Agency Response to Public Comments on EPA's "Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions"

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I. INTRODUCTION

The Points to Consider in the Preparation of the Toxic Substances Control Act (TSCA) Biotechnology Submissions for Microorganisms[1], hereafter referred to as the Points to Consider, broadly outlines information and data that EPA finds useful for conducting risk assessments of intergeneric[2] microorganisms. Although there of other types of notices to the Agency, the most commonly received types of microorganism submissions to the Agency are the Microbial Commercial Activity Notice (MCAN) for intergeneric microorganisms intended for commercial production, and the TSCA Experimental Release Application (TERA) for intergeneric microorganisms intended for introduction to or use in the environment.

The Points to Consider was last updated in 1997 with the promulgation of the Final Biotechnology Rule[3]. In recent years new technologies have emerged in terms of novel microorganisms not foreseen as having TSCA uses, and in dramatically different design and manufacturing systems for these microorganisms. In addition, there have been extensive advances in genetic engineering and genome editing techniques. Acknowledging the need to update the Points to Consider for the emerging technologies and recent biotechnological developments, EPA decided to first address the rapidly developing industry of genetically engineered (GE) eukaryotic microalgae and cyanobacteria, hereafter referred to collectively as algae, for production of biofuels and bioproducts. The specific recommendations for information and data regarding GE algae is the "Algae Supplement" to the existing Points to Consider. The "Algae Supplement" is a companion document being released concurrently with this Agency Response to Public Comments on EPA's "Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions". The "Algae Supplement" aims to assist producers of GE algae in preparing submissions for the Agency.

On September 30, 2015 in Washington, DC, EPA's Office of Pollution Prevention and Toxics (OPPT) hosted a public workshop entitled, "Workshop for Public Comment on Considerations for Risk Assessment of Genetically Engineered Algae"[4]. At this meeting, EPA solicited input from the regulated community and the public regarding recommended information and data that EPA thought applicable for algal biotechnology submissions as described in the "Considerations for Risk Assessment of Genetically Engineered Algae"[5]. Comments received during the workshop and in the associated docket[6], as well as input from other scientific and stakeholder sources, were then incorporated into a second document, "Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions".

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[1] Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms. 1997. (http://www.epa.gov/sites/production/files/2015-08/documents/biotech_points_to_consider.pdf).

[2] Intergeneric microorganism means a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera.

[3] Microbial Products of Biotechnology: Final Regulation Under the Toxic Substances Control Act (62 FR 17910-17958), 1997. (http://www.gpo.gov/fdsys/granule/FR-1997-04-11/97-8669).

[4] https://projects.erg.com/conferences/oppt/2015meeting.htm

[5] https://projects.erg.com/conferences/oppt/docs/Biotech_Workshop_Report_Final_2015-12-21.pdf, Appendix F

[6] EPA-HQ-OPPT-2015-0508

A second public meeting, "Public Meeting and Opportunity for Public Comment on EPA's Draft Algae Guidance for the Preparation of TSCA Biotechnology Submissions" was held on October 27, 2016 in Tempe, AZ to further solicit input from stakeholders and the public[7]. In issuing its "Draft Algae Guidance"[8] for discussion at this public meeting, EPA requested that comments be directed at answering a set of specific Charge Questions (Attachment 1). Comments received during this meeting and those received in the associated docket[9] were addressed and used to develop the final document, "Algae Supplement" to the Points to Consider.

This document is organized to address comments that were responsive to EPA's specific request, as well as those that were not directed at specific questions, but nevertheless provided suggestions to EPA for organizing and preparing the "Algae Supplement". The latter comments are addressed first, followed by the comments that address the charge questions. Only comments received from the 2016 public meeting and those in the associated docket are addressed in this response to comments document. Comments received during the 2015 workshop and in the associated document are not covered here since they were previously addressed in development of the "Draft Algae Guidance" that was discussed at the 2016 public meeting.

Several commenters offered their opinions on policy-related issues such as EPA's scope of coverage for microorganisms. EPA acknowledges these policy-related comments, but they are not addressed here as they are not within the scope of this document.

[7] https://projects.erg.com/conferences/oppt/workshophome.htm
[8] https://projects.erg.com/conferences/oppt/docs/Draft_Algae_Guidance_October2016.pdf
[9] EPA-HQ-OPPT-2015-0508

II. SUMMARY OF COMMENTS AND EPA RESPONSES

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Data - Not all data required	TSCA requires, under Section 5, that submitters provide "any test data in the possession or control of the person giving such notice which are related to the effect of any manufacture, processing, distribution in commerce, use or disposal of such substance…on health or the environment, and a description of any other data concerning the environmental and health effects of such a substance." This section primarily focuses on a risk assessment of the environment in which the algae are cultivated, and potential worker exposure. However, while it is extremely prescriptive, it is not clear whether and when the information listed in the draft guidance may be required from a risk assessment perspective.	1	The "Algae Supplement" is meant to be a brief overview of information that may be useful to EPA in conducting a risk assessment. It is not a list of required information, data, or testing. The "Algae Supplement" presents recommendations for information and data that may apply to any algal strain used in any production system. Not all of the information and data elements outlined in the "Algae Supplement" will be applicable to all TSCA algae submissions. The submitter will need to consider the information elements presented and determine which ones are relevant to their specific strain and production platform.
	Having relevant information which is fit for purpose is a concept ingrained into the risk assessment approach of the EPA's Office of Pesticide Programs (OPP), which, after many years of operating on a mandatory set of test data, has learned that a tailored approach to testing is often more protective, and a more efficient and less wasteful use of resources, including the use of animals for testing. Information that is "fit for purpose" is important for regulating GE algae too, because intergeneric or engineered microalgae, unlike native strains, are bred for highly specific uses and to live in specific cultivation environments. This means that some information will be redundant, and some will not be informative.	2	Unlike OPP's data requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for genetically engineered microorganisms used as microbial pest control agents, the part 725 regulations do not include specific required studies. Therefore, the Points to Consider, and the "Algae Supplement", present a broad range of information and data elements that may apply to any algal strain used in any production system. The "Algae Supplement" is an overview of information that may be useful to EPA in conducting a risk

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			assessment. It is not a list of required data, information, or testing.
	EPA/OPPT should ensure that they are not requiring duplicative information or regulation when other parts of the EPA (OPP) or agencies (OSHA) are the responsible authority. For example, one of the pieces of information listed in the guidance is the use of antimicrobials or pesticides in the media, yet the risk assessment, use, and risk management practices required for these products is already regulated by EPA's Office of Pesticide Programs. It is not entirely clear what bearing this information has on the risk assessment for the microalga strain.	3	If data have previously been developed for another agency, then submitters are required to provide the data as Sections 5(d)(1)(B) and 5(d)(1)(C) of TSCA requires a submitter to provide any information in the possession or control of the person giving such notice if it relates to effects on health or the environment from the microorganism at issue. However, EPA/OPPT would not be regulating the antimicrobial or pesticide used in the media as antimicrobials and pesticides are not under TSCA's jurisdiction and are registered solely by OPP under the Federal Insecticide Fungicide and Rodenticide Act. In addition, the Updated Coordinated Framework of 2017 encourages information sharing among the participating agencies when appropriate. In some cases, there may be a need for coordination with OPP or other agencies with shared interests. Although no formal system exists for the exchange of information among the three regulatory agencies, EPA, the

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			Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA), there is an interagency Biotechnology Working Group organized by the White House's Office of Science Technology and Policy that holds biweekly calls, and a FDA, USDA, and EPA monthly biotechnology conference call. In addition, individuals from the various agencies and offices have clearance for Confidential Business Information (CBI) submitted to other agencies. Regarding the request for whether antimicrobials or pesticides are used in the media, EPA thinks that knowledge of the use of antimicrobials or pesticides speaks to the competitive ability of the GE algal strain. If antimicrobials or pesticides are needed in the ponds under optimal conditions for the alga to fend off contaminating microorganisms or microscopic animals (e.g., rotifers), it may suggest that the GE alga is not so robust in its growth that it would be able to survive and outcompete other microorganisms in the environment if it gets into local surface waters. Also, knowledge of the use of antimicrobials or pesticides informs the analysis of the subject microorganism. This type of information is required to the extent that it enables a microorganism to be accurately and unambiguously identified [725.12 (b)(1) and (2)]. It is important to know whether the subject

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			microorganism has any antibiotic or pesticide resistance genes, and if so, whether they are accurately described.
Data - Decision Tree/Tiered Approach to Data Needs	EPA is requested to consider a tiered, or stratified approach to data requirements, such that algae which do not pose any significant risk (by virtue of the characteristics of the organism or the manner in which they are housed) are not subjected to further exhaustive and unnecessary testing.	4	*This response is applicable to comments # 4, 5, and 6.* EPA/OPPT agrees that in the future it may consider a "tiered/stratified" approach. Such an approach may assist submitters in determining which information and data elements are relevant for their submission(s). However, currently, the science available to determine the potential risks from specific algae is not developed enough to identify "low risk" strains based on substantial evidence of safe use as was done with the Tiered Exemptions for closed system fermentation with industrial "workhorse" microorganisms. The "Algae Supplement" is an overview of information that may be relevant to any algal strain used in a wide variety of production platforms. Thus, not all data elements will be relevant to a specific alga.
	EPA is requested to provide information in this guidance on the experiences and parameters that EPA has developed in relation to microalgae and cyanobacteria, and provide more specific decision-making tools to assist submitters with determining what information to provide from the information list provided, based on knowledge of the organism and how it will be housed.	5	
	Given the array of organisms and production processes, it would be helpful if the Draft Algae Guidance were clear on what information is necessary for the different situations, while also minimizing requirements for redundant or duplicative information where possible. Establishing a decision tree could be helpful to guide a submitter through what information is needed based on the characteristics of the organism and the production system being used.	6	
Data - Taxonomy	EPA is recommended to revisit the section in the draft guidance on recipient microorganism characterization. For example, the use of traditional phenotypic methods is limited by the fact that, as with all organisms, microalgae may contain genes which are not expressed, or are expressed preferentially depending on the environment in which they are kept or nutrient availability. Taxonomy should therefore be based	7	The Recipient Microorganism Characterization does not recommend the use of only phenotypic methods. In A.1. Taxonomy, under a. 3), the paragraph explicitly states that phylogenic methods relying on nucleic acid analyses are the primary means of

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	only on scientific, quantitative methods such as nucleic acid analysis, rather than on a qualitative, observable trait that may change due to external factors or depending on a subjective evaluation of the phenotype. In terms of providing substantiating information on strain identity, companies have found culture collections to have incorrect identification entries as they do not verify the information provided by the original submitter. Further, it has proven to be difficult to correct these incorrect entries with the EPA even after the submitter or third party has provided evidence or published data that shows the identification is incorrect. Such errors impact the protection of intellectual property rights; regulatory review times and substantive requirements; and future submissions. EPA should provide more detailed guidance on the provision of validated identification information and establish a process to correct existing errors so that the outcomes can be accepted and used by EPA.		identification for most taxa. The Agency takes a multifaceted approach in taxonomic classification that incorporates existing literature and knowledge of the engineered microorganism, as well as phenotypic and genotypic data provided by the submitter. EPA acknowledges the commenter's description of the challenges inherent in phenotyping engineered strains of algae, however, the Agency disagrees with the commenter's assertion that phenotypic data should not be used for taxonomic characterization of algae. The Agency believes that a polyphasic approach incorporating genotypic and phenotypic data is optimal as it allows for the greatest degree of flexibility given the wide variety of organisms submitted for review by the agency. Ultimately, as outlined in the Points to Consider, "*As there are no universally applicable methods for identifying microorganisms, it is up to the submitter, or its agent, to select the most appropriate ones for submitted organism(s).*" The "Algae Supplement" has been revised to clarify that a polyphasic approach to taxonomic identification is preferred and a more detailed discussion of taxonomy sites for cyanobacteria and eukaryotic algae has been added.
	With respect to taxonomy in Section III.A. I. we recommend that point three on how to substantiate taxonomy precedes point 2. Point 2 describes ways in which to substantiate	8	As recommended, the order of points 2 and 3 in the taxonomy section were changed in the "Algae Supplement".

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	taxonomy. In addition, section b should be expanded to discuss the currently transitional nature of the classifications, the elements that are captured by the taxonomy, the role of phenotype and genotype in such classifications, and guidance on consulting and tracking developments concerning possible reclassifications as discussed in the published literature, for example. Guidance that "classification could include .... " is not particularly useful compared to providing guidance on what classification should include (Section III.B.2, p. 8).		The taxonomy section, II.A.1.b, has been expanded as requested.
Data - Environmental Fate	Bearing in mind that GE algae are created using proprietary research and development, there is a strong commercial incentive to prevent their escape into the environment where they may end up being taken by a competitor. GE microalgae may be specifically engineered to prevent their survival in the environment for precisely this reason. Furthermore, even if they do escape the confines of their fermenter, photobioreactor, open pond, or other containment and survive, it should not be assumed that there will be an adverse outcome as a result. The EPA should therefore contextualize and justify when information on environmental fate is required, to prevent the development of data which are unnecessary or superfluous to the risk assessment.	9	EPA does not use a default assumption that there will be an adverse effect if an alga survives in the environment. However, the potential of the alga to survive and outcompete indigenous algal species is an important component in evaluating potential ecological effects. Survival information, as required at 40 C.F.R. § 725.155(d)(3)(ii) for both a Microbial Commercial Activity Notice (MCAN) and a TSCA Environmental Release Application (TERA), is considered not only for the specific environment in which the algal strain is being produced/used, but also in other environmental media into which the subject microorganism may potentially be disseminated. Survival data may also be useful even for algae grown in contained systems as there are low level releases from closed system fermenters and the possibility of leakage with PBRs. The assertion that the microalgae are unable to survive outside the pond, PBR, or fermenter may require substantiation, however the type and

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			amount of information will vary case-by-case.
	Environmental fate data are not necessary if the microalgae are unable to survive outside the pond, fermenter, photobioreactor, or other containment technologies. Some of the information described in the draft guidance document may not be necessary for microalga strains that are established to be well-characterized as that term is defined in the regulations.	10	The term "well-characterized" in the 1997 Final Biotechnology Rule refers only to the introduced genetic material criterion in the Tiered Exemptions, or to noncoding regulatory sequences, not to microorganisms. Information on "survival and dissemination of the microorganism under relevant environmental conditions" is required at 40 C.F.R. § 725.155(d)(3)(ii). If the algal strain is to be used uncontained in the environment, survival information helps inform prediction of potential ecological effects. In the absence of information indicating the microorganism has been thoroughly incapacitated in its ability to survive outside the ponds, it is often assumed that it would survive if the strain is an environmental isolate. The lack of the ability of an algal strain to survive outside a pond, PBR or fermenter may require substantiation.
	The key risk assessment questions for algae are strain selection and survival in the receiving environment. If a proposed algae strain is native to the surrounding environment, ecological effects are not anticipated. For strains not native to the surrounding environment, the question becomes one of survival. If a strain is unsuitable for survival in the receiving environment, other risks, such as the potential to produce toxins and the potential for harmful algal blooms or other ecological effects, are mitigated. By design, the conditions in industrial algae production systems are	11	The fate section of the "Algae Supplement" (F. Fate of the GE Alga) includes information related to the survival of the intergeneric microorganisms relative to the unmodified recipient strains. EPA acknowledges that different conditions are used in industrial algae production systems, and therefore, identifies those conditions as useful

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	substantially different from those of the surrounding environment. Temperature, salinity, and other conditions are optimized to maximize productivity and minimize risk of contamination by pests. Strains are also typically optimized to thrive in the system environment, making them unsuitable for survival in the surrounding environment.		information for assessing the likelihood of survival. See Section H.2. and H.3. of the "Algae Supplement" for the specifics of the production system conditions.
Data - Containment	As risk is a function of both hazard and the probability of exposure, EPA should provide clarification on the projected data needs associated with fully contained, uncontained, and partially contained algae production systems along with descriptions of facilities that qualify for each status. Thus, for example, the use of full containment systems (which could be physical, biological or combination of both) should not necessitate exposure or environmental fate data. The definition of contained use used by the EU for the purposes of regulating GE microorganisms provides a fair set of criteria for determining whether or not a system or process is "contained" [1]. It also refers to what the containment system should achieve from the perspective of safety for the general population or the environment, as opposed to specifying the different kinds of production systems that qualify as contained or not [2]. The relevancy and necessity of this information to risk assessment and regulatory decision making is important and should be clarified. *Citations:* *1. EU Contained Use Directive 2009/41/EC* *Contained Use Directive 2009/41/EC and D Glass "Government regulation of the uses of genetically modified algae and other*	12	Currently, the Agency cannot designate specific recommended information for various algae production systems based on whether they are considered contained, partially contained, or uncontained as there may be releases associated with all of them. Even in closed system fermentation, which is typically thought as being a "contained" system, there are some releases of viable microorganisms as aerosols in off-gases and in the disposal of inactivated liquid and solid wastes where the inactivation methods may not be 100% effective. These releases through aerosols and in the liquid and solid waste streams are acknowledged in the 1997 Biotechnology Rule for the Tiered Exemptions, and there are criteria to be met for each of them that are given at 40 C.F.R. § 725.422 to qualify for the exemption. Although EPA is aware of the EU Directive on the contained uses of genetically modified microorganisms, EPA evaluates containment and releases based on its own experience (e.g., data from submitters) as well as from other available information on a case-by-case

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	microorganisms in biofuels and biobased chemical production". 2015 (http://link.springer.com/chapter/10.1007%2F978-3-319-20200-6_2)		basis. Appendix A of the "Algae Supplement" identifies the potential environmental release points for closed-system fermentation, photobioreactors, and open pond algae production systems. Section H of the "Algae Supplement" identifies information that EPA find useful in evaluating containment.
Data - Methodology	The current Draft Algae Guidance document requests available data for potential human health effects of the GE Alga. Specifically, the draft requests data for immunological effects such as skin irritation/rashes and eye irritation. There are currently several internationally validated in vitro methods that provide predictive information on skin and eye irritation and skin sensitization (see summary of specific methods in Appendix H.2) [1-3] that EPA should consider. *Citations:* *1. Reisinger et al. (2015) Toxicology In Vitro 29: 259-270 (http://www.sciencedirect.com/science/article/pii/S0887233314002094)* *2. Johansson et al. (2013) Toxicology In Vitro 27:1163-9 (https://www.ncbi.nlm.nih.gov/pubmed/23032079)* *3. Ramirez et al. (2014) Toxicology In Vitro 28:1482-97 (https://www.ncbi.nlm.nih.gov/pubmed/25172300)*	13	The Points to Consider and the "Algae Supplement" apply to a broad range of microorganisms and production platforms. For some algal species known to cause skin or eye irritation, such testing may be prudent. EPA thanks the commenter for the skin sensitization methodology provided. Section 4(h) of TSCA, as amended by the 2016 Frank R. Lautenberg Chemical Safety for the 21st Century Act Section, states that "the Administrator shall reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animal in the testing of chemical substances or mixtures…". EPA recently published new methodology for skin sensitization - Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing (EPA-HQ-OPP-2016-0093).

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Data - Specific Data	For example, the list includes information on the materials from which an open pond is constructed, or in the case of photobioreactors (PBR), the thickness and tensile strength of the PBR material. EPA should explain why this information is needed --whether and how it informs the degree of containment and probability of release, or whether other criteria that impact risk are being considered and necessitate this type of information. Many companies rely on patented photobioreactors and downstream separation techniques designed by third parties in order to compete more effectively. Companies may not have the market power or ability to influence these designs, or to alter the technical specifications of the materials used to make them.	14	The information on the materials used in construction of PBRs allows EPA to evaluate the integrity of the production system and the potential for breaches in containment. EPA is not requiring or recommending companies request changes from the manufacturers of PBR materials. If submitters make use of PBRs materials manufactured by others, the product description provided by the manufacturer of the PBR materials is useful to EPA for evaluating containment.
Statutory Authority/ Regulations - Statutory Authority	It is strongly suggested that the guidance for submission of technologies, regulated under TSCA, should reflect the recent amendment to TSCA when suggested methods for acquisition of relevant data are described.	15	The 2016 Frank R. Lautenberg Chemical Safety for the 21st Century Act did not specifically affect 40 C.F.R. Part 725 - Reporting Requirements and Review Processes for Microorganisms. The "Algae Supplement" does not include requirements beyond those found at § 725.155 (Information to be included in the MCAN), § 725.160 (Submission of health and environmental effects data), and § 725.255 (Information to be included in the TERA). The "Algae Supplement" is a brief overview of information that may be useful to EPA in conducting a risk assessment.
	The risk assessment guidelines need to be modified to make clear that all kinds of genetic engineering should be covered by the guidelines. The guidelines approach this by recognizing that synthetic DNA even if it copies intragenic DNA may not be identical to the original and thus recommends EPA review of the new organism. The White	16	As quoted, the footnote states "It also covers some of the products produced by such plants, animals, and microbes or their derived products as determined by existing statutes and regulations."

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	House memorandum on the revisions of the coordinating framework makes clear that it intends agencies to include all genetic engineered products, not just those that are intergeneric. The key language is found in the first footnote to the White House memo [1]. While the EPA draft language in B.1. partially addresses this issue. Making clear that ALL genetic engineering is subject to the oversight of the EPA would strengthen the document. *Citation from the Update to the Coordinated Framework:* *1. For the purpose of this memo, "biotechnology products" refers to products developed through genetic engineering or the targeted or in vitro manipulation of genetic information of organisms, including plants, animals, and microbes. It also covers some of the products produced by such plants, animals, and microbes or their derived products as determined by existing statutes and regulations."* *https://www.whitehouse.gov/sites/default/files/microsites/ostp/modernizing_the_reg_system_for_biotech_products_memo_final.pdf*		The Office of Science and Technology Policy memorandum referred to in this comment, which has been archived but can be found on an EPA site (Memorandum from OSTP on Modernizing the Regulatory System for Biotechnology Products) was directed at all agencies under the Coordinated Framework, each with their own statutory authority. This memo does not supersede any granted authorities and EPA/OPPT implements the biotechnology regulations under TSCA, Microbial Products of Biotechnology; Final Regulation Under the Toxic Substances Control Act (40 C.F.R. Parts 700, 720, 721, 723, and 725). EPA/OPPT requires reporting only for intergeneric microorganisms and not all genetically engineered microorganisms or other organisms.
Statutory Authority/ Regulations - Regulations	The draft guidance should include a discussion of how reported strains and the output chemicals are included on the TSCA Inventory, and make this discussion available for public comment, as this is not well understood by stakeholders. A naming system for modified microalgae and cyanobacteria and certain chemical substances that they produce for TSCA Inventory identification purposes is needed via a process that includes input from stakeholders. More specifically, the severe chemical nomenclature challenges facing modem, bio-based, class 2 substances are very similar to those which led EPA to recognize the Soap and Detergent Association Nomenclature (SDA) system.	17	EPA is currently discussing options internally on how best to list microorganisms on the TSCA Chemical Substance Inventory. There is an existing nomenclature system for the listing of class 2 chemicals and unknown or variable composition, complex reaction products and biological materials (UVCBs) on the TSCA Inventory. Information related to the listing of UVBCs is available on the Agency's website

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			(https://www.epa.gov/sites/production/files/2015-05/documents/uvcb.pdf). The listing of traditional chemical substances on the Inventory is beyond the scope of the "Algae Supplement" to the Points to Consider.
	Once an intergeneric strain is listed on the TSCA Inventory, any person may legally manufacture or import the notified strain for a non-exempt commercial purpose. This is easier said than done for several reasons. Because of the level of detail (in terms of technique and sequencing) EPA requires in its submissions, it is rare for companies to make TSCA Inventory determinations without needing to consult the Agency directly. Also, taxonomy designations for microalgae are subject to rapid change as new molecular information is discovered. The agency can disagree with a Submitter's taxonomic classification, with potentially adverse intellectual property consequences if a patent already has been issued under that classification. EPA has yet to develop a formal naming system for listing new, intergeneric strains or many of the output chemicals they produce on the TSCA Inventory.	18	The Agency may disagree with the taxonomic designation provided by a submitter if it differs from the most current accepted taxonomic name for a microorganism. EPA is aware that microbial taxonomy changes and adapts to changes in identification when appropriate. The Agency uses the most recent relevant information and accepted taxonomies. The taxonomy section of the "Algae Supplement" (II.A.1.) provides links to the most current databases for cyanobacteria and eukaryotic microalgae. EPA agrees that it is often necessary to contact the Agency to determine if a microorganism is already on the TSCA Inventory as the characterization of a microorganism is often claimed as Confidential Business Information. Please see the response to comment #17 regarding listing of microorganisms on the Inventory.
	The TERA process could be used to develop the information needed for risk assessments to 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 non-target species [1].	19	EPA agrees that small-scale environmental introductions such as that in TSCA Experimental Release Applications (TERAs) develop information useful for risk assessment at large-scale and later at commercialization-scale.

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	The EPA should develop their guidance document to better demonstrate how the TERA can be utilized by microalgae and cyanobacteria processes that are under development. The EPA should explain how the information thus developed could be integrated into the TSCA biotechnology commercial application notification: In terms of existing practice, EPA regulations already include a mechanism by which outdoor experimentation relating to modified microorganisms can take place in a stepwise approach, with risks assessed as the scale of experimentation increases. The TERA submission, in theory, offers a pathway to exploration of the use of modified microalgae in various production systems, yet TERA approvals for a single strain are not cost-effective to pursue. There is no public guidance on how to successfully obtain approval for a programmatic TERA. As a result, a programmatic TERA process is currently not a viable option to conduct small scale research critical to advance the development of commercial open pond systems and to gather useful data. Programmatic TERA conditions, for example, could specify time periods for the R&D activities, periodic reporting and tiered data development, inactivation and disposal requirements, and real-time monitoring and the use of viable tracking measures such as the use of fluorescent protein markers. A discussion of the ability to submit TERAs for more than one species, and strategies for groupings, would be a useful addition to this guidance. *Citations:* *1. Government Regulation of the Uses of Genetically Modified Algae and Other Microorganisms in Biofuel and Bio-based Chemical Production David J. Glass 2015*		The TERA is not confined to a single microbial strain or a single field test. The TERA can include multiple strains that may be tested multiple times or at multiple sites. The Agency has internally considered the concept of programmatic TERAs and plans to include a discussion of them in the Points to Consider when it is revised in its entirety since programmatic TERAs will apply to all TSCA microorganisms for environmental introductions, not just for outdoor growth of algae. The Agency encourages pre-notice consultations with submitters for discussions of potential environmental release research programs.
	The TSCA law now clearly requires EPA to consider Alternatives when a "chemical (or microorganism) might be restricted or prohibited."	20	As stated in the Comment, the amended TSCA requires EPA to consider alternatives when a chemical might be restricted or prohibited. It is the

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	REQUIREMENTS FOR RISK EVALUATION2 : "C) CONSIDERATION OF ALTERNATIVES.—Based on the information published under subparagraph (A), in deciding whether to prohibit or restrict in a manner that substantially prevents a specific condition of use of a chemical substance or mixture, and in setting an appropriate transition period for such action, the Administrator shall consider, to the extent practicable, whether technically and economically feasible alternatives that benefit health or the environment, compared to the use so proposed to be prohibited or restricted, will be reasonably available as a substitute when the proposed prohibition or other restriction takes effect " EPA should improve this guidance by explicitly requiring the company to review other ways that the chemicals its organism produces can be produced and comparing its products to those other products. In essence, why is this microorganism, the safest and most sustainable way to produce this chemical? Can a non-genetically engineered microorganism produce the same results as the engineered organism under review? This should be an explicit part of the review of alternatives.		Administrator that needs to consider whether technically and economically feasible alternatives that benefit health or the environment, compared to the use so proposed to be prohibited or restricted, will be reasonably available as a substitute when the proposed prohibition or other restriction takes effect. Thus, the consideration of alternatives is done by EPA and is not a requirement of the submitter.
Tiered Exemptions	As this industry grows, EPA should identify genetically enhanced or engineered microalgae that can be included on the list of well-characterized strains eligible for the Tier I exemption under TSCA based on a history of safe use, the nature of the recipient organism and other such characteristics already in place for other well-characterized microorganisms.	21	In the 1997 Final Biotechnology Rule, the Tiered Exemptions from MCAN reporting requirements apply to certain eligible recipient microorganisms used in closed system fermentation. The ten eligible recipient microorganisms, five bacteria and five fungi, are industry "workhorses" with long histories of safe use. No algae currently meet the long history of safe use requirement needed to make the *a priori* determination that they will not present an unreasonable risk to human health and/or the environment. In addition to the use of an eligible recipient microorganism (listed

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			at 40 C.F.R. § 725.420) in the Tiered Exemption, the introduced genetic material must meet four criteria (listed at § 725.421). Furthermore, there are containment and inactivation criteria that must be met (listed at § 725.422). The Tiered Exemptions are solely for use with closed systems and thus algae grown outdoors in open ponds would not meet the containment criteria of the Tiered Exemptions.
	With regard to the exemption from having to file an MCAN for ten species of microorganisms that are well-characterized providing certain other criteria are met, the draft guidance document could point out that no microalgae or cyanobacteria are currently eligible for this exemption. The process and factors that need to be established to advance these genus or species as well-characterized when petitioning for a new exemption listing could also be discussed.	22	The "Algae Supplement" is now an addendum to the Points to Consider and the information and data to be supplied when petitioning the Agency to add a microorganism to the list of eligible recipient microorganisms in the Tiered Exemptions are given at 40 C.F.R. § 725.67. These data will be discussed in more depth when the revisions to the Points to Consider in its entirety are made. Thus, this will not be covered separately in the "Algae Supplement".
Risk Management vs. Risk Assessment	EPA could provide guidance on the use of unpublished and published literature, inclusion and explanation of reported adverse effects, phenotypic changes to look for, exposure points and modeling recommendations, assumptions used in the risk assessments, and the like.	23	An overview of EPA's risk assessment process may be included in the Points to Consider when it is revised in its entirety since EPA conducts risk assessments of many types of microorganisms, not just algae. Such an overview could discuss the use of published vs. unpublished literature. Currently, much of EPA's risk assessment is based on information in the published literature on the characteristics/traits of the recipient

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			microorganism. Then an analysis is made evaluating how the genetic modifications alter those characteristics. Phenotypic changes and resultant adverse effects, if any, vary depending on the introduced genetic material and the methods of genetic construction. In Appendix A of the "Algae Supplement", release points and potential exposures for various algae production systems are provided.
	EPA should provide an explanation of how the data/information/studies will be weighed and integrated into the risk assessment.	24	*This response is applicable to comments # 24 and 25.*
	EPA is requested to include in their GE algae specific protection goals which are essential to the problem formulation approach to risk assessment that is key to scientific and risk-based regulation. This will ensure that the scope of the guidance does not go beyond what is necessary and authorized and will not be duplicative of regulatory activities already being conducted by EPA and other agencies. In addition, protection goals are useful in providing insights into the types of risks potentially posed by the GE Algae that the EPA is evaluating.	25	The "Algae Supplement" does not include information requests beyond the requirements in the regulations at 40 C.F.R. § 725.155 (Information to be included in the MCAN), § 725.160 (Submission of health and environmental effects data) and § 725.255 (Information to be included in the TERA). Rather it is a brief overview of information that may be useful to EPA in conducting a risk assessment. For manufacture to commence without restrictions, or an environmental introduction be allowed, EPA must determine that the proposed manufacture or environmental introduction is not likely to present an unreasonable risk to human health or the environment. EPA conducts risk assessments under the paradigm of Risk = Hazard x Exposure. Thus, the "Algae

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			Supplement" lists information and data that is useful for conducting risk assessments as these elements address potential human health hazards (including those to potentially exposed and susceptible subpopulations), ecological hazards, as well as exposures to consumers, workers, the environment, and the general population as required by TSCA and the Lautenberg amendment. The "Algae Supplement" is not the appropriate venue for a discussion of EPA's risk assessments. An overview of EPA's risk assessment process may be included in the Points to Consider when it is revised in its entirety as this discussion would be relevant for any microorganism used in any TSCA application.
	Regulatory risk assessments for algae should be strain-specific and should focus on whether the introduced genetic modifications change the predicted behavior or risk characteristics of the recipient strain. If the modifications are determined not to alter these characteristics, then the likelihood is low that the use of the modified strain would pose any environmental or safety risks.	26	EPA agrees that a risk assessment for any microorganism needs to be strain specific. EPA conducts a robust literature search on the recipient microorganism and then focuses on whether the introduced genetic modification changes the behavior or characteristics of the recipient.
	ABO urges EPA to continue to allow applicants to rely on genomic or proteomic analysis of proposed recipient strains to address whether such strains produce toxins or might be pathogenic or virulent.	27	Applicants are now and will continue to be allowed to use genomic and proteomic analyses to screen for toxins and/or pathogenicity traits. The Agency agrees that these approaches are appropriate for the risk assessment process. In addition, EPA will continue to conduct robust literature searches on the recipient microorganism to ensure all

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			aspects relating to pathogenicity and potential toxin production are considered in the risk assessment.
	In the interests of transparency, EPA is requested to provide additional information in the guidance on how regulatory decision making is conducted – for example: (i) Clear explanations of the specific risks being addressed by the EPA and descriptions of how those risks are assessed, including the application of models, where used. (ii) The rationale for information listed in the guidance in terms of how it would inform the risk assessment, to supplement the broad categories of information in the current draft. (iii) An explanation of how data / information / studies will be weighed and integrated into the risk assessment.	28	Sections I and II of the "Draft Algae Guidance" that included a discussion of risk assessment are no longer part of the "Algae Supplement" which consists solely of Section III of the "Draft Algae Guidance" that covers the information and data EPA finds useful for conducting risk assessments. When revising the entire 1997 Points to Consider document, EPA plans to elaborate on its risk assessment process. As discussed above in response to comment #23, much of the Agency's risk assessment is based on information in the published literature on the characteristics/traits of the recipient microorganism. Then an analysis is made evaluating how the genetic modifications alter those characteristics. Information on the potential for survival is useful in addressing potential ecological effects.
	Conducting a risk assessment requires consideration of hazard(s) and the probability of exposure. By contrast, risk management reflects the need for risk mitigation strategies and approaches. EPA is seeking to provide guidance on hazard and exposure information as well as information on risk management practices. The National Academy of Science's 1983 "Red Book" [1] recommended that the process of assessing risks should remain separate – though informed by – the process of managing them. This recommendation is routinely applied by	29	The Points to Consider, and thus the "Algae Supplement", attempts to solicit information and data useful to EPA for conducting risk assessments. There is no requirement at 40 C.F.R. § 725.155 (Information to be included in the MCAN) or § 725.160 (Submission of health and environmental effects data) for a submitter to provide information on the use of Personal Protective Equipment (PPE). The "Algae

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	other offices within the EPA and by other regulatory jurisdictions around the world. In the guidance document, and by implication in the agency's own practices, the line between the science based risk assessment step and the policy laden risk management step is blurred. For example, risk assessments should not take into account the use of Personal Protective Equipment (PPE), as this is a risk management tool. Certain risk management practices may not actually be necessary, may add to the burden of information requirements, and may in some cases be better addressed through best practice recommendations. Therefore, if PPE is not necessary based on the risk assessment, then it should not become an obligatory part of the risk management approach even if a company has voluntarily decided to implement it. *Citations:* *1. "A Second Act for Risk Based Chemicals regulation" K Belton and J Conrad; Issues in Science and Technology, National Academies of Sciences, Engineering and Medicine, Fall 2016*		Supplement" does not impose any requirements. Rather it is an overview of information that may be useful to EPA in conducting a risk assessment. EPA assesses risk to workers considering engineering controls described in the MCAN but in the absence of PPE such as gloves and respirators. If risks are preliminarily identified, EPA then considers whether they would be mitigated by the use of PPE. Knowledge of the submitter's selection and use of PPE is considered when such information is provided by the submitter or may be requested as a result of identified risks. In addition, information on PPE is required for a TERA as listed at § 725.255(e)(2)(v).
	It is important to differentiate between risk assessment and risk management. That is, EPA should request specific data from applicants that would be needed to complete a risk assessment (e.g. as outlined in the Considerations document), but not all that information would necessarily lead to a conclusion that a given activity is potentially risky such that risk management and/or monitoring activities should be imposed. For example, data on environmental survival or persistence of a recipient or a modified algae strain could and should be submitted in an MCAN or TERA to the extent available, but it should not be necessary to require monitoring of environmental dispersal in all cases.	30	The Points to Consider, and thus the "Algae Supplement", merely describes information and data EPA has generally found useful for conducting risk assessment and does not require information and data. The risk assessment determines whether risk management measures may be necessary. "Survival and dissemination information under relevant environmental conditions" is information to be included in the MCAN as listed at 40 C.F.R. § 725.155(d)(3)(ii). Likewise, this same information is to be included for a TERA as specified at

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			§ 725.255(d). Survival information is useful for risk assessments as it affects potential ecological effects.
Risks and Benefits of GE Algae	There are tremendous potential benefits that algae-based products can bring to society - from food products and animal nutrition to renewable chemicals, vaccine delivery systems and even a food source for long-term space flights. These algae-based products have the potential to provide breakthroughs in healthier nutrition and reductions in the environmental impact of food and material production.	31	*This response is applicable to comments # 31, 32, 33, 34, and 35.* EPA recognizes that commercial production of algae for numerous products has, and may further, result in many benefits to society. EPA does not generally anticipate harm from commercial GE algae production, but the Agency is responsible for assessing the risks of GE algal strains prior to commercialization or environmental introduction. Risk assessments are done on a case-by-case basis due to the myriad of algal species and introduced traits that are possible. The Agency is familiar with the research articles and reports provided in these comments, as well as other published literature on risk assessment of GE algae. In developing the "Algae Supplement", the Agency made every effort to use the most current and sound science and incorporate relevant information elements into the "Algae Supplement".
	As products and technologies have advanced, the fundamental principle of risk-based regulation of the product, not the process, is as relevant now as before.	32	
	The many years of safe use of microbes and microalgae that these "changes in biotechnology products and production technologies" have enabled should be reflected in this guidance document and within EPA's ongoing regulatory process. As the benefits of these technologies have become apparent, EPA should not act to stymie their development by an overly precautionary approach which anticipates harm, when to date evidence from commercial production systems is indicative of the contrary.	33	
	The National Academies of Science 2012 review of algal biofuels sustainability [1] found no sustainability concerns of genetically engineered organisms. Neither algae nor the biotechnology regulated under the proposed guidance represents an inherent risk. Algae are a ubiquitous, vital component of terrestrial, aquatic and marine ecosystems, and nature's original pollution mitigation technology. Example.1 - Industrial algae production in the U.S. has a more than 40-year track record of safety. Large-scale (several hundred tons of biomass produced) open pond production facilities exist for Spirulina (Earthrise Nutritionals LLC in California) and Haematococcus (Cyanotech Corp. in Hawaii).	34	

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	Nannochloropsis has been produced heterotrophically in biofermentors at commercial scale in the U.S. for over a decade – first by DSM and now a growing list of companies including TerraVia, ADM and Alltech. There is also a number of smaller facilities producing a variety of strains of microalgae in closed photobioreactors (PBRs), greenhouse enclosed ponds, and open air ponds, both lined and unlined. Example.2 - Genetically engineered (GE) algae have been safely tested experimentally in open air conditions under TSCA Experimental Release Applications (TERAs) R-13-0003 through -0007 based on EPA's determination that these tests did not present unreasonable risks to human health or the environment. Microbial Commercial Activity Notice (MCAN) applications for GE algae strains from ABO members Algenol, Joule and TerraVia have also been approved by EPA, further reinforcing the safety of industrial algae strains. *Citations:* *1. Sustainable Development of Algal Biofuels in the United States, National Academies Press, 2012*		
	The 1989 study convened by the National Academy of Sciences [1] concluded that risks of modified organisms do not differ from those posed by similar uses of naturally occurring microorganisms. Henley et al. (2012) [2] also concluded that most GM algal traits are unlikely to confer a selective advantage in nature, and thus would rapidly diminish, resulting in low ecological risk. *Citations:* *1. Tiedje JM, Colwell RK, Grossman YL, Hodson RE, Lenski RE, Mack RN, Regal PJ: The Planned Introduction of Genetically Engineered Organisms - Ecological Considerations and Recommendations. Ecology 1989, 70(2):298-315.* *2. Henley, W.J.; R.W. Litaker; L. Novoveska; C.S. Duke; H.D. Quemada and R.T. Sayre. 2013. Initial risk assessment of genetically modified microalgae for commodity scale biofuel cultivation. Algal research 2:66-77.*	35	

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Format and Improvements	The differences between eukaryotic algae (microalgae) and prokaryotic algae (cyanobacteria) make their inclusion in the same guidance document more challenging to manage. Separate sections should be developed in this guidance for cyanobacteria and microalgae to describe the considerations distinct to each group. Grouping these classes together has the potential to cause confusion on many aspects of reporting. The types of manufacturing operations that could employ the use of either group may be similar in their design. Nevertheless, the differences in life cycle, strategies for genetic manipulation, and the associated risk assessments will likely lead to different information needs on a case-by-case basis.	36	*This response is applicable to comments # 36, 37, and 38.* As is the case with the Points to Consider document, which was written to cover any microorganism (e.g., bacteria, fungi, viruses, protists) that could be used in any applications subject to TSCA oversight, the "Algae Supplement" is written broadly enough to encompass information and data that is useful for either cyanobacteria or eukaryotic microalgae. Submitters should consider the various information and data elements and determine which ones are relevant to their algal strain. Cyanobacteria and eukaryotic microalgae are grouped together predominately because of the similarity in manufacturing designs (e.g., outdoor growth in PBRs or open ponds). Separating out the two in different documents would result in unnecessary repetition. The section on risk assessment that was in the Draft Algae Guidance is no longer part of the "Algae Supplement" to the Points to Consider. A discussion of EPA's risk assessment process may be included when the Points to Consider is revised in its entirety.
	Separate sections should be developed in this guidance for cyanobacteria and microalgae to describe the considerations distinct to each group.	37	
	Overall, the discussion on risk assessment should more specifically describe considerations specific to microalgae and cyanobacteria.	38	
	It is difficult to reconcile the organization of the draft guidance with the corresponding information requirements in the regulations. This makes it difficult to understand when or where to apply the guidance being provided. Therefore, the	39	*This response is applicable to comments # 39, 40, 41, and 42.*

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	guidance document should more closely track the stages of commercial development and information requirement sections in the TSCA biotechnology rules. The original Points to Consider closely follow a progression of commercialization (bona fide submissions, research and development (R&D), and premanufacture notification) and the specific information requirements in Part 725. To maintain consistency, the same format should be used here. To be a standalone document that can be used independently from the original Points to Consider, the guidance needs more upfront discussion of the administrative matters for these types of submissions, including explicit statements regarding how prenotice consultation can help a potential submitter address issues unique to these submissions. With respect to guidance on the information requirements, the document should reference specific regulatory sections for making bona fide, R&D, and MCANs submissions.		Since the "Algae Supplement" is now an appendix within the Points to Consider, Sections I and II of the "Draft Algae Guidance" have been deleted and the only remaining section from that document, analogous to the Points to Consider (Section IV.), is the "Information Needs for an Algal Risk Assessment". As a result, it follows the same format as Section IV. of the Points to Consider document. In addition, all of the specific information requirements on the microorganism for an MCAN found at 40 C.F.R. § 725.155 and § 725.160 are contained within the "Algae Supplement". Likewise, all of the specific information requirements on the microorganism for a TERA at § 725.255 are contained within the "Algae Supplement". The "Algae Supplement", like the Points to Consider, is formatted to assist in obtaining information that structurally fits into the format of our risk assessments even though it may not follow the same order of information requirements in the regulatory text.
	The challenge before the agency is to provide useful insights specific to these submissions while incorporating generally applicable guidance from the original Points to Consider document for this guidance document to be used independently. In Section I, subsections B and D should be consolidated and subsection C should be deleted because this information is not directly relevant, it is already known, and inclusion will render the document out-of-date quickly.	40	
	With respect to TERAs. references to specific information requirements in 40 C.F. R. § 725.255 are currently missing from the document. Please ensure that the organization structure of this document references and tracks the information criteria required for these submissions in EPA's regulations at 40 C.F.R. Part 725.	41	
	With respect to MCANs, references to specific information requirements in 40 C.F.R. § 725.155 et seq. are currently missing from the document. Please ensure that the organization structure of this document references and tracks the information required for these submissions in EPA' s	42	

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	regulations at 40 C.F.R. Part 725. Persons who intend to manufacture or import a new microorganism (i.e., one not listed on the TSCA Inventory) for a non-exempt commercial purpose must submit an MCAN to EPA at least 90 days prior to such manufacture or import. EPA may extend the notification period to 180 days. Submitters typically provide the required information in narrative form, according to the order it is described at 40 C.F.R. § 725.155-160. This includes: Submitter identification; Strain identity information, including a description of the recipient and new strain, genetic construction, and phenotypic and ecological characteristics. The required identity information includes a taxonomic designation, and information on phenotype and genotype is required to the extent that it enables the new strain to be accurately and unambiguously identified; A description of the manufacturing process and downstream processing; Byproducts of manufacture, processing, use, and disposal of the new strain; Total production volume; Use information; Description of worker exposure and environmental release; and All health and environmental effects data in the possession or control of the submitter.		
	The specific regulations governing MCAN submissions should be cross-referenced in the document, where appropriate, to aid in understanding the needs of the underlying information requirements. In particular, there is a statement in the draft guidance document that "Under TSCA, there is no specified list of information and/or data elements for a microorganism" (Section II, ¶ 4, p. 5) which should be taken out or modified to reference these regulatory sections. In addition, the manner in which the information is presented in the guidance is currently confusing for submitters because information that must be provided by regulation is not distinguished from data which can facilitate agency reviews when submitted voluntarily.	43	Since the “Algae Supplement” is now an appendix in the Points to Consider, the administrative portion of the Points to Consider still pertains to this “Algae Supplement” and thus does not require cross-referencing. The introduction of the former “Draft Algae Guidance” document containing this statement has been eliminated from the final “Algae Supplement”.

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	The information in the appendix describing current algal manufacturing processes provides important context, and it would be more useful if placed in Section III H. "MANUFACTURING PROCESS DESCRIPTIONS & PRODUCTION VOLUMES". More generally, certain processes involve secretion of the chemical substance that is being produced while other strains will store up the desired commercial product such that processing involves lysing of the cells and purification steps. This distinction in production and the relevance if any to EPA risk assessments is not brought out in the current draft. In addition, the identification of potential points of release into the environment for each method of production is useful. Placeholders for evaluating the fate of scrubber water, return water, and biomass should always be included as these two release points typically are evaluated in agency risk assessments. Transport of seed stock is common to all systems and should not be included in one description and left out of another.	44	The original Appendix to the "Draft Algae Guidance" on the current manufacturing processes used for algae is now Appendix A of the "Algae Supplement". Inclusion of this lengthy attachment within the information and data elements would confuse the basic information EPA recommends that submitters provide. However, in the "Algae Supplement", Appendix A is now referenced in Section II.H. "Manufacturing Process Description and Production Volume" to ensure the reader is aware of this useful information while considering the information and data elements under this section.
	EPA uses different adjectives throughout the document to describe the required risk assessment. such as 'integrated' (p. 4) "scientifically credible' (p. 5), 'robust (p. 5). "regulatory" (p. 6). None of these are particularly helpful in terms of guiding Submitters regarding particular information needs. We suggest these discussions on risk assessment be consolidated.	45	Sections I and II of the original "Draft Algae Guidance" have been eliminated from what is now the "Algae Supplement", and thus this comment is no longer applicable. The Agency plans to include a discussion of risk assessment when the Points to Consider is revised in its entirety.
	Sections of the document seem repetitive, in particular the purposes of the different lists of information provided are not clear and they seem to contain the same information. The listings provided should where necessary state that they are illustrative, rather than exhaustive, and may not apply in every case (e.g., gram reaction is not observed in all microalgae on p. 7, fate is not included in the list on page 9, CO2 source is not listed on page 14). Many of the items listed in Section III, "General Description and Characterization") are not specific	46	The "Algae Supplement" was designed to cover both cyanobacteria and eukaryotic microalgae, and thus, was broadly written to contain data elements that may be relevant for either. All data elements may not apply to all algal strains. The submitter will need to consider the information elements presented and determine which ones are relevant to their specific strain and

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	to microalgae or cyanobacteria, and could be consolidated and prioritized.		production platform. Acknowledgement that not all data elements are relevant to all algal strains has been added in Section I.D. of the "Algae Supplement". The Gram reaction needs to remain just as it is in the Points to Consider. The list on p. 9 of the original "Draft Algae Guidance" (Section III A 2) is the General Description and Characterization to cover both cyanobacteria and eukaryotic microalgae. The fate is now addressed in Section F of the "Algae Supplement". The source of the CO_2 is requested in Section H, "Manufacturing Process Descriptions and Production Volumes".
	EPA should include guidance on how to construct structurally representative generic names. The guidance on confidential business information (CBI) in the original Points to Consider document stands updating in this and other respects, based on recent changes to the law. EPA should address these changes in this draft guidance document, and it should reference CBI considerations specific to these submissions, such as when monitoring information will be considered health and safety data and how patent protection may affect a CBI claim.	47	Since the "Algae Supplement" is an appendix in the Points to Consider document, inclusion of a separate CBI section in the "Algae Supplement" based upon the Lautenberg amendment to TSCA would be contradictory to the language already in the Points to Consider. EPA plans to update the entire Points to Consider document to address microorganisms not previously considered and for novel genetic engineering and genome editing techniques. In addition, the Agency will be updating the CBI substantiation language and other provisions of the Frank R. Lautenberg Chemical Safety for the 21st Century Act in the upcoming revision.
	To be exempt from notification R&D activities must be conducted inside a structure and the containment requirements	48	As previously stated, the final document EPA has developed on recommended

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	found at 40 C.F.R. § 725.234 need to be followed. Particular guidance associated with these specific research activities should be provided. Areas for guidance include the use of closed photobioreactor systems, greenhouses, methods and documentation of inactivation, and waste disposal. Given that there are several academic institutions whose research figures prominently in this field that may receive federal funding, it may be appropriate to include a description of this exemption as well.		information and data for algae submissions is now the "Algae Supplement" to the Points to Consider document. Discussions of exemptions from reporting requirements is outside the scope of this guidance for submitters of MCANs or TERAs. The Points to Consider document discusses the exemptions from TSCA Section 5 reporting requirements for Research and Development. In some cases, PBRs may meet the TSCA definition of "structure" and thus be exempt at the R&D stage (40 C.F.R. § 725.234 - Activities conducted inside a structure). There is also an exemption at the R&D stage for activities subject to the jurisdiction of another Federal Agency (40 C.F.R. § 725.232). The Agency encourages pre-notice consultations from companies to address uncertainties regarding their reporting requirements.
	More guidance on the evaluation for potential releases to the environment in TERA submissions would be useful. The information provided in section G on page 13 is simply a list rather than guidance. Additionally, a discussion on the potential usefulness of environmental monitoring via the use of microbiome or metagenomics analysis would be helpful. Recent advances in sequencing technologies have greatly increased the amount of useful data regarding both members of environmental microbial communities as well as their relative abundances, while at the same time reducing the time and cost associated with such analyses. This data could greatly aid EPA in its risk assessment process, especially at the smaller scale of a TERA submission, regarding the	49	Appendix A in the former "Draft Algae Guidance", is now Appendix A of the current "Algae Supplement". It provides diagrams with potential points of releases to the environment for closed system fermenters, PBRs, and open pond production systems. The Agency is receptive to receiving microbiome and metagenomics analyses to aid in assessing the survival and competitiveness of a GE algal strain used in the environment in a TERA application.

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	relative risk of either intentional or unintentional release of GE organisms into the environment.		
	With regard to genetic constructs, among the useful addition to the guidance are illustrations of genetic constructs that can be provided to facilitate EPA's reviews. Other areas of guidance that may be beneficial to include are those which need to be taken into account under the new safety standard. For example, with respect to a microalgae or cyanobacteria being found "free of toxin encoding sequences" means that the introduced genetic material must not contain a functional portion of any of EPA's listed toxin encoding sequences at 40 C.F.R. § 725 .421 (d)(2). A "functional portion" means any sequence which codes for a polypeptide that directly or indirectly contributes to toxic effects in humans, binds a toxin or toxin precursor to target human cells, or facilitates intracellular transport of a toxin in target human cells. A direct toxin encoding sequence would be one whose encoded polypeptide is directly toxic to target cells. An indirect sequence to avoid is one whose encoded polypeptide is not directly toxic to target cells, yet still adversely affects humans, e.g., the portion of botulism toxin that blocks release of acetylcholine. There are a number of databases that can be consulted. An illustrative list is provided in appendix 2 to these comments. Also, a discussion of what EPA looks for when reviewing the use of antibiotic resistance markers (ABRs) and guidance on their use and removal would be a useful addition to this guidance document.	50	Since the "Algae Supplement" is now an appendix in the Points to Consider document, the Agency will not be providing new genetic construct illustrations in the "Algae Supplement" since they exist in the Points to Consider. EPA will be revising its Attachment 4 of the Points to Consider that has illustrations of genetic constructs when it is revising the entire Points to Consider document. The "free of toxin encoding sequences" in this comment is being taken out of context. This "free of toxin encoding sequences" phrase is the fourth criteria for the introduced genetic material specifically in the 5(h)(4) Tiered Exemption at 40 C.F.R. § 725.421(d). No cyanobacteria or microalgae are eligible recipient microorganisms in the Tiered Exemptions, so these criteria do not apply. However, in its risk assessment the Agency does evaluate the potential for cyanotoxin and phycotoxin production by the GE alga. EPA thanks the commenter for the appendix listing the databases. With regards to the use of antibiotic resistance marker genes, the Agency will have a discussion on this topic when it revises the Points to Consider in its entirety to discourage the presence of

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			genes in production microorganisms that encode resistance to clinically important antibiotics.
	With respect to environmental fate and transport an explanation on what EPA would like submitters to address under point 5 on page 11 is respectfully requested, but may be more appropriate to incorporate into the separate section on fate and transport considerations beginning on page 12 of the draft document. The fate section should reference the need to explain how these strains are disadvantaged in the environment as well as advantaged, as the former is more likely to be the case. In addition to the identification of the factors in this section, which are useful, EPA should add guidance on how these factors can influence the risk assessment. EPA should add an expanded discussion on the usefulness of inactivation data in these submissions. An example protocol is provided in Appendix 1 to these comments. While there are other ways to manage risk without an inactivation step, inactivation is frequently used and data demonstrating its effectiveness when voluntarily provided often facilitates agency reviews.	51	Point 5 in Section E. "Potential Ecological Effects" is not related to fate and transport, but rather to environmental effects. This point aims to assist in the evaluation of the potential effects of algae on biogeochemical cycles – specifically, the carbon cycle. In contrast, the fate section requests information on how the intergeneric microorganism survives relative to the unmodified recipient strain. This section is where submitters can provide information on whether the genetic modifications have resulted in a strain with more robust growth or a debilitated strain. Knowledge of the inability of an algal strain to survive in the environment would lessen concerns for potential exposures, and thus, potential effects. Language has been added to Section II.F. of the "Algae Supplement". Inactivation data help the Agency estimate releases of the live microorganism into the environment in the liquid and solid waste streams. The Agency appreciates the protocol provided in the appendix with the comment. When revising the entire Points to Consider document, EPA plans to elaborate on inactivation methods and cell kill curves.
	The EPA should provide examples or guidance on the type of studies, test data and methodologies it considers sufficient to	52	Part 725 does not have prescribed data requirements or test methods that must

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	develop the information it needs. Test methods should be widely available, reliable and replicable. Scientific methodology requires observational and experimental data that are authentic and of known measurement error; experimental variables that are relevant to the hypotheses being tested; the control of externalities that may confound observations and experimental results; and reproducibility by other performers or counterfactual verification.		be used, although for testing traditional chemicals and microbial pest control agents, OCSPP has many Harmonized Test Guidelines. The 880 series for "Biochemicals Test Guidelines", and the 885 series for "Microbial Pesticide Test Guidelines" may be useful for microorganisms and/or their products. However, EPA does not dictate the methodology a company may use to develop test data. Pre-notice consultations with the Agency are encouraged.
Charge Questions - Survival and Competition	The characteristics that are being engineered and synthesized for industrial applications, are precisely those that could very likely lead to invasiveness and HABs. While industry and researchers frequently proclaim that released engineered microalgae will not likely survive in the wild, that claim has not been supported by data or analysis. Indeed, it is rapid growth, resistance to stress, ability to withstand industrial conditions, secretion of compounds that are essentially toxic to predators - all these are characteristics that commercial producers want to engineer, and all are characteristics that would render them potentially invasive and disruptive.	53	*This response is applicable to comments # 53, 54, and 55.* The "Algae Supplement" provides an extensive list of pertinent information and data elements for GE algae. Concerns such as potential invasiveness and the ability to form harmful algal blooms (HABs) are addressed in risk assessments conducted by the Agency.
	Climate change now presents us with unprecedented context. Predictions about the future of ecosystems are increasingly uncertain, but we can predict ongoing warming, and expanding demands for products of agriculture and forestry (hence more fertilizer use). The conditions favoring algae blooms and shifting algae productivity are already increasing, and it is safe to assume there will be consequences we cannot now predict. The scale of those consequences is illustrated by the following example: recently the EU Geosciences Union reported that smaller plankton species (nano) thrive in elevated CO2 and outcompete larger species. These larger	54	

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	species are responsible for the "ocean pump" that carries C to depths (hence overall reduction in ocean CO2 uptake) and larger species emit dimethyl sulphide which is key to cloud formation and therefore cooling[1]. *Citations:* *(1) European Geosciences Union, 2013. Tiny Plankton Could Have Big Impact on Climate. (http://www.egu.eu/news/76/tiny-plankton-could-have-big-impact-on-climate/)*		
	The vast realm of new discoveries and shifting dynamics indicate our understanding of microalgae and ecosystems is embryonic, at best and not sufficient to effectively assess environmental impacts of releases. Estimates are that we have so far only identified about half of existing species of cyanobacteria [1], yet we are now essentially creating organisms that are new to nature - not designed by evolution and natural selection but rather designed by biotechnologists seeking commercially profitable chemicals and compounds. *Citations:* *(1) Nabout et al 2013. Biodiversity and Conservation. How Many Species of Cyanobacteria Are There? Using a Discovery Curve to Predict Species Number*	55	
	Snow A. and Smith V. pose a number of "worst case scenarios" and open- ended questions regarding the potential for GE algae to escape and disperse into the environment [1]. These are hypothetical and suppositional in nature and assume negative impacts in all cases. However, the authors also add that evidence suggests that the persistence of free living algae is not expected to have unwanted consequences, and they cite literature that speaks to the likelihood of GE algae being unable to compete against natural forms in the environment precisely because of the specific nature of their genetic modification. Henley et al concluded that in general, most target GM algal traits are unlikely to confer a selective	56	*This response is applicable to comments # 56 and 57.* EPA is familiar with these articles on the risk assessment of GE algae referred to in the comment. EPA agrees that strain selection and survival are key factors that should be considered in its risk assessments of GE algae. The Agency does evaluate the potential for a strain to survive in the environment in which it is used and in environments into which it

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	advantage in nature, and thus would rapidly diminish, resulting in low ecological risk [2]. They further stated that genetic and mechanical containment, plus conditional matching of GM algal traits to unnatural cultivation conditions, further reduces risk. Bearing in mind that GE algae are created using proprietary research and development, there is a strong commercial incentive to prevent their escape into the environment where they may end up being taken by a competitor. GE microalgae may be specifically engineered to prevent their survival in the environment for precisely this reason. *Citations:* *1. Snow, A. and Smith, V. Genetically engineered algae for biofuels: A key role for ecologists. Bioscience (2012) 62 (8): 765-768.* *2. Henley, W.J.; R.W. Litaker; L. Novoveska; C.S. Duke; H.D. Quemada and R.T. Sayre. 2012 Initial risk assessment of genetically modified microalgae for commodity scale biofuel cultivation. Algal research 2:66-77*		may be disseminated. The inability to survive outside the pond environment may require substantiation depending on the algal strain and the genetic modifications, however the type and amount of information will vary case-by-case.
	The key risk assessment questions for algae are strain selection and survival in the receiving environment. If a proposed algae strain is native to the surrounding environment, ecological effects are not anticipated. For strains not native to the surrounding environment, the question becomes one of survival. If a strain is unsuitable for survival in the receiving environment, other risks, such as the potential to produce toxins and the potential for harmful algal blooms or other ecological effects, are mitigated. By design, the conditions in industrial algae production systems are substantially different from those of the surrounding environment. Temperature, salinity, and other conditions are optimized to maximize productivity and minimize risk of contamination by pests. Strains are also typically optimized to thrive in the system environment,	57	

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	making them unsuitable for survival in the surrounding environment.		
Charge Questions - Food Web Dynamics	A key paper on this issue, by Kevin Flynn et al, is titled "Monster Potential Meets Potential Monster" [1]. The authors engaged in a rigorous modeling exercise to assess those claims and show quantitatively that it is indeed possible to engineer microalgae to dramatically increase productivity for commercial purposes (monster potential), however: "simulations indicate that the ideal GM microalgae for commercial deployment, could, on escape to the environment, become a harmful algal bloom species par excellence… this is because an organism able to produce carbohydrate and lipid at high doses will obtain a stoichiometric composition that will be far from optimal as food to support zooplankton". In other words, their growth will not be held in check because they will not "taste good" to predators. "A strong argument can be made for the regulation of GE microalgae at an international level because of the potential for damage to have global consequences, echoing recent concerns over geoengineering." As an example, the EU Geosciences Union (2013) [2] reported that smaller plankton species thrive in elevated CO2 and outcompete larger species. These larger species play a role in ocean CO2 uptake as well as cloud formation and, therefore, cooling. *Citation:* *1. Flynn KJ, Mitra A, Greenwell HC, Sui J: Monster potential meets potential monster: pros and cons of deploying genetically modified microalgae for biofuels production. Interface Focus 2013, 3(1) (http://rsfs.royalsocietypublishing.org/content/3/1/20120037)* *2. European Geosciences Union, 2013. Tiny Plankton Could Have Big Impact on Climate. (http://egu.eu/news/76/tiny-plankton-could-have-big-impact-on-climate*)	58	The Agency appreciates the submission of these resources. The potential effects of GE algae on food web dynamics is considered by the Agency and is addressed in Section II.E.7. of the "Algae Supplement", Potential Effects on Microbial Food Web/Trophic Level Changes.

Topic	Comments	#	Agency Response
Charge Questions - Horizontal Gene Transfer	No comments received.		
Charge Questions - Releases and Worker Exposure	EPA should consider providing a table for how to assess worker exposure. Prior EPA guidance on ethanol production provided such a table that illustrates the number of workers, types of work tasks, and estimates of daily and annual exposure, all of which should be addressed in a submission to the agency.	59	*This response is applicable to comments # 59 and 60.* EPA is not able to estimate the number of workers, the types of work tasks, or the duration of exposure for all algae production systems as they may widely differ among submitters. However, as requested, a table that specifies the type and duration of exposure that would be helpful if filled out by companies for their specific manufacturing platform has been added in the occupational exposure section of the "Algae Supplement" (see Table 1 in Section II. I. 1.).
	EPA should consider providing a table for how to assess worker exposure.	60	
	We must honestly acknowledge that it is simply not possible to contain engineered or synthetic microalgae, especially given their very small size. They will escape from facilities. Workers will be exposed. This is very clearly the case for open pond cultivation – but it is also the case for photobioreactors and other processes that have generally been considered "contained". Industrial production systems are operating on a very large scale, and workers are usually not trained in biosafety. Engineered organisms are in some cases transported from point of production to point of industrial application. Accidents happen. The high likelihood of environmental release is precisely why we are addressing the potential impacts on ecosystems. Once released microalgae are capable of being transported over long distances via water	61	EPA does evaluate both worker exposure and environmental releases for all types of production systems used for GE algae. When appropriate, workers wear personal protective equipment (PPE) to minimize exposures. EPA acknowledges that some algae grown outdoors may be transported for long distances. Therefore, EPA evaluates the potential for survival of the algal strain and the potential ecological effects that may result if a GE alga was to disseminate and establish in the environment.

Topic	Comments	#	Agency Response
	and air. Their introduction into nature has the potential for serious negative consequences that would be potentially impossible to reverse.		
	Section IV.F of EPA's Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms identifies the information about a containment system that should be provided to assess the potential for release of modified microorganisms from the containment system. The existing guidance in Section F has proven easily adaptable for contained photobioreactors. Companies such as Joule and Algenol have been able to utilize this guidance to successfully submit MCANs describing cyanobacteria photobioreactors to EPA's satisfaction.	62	EPA thanks the commenter for stating that Section F of the Points to Consider, is useful and easily adapted to contained bioreactors. The information requests in Section IV.F. of the Points to Consider were retained in Section I - Exposures to the GE Alga, in the "Algae Supplement". The Points to Consider, and thus the "Algae Supplement" were written for broad coverage of various manufacturing platforms and the information received from submitters allowed EPA to evaluate various containment systems and estimate environmental releases in the risk assessments.

Comment Affiliations

Comment ID	Source	Comment Numbers
EPA-HQ-OPPT-2015-0508-0025	Biofuelwatch	53-55, 58, 61
EPA-HQ-OPPT-2015-0508-0026	Physicians Committee for Responsible Medicine (PCRM)	13, 15
EPA-HQ-OPPT-2015-0508-0027	Keller and Heckman LLP	8, 17, 18, 22, 23, 36-51, 59, 60
EPA-HQ-OPPT-2015-0508-0028	Biotechnology Innovation Organization (BIO)	1-5, 7, 9, 10, 12, 14, 19, 21, 24, 25, 28, 29, 32, 33, 35, 52, 56
EPA-HQ-OPPT-2015-0508-0029	TerraVia Holdings, Inc.	6, 31
EPA-HQ-OPPT-2015-0508-0030	International Center for Technology Assessment (ICTA)	16, 20
EPA-HQ-OPPT-2015-0508-0031	Algae Biomass Organization (ABO)	11, 26, 27, 30, 34, 57, 62

III. ATTACHMENT 1 – CHARGE QUESTIONS

I. Questions on Considerations of Ecological Effects of Genetically Engineered Algae

Genetically engineered (GE) microorganisms have the potential to affect the ecological dynamics of the natural environment. It has been suggested that there are several areas of ecological impacts that merit evaluation: competition, harmful algal blooms (HABs), food web dynamics, horizontal gene transfer, and releases (Henley et al., 2013[10]). The following charge questions are designed to obtain public input about which components of these areas warrant specific consideration by EPA.

1. Survival and Competition

a. What types of survival and competition studies should EPA consider when GE algae are commercially grown in photobioreactors or open ponds?
b. Please comment on whether microcosm studies using potential receiving waters are necessary for evaluating potential competitiveness and other ecological effects, or whether standardized aquatic microcosms are adequate.
c. For open ponds systems, please identify and provide rationale for other ecological endpoints that should be considered when evaluating the potential effects of GE algae.
d. When evaluating ecological hazards, apart from the ability of GE algae to outcompete indigenous species, please identify and provide rationale for other factors, if any, that may promote HABs in GE algae production.

2. Food Web Dynamics

a. Please identify and provide the rationale for any useful data that EPA should consider when evaluating the potential effects of open pond GE algae on food web dynamics.
b. Please identify and provide the rationale for any useful endpoints (e.g., biodiversity, growth, reproduction) that EPA should consider when evaluating trophic level effects due to the presence of GE algae.

3. Horizontal Gene Transfer

a. It is hypothesized that horizontal gene transfer (HGT) could occur via exchange of genetic material through various modes of transfer. Please comment on whether our current Points to Consider or Draft Algae guidance sufficiently address HGT issues in cyanobacteria and eukaryotic algae.
b. Please identify and provide the rationale for any other information that EPA should consider when evaluating the risks of HGT in GE algae.

4. Releases and Worker Exposures

a. Based on current technological advances in process monitoring, please identify and provide the rationale for release and worker exposure estimates that EPA should use in the exposure assessment for algal systems (e.g., photobioreactors, open ponds).

[10] Henley, W.J., R.W. Litaker, L. Novoveska, C.S. Duke, H.D. Quemada, and R.T. Sayre. 2013. Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res. 2:66-77.

b. Please comment on which specific process parameters or unit operations should be routinely evaluated to detect these potential releases and exposures.