United States Biostimulant Industry Recommended Guidelines to Support Efficacy, Composition, and Safety of Plant Biostimulant Products

Developed by the Biostimulant Industry Workgroup, a collaboration of the Biological Products Industry Alliance (BPIA) and The Fertilizer Institute (TFI).

Acknowledgement

The development of this document began in January 2020 with the release of a report from the United States Department of Agriculture (USDA), in consultation with the United Stated Environmental Protection Agency (EPA), to the United States Congress and the President of the United States on Plant Biostimulants. The Biostimulant Industry Workgroup (BIW) established teams of volunteers to draft each section. Over the course of two years, hundreds of hours were volunteered by industry representatives of the BPIA and TFI with significant input received from reviewers representing the Association of American Plant Food Control Officials (AAPFCO,) Academia, commodity organizations, regulatory professionals, and other industry associations. The BIW wishes to extend its heartfelt thanks to all who contributed their time, experience, and knowledge to this effort, which we believe will serve to further the credibility and use of these valuable products.

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Introduction

There is a growing interest by farmers, growers, and consumers to produce healthy food utilizing sustainable agriculture principles and by urban architects and residents to utilize green technology. One of the valuable tools for sustainable crop production as well as for professional landscape and residential uses are plant biostimulants. These products are not fertilizers or pesticides. They are a unique category of products that improve natural plant nutritional processes, which can result in improved plant health, tolerance to environmental stresses, and enhanced overall plant growth, quality, and yield. Biostimulant products can increase the uptake and utilization of native and applied nutrients, thus reducing the potential for off-farm nutrient runoff into rivers, lakes, and streams or loss to the atmosphere as greenhouse gasses. Plant biostimulants can also contribute to yield and quality without increasing applied fertilizer, water, or planted acres, thus, sustainably enhancing the efficient use of these inputs and natural resources.

Plant biostimulants represent a broad category of products from microbial inoculants or their metabolites through the plant and algal extracts, complex carbon-based natural deposits, and their extracts like humic and fulvic acids, protein hydrolysates, to purified single molecules derived from natural or synthetic sources. They can be used for conventional and, potentially, organic crop production, as well as non-agricultural, turf, and ornamental applications. Innovation and new biostimulant product development are accelerating by public and private institutions and companies of all sizes throughout the world. While estimates of the industry's global growth vary, plant biostimulants are expected to become a ~3-billion-dollar market by 2025 (DunhamTrimmer 2021).

Today, plant biostimulant products and technologies face regulatory challenges that can limit their use, thus reducing the benefits these products offer. The global regulations of plant biostimulants vary greatly. In Canada, India, and member states of the European Union, the term "biostimulant" has been defined and regulatory requirements to verify product efficacy, safety and composition have been established or are in development.

The United States Department of Agriculture (USDA) proposed definition for the term is: "A plant biostimulant is a substance(s), microorganism(s), or mixtures thereof, that, when applied to seeds, plants, the rhizosphere, soil or other growth media, act to support a plant's natural nutrition processes independently of the biostimulant's nutrient content. The plant biostimulant thereby improves nutrient availability, uptake, or use efficiency, tolerance to abiotic stress, and consequent growth, development, crop quality or yield." (USDA 2019) However, the regulatory path for biostimulants has not been

established, thus preventing developers to register products according to their intended use, benefits, and safety.

Technology developers, depending on the product's composition and intended use, must either register their product as a fertilizer, soil amendment, beneficial substance, or inoculum with the Department of Agriculture in every state in which they intend to sell the product or as a plant regulator (i.e., pesticide) with the United States Environmental Protection Agency (EPA). Registering a product under state fertilizer regulations requires minimal to no data or information to support a product's efficacy, composition, or safety. However, if federally registered as a plant regulator with EPA, data attesting to product composition and human and environmental safety are required to be submitted under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) 7 U.S.C. §§ 136 et seq. While the EPA typically does not require applicants to submit efficacy data, the Agency reserves the right to require, on a case-by-case basis, submission of such data for any pesticide product registered or proposed for registration.

In the absence of unified state or federal requirements for biostimulants, the biostimulant Industry is recommending the following guidelines as a general, science-based framework that can be voluntarily employed by technology developers and marketers, and utilized by regulatory agencies to demonstrate the efficacy, composition, and safety of plant biostimulant products sold in the United States. The industry believes the use of these guidelines will strengthen the credibility of individual plant biostimulants and, by extension, the product category with regulators, consumers, and growers.

This document and guidelines were developed by the Biostimulant Industry Workgroup (BIW), a collaboration of The Biological Products Industry Alliance (BPIA) Biostimulant Innovation Committee and The Fertilizer Institute (TFI) Biostimulant Council. They are the result of hundreds of volunteer work hours contributed by dozens of biostimulant industry subject matter experts with feedback from academic, regulatory, commodity organizations, and other stakeholders.

The guidelines are intended to be updated periodically to encompass advances documented in the scientific literature, the development of International Standards Organization (ISO) or other standard methods, and relevant regulatory guidance from EPA, USDA, the American Association of Plant Food Control Officials (AAPFCO) and international organizations such as Organization for Economic Cooperation and Development (OECD), and the Food and Agriculture Organization (FAO) of the United Nations.

This document is organized into four sections: I. Recommended Guidelines for Verification of Efficacy Claims for Plant Biostimulants, II. Recommended Guidelines for Verification of Composition Claims for Plant Biostimulants, III. Recommended Guidelines for Assessing the Safety of Plant Biostimulants and IV. Considerations in Using the Guidelines.

I. Recommended Guidelines for Verification of Plant Biostimulant Efficacy Claims

This Section provides guidelines for verification of plant biostimulant efficacy claims. The claims should be consistent with the benefits described in the definition of a plant biostimulant. They can be substantiated with experimental evidence through research conducted under commercial or alternative growing systems by measuring specific physiological outcomes, or differences in plant growth, quality, or yield compared to an appropriate experimental control.

Note: It is recommended that companies Refer to "EPA Draft Guidance for Plant Regulator Products and Claims, Including Plant Biostimulants" to identify appropriate efficacy claims for the product and use the same language on the product label, presentation abstracts, and/or marketing material.

Verification of plant biostimulant efficacy claims using one of three methods

A. Association with relevant published literature.
    1. Identify product claims *(use the exact wording that appears on the product label)*.
    2. Provide data supporting stated product claims on the label and other product literature.
        a. Identify product ingredients and/or properties.
        b. Provide recommended rates of application.
        c. Provide peer-reviewed scientific literature supporting the efficacy of the specific product composition at recommended application rates.
B. Research conducted using scientifically recognized methods as described below.
C. Utilize a combination of research test results and relevant published literature.

Note: It is not recommended to rely solely on consumer testimonials of the product, presentation abstracts, or marketing material.

Guiding principles for data generation to verify efficacy claims

A. Experimental design: The experimental methodology/protocol should include an appropriate experimental layout, experimental unit, replications, randomization, control treatments for reference, and comparison and response measures that directly support the product claim. The appropriate number of replicates should depend upon the amount of experimental variation, the number of treatments, and the size of the treatment difference to be detected.

B. Select control treatments to substantiate efficacy claims:

    1. Must include an untreated (negative) control comprised of treatment without the biostimulant material for which the claim is being tested.
    2. May include positive controls, such as a comparative standard product, which exhibits known effects like those being claimed.
    3. If applicable, control of plant food composition (e.g., N, P, K, secondary, or micronutrients) for products combining biostimulant with plant food to demonstrate the efficacy of the biostimulant component alone.

4. When possible, for experimental designs where a challenge condition is used, control data should be generated in the absence of the challenge condition (e.g., drought, heat, reduction of input).

C. Location of research trials:

1. Locations for research tests should be selected depending upon the 1) proposed claim, 2) target growing systems, and 3) sensitivity of the product and claim to soil and environmental conditions.
2. Research tests to support claims for yield or quality improvements should be conducted using the commercial or target growing system:
    a. Field research small plots
    b. Farmer's or grower's fields – strips, sections
    c. Greenhouse production settings
    d. Urban landscape
    e. Others – as determined by product and use
3. Other claims may be supported by tests conducted in alternate growing systems:
    a. Research greenhouses
    b. Laboratory growth chambers
    c. Controlled conditions field trials (shaded plots vs. unshaded; different watering systems, etc.)
    d. Others - as determined by product and use
4. Data generated outside the US may be acceptable provided they meet the following criteria:
    a. Support efficacy claims allowed in the US.
    b. Are applicable to the target growing practices in US.
    c. Comply with the guidelines described in this document.
    d. For agricultural land production, claims of yield and quality, a minority share (<50%) of data generated outside of the US is acceptable provided the way the data were generated complies with the methods described in this document and agronomic practices used match the desired use pattern in the US.
    e. For yield and quality efficacy claims in commercial greenhouse production, data produced outside the US is acceptable provided greenhouse practices are like those used in US commercial settings.

D. Selection of test crops:

1. Use crop(s) and/or crop groupings supporting your crop(s) application rates, and efficacy claims. In each case provide rational for the proposed grouping and provide data for minimum two representative crops for the group.
    a. taxonomic rank (e.g., Fabaceae/legumes, Poaceae/cereal, etc.)
    b. organ of interest (e.g., leafy vegetables, bulb, tuber, fruit, flower, etc.)
    c. life cycle (e.g., annual, perennial, etc.)
    d. resistance to stress (e.g., crops sensitive to low temperature, to drought, etc.)
    e. product use (e.g., ornamental flowers, lawns, etc.)
    f. other – define

2. Number of research trials/tests (locations, seasons). Identify and substantiate the number of trials/tests, locations and seasons depending on the product claims, target growing systems, expected product performance in different soil and environmental conditions.

E. Statistical analysis:

1. Research tests and data must include statistical analysis appropriate to the experimental design and test objectives. Consultation with a statistician on design and analysis is recommended.
2. The main objective of the data analysis is to estimate the magnitude of the difference between the various treatments and to provide a measure of variability of those estimates.
3. The decisions of acceptability or rejection of the treatment should not be based on p-value alone. P-values should be taken as a continuous measure of evidence against the null hypothesis and p-values greater than 0.05 may be acceptable depending on study objectives.
4. Approval or rejection of a product claim should be based on at least two criteria.
    a. Estimation of economic or biological benefit of a treatment/claim to the crop/grower using the best available descriptive statistic(s).
        i. A full and complete reporting of descriptive statistics that are relevant to the study question is a fundamental component of reporting any data set.
        ii. The descriptive statistics should fully disclose the magnitude of treatment effects and variability.
        iii. Examples of descriptive statistical test procedures include:
            i. A measure of central tendency, e.g., mean, median, mode, weighted mean, mean adjusted for other factors (e.g., across soil types) or covariates (e.g., rainfall, temperature, etc.).
            ii. A measure of variation: range, variance, standard deviation, coefficient of variation, or quartiles.

Note: The environmental conditions and plant species involved in the estimate will strongly affect the descriptive statistics and should be clearly stated to understand the limitations of the estimates.

5. Estimation of treatment effect relative to variability using inferential statistical methods.
    a. If tests are of exploratory nature, p-value thresholds should not be used. Instead, other methods, such as confidence intervals, may be more appropriate. Confidence intervals are particularly informative to provide the range of values that the true mean could take that would be compatible with the data. For example, the endpoints of a confidence interval of treatment difference can be interpreted based on the practical implications of the range of values.
    b. Examples of statistical models from which inferential statistics may be derived:
        i. T-test and ANOVA (Analysis of Variance) to compare two and more independent treatments, respectively
        ii. Regression to predict the relationship between a set of independent variables and the response variable
        iii. Linear mixed model to make broad-sense inference (e.g., across years/locations) about treatment effects while accounting for non-independent observations
        iv. Bayesian statistical models

c. p-value thresholds are permissible if they are used judiciously. For example, if a study is set up to investigate a primary hypothesis to be tested with a pre-specified method of analysis, which includes a justified significance level (alpha), alternative hypothesis, and any adjustment for multiplicity, comparing the p-value to alpha would provide a piece of objective information for decision.

Additional Considerations

In situations where a product meets all other criteria (e.g., safety, identification, characterization) required for a biostimulant, but is unable to completely fulfill the efficacy claim requirements set forth in this document, the product may be marketed for a period of two years while additional studies are conducted to substantiate efficacy claims. An example of when a product is unable to meet the efficacy claim requirements includes demonstration of an agronomically favorable data trend in support of the efficacy claim, but the inability to estimate effect of the treatment due to variability.

II. Recommended Guidelines for Verification of Plant Biostimulant Composition

This Section provides guidelines for verification of plant biostimulant composition testing using scientifically recognized methods. It also addresses guidelines for testing of potential contaminants such as heavy metals, microbial pathogens and other substances considered pollutants/impurities.

Plant biostimulants can be placed into five compositional categories:

A. Microbial Based Biostimulants

1. Live Microbial Products (e.g., *Rhizobacter sp., Bacillus sp., Azotobacter sp., Azospirillum sp., Glomus sp., Trichoderma sp.,* etc.)
2. Complex Products Based on Non-Living Microorganisms and Their Metabolites

B. Algal or Plant Extract Biostimulants

1. Aquatic Plant Extracts (e.g., derived from macroalgae such as *Ascophyllum sp., Ecklonia sp. Fucus sp., Kappaphycus sp., Laminaria sp., Sargassum sp., Ulva sp.,* etc.)
2. Microalgal Extracts (e.g., derived from microalgae such as *Chlorella sp., Spirulina sp.,* etc.)
3. Higher Plant Extracts (e.g., derived from plants such as *Allium sp., Brassica sp., Digitalis sp., Lupinus sp., Lycopersicon sp., Medicago sp.,* etc.)

C. Complex Carbon-Based Biostimulants

1. Mined natural deposits (Humic substances) primarily composed of three fractions (humic acids, fulvic acids, and humin). Sources of humic substances are commercially harvested from terrestrial deposits which include, but are not limited to, leonardite, oxidized lignite, oxidized sub-bituminous coals, humalite, carbonaceous shales (including humic shale), peat, and sapropel.
2. Other Complex Carbon-Based Residuals and Extracts (e.g., vermicompost/worm castings, compost waste materials, biochar etc.), or liquid extracts derived from these materials (e.g., compost tea, etc.).

D. Protein hydrolysate Biostimulants (containing peptides and free amino acids) - derived from plant, animal, or microbial protein feedstock

    1. Manufactured by chemical hydrolysis.
    2. Manufactured by enzymatic hydrolysis.

E. Defined Molecules Purified from Minerals, Plants, Animals, Microbes, or obtained by Synthesis. These may include:

    1. Organic molecules (e.g., amino acids, polyamines, polyphenols, betaines, oligosaccharides, alginates, carboxylic acids, fatty acids, chitin, chitosan etc.)
    2. Minerals not recognized as plant nutrients (e.g., silicon, selenium, etc.).

Guidelines to support product composition

A. Identify product composition (*use exact wording that appears on the product label in the Guaranteed Analysis section*). List the guaranteed substances(s) or the name(s) of the microbial organism(s) and its taxonomic classification up to the strain level (genus, species and strain identified, if applicable).

B. State the minimum amount of the guaranteed substance(s) in the final product in percentage weight by weight. For live microbial biostimulants state the minimum guaranteed amount of the claimed organism in recognized units of potency (e.g., Colony Forming Units CFU/g, percentage of weight, or other appropriate expression of composition).

C. Provide a method(s) for identification of the guaranteed substance(s). Analytical methods for identification of guaranteed substance(s) and/or their components vary greatly. Any method provided must be repeatable under common laboratory conditions. Preferably, when available, internationally recognized methods (e.g., ISO, AOAC, EPA, OECD) should be chosen.

D. Provide a derivation statement for each guaranteed substance(s). If applicable, identify the source of raw material (e.g., species of microbe, plant or animal).

E. Combination products containing mixtures of substances from multiple biostimulant product categories should follow requirements and methodologies for each claimed category.

F. If applicable, guarantee plant food ingredients in the product, either added or inherent (e.g., N, P, K, secondary or micronutrients) using general guidelines for fertilizer claims.

Composition guidelines for specific biostimulant groups

A. Live Microbial Products

    1. Describe the method used to obtain the taxonomic classification and provide an associated report, study, or publication of the same.
    2. Provide a method for identification of the microbial organism in the product (e.g., 16S rDNA sequencing, genome-based ANI scoring, etc.). Any method provided must be well established and repeatable under common laboratory conditions.

3. Describe the origin of each microbial biostimulant organism (state and county, or country, of origin) and its history (e.g., any genetic modifications to the strain).
4. Demonstrate the microbial biostimulant organism is not a human, plant or animal pathogen (e.g., published literature, clearances for free movement, etc.)
5. State the known shelf-life stability or expiration date of the product, as applicable.
6. To the extent practical, a sample of each microbial biostimulant organism should be maintained on deposit in a nationally/internationally recognized culture collection (e.g., Budapest Treaty on the International Recognition of Deposit of Microorganisms for the Purposes of Patent Procedure), or provide an explanation of why deposition is not possible.
7. Confirm the Convention on Biodiversity status of microorganisms derived from non-U.S. countries.

B. Complex Products Based on Non-Living Microorganisms and Their Metabolites

1. Provide the name of the source microbial organism and its taxonomic classification up to the strain level (genus, species, and strain identifier, if applicable).
2. Describe how the microbial organism is rendered non-viable or inactivated and/or demonstrate that the product does not contain viable source of microorganism.
3. Provide a guarantee of an identifying compound resulting from the production process, if applicable.

C. Algal or Plant Extracts

1. Provide the name of the primary plant/algal/microalgal species used in the manufacture of the biostimulant
2. Provide a guarantee of an identifying compound chosen to demonstrate the presence of the particular extract (i.e., mannitol, alginic acid, ulvan, fucoidan, betaine, etc.).

Guideline for demonstrating absence of contaminants in plant biostimulants

A. Heavy metal contaminants (to be tested if there is a risk of contamination).

1. Heavy metals in fertilizing products (As, Cd, Co, Cr, Cu, Pb, Hg, Ni, Mo, Se and Zn)
2. Methodology used should follow already accepted methods such as EPA, ISO, or AOAC
3. Maximum permitted levels should follow established limits (e.g., Washington State Department of Agriculture, https://agr.wa.gov/departments/pesticides-and-fertilizers/fertilizers/metals-analysis-requirement).

B. Microbial pathogens (to be tested if there is a risk of contamination).

1. Microbial contaminants are not to exceed established limits (Institute of Medicine and National Research Council. 2003. *Scientific Criteria to Ensure Safe Food*. Washington, DC: The National Academies Press. https://doi.org/10.17226/10690.)

Salmonella	Absence in 25 g or 25 mL
E. coli	< 10 CFU or MPN in 1 g or 1 mL
Fecal coliforms	< 10 CFU or MPN in 1 g or 1 mL

2. Other microbial contaminants in fermented products not to exceed the following limits:

Shigella species	Absence in 25 g or 25 mL
Vibrio cholera enrichment	Absence in 25 g or 25 mL
Enterococci	< 10 CFU or MPN in 1 g or 1 mL

Methodologies used should follow already accepted (Institute of Medicine and National Research Council. 2003. *Scientific Criteria to Ensure Safe Food*. Washington, DC: The National Academies Press. https://doi.org/10.17226/10690.)

C. Other contaminants

Additional contaminants in products that may require testing include toxic metabolites, environmental contaminants, antibiotic residues, or pesticide contaminants. Testing for such contaminants may be recommended on a case-by-case basis due to potential exposure during product development, from raw material source or manufacturing processes.

III. Recommended Guidelines for Conducting a Plant Biostimulant Safety Assessment

Introduction

Human and environmental safety assessments of plant biostimulants and other non-pesticidal crop inputs, e.g., fertilizers, soil amendments and plant inoculants are not conducted by individual US state regulators as part of the registration process. It is therefore incumbent upon the product developer to verify the safety of their products before commercializing. The biostimulant industry recommends a decision tree approach be used as a straight-forward mechanism for product developers to transparently assess the human and environmental safety of a plant biostimulant guaranteed substance (GS).

Decision trees for two general product classes were developed: (1) extracts, acids (e.g., organic amino, fulvic, humic, fatty, etc.), minerals, and other substances; and (2) living microorganisms, which are illustrated in Figures 1 and 2, respectively. Each of the decision trees will be discussed in more detail and further recommendations provided.

Some registrants of plant biostimulants and related products register products in countries which require a formal human and environmental safety assessment as a condition of registration and commercialization. In this case, a safety assessment of a GS may be satisfied based on that of a competent regulatory body. Section II of this chapter provides several examples of how to determine if a GS qualifies for this pathway.

The first step in conducting a safety assessment is characterization of the GS by clearly describing the substance and/or identifying the specific microorganism. Reliable and sufficient information may be available in the toxicological and ecological sections of a safety data sheet (SDS) from the supplier. However, if unavailable or judged unreliable or insufficient, a review of the scientific literature and other

available open-source information should be performed. Guidance on the characterization of a viable microorganism is discussed in Section III, additional guidance on identifying the risk group classification of a microorganism is provided in Section IV.

Criteria are established for conducting a literature search in Section V, and guidelines are provided for how to summarize and present the results of a literature search to demonstrate safety. A literature search will rely on scientific peer-reviewed open literature. Other sources might include a review of the GS from competent regulatory authorities from other uses such as a pesticide and food or feed additive. Similarly, criteria for demonstrating there is "sufficient data available" to address safety are provided in Section VI.

If sufficient information on human and environmental safety of a GS or microorganism is unavailable from literature or other sources, supporting data or a scientifically sound rationale to address the potential concern should be developed. While recognizing plant biostimulants are not pesticides, study protocols have been published by EPA, OECD, and other regulatory bodies to assess the safety of substances and microorganisms used in agriculture. Companies may consider these methods when developing protocols for their own microbial (Table 3) or other products (Table 4) or rationale to support its safety.

The decision tree approach characterizes the safety of a GS. The safety of the co-formulants in end-use products is expected to be characterized by the manufacturer under Occupational Safety and Health Administration or Global Harmonization Standard guidelines and in SDS development.

Where data do not need to be developed and the guaranteed substances have passed the safety assessment, label statements for end-use products regarding precautions, first aid and use of personal protective equipment will be tied to SDS statements and supporting information. Label guidelines will be developed to provide a standard format and reporting of precautions, first aid and personal protective equipment.

A GS with demonstrated safety concerns or significant adverse effects (toxicity) observed, which cannot be mitigated by reasonable steps such as Personal Protective Equipment or specific use restrictions, will fail to pass the safety assessment.

Figure 1. Decision tree for assessing the human health and environmental safety of plant biostimulant derived from extracts, minerals, acids and other guaranteed substances.

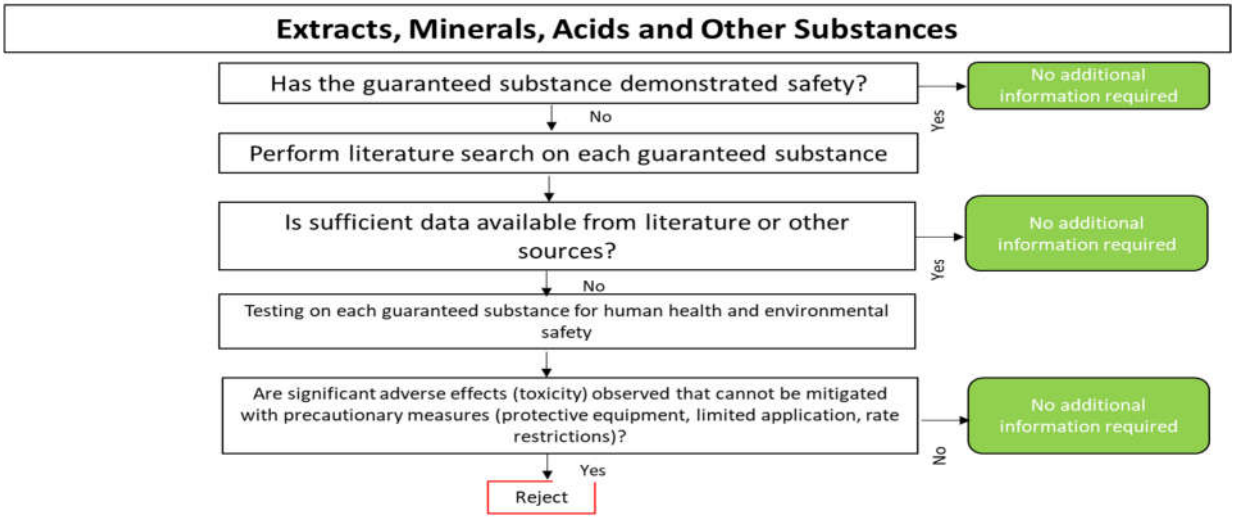
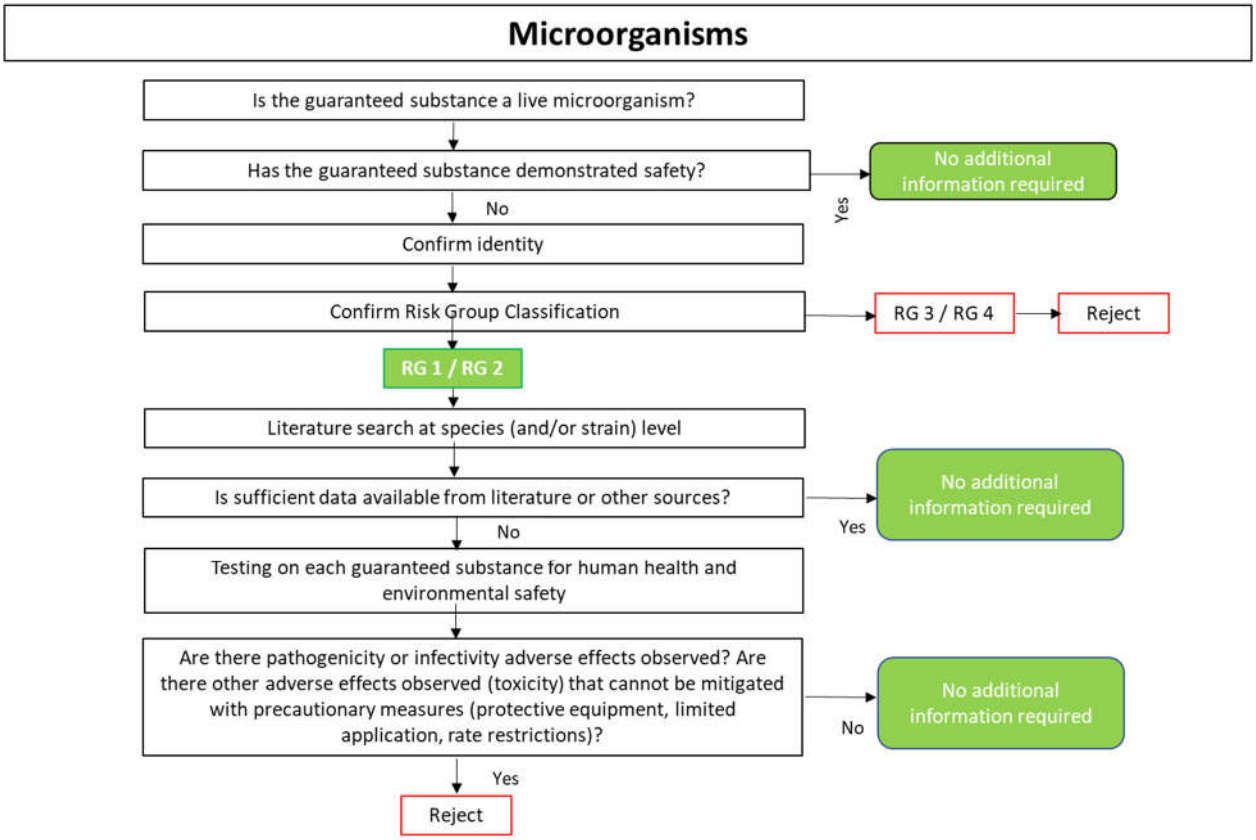


Figure 2. Decision tree for assessing the human health and environmental safety of microorganisms as plant biostimulant guaranteed substances.



Determining if a Guaranteed Substance Requires a Safety Assessment

A GS recognized as safe by a competent regulatory body would be exempted from the safety assessment. Several examples are provided below, which are more focused on human safety.

If the human and environmental impacts of a GS have been evaluated and deemed safe by a competent regulatory body, they would be exempted from further assessment. Several examples and their hyperlinks are provided below.

A. US Food and Drug Administration (FDA) Generally Recognized as Safe (GRAS) and food additive listings:
    1. https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
    2. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=182
    3. https://www.fda.gov/food/food-additives-petitions/food-additive-status-list
B. US Environmental Protection Agency (EPA) inert with tolerance exemption for food use:
    1. https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1::
C. European Food and Safety Authority (EFSA) Qualified Presumption of Safety (QPS) and assessed substances with no risk for food and feed:
    1. https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps
    2. http://www.efsa.europa.eu/
D. EU Commission on Food Additives:
    1. https://ec.europa.eu/food/safety/food_improvement_agents/additives/database_en
E. Codex Standard for Food Additives:
    1. http://www.fao.org/gsfaonline/docs/CXS_192e.pdf
    2. http://www.fao.org/gsfaonline/additives/index.html
F. Flavor and Extracts Manufacturers Association (FEMA) GRAS list
    1. https://www.femaflavor.org/gras

Guidance on Microbial Identification Methods and Reporting

Provide a method for identification of the microbial organism in the product (e.g., 16S rDNA sequencing, genome-based ANI scoring, etc.). Any method provided must be well established and repeatable under

common laboratory conditions. Refer to Chapter 2. Guidance for Verification of Plant Biostimulant Composition Claims for additional information.

Guidance on Identifying the Risk Group Classification

World Health Organization (WHO) Classification of Infective Microorganisms by Risk Group (2004) classifies the agents in that country by risk group based on pathogenicity of the organism, modes of transmission and host range of the organism. These may be influenced by existing levels of immunity, density and movement of host population presence of appropriate vectors and standards of environmental hygiene. https://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf

A. WHO Basis for Risk Grouping: Each country

1. Availability of effective preventive measures - Such measures may include prophylaxis by vaccination or antisera; sanitary measures, e.g., food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.

2. Availability of effective treatment - Effective treatment includes passive immunization and post exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of the emergence of resistant strains. It is important to take prevailing conditions in the geographical area in which the microorganisms are handled into account.

    a. WHO Risk Group 1 (no or low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease.

    b. WHO Risk Group 2 (moderate individual risk, low community risk). A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.

    c. WHO Risk Group 3 (high individual risk, low community risk). A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

    d. WHO Risk Group 4 (high individual and community risk). A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Note: Individual governments may decide to prohibit the handling or importation of certain pathogens except for diagnostic purposes.

B. Additional websites where the risk group of an organism can be found:

1. American Biological Safety Association (ABSA) https://my.absa.org/Riskgroups

   - Click on one of the *Quicklinks* and lands on page with list of genus names, then click on genus of interest and then scroll to find specific species name of interest
   - Or enter genus/species in the "Search Database" box

2. Federal Institute for Occupational Safety and Health (BAUA)

   - Classification of prokaryotes (bacteria and archaea) into risk groups

     https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische Regeln/Regelwerk/TRBA/TRBA-466.html

Note: Link leads to page where the document titled TRBA 466 "Einstufung von Prokaryonten (Bacteria und Archaea) in Risikogruppen" is found. The document can be viewed online or downloaded in German language. You can go to the English page by clicking on English at the top of the page; however, the English document may not be the most current version. Recommend checking the German language list if your genus/species is not found on the English language list. The microorganism's names are the recognized taxonomic names in both files.

   - Classification of fungi into risk groups

     https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRBA/TRBA-460.html

Note: Link leads to page where the document titled TRBA 460 Einstufung von Pilzen in Risikogruppen is found. The document can be viewed online or downloaded. Note that the document is only available in German. The microorganism's names are the recognized taxonomic names.

3. DSMZ List of Prokaryotic names with Standing in Nomenclature https://lpsn.dsmz.de/

   - Click on Advanced Search (left side of page)
   - Type in your genus and species
   - Select risk group ("All Risk Groups", 1, 2 or 3)

Note: In the event a microorganism is not listed in any Risk Group, it is recommended that a case for a likely Risk Group classification be made using literature or other available data.

Guidance on literature searches and summarizing literature searches

The goal from a literature review is to determine if there are sufficient scientific data and information on the active substance in question on human and environmental safety. Following the decision tree, if it is determined that there are not sufficient scientific data and information, testing must be completed on each guaranteed substance for human health and environmental safety. If there are enough scientific

data and information to justify the claim that the specific guaranteed substance is not detrimental to human or the environment, no further testing is required.

A. Information sources

A thorough and extensive retrieval of literature is central to address the key terms with as little bias as possible. The source of information for the literature search should be identified and documented. Commonly used sources include:

https://apps.webofknowledge.com/

https://pubmed.ncbi.nlm.nih.gov/=

Google Scholar https://scholar.google.ca/

B. Literature search strategy

The guaranteed substance must be included in the search. The search strategy is an ad hoc combination of search terms designed to retrieve as many literature hits as possible and with relevance to the review question. Structure the search by:

- Selecting key elements (i.e., phrases, words) to be used in the search
- Identifying search terms that capture the key elements
- Defining use of Boolean operators and truncation to broaden or narrow the search

The search terms should represent the key elements by considering synonyms, abbreviations, changes in terminology over time and spelling variants (e.g., British and US English variants). Include the relevant Boolean operators.

Example: A search contains *Brevibacillus validus*, then relevant search terms would be: *Brevibacillus validus*, *Bacillus validus*, *B. validus*

Use of boolean operators may refine the search, e.g., the question: "Is *Brevibacillus validus* associated with infectious disease?" contains the key elements (*Brevibacillus validus*) and (infectious diseases).

Useful search terms and Boolean operators would be: *Brevibacillus validus* AND infectious diseases. Further variations could be introduced by using a truncated word in combination with '*' (wildcard), e.g., infect* and disease*

It is possible to limit the search to specific years, but a sound justification should be provided.

Finally, it is important to consider how broad the search terms should be applied. In general, it is recommended to use a broad search for the initial screening.

The date that the literature search was conducted should be well documented. If needed, the researcher can go back and perform an additional literature search starting from the last retrieval date.

C. Keywords to be used in the literature review

Each active substance should be used in the search term, along with the following descriptors, or a combination of the following. Suggested search terms include, but are not limited to:

- Health
- Environment
- Human
- Marine
- Toxic
- Ecotox
- Safety
- Pathogenic

D. Selection of literature to include in review

Once the literature search is completed, the articles can be screened to assess and select relevant literature to include in the review. The quality and validity of the literature should also be considered.

The literature selection process is performed by screening of titles and abstracts for key terms. A screening checklist based on the key elements of the question is useful for this purpose. Based on the screening of the title and abstract of each record, a decision is made to include or exclude the record from further review.

Once the screening has been completed a full-text examination of the selected records is necessary to make a definite decision on whether a record should be included or excluded from the review. See Section VI for a checklist of relevance and reliability criteria.

In the list of records for full-text examination it should be noted for each record whether it is 'relevant' or 'not relevant'.

E. Presenting the findings

The information from the literature included in the review should be presented in a structured way, such as in a summary table, along with copies of the literature included in the table. A conclusion should also be made about the key terms in support of the safety assessment.

F. References

Application of systematic review methodology to food and feed safety: Application of systematic review methodology to food and feed safety assessments to support decision making, EFSA guidance 2010.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1637

Criteria for demonstrating "sufficient data available"

A. Guidance on how to ensure information available on a GS is sufficient (e.g., from literature or other sources).

   Two key concepts to address when incorporating data to satisfy a safety assessment: relevance and reliability. These are defined in Klimisch et al. (1997) and Moermond et al. (2016) as:

   - Relevance – the extent to which data are suitable for the purpose of an assessment
   - Reliability – "the inherent quality of an effect value in a test report or publication relating to: 1) a clearly described experimental design to allow for the study to be repeated independently, 2) the way the experimental procedures were performed, and 3) the reporting of the results to provide evidence of the reproducibility and accuracy of the findings." (Moermond et al., 2016)

B. Criteria for relevance and reliability

   The criteria for relevance and reliability should be summarized (see example below). Only studies that are considered relevant should be assessed for reliability.

Table 1. Selection criteria for relevance

• Is the test species/system representative of a species of concern?
• Is the route and magnitude of exposure fit for purpose?
• Is the selected endpoint provided and relevant for the protection goal (e.g., survival, growth, reproduction for ecological risk assessment)?
• Is the test substance representative of the substance under evaluation?
• In the case of reports on known *<strain name>* pathogens in a certain non-target organism, is there any relevance for *<strain name>?*
• Are there other aspects of the study that render the findings irrelevant for assessing human health/environmental risks?

Table 2. Selection criteria for reliability (minimum information reported e.g.)

- Was the test item adequately described (purity, composition, origin)?
- Were the test levels measured (if feasible and appropriate)?
- Was the test species identified properly (species, strain, sex, age)?
- Was there clear and comprehensive description of material and methods, including location, replicates, test conditions, and were they appropriate to the objective of the investigation?
- Was feeding of test animals appropriate to maintain health (depending on duration of the study)?
- Was duration of exposure defined?
- Was data on chemical and physical test conditions (pH, conductivity, light intensity/cycle, temperature, etc.) provided, and was it appropriate to the test organism?
- Were dose/concentration relationships described, if applicable?
- Were control groups used, adequately described, and performance acceptable?
- Were the endpoints (body weight, length, survival, etc.) defined?
- Were results and their derivation presented in a clear and appropriate manner (e.g., statistical determination of endpoint values)?
- How close was the method to a validated test guideline?
- Can the effects be ascribed to the defined chemical exposure?
- Can the presence and absence of toxicological effects be determined?

C. Criteria for the evaluation of open literature

When reviewing publications in the public domain for relevance and reliability, consider the following

- The study results are presented as a full article (*i.e.*, not an abstract).
- The paper is the primary source of the data.
- The information is publicly available.
- The language of the article is not an impediment to the interpretation of the findings.

Note: The ToxRTool is an application used to evaluate the reliability of data/studies/publications. It is focused on toxicology in vivo and in vitro data. It can be accessed at this site https://ec.europa.eu/jrc/en/scientific-tool/toxrtool-toxicological-data-reliability-assessment-tool. A paper by Moermond et al. (2016) has described how this approach can be used for evaluating ecotoxicology studies.

D. References

Klimisch H-J., Andreae M, Tillmann U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Reg Toxicol Pharmacol 25:1–5.

EFSA] European Food Safety Authority Scientific Committee. 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA J 9:2092. 49 p. DOI:10.2903/j.efsa.2011.2092

Moermond C, Beasley A, Breton R, Junghans M, Laskowski R, Solomon K, Zahner H. 2017. Assessing the reliability of ecotoxicological studies: An overview of current needs and approaches. Integr Environ Assess Manag 13:640–651.

Ruden C, Adams J, Ågerstrand M, Brock TCM, Buonsante V, Poulsen V, Schlekat CE, Wheeler JR, Henry TR. 2017. Assessing the relevance of ecotoxicological studies for regulatory decision-making. Integr Environ Assess Manag 13:652–663.

Moermond C, Kase R, Korkaric M, Agerstrand M. 2016. CRED: Criteria for reporting and evaluating ecotoxicity data. Environ Toxicol Chem 35:1297–1309.

Table 3. US EPA/ OECD Guidelines for Assessing Microbial Safety

Study	US EPA/OECD Guideline	Guaranteed Substance	Formulation	Example Rationale
Acute Oral Toxicity/Pathogenicity	885.3050	X[1]		• Generate and evaluate data alternative to the study on the guaranteed substance's toxicity to determine if a scientifically viable rationale is a valid option. • Guaranteed substance is highly volatile. • Guaranteed substance is not friable, and particles are too large to be ingested; or the product design prevents oral exposure. • Generate or make available data on other routes of exposure regarding pathogenicity.
Acute Pulmonary Toxicity/Pathogenicity	885.3150	X[1]		• Generate and evaluate data alternative to the study on the guaranteed substance's toxicity to determine if a scientifically viable rationale is a valid option. • Generate or make available data on other routes of exposure regarding pathogenicity.
Acute Eye Irritation	870.2400/405		X[2]	• Generate or make available data to support formulation is not an eye irritant.
Dermal Irritation	870.2500/404		X[2]	• Generate or make available data to support formulation is not dermal irritant.
Dermal Sensitization	870.2600/429		X[2]	• Product does not result in repeated dermal exposure under conditions of use. • Generate or make available data to support formulation is not dermal sensitizer. • The guaranteed substance is a known sensitizer.
Toxicity/Pathogenicity – Aquatic Organisms	885.4240;885.4200	X[3]		• No exposure to aquatic organisms including threatened and endangered species. • Generate or make available data that guaranteed substance shows no pathogenic/toxic effects on aquatic organisms.
Toxicity/Pathogenicity-Bees, Non-Target Arthropods	885.4380; 885.4340 213;214[4]	X[5]		• No exposure to bees or non-target arthropods including threatened and endangered species. • Generate or make available data/ literature that guaranteed substance shows no detrimental impacts on bees, closely related species and/ or non-target arthropods. • Exposure of bees and non-target arthropods is negligible or minimal.
Toxicity - Soil Organisms (earthworms)[6]	OECD 222	X		• No exposure to soil organisms including threatened and endangered species. • Generate or make available data that guaranteed substance shows no pathogenic/toxic effects to soil organisms.
Toxicity/Pathogenicity-Birds	885.4050	X		• No exposure to birds including threatened and endangered species. • If exposure of birds and mammals is expected to be minimal or negligible generate or make available data/literature to show no pathogenic/toxic effects to birds and mammals.

[1]Route of administration dependent upon the relevant route of exposure.

[2]If co-formulants have known irritating or sensitization properties.

[3]Conditional based on exposure to aquatic organisms.

[4]For insects, chronic non-target arthropod guidelines for chemicals may be applicable (IOBC guidelines for *Aphidius* and *Typhlodromus*).

[5]Conditional based on any treatment that results in drift or dust generation (example: spray applications and seed treatments).

[6]Given the relationship between earthworms and soil micro-organisms, testing becomes more relevant as the live microorganisms deviate from the wild type / naturally occurring (*e.g.*., genetically altered).

Note: Additional studies may be considered pending literature evaluation and any known toxins produced by microbes (in silico analysis may be appropriate).

Table 4. US EPA/ OECD Guidelines for Assessing Safety of Extracts, Minerals, Acids, and Other Substances

Study	US EPA/OECD Guideline	Guaranteed Substance	Formulation	Example Rationale
Acute Oral Toxicity/Pathogenicity	870.1100/425	X		• Generate and evaluate data alternative to the study on the guaranteed substance's toxicity to determine if a scientifically viable rationale is a valid option. • Guaranteed substance is highly volatile. • Guaranteed substance is not friable, and particles are too large to be ingested; or the product design prevents oral exposure. • Generate or make available data on other routes of exposure regarding pathogenicity.
Acute Inhalation toxicity	870.1300/403	X		• Generate and evaluate data alternative to the study on the guaranteed substance's toxicity to determine if a scientifically viable rationale is a valid option. • Generate or make available data on other routes of exposure regarding pathogenicity.
Acute Eye Irritation	870.2400/405		X[1]	• Generate or make available data to support formulation is not eye irritant1.
Dermal Irritation	870.2500/404		X[1]	• Generate or make available data to support formulation is not dermal irritant.
Dermal Sensitization	870.2600/429		X[1]	• Product does not result in repeated dermal exposure under conditions of use. • Submit data to support formulation is not dermal sensitizer. • The substance is a known sensitizer.
Toxicity – Aquatic Organisms	850.1010/202 850.1075/203	X[2]		• No exposure to aquatic organisms including threatened and endangered species. • Generate or make available data that guaranteed substance shows no pathogenic/toxic effects on aquatic organisms.
Toxicity- Bees, Non-Target Arthropods[3]	OECD 213;214[3]	X[4]		• No exposure to bees or non-target arthropods including threatened and endangered species. • Generate or make available data/ literature that guaranteed substance shows no detrimental impacts on bees, closely related species and/ or non-target arthropods. • Exposure of bees and non-target arthropods is negligible or minimal.
Toxicity- Birds	850.2100/223	X		• No exposure to soil organisms including threatened and endangered species. • Generate or make available data that guaranteed substance shows no pathogenic/toxic effects to soil organisms.

[1]If co-formulants have known irritating or sensitizing properties.

[2]Conditional based on exposure to aquatic organisms.

[3]For insects, chronic non-target arthropod guidelines for chemicals may be applicable (IOBC guidelines for Aphidius and Typhlodromus).

[4]Conditional based on foliar application.

[1] EPA OPP Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products (Acute Oral, Acute Dermal, Acute Inhalation, Primary Eye, Primary Dermal, and Dermal Sensitization), March 1, 20

IV. Using the Guidelines

As described in the introduction, this guidance document was developed by the Biostimulant Industry Workgroup (BIW) to support the efficacy, composition, and safety of plant biostimulant products in the United States. Such data and information are not required when registering non-pesticidal products at the individual state level. Consequently, while these, science-based guidelines are not intended as "requirements," product developers are encouraged to consider employing them to strengthen the overall credibility of their plant biostimulant and by extension, the category, with regulators, growers, and consumers.

In employing the guidelines for the development of a new biostimulant product, companies can view them as best practices and determine how and what aspects will be implemented based upon resources, use pattern and the product itself. For example, when planning studies to demonstrate the benefits a product can provide, a company may consider the recommended experimental design, crops, number of trial locations, or methods of statistical analysis appropriate for their situation. Doing so will strengthen the veracity of product claims and increase credibility with growers, regulators, and other stakeholders.

Similarly, depending upon the type of product, microbial, extract, protein hydrolysate, amino acid or other, documentation of the methodology used to verify the number of microbes in their product, or the percentage of a particular guaranteed ingredient is useful. A company able to document such information, will provide greater confidence to both consumers and regulators on the quality and consistency of their product. Demonstrating a lack of heavy metal contaminants or microbial pathogens will likewise increase confidence that the product is not increasing environmental or human exposure to harmful materials or organisms.

The safety assessment decision trees provide companies a methodology to follow that can serve to provide confidence in the overall safety of their product and address potential concerns or limit liability in the case issues are raised. Demonstration of minimal or no environmental or human risk of these non-pesticidal products in addition to proven efficacy serves to underscore the benefit of these products, especially as technology continues to evolve, novel organisms are identified and overall use of biostimulants expands.

In conclusion, these guidelines provide a science-based framework for plant biostimulant companies to document and communicate the performance, composition, and safety of their products to US growers, regulators, consumers, and other stakeholders. Although voluntary, product developers are encouraged to adopt them, which will further strengthen the credibility and use of plant biostimulants and this unique category of plant input.