United States Biostimulant Industry Recommendations to Assess the Efficacy, Composition, and Safety of Plant Biostimulant Products

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Abstract

Plant biostimulant products are gaining significant traction as valuable tools in the agricultural and horticultural sectors, facilitating sustainable crop production and environmentally conscious operations while bettering plant growth, quality, and yield. These unique products cannot be classified as fertilizers, nor do they work directly on pests like pesticides. Working to enhance natural plant processes, they offer invaluable aid to producers, sustainably improving the efficiency of inputs and natural resources. Unlike widely used agrochemicals and traditional fertilizers, plant biostimulant products lack consistent regulatory oversight in the United States (U.S.), thus creating uncertainty for product developers and limiting commercialization and adoption.

In this policy commentary, members of the Biostimulant Industry Workgroup (BIW) recommend science-based criteria to verify plant biostimulant product claims in the U.S. It is anticipated that the application of these principles by biostimulant product developers will provide users, regulators, and other stakeholders in the U.S. with greater confidence in their product's ability to perform as claimed, that its contents are consistent with its labeling, and the human and environmental safety of the product has been considered. The objective is to strengthen the credibility of individual biostimulant products and the category as a whole.

Keywords: plant biostimulant products, biostimulants, recommendations, efficacy, composition, safety

1. Introduction

There is a growing interest by farmers, growers, and consumers to produce healthy food utilizing sustainable agricultural principles and by urban architects and residents to utilize green technology [15]. Plant biostimulants are one of the valuable tools helping farmers and growers achieve sustainable crop production, along with professionals and homeowners implementing environmentally friendly practices [19, 24].

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A Plant biostimulant as adopted by the Association of American Plant Food Control Officials (AAPFCO) at their Summer Annual Meeting on August 2, 2022 in St. Louis, Missouri and consistent with the definition proposed in a report to the President and Congress by the United States Department of Agriculture [27] 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". Further, to qualify as a plant biostimulant, a substance that meets this definition must also have an intended use and label claims consistent with the definition.

Plant biostimulants are not fertilizers, i.e. macro or micro plant nutrients essential for plant growth [26] or pesticides defined in the U.S. Federal Insecticide, Fungicide, and Rodenticide Act as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, used as a plant regulator, defoliant, or desiccant, and any nitrogen stabilizer [34].

Plant biostimulants are a unique category of products that can contribute to yield and quality improvements without increasing applied fertilizer, water, or planted acres, thus, sustainably enhancing the efficient use of these inputs and natural resources [5, 19]. Plant biostimulants can also 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 [3, 5, 11].

Plant biostimulants encompass a broad category of products from microbial inoculants or their metabolites to plant and algal extracts, complex carbon-based natural deposits and their extracts like humic and fulvic acids, protein hydrolysates, and purified single molecules derived from natural or synthetic sources [5]. They can be used for conventional and, potentially, organic crop production and non-agricultural, turf, and ornamental applications.

The development of new and innovative biostimulant products is being accelerated by public and private institutions and companies of all sizes worldwide. While the industry's global growth estimates vary, plant biostimulants are expected to become a ~3-billion-dollar market by 2025 [15].

Plant biostimulant products currently face regulatory challenges in the U.S. that can limit their use, thus reducing the benefits these products offer. While a product may be considered a biostimulant, there is not an abundantly clear, single, unified, science-based regulatory path for such products in the U.S., thus preventing developers from registering products according to their intended use, composition, and specific benefits. Whereas in 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. [6, 16].

Depending on the biostimulant product's composition and intended use, technology developers 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 U.S. 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. Furthermore, differing state regulations and definitions may result in the same product being considered a soil amendment in one state, a microbial inoculant in another, a fertilizer with nutrient guarantees that provide no nutritional value to the crop in another and in still other states no registration may be necessary for sale.

If federally registered as a plant regulator with the EPA, data attesting to product composition and human and environmental safety must be submitted under the Federal Insecticide, Fungicide, and Rodenticide Act 7 U.S.C. §§ 136 et seq [34]. While the EPA typically does not require applicants to submit efficacy data, the Agency reserves the right to demand, 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 U.S. biostimulant industry endorses the following recommendations as a general, science-based framework that can be voluntarily employed by technology developers and marketers to demonstrate the efficacy, composition, and safety of plant biostimulant products sold in the U.S. The industry believes following these recommendations will strengthen the credibility of individual plant biostimulants by providing users, regulators and other stakeholders with greater confidence in the product's ability to perform as claimed, that it contents are consistent with its labeling and the human and environmental safety of the product has been considered. The industry recognizes that some of these recommendations may not be relevant for plant biostimulant products for which there is already a well-established record of safety and performance based on decades of commercial use.

This document and recommendations were developed by the Biostimulant Industry Workgroup (BIW), a collaboration of The Biological Products Industry Alliance (BPIA) Biostimulant Innovation Committee and The Fertilizer Institute Biostimulant Council (TFIBC). The content results from 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 recommendations are intended to be updated periodically by BPIA and TFIBC to encompass documented advances in the

scientific literature, the development of International Standards Organization (ISO) or other standard methods, and relevant regulatory guidance from the EPA, USDA, 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:

- Recommendations to Verify Plant Biostimulant Efficacy Claims
- Recommendations to Verify Plant Biostimulant Composition Claims
- Recommendations to Verify Plant Biostimulant Safety Claims
- Considerations in Using the Recommendations

2. Recommendations to Verify Plant Biostimulant Efficacy Claims

All product efficacy claims being made and verified should be consistent with the benefits described in the definition of a plant biostimulant. Verification should be done for the minimum recommended application dose that achieves the desired result or effect [22].

To establish credibility, relying solely on consumer testimonials of the product, presentation abstracts, or marketing material is not recommended. Instead, plant biostimulant efficacy claims should be verified via one of three different methods.

(1) Association of the stated claim(s) with relevant published literature.
(2) Data generation via research conducted using scientifically recognized methodology.
(3) Utilization of a combination of relevant published literature and research test results.

Following recommendations offered in Rouphael and Colla [24], it is logical to start the verification process with published literature and existing data. Once existing literature and data are procured, complement the information as necessary with experimental data collected from carefully designed research.

2.1. Recommendations for Associating Published Literature to Verify Efficacy Claims

Efficacy claims can be substantiated by conducting a thorough literature review of relevant scientific literature and associating the claims with previously published data. Published literature may verify specific product claims, product ingredients, the product's mode of action, and/or application rates, thus circumventing the need for novel data generation.

When associating claims with previously published data, it is imperative to identify all parameters verified using relevant published scientific literature. Identify the product claims [33] using the exact wording on the product label, identify product ingredients and/or properties, and specify recommended product application rates.

To verify efficacy claims, the manufacturer/ developer may provide peer-reviewed scientific literature supporting stated product label and associated product literature claims as well as published literature supporting the efficacy of the specific product composition at recommended application rates.

Recommendations for associating published literature to verify efficacy claims are less stringent and rigorous than those employed for verifying plant biostimulant safety claims. Those recommendations are defined in Section 4.5, "Recommendations on Conducting and Summarizing Literature Searches," and may be used to strengthen and add credibility to the efficacy claims literature search.

2.2. Recommendations for Data Generation to Verify Efficacy Claims

Efficacy claims can be substantiated with experimental evidence gathered using proper experimental design under commercial or alternative growing systems such as greenhouse, controlled environment chambers, etc. The research will measure specific physiological outcomes or differences in plant growth, quality, or yield and compare the collected data to appropriate experimental control treatments.

Manufacturers must provide sufficient data to be credible without the process being needlessly burdensome. The amount of data required to support a claim depends on the breadth of the claim itself. Narrower claims require less data than claims making broader statements.

The experimental methodology/design should be adapted to the specific agronomic or horticultural situation and include all pertinent experimental units fashioned in an appropriate, randomized experimental layout, with replications and control treatments for reference to avoid bias and meet specified objectives. Observed comparisons and response measures should directly support the product claim(s). Consultation with a statistician on experimental design is strongly recommended.

2.2.1. Recommendations for Determining the Number of Trials and Replicates.

To quantity, the number of research trials or test numbers needed, consider the number of trials/tests, locations, and seasons necessary depending on the product claims, target growing systems, and expected product performance in different soil and environmental conditions. When appropriately designed, a single trial may demonstrate multiple product claims.

The number of replicates should depend upon the experimental variation, the number of treatments and the size of the treatment difference to be detected. Enough replicates are needed to reduce the data variability and increase the chance of observing differences between treated and untreated plots [22].

2.2.2. Recommendations for Selecting Control Treatments.

Careful selection of control treatments to substantiate the efficacy claims is essential for credible experimental design. Control

treatments isolate the effect of independent variables and are necessary to establish a cause-and-effect relationship.

- The experimental design must include a negative control untreated by the biostimulant material for which the claim is being tested.
- The experimental design may include a positive control, such as a comparative standardized product, which exhibits known effects like those being claimed.
- When verifying biostimulant products containing plant food, include a control treated with an identical plant food composition (e.g., N, P, K, secondary, or micronutrients) to demonstrate the efficacy of the biostimulant component alone.
- When a challenge condition is used (e.g., drought, heat, reduction of input) in the experimental design, control data should be generated in the absence of the challenge condition if possible.

2.2.3. Recommendations for Choosing Suitable Test Crops.

When selecting suitable test crops, use crop(s) and/or crop groupings supporting your efficacy claims and crop(s) application rates. In each case, provide the rationale for the proposed grouping and provide data for a minimum of two representative crops for the group.

Crops may be grouped by taxonomic rank (Fabaceae/legumes, Poaceae/cereal, etc.), organ of interest (leafy vegetables, bulb, tuber, fruit, flower, etc.), life cycle (annual, perennial, etc.), resistance to stress (crops sensitive to low temperature, drought, etc.), or product use (ornamental flowers, lawns, etc.). Other groupings may be used if the grouping significance is defined.

2.2.4. Recommendations for Selecting Research Locations.

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.

Research tests to support yield or quality improvements claims should be conducted in a commercial setting or the target growing system. Locations may include a greenhouse production setting, strips or sections of farmer's or grower's fields, field research small plots, or an urban landscape setting.

Research tests to support other claims should be conducted in alternate growing systems such as research greenhouses, laboratory growth chambers, or field trials under controlled conditions (shaded plots vs. unshaded, different watering systems, etc.).

In the event none of these locations are applicable, others may be used as determined by the product and its intended use.

2.2.5. Recommendations for Utilizing International Research Locations.

Data may be generated at locations outside the US, provided it meets the following criteria:

- Practices used must match those employed in the US.
- Data supports efficacy claims allowed in the US.
- Data is applicable to the target production practices in the US.
- All aspects of the data generation comply with the recommendations described in this document.

For yield and quality efficacy claims pertaining to agricultural land production, a minority share (<50%) of data generated outside the US is acceptable, provided the data generation meets the preceding criteria.

2.2.6. Recommendations for Conducting Appropriate Statistical Analysis.

Following the research conclusion, statistical analysis relevant to the experimental design and test objectives must be performed on the research tests and data. *Consultation with a statistician on analysis is strongly recommended.*

The main objective of the data analysis is to estimate the magnitude of the difference between the various treatments and provide a measure of the variability of those estimates.

Approval or rejection of a specified product claim should be based on the following two criteria at a minimum:

(1) Estimation of the economic or biological benefit of a treatment/claim to the crop/grower using the best available descriptive statistic(s)*. The descriptive statistics should fully disclose the magnitude of treatment effects and variability—a complete reporting of descriptive statistics relevant to the study question is a fundamental component of data set reporting.
Suitable descriptive statistical test procedures include a measure of central tendency and variation. Central tendency may be measured using mean, median, mode, weighted mean, or mean adjusted for other factors (e.g., across soil types) or covariates (rainfall, temperature, etc.). Range, variance, standard deviation, coefficient of variation, or quartiles may be used to measure variation.

*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.

(2) Estimation of the treatment effect(s) relative to variability using inferential statistical methods. Inferential statistics may be derived from statistical models such as T-test and ANOVA (Analysis of Variance) to compare two or more independent treatments. Regression may be used to predict the relationship between a

set of independent variables and the response variable. Linear mixed models can make a broad-sense inference (e.g., across years/locations) about treatment effects while accounting for non-independent observations. Use Bayesian statistical models to make inferences about conditional probabilities.

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 accepted depending on the study objectives.

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 a decision.

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, providing the range of values 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.

2.3. Recommendations for Additional Considerations

In situations where a product meets all other criteria (e.g., safety, identification, and characterization) required for a biostimulant but is unable to fulfill the efficacy claim requirements outlined in this document altogether, the company should continue development of efficacy data to support the product claims before pursuing initial commercialization.

An example of when a product cannot meet the efficacy claim requirements includes the demonstration of an agronomically favorable data trend in support of the efficacy claim but the inability to estimate the effect of the treatment due to variability.

3. Recommendations to Verify Plant Biostimulant Composition Claims

This section provides recommendations for verifying plant biostimulant composition using scientifically recognized methods. It also addresses recommendations for testing potential contaminants such as heavy metals, microbial pathogens, and other substances considered pollutants or impurities.

3.1. Recommendations to Support Verification of Product Composition

The first verification step is to describe the product composition using the exact wording on the product label in the Guaranteed Analysis section. List each guaranteed substances (GS) or the name(s) of the microbial organism(s) and each

taxonomic classification up to the strain level (genus, species, and strain identified, if applicable).

State the minimum amount of each GS in the final product in percentage weight by weight (% w/w). 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 expressions of composition).

Provide the method(s) utilized to identify the guaranteed substance(s). Analytical methods for identifying guaranteed substance(s) or their components vary greatly. When available, internationally recognized methods (e.g., ISO, EPA, OECD) should be chosen. Any process provided must be repeatable under standard laboratory conditions.

Provide a derivation statement for each GS detailing the sources of all guaranteed primary, secondary, or micronutrients. If applicable, identify the source of raw material (e.g., species of microbe, plant, or animal).

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.

3.2. Recommendations to Verify the Composition of Specific Biostimulant Groups

Plant biostimulants can be placed into five compositional categories. Particular recommendations vary between the individual biostimulant groups, making it imperative to follow the specific recommendation based upon the GS being verified. (1) *Microbial-based biostimulants*, including live microbial products such as species and non-pesticidal strains of *Rhizobacter*, *Bacillus*, *Azotobacter*, *Azospirillum*, *Glomus*, *Trichoderma*, etc., and complex products based on non-living microorganisms and their metabolites.

(2) *Algal or plant extract biostimulants*, including aquatic plant extracts derived from macroalgae species such as *Ascophyllum*, *Ecklonia*, *Fucus*, *Kappaphycus*, *Laminaria*, *Sargassum*, *Ulva*, etc., microalgal extracts derived from microalgae species such as *Chlorella*, *Spirulina*, etc., and higher plant extracts derived from plant species such as *Allium*, *Brassica*, *Digitalis*, *Lupinus*, *Lycopersicon*, *Medicago*, etc.

(3) *Complex carbon-based biostimulants*, including mined natural deposits (humic substances) primarily composed of three fractions (humic acids, fulvic acids, and humin). This category also includes other complex carbon-based residuals and extracts

(vermicompost/worm castings, compost waste materials, biochar, etc.) or liquid extracts derived from these materials (compost tea, etc.).

(4) *Protein hydrolysate biostimulants* contain non-pesticidal peptides and free amino acids derived from plant, animal, or microbial protein feedstock. They can be manufactured by chemical or enzymatic hydrolysis.

(5) *Defined molecules purified from minerals, plants, animals, and microbes or obtained by synthesis* may include organic molecules (amino acids, polyamines, polyphenols, betaines, oligosaccharides, alginates, carboxylic acids, fatty acids, chitin, chitosan, etc.) and minerals not recognized as plant nutrients (silicon, selenium, etc.).

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

3.2.1. Recommendations to Verify the Composition of Live Microbial Products.

- Describe the method used to obtain the taxonomic classification and provide an associated report, study, or publication.
- Provide a method for identifying the microbial organism in the product (16S rDNA sequencing, genome-based ANI scoring, etc.). Any method provided must be well established and repeatable under standard laboratory conditions.
- 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).
- Demonstrate the microbial biostimulant organism is not a human, plant, or animal pathogen (published literature, clearances for free movement, etc.)
- State the known shelf-life stability or expiration date of the product, as applicable.
- 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.
- Confirm the Convention on Biodiversity status of microorganisms derived from non-U.S. countries.

3.2.2. Recommendations to Verify the Composition of Algal or Plant Extracts.

- Provide the name of the primary plant/algal/microalgal species used in manufacturing the biostimulant.
- Provide a guarantee of an identifying compound chosen to demonstrate the presence of the

particular extract (mannitol, alginic acid, ulvan, fucoidan, betaine, etc.).

3.2.3. Recommendations to Verify the Composition of Complex Products Based on Non-Living Microorganisms and Their Metabolites.

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

3.3. Recommendations to Demonstrate the Absence of Contaminants in Plant Biostimulants

If there is a risk of product contamination, the plant biostimulants must be tested for contaminants, and results must fall within established acceptable limits.

To verify the biostimulant products pose no threat of heavy metal contamination, they should be tested for all components that may be in fertilizing products, such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), molybdenum (Mo), selenium (Se), and zinc (Zn).

The methodology should follow already accepted methods such as EPA, ISO, or AOAC. The maximum permitted levels should follow established limits like those set by the Washington State Department of Agriculture [36].

For microbial contaminant testing, the methodologies used should follow already accepted methods. Contaminants are not to exceed established limits set forth by the Institute of Medicine and National Research Council [17].

Biostimulant products may require testing for additional contaminants, including toxic metabolites, environmental pollutants, antibiotic residues, or pesticides. Testing for such contaminants may be recommended on a case-by-case basis due to potential exposure during product development from the raw material source(s) or manufacturing processes.

4. Recommendations to Verify Plant Biostimulant Safety Claims

During the registration process of plant biostimulants and other non-pesticidal crop inputs, e.g., fertilizers, soil amendments, and plant inoculants, human and environmental safety assessments are not conducted by individual US state regulators. It is, therefore, incumbent upon the product developer to assess and verify the safety of their products before commercializing.

4.1. Determining if a Guaranteed Substance Requires a Safety Assessment

The first step is the characterization of the GS by clearly describing the substance or identifying the specific microorganism. Reliable and sufficient information may be available in the supplier's toxicological and ecological sections of a safety data sheet (SDS).

If a GS has already been registered and commercialized in a country where a formal human and environmental safety assessment was a condition of registration, it has been recognized as safe by a competent international regulatory body. It, therefore, would be exempted from the safety assessment.

A GS will also be exempted from a further safety assessment if its human and environmental impacts have been evaluated and deemed safe by a competent regulatory body. Many such regulatory agencies have online listings or searchable databases with products they have considered to be safe.

- US Food and Drug Administration (FDA) Generally Recognized as Safe (GRAS) [28] and food additive listings [29].
- US Environmental Protection Agency (EPA) inert ingredients with tolerance exemption for food use [35].
- European Food and Safety Authority (EFSA) Qualified Presumption of Safety (QPS) and assessed substances with no risk for food and feed [10].
- European Union Commission on Food Additives [7].
- Codex Standard for Food Additives [2].
- Flavor and Extracts Manufacturers Association (FEMA) Generally Recognized as Safe (GRAS) list [14].

4.2. Recommendations on Using a Decision Tree Approach for a Safety Assessment

If a safety assessment is required, the biostimulant industry recommends that product developers use a decision tree approach as a straightforward mechanism to transparently evaluate and characterize a guaranteed substance's human and environmental safety.

Decision trees for two general product classes were developed:

(1) extracts, acids (e.g., organic amino, fulvic, humic, fatty, etc.), minerals, and other substances (Figure 1).
(2) living microorganisms (Figure 2).

The two decision trees follow similar yet slightly different protocols, as live microorganisms require more in-depth verification. If the information on demonstrated safety is unavailable or judged unreliable or insufficient, a review of the scientific literature and other available open-source information should be performed.

If sufficient information on a GS or microorganism's human and environmental safety is unavailable from literature or other sources, supporting data or a scientifically sound rationale to address the potential concern should be developed.

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

4.3. Recommendations on Microorganism Identification Methods and Reporting

Microbial organism identification must be performed using a well-established method (e.g., 16S rDNA sequencing, genome-based ANI scoring, etc.) and repeatable under standard laboratory conditions. Refer to Section 2. "Recommendations to Verify Plant Biostimulant Composition Claims," for additional information.

4.4. Recommendations on Identifying the Microorganism Risk Group Classification

Microorganisms are classified into Risk Groups according to the degree of risk of infectivity, pathogenicity, the availability of preventive measures and effective treatments, and potential damage to the environment. Risk Groups correlate to, but do not always equate with, biological safety levels. Biosafety levels prescribe the work practices, engineering controls, personal protective equipment, and facility requirements required for working with biological agents. To determine the risk group classification, utilize the recommended classification set forth by the World Health Organization (WHO). The 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.

Microbial organisms classified as Risk Group 1 (RG1) or Risk Group 2 (RG2) continue through the decision tree onto the associated literature search. Risk Group 3 (RG3) and Risk Group 4 (RG4) organisms are deemed unsafe and automatically rejected for use.

- WHO Risk Group 1 (no or low individual and community risk): A microorganism that is unlikely to cause human disease or animal disease.
- 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 spreading infection is limited.
- 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.
- WHO Risk Group 4 (high individual and community risk): A pathogen

that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not typically available.

Risk group factors may be influenced by existing levels of immunity, density, and movement of the host population, presence of appropriate vectors, and standards of environmental hygiene. In each country, the availability of effective preventative measures and effective treatment must also be considered.

Preventative 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.

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 crucial to consider prevailing conditions in the geographical area where the microorganisms are handled.

Additional information on organism risk can be found through the American Biological Safety Association (ABSA) [1] and the DSMZ List of Prokaryotic names with Standing in Nomenclature [4]. The Federal Institute for Occupational Safety and Health (BAUA) classifies prokaryotes [8] and fungi [13] into risk groups.

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.

Regardless of the risk category classification, individual governments may prohibit the handling or importation of specific pathogens except for diagnostic purposes.

4.5. Recommendations on Conducting and Summarizing Literature Searches

A literature review aims to determine if there is sufficient scientific data and information on the human and environmental safety of the active substance in question. Following the decision tree, no further testing is required if there is enough scientific data and information to justify the claim that the specific GS is not detrimental to humans or the environment. If it is determined that there is not sufficient scientific data and information, testing must be completed on each GS for human health and environmental safety.

A thorough and extensive retrieval of scientific peer-reviewed open literature using systematic review methodology [8, 9] is central to addressing 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 Web of Science, PubMed, and Google Scholar. Other sources might consist of a review of the GS from competent regulatory authorities for other

uses such as a pesticide and food or feed additives.

4.5.1. Recommendations for Structuring a Literature Search.

When conducting a literature search, the name of the GS must be included in the search. The search strategy is an ad hoc combination of search terms relevant to the review question designed to retrieve as many literature hits as possible. Generally, it is recommended to use a broad search for the initial screening and then narrow it accordingly.

Structure the search by 1) selecting key elements (i.e., phrases, words) to be used in the search, 2) identifying search terms that capture the key elements, and 3) defining the 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). Each active substance should be used in the search term, along with the following descriptors or a combination. Suggested search terms include but are not limited to health, environment, human, marine, toxic, ecotoxicity, safety, and pathogenic.

Example:

- A search contains the key element *Brevibacillus validus*; therefore, the relevant search terms would be *Brevibacillus validus*, *Bacillus validus*, and *B. Validus*.
- Further helpful search terms would be “infectious diseases.”
- Boolean operators would be *Brevibacillus validus* AND infectious diseases. The use of Boolean operators may refine the search. For instance, the question, “is *Brevibacillus validus* associated with infectious disease?” contains the key elements (*Brevibacillus validus*) and (infectious diseases).
- Further variations could be introduced by using a truncated word combined with ‘*’ (wildcard), e.g., infect* and disease*.

4.5.2. Recommendations for Literature Screening and Selection.

Once a thorough literature search is completed, the amassed articles must be screened for consideration in the review.

The literature selection process is performed by reviewing article titles and abstracts for key terms. A screening checklist based on the key elements of the question at hand is helpful 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 decide whether a record should be included or excluded from the review. Consider both the quality and validity of the relevant literature. See Tables

1 and 2 for a checklist of relevance and reliability criteria.

The list of records for the full-text examination should note whether each record is "relevant" or "not relevant."

4.5.3. Recommendations for Summarizing the Literature Review.

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 regarding the key terms supporting the safety assessment.

The date that the literature search was conducted should also be well documented. If needed, the researcher can perform an additional literature search starting from the last retrieval date. Limiting the search to specific years is possible, but a sound justification should be provided.

4.5.4. Criteria for Demonstrating "Sufficient Data Available."

For data to be deemed sufficient and utilized to satisfy a safety assessment, two key concepts must be addressed: relevance and reliability (Table 1 and 2, respectively). These are defined in Rudén et al. [25], Klimisch et al. [18], and Moermond et al. [20].

Regarding relevance, look at the extent to which the data is suitable for the safety assessment. Only studies that are considered relevant should be assessed for reliability.

Regarding reliability, consider "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" [21].

When reviewing publications in the public domain for relevance and reliability, consider if the study results are presented as a full article (i.e., not an abstract), if the paper is the primary source of the data, if the information is publicly available, and if the language of the article is not an impediment to the interpretation of the findings.

For assistance in reviewing and evaluating ecotoxicology studies, the ToxRTool [18] should be utilized following the approach described by Moermond et al. [21].

4.6. Recommendations on Developing Study Protocols or Scientifically Sound Rationale

Study protocols have been published by EPA [32], OECD, and other regulatory bodies to assess the safety of substances and microorganisms used in agriculture. While recognizing plant biostimulants are not pesticides, companies are recommended to consider these methods when developing protocols or rationale to support the safety of their substances (Table 3) or microorganisms (Table 4).

4.7. Recommendations on Labeling Guidelines and Developing Guaranteed Substance Safety Literature

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 Globally Harmonized System of Classification and Labelling of Chemicals [30] guidelines and appropriate Safety Data Sheets (SDS) development [31].

Where data does 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.

5. Considerations in Using these Recommendations

As described in the introduction, these recommendations were 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 recommendations are not intended as "requirements," product developers are encouraged to employ them to strengthen the overall credibility of their plant biostimulant and, by extension, the category with regulators, growers, and consumers.

In employing the recommendations for developing a new biostimulant product, companies can view them as best practices and determine how and what aspects will be implemented based on resources, use patterns, and the product itself. For example, when planning studies to demonstrate a product's benefits, a company may consider the recommended experimental design, crops, number of trial locations, or statistical analysis methods 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 product type, 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 proper. A company able to document such information will provide greater confidence to consumers and regulators on their product quality and consistency. Demonstrating a lack of heavy metal contaminants or microbial pathogens will 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 provide confidence in their product's overall safety. Demonstrating 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 widespread use of biostimulants expands.

6. Conclusion

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

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Appendix A - Figures

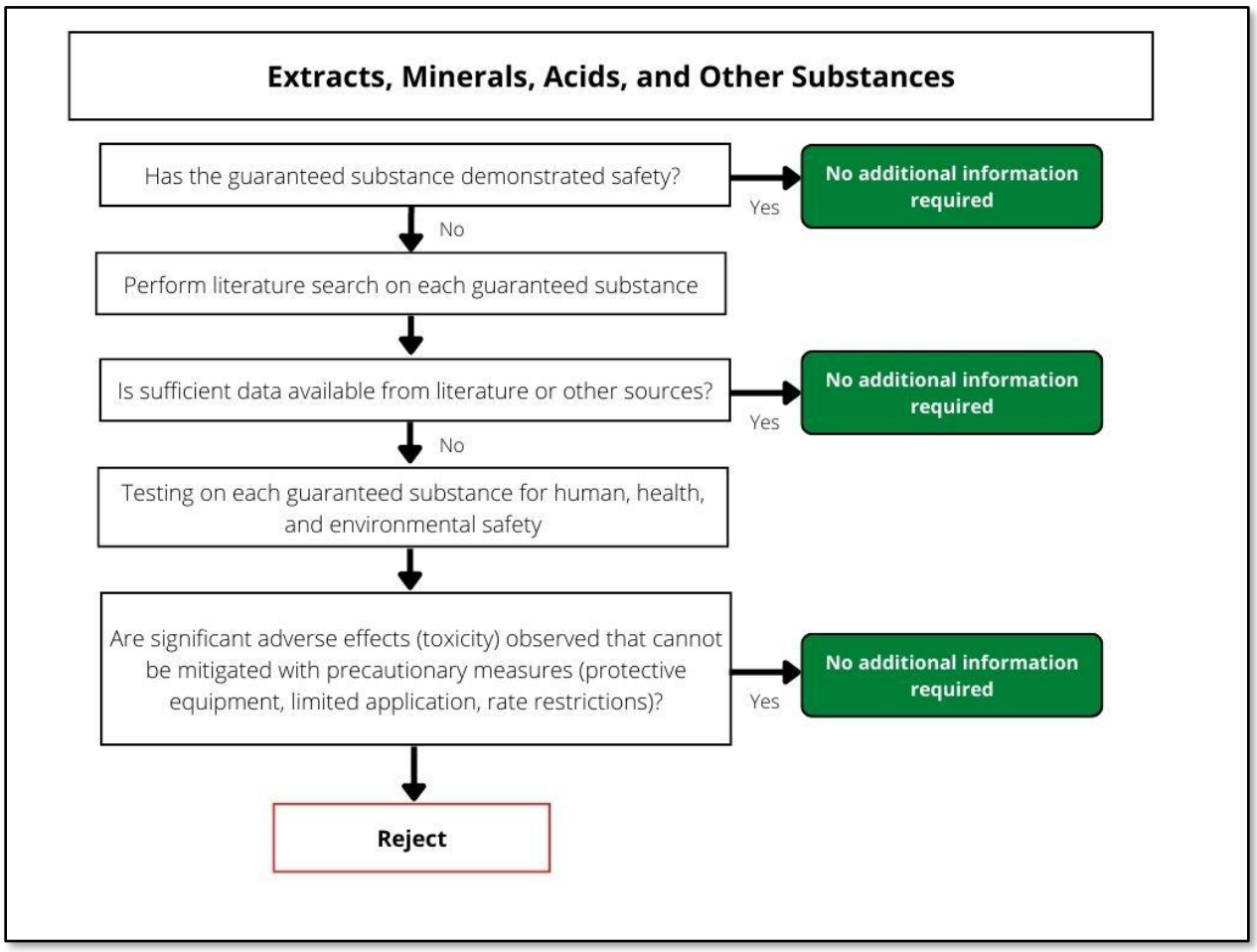

Figure 1. Decision tree for assessing the human health and environmental safety of plant biostimulant derived from extracts, minerals, acids, and other guaranteed substances.

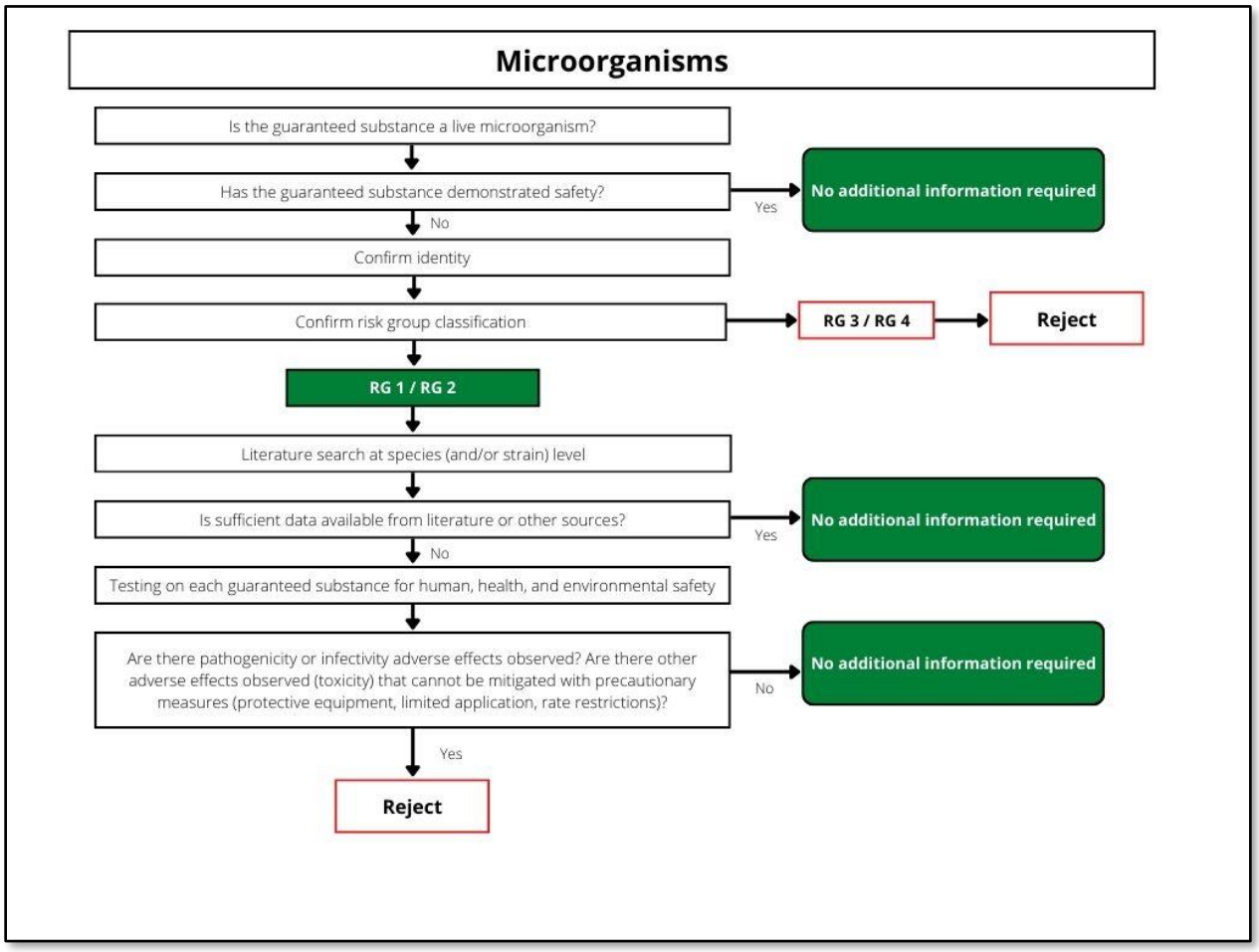

Figure 2. Decision tree for assessing the human health and environmental safety of microorganisms as plant biostimulant guaranteed substances.

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 appropriately identified (species, strain, sex, age)?
- Was there a clear and comprehensive description of material and methods, including location, replicates, and test conditions, and were they appropriate to the objective of the investigation?
- Was feeding of test animals appropriate to maintain health (depending on the duration of the study)?
- Was the 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 clearly and appropriately (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?

Table 3: 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 irritant.[2]
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.

[2] 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, 2022

Table 4: 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 microorganisms, 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).