic Substances Control Act
USDA	U.S. Department of Agriculture

References

Adrio J-L, Demain AL (2010) Recombinant organisms for production of industrial products. Bioengineered 1(2):116–131

Alexander M (1985) Genetic engineering: ecological consequences. Issues Sci Technol 1(3):57–68

Bergeson LL, Auer CM, Peveler RD (2012) TSCA and the regulation of renewable chemicals. Ind Biotechnol 8(5):262–271

Bergeson LL, Auer CM, Hernandez O (2014) Creative adaptation: enhancing oversight of synthetic biology under the toxic substances control act. Ind Biotechnol. doi:10.1089/ind.2014.1532

Buschke N, Schafer R, Becker J, Wittmann C (2013) Metabolic engineering of industrial platform microorganisms for biorefinery applications–optimization of substrate spectrum and process robustness by rational and evolutive strategies. Bioresour Technol 135:544–554

BusinessWire (2013) Mascoma announces FDA favorable review of its next generation bioengineered yeast, TransFerm Yield+. http://www.businesswire.com/news/home/20130618006024/en/Mascoma-Announces-FDA-Favorable-Review-Generation-Bioengineered#.VDgUpyldW6U. Accessed 10 Oct 2014

Cao Y, Cao Y, Lin X (2011) Metabolically engineered Escherichia coli for biotechnological production of four-carbon 1,4-dicarboxylic acids. J Ind Microbiol Biotechnol 38(6):649–656

Cao Y, Zhang R, Sun C, Cheng T, Liu Y, Xian M (2013) Fermentative succinate production: an emerging technology to replace the traditional petrochemical processes. Biomed Res Int 2013:723412

Chen Y, Nielsen J (2013) Advances in metabolic pathway and strain engineering paving the way for sustainable production of chemical building blocks. Curr Opin Biotechnol 24(6):965–972

Chen C-H, Sassa Y, Suda E, Watanabe KN (2006) Biosafety system frameworks for living modified organisms in Japan and Taiwan. Plant Biotechnol 23(5):539–546. doi:10.5511/plantbiotechnology.23.539

Chen X, Zhou L, Tian K, Kumar A, Singh S, Prior BA, Wang Z (2013) Metabolic engineering of Escherichia coli: a sustainable industrial platform for bio-based chemical production. Biotechnol Adv 31(8):1200–1223

Colin VL, Rodriguez A, Cristobal HA (2011) The role of synthetic biology in the design of microbial cell factories for biofuel production. J Biomed Biotechnol 2011:601834

CTNBIO (2014) Commercial approvals: microorganisms. http://www.ctnbio.gov.br/index.php/content/view/14610.html. Accessed 22 Oct 2014

Dana GV, Kuiken T, Rejeski D, Snow AA (2012) Synthetic biology: four steps to avoid a synthetic-biology disaster. Nature 483(7387):29

Danish K, Epifani LE, Zevin A (2014) Inventory of Federal Regulations Affecting Biofuels other than the Renewable Fuel Standard. VanNess Feldman, LLP. http://bipartisanpolicy.org/sites/default/files/files/VNF_Biofuels.pdf. Accessed 16 Oct 2014

Darch H, Shahsavarani A (2012) The regulation of organisms used in agriculture under the Canadian Environmental Protection Act, 1999. In: McHughen A, Wozniak CA (eds) Regulation of agricultural biotechnology: the United States and Canada. Springer, Dordrecht, pp 137–145. doi:10.1007/978-94-007-2156-2_8

Davison J (2005) Risk mitigation of genetically modified bacteria and plants designed for bioremediation. J Ind Microbiol Biotechnol 32(11–12):639–650

de Jong B, Siewers V, Nielsen J (2012) Systems biology of yeast: enabling technology for development of cell factories for production of advanced biofuels. Curr Opin Biotechnol 23(4):624–630. doi:10.1016/j.copbio.2011.11.021

Dellomonaco C, Fava F, Gonzalez R (2010) The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb Cell Fact 9:3

Eggers B, Mackenzie R (2000) The Cartagena protocol on biosafety. J Int Econ Law 3(3):525–543. doi:10.1093/jiel/3.3.525

Environment Canada (2014) Biotechnology (living organisms) risk assessment decisions. http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=8AD6A8C1-1. Accessed 22 Oct 2014

Enzing CN, Nooijen A (2012) Algae and genetic modification. Research, production and risks, COGEM

European Union (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018. Accessed 23 Sept 2014

European Union (2009) Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:125:0075:0097:EN:PDF. Accessed 23 Sept 2014

Glaser A, Glick P (2012) Growing risk: addressing the invasive potential of bioenergy feedstocks. National Wildlife Federation, Washington, DC

Glass DJ (1991) Chapter 10: Impact of government regulation on commercial biotechnology. In: Ono RD (ed) Business of biotechnology, Newnes, Boston, pp 169–198, doi:http://dx.doi.org/10.1016/B978-0-7506-9119-2.50017-4

Glass DJ (1995) Biotic effects of soil microbial amendments. In: Rechcigl JE (ed) Soil amendments: impacts on biotic systems. Lewis Publishers, Boca Raton, pp 251–303

Glass DJ (2003) Regulation of the commercial uses of microorganisms. In: Encyclopedia of environmental microbiology, Wiley, New York. doi:10.1002/0471263397.env018

Golden JS, Handfield RB (2014) Why biobased? Opportunities in the emerging bioeconomy. U.S. Department of Agriculture, http://www.biopreferred.gov/files/WhyBiobased.pdf. Accessed 13 Oct 2014

Gressel J, van der Vlugt CJB, Bergmans HEN (2013) Environmental risks of large scale cultivation of microalgae: mitigation of spills. Algal Res 2(3):286–298, http://dx.doi.org/10.1016/j.algal.2013.04.002

Gressel J, van der Vlugt CJ, Bergmans HE (2014) Cultivated microalgae spills: hard to predict/easier to mitigate risks. Trends Biotechnol 32(2):65–69. doi:10.1016/j.tibtech.2013.11.003

Gupta A, Falkner R (2006) The influence of the Cartagena protocol on biosafety: comparing Mexico, China and South Africa. Global Environ Polit 6(4):23–55. doi:10.1162/glep.2006.6.4.23

He MX, Wu B, Qin H, Ruan ZY, Tan FR, Wang JL, Shui ZX, Dai LC, Zhu QL, Pan K, Tang XY, Wang WG, Hu QC (2014) Zymomonas mobilis: a novel platform for future biorefineries. Biotechnol Biofuels 7:101

Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT (2013) Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res 2(1):66–77, http://dx.doi.org/10.1016/j.algal.2012.11.001

Hong KK, Nielsen J (2012) Metabolic engineering of Saccharomyces cerevisiae: a key cell factory platform for future biorefineries. Cell Mol Life Sci 69(16):2671–2690

Jang YS, Park JM, Choi S, Choi YJ, Seung Do Y, Cho JH, Lee SY (2012) Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. Biotechnol Adv 30(5)):989–1000

Jones CS, Mayfield SP (2012) Algae biofuels: versatility for the future of bioenergy. Curr Opin Biotechnol 23(3):346–351. doi:10.1016/j.copbio.2011.10.013

Krimsky S (1985) Genetic alchemy: the social history of the recombinant DNA controversy. The MIT Press, Cambridge, MA

Kung Y, Runguphan W, Keasling JD (2012) From fields to fuels: recent advances in the microbial production of biofuels. ACS Synth Biol 1(11):498–513. doi:10.1021/sb300074k

Larkum AW, Ross IL, Kruse O, Hankamer B (2012) Selection, breeding and engineering of microalgae for bioenergy and biofuel production. Trends Biotechnol 30(4):198–205. doi:10.1016/j.tibtech.2011.11.003

Lennen RM, Pfleger BF (2012) Engineering Escherichia coli to synthesize free fatty acids. Trends Biotechnol 30(12):659–667

Lennen RM, Pfleger BF (2013) Microbial production of fatty acid-derived fuels and chemicals. Curr Opin Biotechnol 24(6):1044–1053

Menetrez MY (2012) An overview of algae biofuel production and potential environmental impact. Environ Sci Technol 46(13):7073–7085. doi:10.1021/es300917r

Nielsen J, Larsson C, van Maris A, Pronk J (2013) Metabolic engineering of yeast for production of fuels and chemicals. Curr Opin Biotechnol 24(3):398–404

Nozzi NE, Oliver JW, Atsumi S (2013) Cyanobacteria as a platform for biofuel production. Front Bioeng Biotechnol 1:7

OSTP (1986) Coordinated framework for regulation of biotechnology. Fed Regist 51:23302–23393
Peralta-Yahya PP, Keasling JD (2010) Advanced biofuel production in microbes. Biotechnol J 5(2):147–162
Radakovits R, Jinkerson RE, Darzins A, Posewitz MC (2010) Genetic engineering of algae for enhanced biofuel production. Eukaryot Cell 9(4):486–501. doi:10.1128/ec.00364-09
Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ (2008) A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol 19(5):430–436. doi:10.1016/j.copbio.2008.07.008
Rosgaard L, de Porcellinis AJ, Jacobsen JH, Frigaard NU, Sakuragi Y (2012) Bioengineering of carbon fixation, biofuels, and biochemicals in cyanobacteria and plants. J Biotechnol 162(1):134–147. doi:10.1016/j.jbiotec.2012.05.006
Ryan C (2009) Cultivating clean energy: the promise of algae biofuels. National Resources Defense Council, Washington, DC
Sayler GS, Ripp S (2000) Field applications of genetically engineered microorganisms for bioremediation processes. Curr Opin Biotechnol 11(3):286–289
Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480(1–2):1–9
Slating TA, Kesan JP (2012) A legal analysis of the effects of the Renewable Fuel Standard (RFS2) and Clean Air Act on the commercialization of biobutanol as a transportation fuel in the United States. GCB Bioenergy 4(2):107–118. doi:10.1111/j.1757-1707.2011.01146.x
Snow AA, Smith VH (2012) Genetically engineered algae for biofuels: a key role for ecologists. Bioscience 62(8):765–768. doi:10.1525/bio.2012.62.8.9
Tiedje JM, Colwell RK, Grossman YL, Hodson RE, Lenski RE, Mack RN, Regal PJ (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70(2):298–315
Trentacoste EM, Martinez AM, Zenk T (2014) The place of algae in agriculture: policies for algal biomass production. Photosynth Res. doi:10.1007/s11120-014-9985-8
Tribe D (2012) Gene technology regulation in Australia: a decade of a federal implementation of a statutory legal code in a context of constituent states taking divergent positions. GM Crops Food: Biotechnol Agric Food Chain 3(1):21–29
Urgun-Demirtas M, Stark B, Pagilla K (2006) Use of Genetically Engineered Microorganisms (GEMs) for the bioremediation of contaminants. Crit Rev Biotechnol 26(3):145–164. doi:10.1080/07388550600842794
USDA (1987) Introduction of genetically engineered organisms. Fed Regist 52:22892–22915
USDA (1993) Notification procedures for the introduction of certain regulated articles. Fed Regist 58:17044–17059
USDA (1997) Simplification of requirements and procedures for genetically engineered organisms. Fed Regist 62:23945–23958
USDOE (2010) National algal biofuels technology roadmap. U.S. Dept. of Energy, Office of Energy Efficiency and Renewable Energy, Washington, DC
USDOE (2013) Replacing the whole barrel to reduce U.S. dependence on oil. http://www.energy.gov/sites/prod/files/2014/04/f14/replacing_barrel_overview.pdf. Accessed 20 Oct 2014
USEPA (1997a) Fact sheet: commercialization of Sinorhizobium (Rhizobium) Meliloti, RMBPC-2. http://www.epa.gov/biotech_rule/pubs/factdft6.htm. Accessed 23 Sept 2014
USEPA (1997b) Microbial products of biotechnology; final regulation under the Toxic Substances Control Act. Fed Regist 62:17910–17958
USEPA (1997c) Points to consider in the preparation of TSCA biotechnology submissions for microorganisms. http://www.epa.gov/oppt/biotech/pubs/pdf/ptcbio.pdf. Accessed 23 Sept 2014
USEPA (1997d) Regulatory impact analysis for the regulation of microbial products of biotechnology: the regulated community. http://www.epa.gov/oppt/biotech/pubs/ria/ria013.htm. Accessed 23 Sept 2014

USEPA (2012) Microorganisms; general exemptions from reporting requirements; revisions to recipient organisms eligible for tier I and tier II exemptions. Fed Regist 77:54499–54511

USEPA (2014) TSCA Biotechnology notifications, FY 1998 to present. http://www.epa.gov/biotech_rule/pubs/submiss.htm. Accessed 23 Oct 2014

USFDA (2014) Generally Recognized as Safe (GRAS) notification program. http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/default.htm. Accessed 23 Sept 2014

Viebahn M, Smit E, Glandorf DM, Wernars K, Bakker PHM (2009) Effect of genetically modified bacteria on ecosystems and their potential benefits for bioremediation and biocontrol of plant diseases – a review. In: Lichtfouse E (ed) Climate change, intercropping, pest control and beneficial microorganisms, vol 2, Sustainable Agriculture Reviews. Springer, Dordrecht, pp 45–69. doi:10.1007/978-90-481-2716-0_4

Work VH, D'Adamo S, Radakovits R, Jinkerson RE, Posewitz MC (2012) Improving photosynthesis and metabolic networks for the competitive production of phototroph-derived biofuels. Curr Opin Biotechnol 23(3):290–297. doi:10.1016/j.copbio.2011.11.022

Wozniak C, McClung G, Gagliardi J, Segal M, Matthews K (2012) Regulation of genetically engineered microorganisms under FIFRA, FFDCA and TSCA. In: McHughen A, Wozniak CA (eds) Regulation of agricultural biotechnology: the United States and Canada. Springer, Dordrecht, pp 57–94. doi:10.1007/978-94-007-2156-2_4

Wrubel RP, Krimsky S, Anderson MD (1997) Regulatory oversight of genetically engineered microorganisms: has regulation inhibited innovation? Environ Manage 21(4):571–586

Yamanouchi K (2005) Regulatory considerations in the development and application of biotechnology in Japan. Rev Sci Tech 24(1):109–115

Yu C, Cao Y, Zou H, Xian M (2011) Metabolic engineering of Escherichia coli for biotechnological production of high-value organic acids and alcohols. Appl Microbiol Biotechnol 89(3):573–583

Zhang F, Rodriguez S, Keasling JD (2011) Metabolic engineering of microbial pathways for advanced biofuels production. Curr Opin Biotechnol 22(6):775–783

http://www.springer.com/978-3-319-20199-3

Algal Biorefineries
Volume 2: Products and Refinery Design
Prokop, A.; Bajpai, R.K.; Zappi, M.E. (Eds.)
2015, VIII, 557 p., Hardcover
ISBN: 978-3-319-20199-3