Regulatory Considerations for Probiotic Path-to-Market

Prepared by:

James Akingbasote, PhD

Samir Samman, PhD

Andrew Charrette, MSc

Corey Hilmas, MD, PhD

Najla Guthrie, MSc

KGK Science

Premium
Clinical Research &
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About KGK Science

KGK Science is a premium contract research organization (CRO) offering high-quality clinical research trials and expert regulatory support for the nutraceutical, cannabis and hemp industries.

Over 23 years, KGK has successfully helped hundreds of companies with custom designed clinical trials and claim substantiation strategies that move products efficiently into global markets. Equipped with state-of-the-art technologies, novel research techniques, and a seasoned team of industry experts, KGK Science remains at the forefront of our industry, consolidating scientific, clinical, commercial, and regulatory expertise with innovation and agility to serve the expanding needs of global business and consumers.

Key Messages

- Understanding the intersection of the science and regulation of probiotics is crucial to developing a successful path to market and reducing regulatory risk.
- Major differences exist in applicable US and Canadian regulations that govern the introduction of new probiotics into the respective jurisdiction.
- Companies considering bringing probiotic products into the market should hire a consultant to navigate the complex science and regulatory pathways.

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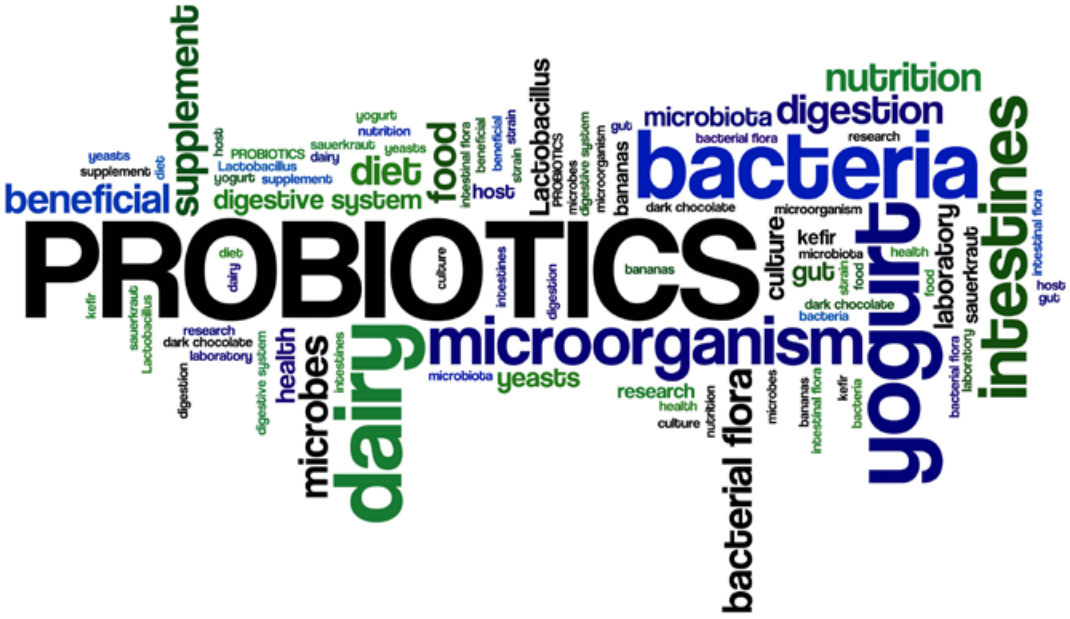
Probiotics

Microorganism, which are now known as probiotics, have been researched for over 100 years with the very early observations suggesting that "bifid" bacteria may be used to overcome diarrhea in children. Probiotics are defined by WHO/FAO as live microorganisms which when consumed in adequate amounts as part of food confer a health benefit on the host [1]. In 2014 the International Scientific Association for Probiotics and Prebiotics fine-tuned the definition of probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [2]. Although the definition has evolved over the years, it is clear that the term probiotic is restricted to products that contain live microorganisms and in sufficient doses to exert a health benefit. While US FDA does not formally define the term "probiotic," the term can be used on labels, but the Agency prefers to use "live microbial ingredients" when referring to probiotic dietary ingredients used in supplements.

To meet the criteria of a probiotic, the ingested microorganisms should be resistant to the actions of gastric juices and bile acids and be able to thrive within the gut microbiota. The most common probiotic microorganisms are Gram-positive bacteria obtained primarily from *Lactobacillus* and *Bifidobacterium* species.

> **"Probiotics are live organisms which when consumed in adequate amounts as part of food confer a health benefit on the host."**
>
> *World Health Organization*



The Gut Microbiota

Gut microbes contribute to human health through enhancement of immunity, metabolic and neurologic functions [3]. Gut microbiota is responsible for fermentation of non-digestible food material in the gut, termed dietary fiber or "prebiotics,' and the resulting products of fermentation are utilized by intestinal epithelia to encourage growth of more microorganisms. The microbiota is the community of microorganisms housed in the organism, while the microbiome refers to the genetic aggregate of all microbiota residing on or within human tissues and biofluids along with the anatomic sides in which they reside. Approximately 100 trillion microorganisms (bacteria, viruses, fungi, yeast and protozoa) exist in the human gut, and increasingly, the microbiome is now considered its own organ of the body. The human microbiome encodes many more genes than the human genome, producing thousands of metabolites, which complement many of the functions of the host, consequently influencing human health, including reduction in rates of obesity and insulin resistance [3]. It is becoming increasingly clear that having the right complements of microorganisms is important for maintaining good health. Dysbiosis, possessing a lower diversity of gut microorganisms, is thought to play a significant role in the development of disease states encountered in the human life cycle (Figure 1). Obesity, autoimmune diseases, cardiometabolic conditions, and infections/inflammation in vulnerable populations (e.g. elderly, infants) can result from dysbiosis or an imbalance in bad/good bacteria. Dysbiosis promotes these adverse health effects by a variety of mechanisms including altered gut hormone regulation, insufficient microbiome diversity to crowd out "bad" bacteria, and proinflammatory mechanisms [4]. Thus as the composition of the gut microbiota changes with age and health status, the chances of disease-related dysbacteriosis or dysbiosis increase [3,4].

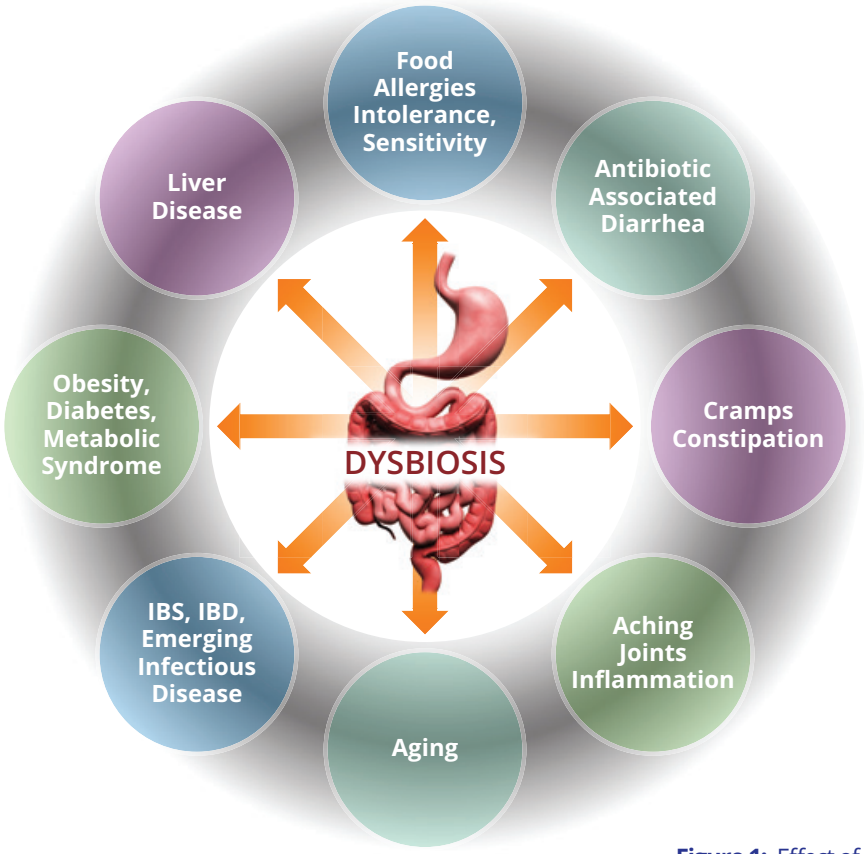


Figure 1: Effect of Dysbiosis on Human Health

Probiotics and the Human Diet

Probiotic organisms have been an integral part of human diet for millennia. Probiotics can be traced back to the first use of cheese and fermented products by the Greeks and Romans who recommended their consumption for children and adults recovering from sicknesses. Communities around the world have relied on microorganisms for food production including the fermentation of a vast range of food products which confers on them properties that either enhance their shelf-life and other properties that make them suitable as food. From cheese and yoghurt in most of the middle-eastern countries, Asia, and Western societies to food products like ogi in the southern hemisphere, micro-organisms play an important role in human diet [Box 1] [5].

Fermented or cultured dairy products are a major source of probiotics. The constituent bacteria either occur naturally in these foods or have been added during preparation. Probiotics are also available as dietary supplements [6].

Of note is the fact that most probiotic organisms that are used in food production are part of the human gut microbiota. For example, *Bifidobacterium* species are among the first organisms to colonize the human gut after birth where they provide immune protection to the host and help in digestion of human milk oligosaccharides. Prominent among food-borne probiotics are *Bifidobacterium bifidum, B. lactis, B. breve, B. longum,* and *B. infantis* that have been found in foods such as yoghurt, cheese, and Kimchi. Other common strains of probiotics which have found their way into the human food chain are *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. gasseri, and L. plantarum; Enterococcus faecium, and the yeast Saccharomyces boulardii [6].*

Box 1: Some Examples of Probiotic-containing Food Consumed Globally

Africa:	Ogi, iru, *incwancwa, Amasi*
South and Central America:	Almidón agrio, chicha, champús, and masa agria
Asia:	Kombucha, Kefir, Buttermilk, Bánh cuốn
Europe:	Cheese, Yoghurt, Hákarl, Žinčica, Surströmming

Current State of the Probiotic Industry

There is a global increase in demand for probiotic products. Currently, the probiotic market has an estimated value of USD 41 billion and is projected to increase to USD 76 billion by 2024, registering an annual growth rate of more than 10% during the forecast period (2019 - 2024) (Figure 2). The probiotic market is driven by the robust demand for health-based products, among consumers, especially by the younger generation [7]. Aside from the food and the dietary supplement industries, there is growing interest in the sports nutrition industry where scientific evidence is pointing to the potential of probiotics as being efficacious in enhancing endurance and improving inflammatory conditions in some athletes (see below).

To key in to this projected growth in the probiotics space, how can companies meet regulatory requirements to ensure success in bringing products to market?

Projected Increase in the Probiotic Market Value

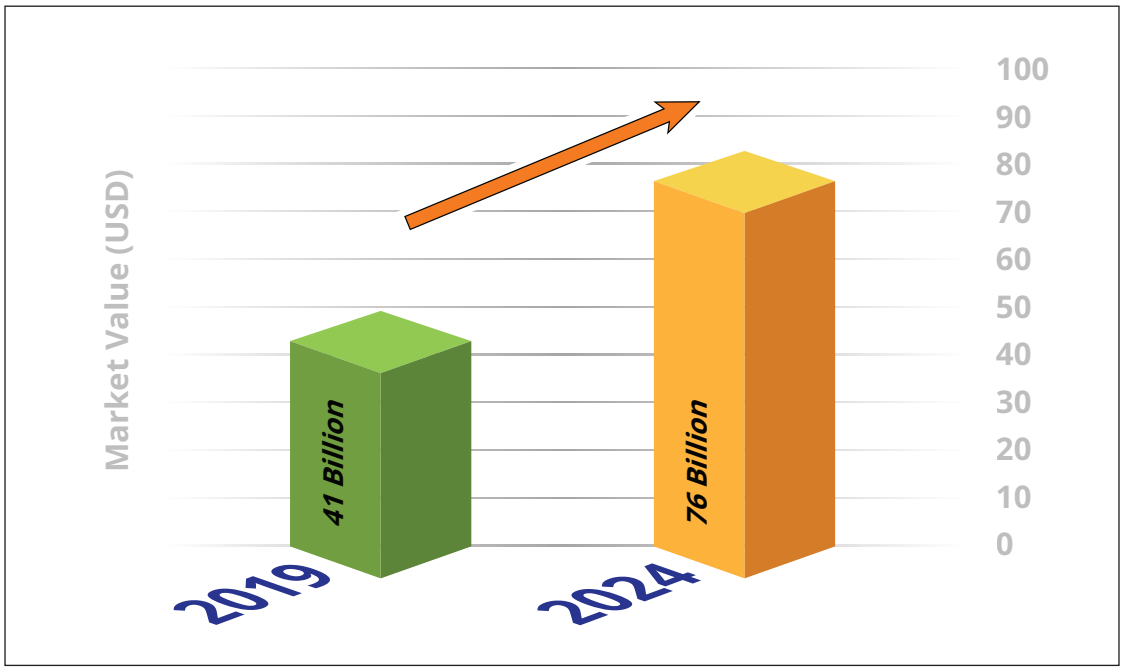


Figure 2: Current Market Value of the Probiotic Industry and its Projected Growth up to 2024

The Regulatory Landscape

A key strategy for success is an understanding of the regulatory landscape of the country where the product is being introduced. The focus of the ensuing discussion will be the path to market for probiotics in the USA and Canada. To a large extent, the requirements in these jurisdictions are similar: to provide a high level of evidence to define the ingredient and demonstrate its safety. However, there are specific areas of important difference in the regulation of the US Food and Drug Administration (FDA) and Health Canada that relate to new live microbial ingredients and their routes to market (Box 2).

Box 2: Pathways to Marketing Probiotics in the US and Canada

New Dietary Ingredient Notification (NDIN)

Generally Recognized As Safe (GRAS)

Food Additive Petition

Natural Health Product (NHP)

Foods/Novel Foods

The Regulatory Landscape

A major difference between FDA and Health Canada are the potential routes available to companies. For instance, in the US, a probiotic can be marketed and sold as a dietary supplement through incorporation of either 1) an old, dietary ingredient (grandfathered, pre-DSHEA dietary ingredient); or 2) a new dietary ingredient (NDI), requiring pre-market notification to FDA. If the ingredient is a live microbial not listed in the Codified Federal Regulation's (CFR's) food additives list[1] or in FDA's GRAS Notice Inventory,[2] one can opt for a GRAS (Generally Recognized As Safe) conclusion or food additive petition to enter the market. The NDI route is a notification system that relies on the ability of the notifier to show reasonable expectation of safety under the conditions of use in labeling. If the new dietary ingredient has never been marketed and sold in interstate commerce prior to October 15, 1994, it is considered "new" requiring a notification to FDA. NDIs must be filed to FDA within 75 days of going to market with identity information and toxicology information that is the basis for concluding a reasonable expectation of safety for the NDI.

GRAS conclusions are safety reviews conducted by "experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures to be safe under the conditions of its intended use." Fundamental to all GRAS conclusions is the criterion that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.[3][8] Furthermore, GRAS conclusions require the recognition of safety through scientific procedures based upon application of generally available and accepted scientific data, information, or methods, which ordinarily are published. There is a requirement that the studies used as the basis for the GRAS conclusion must be published, but this does not imply that it must be published in a journal. The studies must be made available to the general public in some way. A GRAS conclusion can be either submitted to the Agency (FDA-affirmed GRAS notice or conclusion) or not submitted to FDA (self-GRAS or independent GRAS conclusion). The final pathway is one that alters the Codified Federal Regulations through filing a food additive petition. This route petitions the Agency to amend the Federal Food, Drug, and Cosmetic Act to add this petitioned food to the list of food additives.

Because GRAS conclusions should be made public, both independent GRAS conclusions and FDA-affirmed GRAS notices do not fully protect the intellectual property investment of a manufacturer or distributor. Food additive petitions are also public submissions where FDA allows for stakeholders to comment on the submitted data. Self-affirmed or independent-GRAS conclusions provide more IP protection than an FDA-affirmed GRAS notices or food additive petitions. In many cases, companies would wait for another company's FDA-affirmed GRAS notice or food additive petition to be filed to the federal docket before completing their own GRAS conclusion or FDA-affirmed GRAS notice to the Agency. NDIs offer the greatest IP protection because the NDI safety dossier can be fully redacted when submitted.

In Canada, products can enter the market as a Natural Health Product [9] and a product cannot be marketed until Health Canada is fully satisfied that the product meets all the relevant criteria. This is similar to the FDA's food additive petition or FDA-affirmed GRAS conclusion pathway. It differs markedly from FDA's NDI notification pathway and independent GRAS conclusion pathway. NDIs are a notification pathway. Independent GRAS conclusions do not involve submission or notification to a federal health authority.

Table 1 (see next page) shows some key similarities and differences between regulations that affect probiotics in US and Canada.

1. https://www.fda.gov/food/food-additives-petitions/food-additive-status-list
2. https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices
3. See 221 CFR 1170.30(a).

Table 1: Comparison of US vs Canada Regulations for Probiotics

	United States	Canada
Regulatory Definition for Probiotic	No definition, FDA uses the term "live microbial ingredient"	Uses WHO definition
Statement of identity required for probiotic products?	Yes, manufacturers and own label distributors must include a statement of identity for their live microbial ingredient-containing food product ✓	Yes, NHP license holders must attest to the identity of their probiotic ingredient and state on label. Foods making probiotic claims must also include identity statement. ✓
Can "Probiotic" be permitted on food labels?	Yes, manufacturers may declare "Probiotic Supplement" as the statement of identity to describe ingredients contained in the product ✓	Yes, manufacturers may use the term "Probiotic" or similar but must also include probiotic, function or health claim ✓
Probiotics include what category of product	Dietary Supplements or conventional foods ✓	Natural Health Products and conventional food ✓
Nutrition information/Listing of Ingredients	Declared under the heading "Supplement Facts" [4] ✓	Declared under the heading "Nutrition Facts" for foods. Ingredient listed for NHPs [5]. ✓
Listing of ingredient quantity	The total quantitative amount by weight per serving in metric units must be declared for each live microbial ingredient, unless individual ingredients are listed as part of a "probiotic blend" ✓	Total quantitative amounts per serving in colony-forming units (CFUs) should be declared for each probiotic ingredient. ✓
Are Colony Forming Units (CFUs) allowed on probiotic labels?	Yes, CFUs per serving can be voluntarily added to the label, as long as the quantitative amount by weight per serving in metric units is also declared ✓	Yes, this is the standard for declaring quantity of probiotic ingredients per serving of the products ✓
Pre-Market Authorization or Notification of Novel or New Ingredient	Either New Dietary Ingredient Notification, GRAS conclusion, or Food Additive Approval required for any new live microbial ingredient ✓	Premarket review required for all NHPs. Product license required before marketing any NHP. For a new organism, a new-substance notification to Environment Canada is required. For foods, novel live microbial ingredient may qualify as a "novel food" and require premarket review ✓

4. Manufacturers may choose to market probiotic products destined for the US market as conventional foods even when in forms thought to be reserved for pharmaceuticals and dietary supplements (e.g. capsules, caplets, gel caps, tablets, sachet packet). Therefore, probiotics in the US market can be declared as conventional foods with the heading "Nutrition Facts" in the US, but the ingredients, including live microbials, must be approved food additives or GRAS for use per the intended use of the product. In the US, the form (capsule, tablet, gelcap, sachet packet, etc.) does not determine whether the product is a conventional food or dietary supplements.
5. NHP labelling regulations will be changed in 2020 to include a NHP facts panel

The Regulatory Landscape

Table 1: Comparison of US vs Canada Regulations for Probiotics (Continued)

	United States	Canada
Mandatory Efficacy or Health Claims	Structure function claims, while not mandatory to be present on a product, are required by law to be substantiated with efficacy data.[6] Health Claims and Qualified Health Claims require Citizens Petitions to FDA with efficacy data.[7] ✓	In order to meet the definition of a probiotic an efficacy/health claim is required. All licensed NHPs require a claim to be included as the recommended use. ✓
Expiry Dating on Finished Product	No mandatory requirement, however, most companies voluntarily disclose expiry, shelf-life, "use by" or "best if used by" dating or adopt 2-year shelf-life beyond date of distribution ✗	Expiration date required on all NHPs and certain foods. Best before date required on foods that perish in 90 or less. ✓
Conditions of Use	Yes, conditions of use should be disclosed to communicate intended use of the product and indicate target populations (e.g. infants, children, etc.) that should not consume the product ✓	Yes, conditions of use are required as part of the NHP product license application and label ✓
Storage Conditions	Storage conditions are not mandatory, but they should be disclosed to consumers if they affect stability of the product ✓	Storage conditions are mandatory on foods requiring best before date and NHPs if recommended conditions exist. ✓
Requirement for declaration of cryoprotectants and lyophilization ingredients?	Yes, while most processing aids MAY be exempt (because they have no technical or functional effect, are removed before packaged in finished form, are converted into constituents normally present in the food, OR are present in insignificant levels), cryoprotectants and lyophilization ingredients have a functional or technical effect.[8] ✓	There is a requirement to declare the amount of cryoprotectant if it is at a detectable level in the final product or if it a priority allergen. ✓

6. Statements of nutritional support on probiotics, including "helps support intestinal/gastrointestinal health, are considered structure function claims. Structure function claims are permitted on US dietary supplement products as long as the advertiser possesses substantiation for the claim.
7. No health claims or qualified health claims have been approved for probiotic live microbial ingredients by US FDA. Health claims are approved disease claims and involve evaluation to the Significant Scientific Agreement (SSA) standard, which typically requires two RCTs.
8. Most processing aids are not required to be declared in the ingredients list on the food label because processing aids, by definition, have no technical or functional effect in the finished food OR because they are not present OR are present at insignificant levels in the finished food. Cryoprotectants and lyophilization ingredients do have a functional effect in a finished food because they serve a functional effect for the ingredient, which is considered a food in the US. In accordance with 21 FR 101.100(a)(4), added processing aids are considered present in an insignificant amount only if no detectable amount of the agent is present in the finished food. In other words, if you can detect it through chemical testing, OR it is there as a technical or functional effect, then these processing aids should be declared.

	United States	Canada
Food Allergens	The Food Allergen Labeling and Consumer Protect Act of 2004 requires declaration of the 8 FALCPA-designated allergens (milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) [9] ✓	Health Canada requires that any Priority Allergens (milk, eggs, fish, mustard, crustacean and molluscs, tree nuts, peanuts, wheat and triticale, sesame seeds, sulphites and soy are declared for NHPs and Foods ✓
Preventive Controls and GMPs for probiotic ingredient suppliers?	Yes, the 2011 Food Safety Modernization Act (FSMA) gave FDA the authority to evaluate ingredient suppliers through inspectional authorities and mandate regulatory requirements directed at quality of the ingredient supply chain? ✓	NHP ingredients must meet GMP standards for quality. Safe Food for Canadians Act and Regulations (2019) implemented supply chain preventative controls for foods ✓
Good Manufacturing Practices for Probiotic Finished Products?	The dietary supplement cGMP final rule of June 2007 addressed finished product quality and requires probiotic supplements to meet established specifications for identity, purity, strength, composition and limits on contaminants. ✓	NHP must be manufactured or imported by licensed sites which are required to meet GMP standards. ✓

9. As an example, casein and whey can offer survival benefit to certain encapsulated strains in products. Therefore, if the ingredient casein, whey protein or both are present in the probiotic product, the product must contain the phrase "Contains milk" below the ingredients listing of the nutrition information.

Regulation of Probiotics in the US

In the United States, the main routes to bring a probiotic product to market are New Dietary Ingredient (NDI) notification, Generally-Recognized-as-Safe conclusion and Food Additive petition. This section describes the process of making an NDI notification; it further summarizes how a GRAS conclusion is made for the addition of an ingredient into food.

What is an NDI Notification?

New Dietary Ingredients are defined in section 413 of the Federal Food Drug and Cosmetic Act (FD&C Act) as those ingredients that have not been used in food in the USA prior to the enactment of the 1994 Dietary Supplement Health and Education Act (DSHEA) [10]. The FD&C Act requires that manufacturers and distributors who wish to market dietary supplements that contain "new dietary ingredients" notify the FDA about these ingredients. The notification must include information that is the basis on which the manufacturer has concluded that a dietary supplement containing a new dietary ingredient will reasonably be expected to be safe under the conditions of the recommended intake.

An NDI notification is not required however, if there is evidence of the ingredient's use prior to enactment of the 1994 DSHEA Act. Examples of evidence include product labels naming the probiotic ingredient, or search outputs of scientific databases to verify whether the probiotic strain is a new dietary ingredient or whether there are reports of its use prior to 1994.

How are Probiotics Categorized Under the NDI Regulation?

The FD&C Act defines [10] a dietary ingredient as one of the following [11]: (A) vitamin, (B) mineral, (C) herb or other botanical, (D) amino acid, (E) A dietary substance for use by man to supplement the diet by increasing the total dietary intake, or (F) A concentrate, metabolite, constituent, extract, or combination of any ingredient described in (A), (B), (C), (D), or (E). FDA does not have a separate regulatory category or definition for dietary ingredients consisting of live or viable microorganisms. Probiotics are therefore considered under category E [11] which consist of dietary substances for use by man to supplement the diet by increasing the total dietary intake. New Dietary Ingredients have a specific adulteration provision in DSHEA. The adulteration standard [12] applies to all dietary supplements that contain an NDI, even in situations when no notification is required because the supplement contains only dietary ingredients that have not been chemically altered. A dietary supplement containing an NDI is considered adulterated (by default) unless there is adequate information to provide reasonable assurance that the NDI does not present a significant or unreasonable risk of illness or injury.

10. As defined in section 201(ff)(1) of the FD&C Act (21 U.S.C. 321(ff)(1))
11. As defined in section 201(ff)(1)(E) of the FD&C Act (21 U.S.C. 321(ff)(1)(E))
12. 21 U.S.C. 342(f)(1)(B)

Evaluation of the NDI Notification and Safety Considerations

Any NDI notification is assessed for identity and safety. Some of the main questions to be answered in the assessment of an NDI notification for probiotics strain include evidence of the absence of the following: genetic elements encoding virulence factors, toxins, functional and transferable antibiotic resistance genes, production of antimicrobial substances, and genetically-modified organisms using rDNA techniques, and whether the ingredient has a history of safe use in food.

In evaluating the identity of the live microbial ingredient, FDA's scientists look for a detailed description of the organism, such as the strain, genetic elements and absence of toxins (as indicated above), the methods used to establish strain identity and its relationship to other strains of the same species which are present in food or dietary supplements. Other required information includes the method of manufacturing of the product and its shelf-life.

Safety assessment seeks information on the tolerability of the organism when ingested by humans. In particular, evidence from clinical trials carried out in a similar population as the intended market. For example, if the product is proposed for use in adults 18 years and above, the clinical trial study which is submitted as evidence of safety must have been conducted in people of equivalent age range. The same applies to other aspects of the product like the serving size and frequency of consumption.

After having submitted an NDI notification, the Agency carries out a thorough assessment of the ingredient for identity and safety. Different aspects of FDA rules applies in the assessment of the entire notification. For example, Title 21 CFR 190.6 (Code of Federal Regulation) stipulates that certain administrative information regarding the ingredient, its manufacturer/supplier etc. be supplied. Failure to provide any part of the required information and check all the relevant boxes can result in what is termed Incomplete Letter (or ICL). Figure 3 below shows the possible types of response that can be received from an NDI notification.

FDA RESPONSE	MEANING
AKL	Acknowledgement Letter No objection letter, "Good Day" letter (= Company has Notified FDA)
IAL	Inadequate Letter FDA has been notified but Agency has comments, or objects to identity, safety, or both (= Company has Notified FDA)
ICL	Incomplete Letter (= Company has failed to Notify FDA)
NDL	Not a Dietary Ingredient Letter FDA does not consider it to be dietary ingredient (= Company has failed to Notify FDA)
Other	RESET letter. FDA has reset the 75-day time frame to respond to the NDI due to a major amendment filed with the NDI

Figure 3: Possible FDA Response to NDI Notification

Regulation of Probiotics in the US

How to Achieve a Successful Notification

The likelihood of a successful notification (code AKL) is dependent on the provision of key data. For instance, if the notification includes data from preclinical studies, such a study must include analysis of all markers of safety (hematology, blood chemistry, adverse reaction, vital signs and other physiologic parameters); and an appropriate safety factor must be applied that considers the study design (acute studies or subchronic studies) and the uncertainly factor relating to intraspecies variability between humans and the study model (typically a factor of 10 when extrapolating from rodent models to humans). Applying the appropriate safety factor ensures that sensitivity between the test model and the intended users (humans) is considered. This enables the estimation of the margin between the proposed serving size (dose) of the product and the highest safe dose (or NOAEL. See below) in the test model. Many submissions are rejected because of failure to provide data on the maximum tolerated dose – a study usually conducted in animal models. It is important to know the dose that results in lethality in order to calculate the maximum dose (or serving size) before the appearance of adverse effects. This is known as the No-Observed-Adverse-Effect Level (NOAEL). The equivalent of this in clinical studies are tolerability studies which are essential for some types of ingredients.

In order to ensure a successful NDI notification, experienced consultants such as those at KGK Science combine the evidence from the literature with well-designed studies and follow a well-structured and incisive approach in analyzing ingredients (Figure 4). This approach has led to many successful NDI notifications with AKL responses.

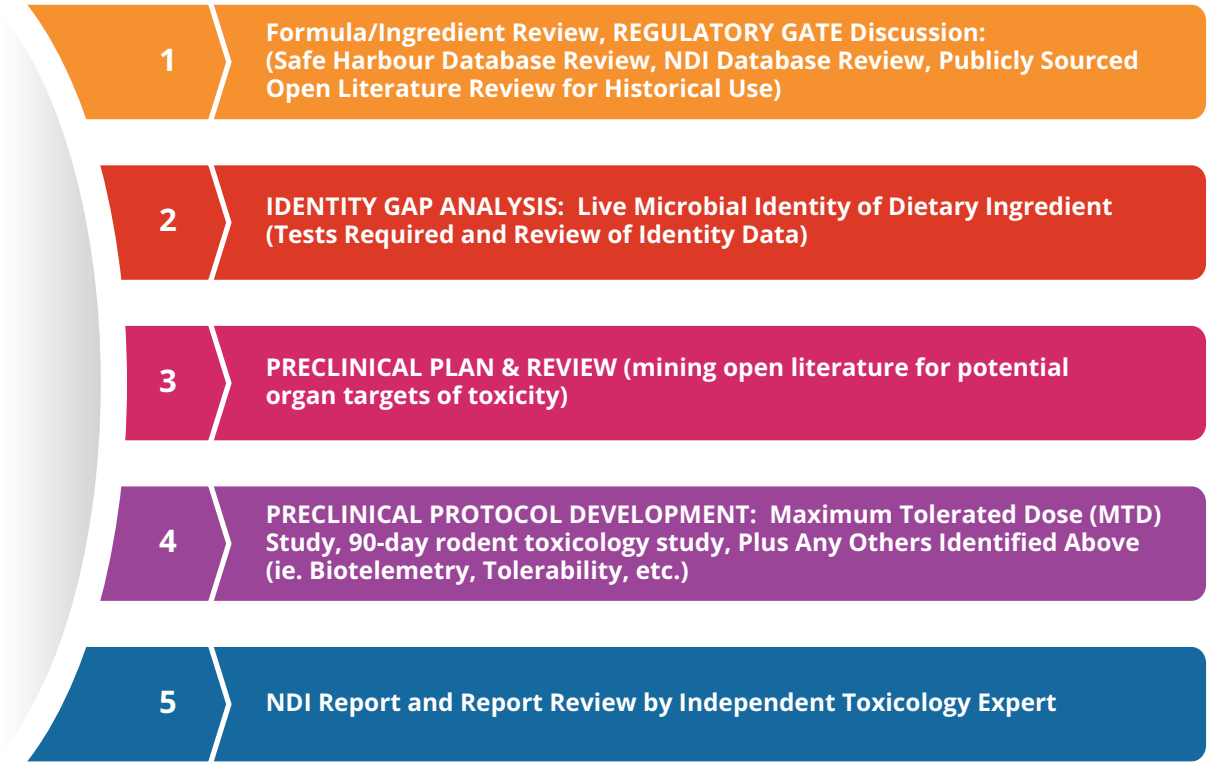


Figure 4: NDI Pathway to Market

Benefits of Making an NDI Notification

Gaining a successful NDI notification produces a high level of confidence by consumers in a given product since it has passed through the rigorous FDA regulatory process. Other benefits of a successful NDI notification include: market exclusivity (patent the NDI Number), Intellectual Property protection, and reduced regulatory vulnerability. In the presence of a successful NDI notification, the burden shifts to FDA to show that the ingredient is unsafe. Without notifying, the NDI can be deemed adulterated for failure to submit an NDI notification and lacking any safety data to show otherwise.

GRAS Conclusions for Live Microbial Ingredients Used in Food

Generally Recognized as Safe (GRAS) is an important route to market a new probiotic product in food in the US [8]. Sections 201 (s) and 409 of the Federal Food, Drug, and Cosmetic Act sets how to determine that an ingredient is GRAS. The provision states:

> **"Any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excepted from the definition of a food additive."**

The provisions stated above and FDA regulation in the 21 CFR 170.3 and 21 CFR 170.30 determine that a food substance may be GRAS either through scientific procedures or, for a substance used in food before 1958, through experience based on common use in food. General recognition of safety is required through scientific evidence at the same quantity and quality as is required to obtain approval of the substance as a food additive[13]. General recognition of safety is based on the application of available and accepted scientific data, which ordinarily are published. Recognition of safety can also be achieved through experience based on common use of the ingredient in foods [14], and requires a substantial history of consumption for food use by a significant number of consumers.

The same level of evidence required for NDI is needed to determine the GRAS status of a probiotic organism. There are two main ways that a GRAS dossier can be presented. One way is by directly notifying the FDA of the GRAS status of a given ingredient. FDA will evaluate the ingredient and determine whether the probiotic ingredient qualifies for GRAS status. Another route is the self-affirmed GRAS determination which is identical to the process described above with the exception that notification to FDA is not required. In both cases, the notifier needs to demonstrate similarity between the ingredient being proposed for a GRAS status and the one with either substantial history of safe use or assessed by scientific methods. Similar to NDI, information on the identity and safety of the probiotic ingredient has to be provided. The conditions of use of the food ingredient is expected to be exactly the same as what has been historically used in food or shown in scientific journals.

13. 21 CFR 170.30(b)
14. 21 CFR 170.30(c) and 170.3(f)

Regulation of Probiotics in Canada

The path to market in Canada for probiotic strains proceeds either as an ingredient of a natural health product (NHP) or in a food. This will be discussed in greater detail in the sections that follow. Meanwhile, there is an initial regulatory requirement that companies producing or importing probiotics into Canada need to consider.

New Substance Notification

If the probiotic strain is entirely new to Canada, the impact of this strain on humans, the environment and ecological diversity needs to be considered [12]. Environmental preservation and minimizing risks to Canadians from environmental pollution, fall under the provision of the Canadian Environmental Protection Act (CEPA 1999). CEPA requires that substances be identified and assessed, prior to market introduction, to determine whether they are "toxic" or potentially toxic to human health, the environment, or to its biological diversity. This premarket assessment requirement encompasses chemicals, polymers and living organisms. If the substance is not listed in the Domestic Substances List (DSL) [15], it must be notified according to the provision of CEPA 1999.

A New Substance Notification (NSN) details information regarding the identity, manufacturing process and controls and hazard assessments done on the substance. For microorganisms, notifiers are required to identify the strain by phenotypic and genotypic characterization. NSNs are assessed jointly by Environment Canada and Health Canada to determine whether there is a potential for adverse effects of the substance on human health, the environment or its biological diversity. After the trans-departmental assessment of the notification [13], the notifier will be advised as to whether there is suspicion the organism is toxic or capable of becoming toxic as described in Table 2.

Table 2: Health/Environment Canada Response to New Substance Notifications and their Corresponding Explanations

Health/Environment Canada Response	Explanation
A	Organism not suspected of being toxic or capable of becoming toxic
B	Suspicion that organism is toxic or capable of becoming toxic
C	Limiting the purpose for which a substance may be used
D	Suspicion that a significant new activity in relation to the organism may result in it becoming toxic

15. The DSL is an inventory of substances commercially used in Canada that is the standard by which a substance is deemed to be new. It was initially based on substances used commercially between 1984 and 1986 and is added to via New Substance Notifications (https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/substances-list/domestic.html).

If the organism is suspected to be toxic or capable of becoming toxic (response B), the following may be required: (i) controls on import and manufacture, or (ii) prohibition pending additional information. Depending on the outcome, the purpose of the substance may be limited (response C). Finally, a possible eventuality is suspicion that a significant new activity [16] in relation to the organism may result in it becoming toxic, and a new activity notice would be issued. Therefore, once a probiotic strain has been deemed as safe for human use and for introduction into the environment, a submission can proceed to introduce the product into the market as a natural health product (NHP) or as a food.

Natural Health Products and Probiotics

According to the *Natural Health Product Regulations* [9], an NHP is defined as a substance or a combination of substances that are listed in Schedule 1. Probiotics feature on the list of ingredients in Schedule 1. NHPs are further defined as a homeopathic medicine or a traditional medicine that is used in the treatment or prevention of a disease, disorder or abnormal physical state or its symptoms in humans.

As indicated by the name, NHPs are expected to provide beneficial health effects to the consumers. The same is also true of probiotics by their definition. Health Canada employs a similar definition for the term probiotic as the World Health Organization; live microorganisms which when administered in adequate amounts confer a health benefit to the host. It is required, therefore, that probiotics, when included in an NHP, must confer a health benefit. This includes "…benefit to the microbiota indigenous to humans" [17]. The NHP regulations [18] provide that an NHP cannot be marketed in Canada without being licensed, for which a Natural Product Number (NPN) is issued. This premarket review of all NHPs encompasses assessment of the safety, quality and efficacy of the probiotic finished product.

There are well-detailed guidelines as to how to assemble and submit a Product Licence Application (PLA). The first step entails determining the category of ingredient. These are shown in Table 3. As indicated above for NDI, the main considerations, regardless of Class, are identity and safety. A PLA however, also requires the assessment of efficacy as a health benefit is a mandatory element of NHP licensing.

16. For living organisms, "significant new activity" is defined as a new use of the organism that results in significantly different release of the organism (amount, method) into the environment than what was previously approved in an NSN.
17. Natural Health Product Regulation section 1(1)
18. Section 4 (1): Subject to subsections (2) and (3)

Regulation of Probiotics in Canada

Table 3: Category of Ingredient for the Purpose of a Canadian Product Licence Application

Class	Explanation	Review Time
I	Organism complies with the Compendium of Monographs [14] for natural health product ingredients.	**60 Days**
II	The product is a combination of two or more ingredients as listed in Compendium of Monographs.	**90 Days**
III	Applies to any of the following: • New ingredient(s), not listed in the Monograph • Masterfile is being used to support safety, efficacy and quality of the ingredient • The ingredient is listed but its intended use differs in terms of dose and/or claims being made.	**210 Days**

Product Licence Applications (PLA) for Probiotics

In submitting a PLA, issues of identity and safety are paramount. The identity of the organism includes information on the species and strain, and quantification (in colony-forming units), a complete assessment of virulence properties such as antibiotic resistance profile, virulence factor production, and toxigenic activity. Demonstrating a history of safe use in food and NHP is advantageous, and methods such as Genomic Alignment Analysis are useful to compare the strain of interest with those of the same species particularly those that have been approved for use in food and NHPs. Health Canada's Compendial Monograph on Probiotics lists microorganisms that have been pre-cleared for use in NHPs up to the species level. A Class I and II PLA attests to complying with the requirements of the monograph which includes characterizing the probiotic up to strain level as part of the quality testing. Attesting to pre-cleared information reduces the PLA review time required by Health Canada, leading to an expedited issuance of the licence. Microorganisms that are not listed in the Probiotic Monograph may be included in an NHP, but a full review of the quality, safety and efficacy information is required. These are considered Class III PLAs, as are the applications for any NHP that does not completely comply with a Compendial Monograph standard.

A significant portion of all Classes of PLAs includes the attestation to the NHP being manufactured according to Good Manufacturing Practices (GMP). The Canadian standard for GMP is very similar to that used in the US and involves establishing specifications that ensure that quality batch testing verifies the product is consistent and safe. Class III PLAs may require that some of the details of the testing specifications are submitted.

As described above for NDI notifications, demonstration of safety in Class III PLAs includes data from high quality studies in animal models for the determination of NOAEL. Evidence also needs to be provided for safety in humans, which can be based on peer-reviewed published literature on the same or similar organism.

All NHPs must have at least one health claim. These are broadly categorized as claims by health conditions (e.g. diabetes), health effects and general health claims [15]. Claims pertaining to probiotic NHP will likely fall under the category of claims by health effects that will allow statements as to whether the product diagnoses, treats, cures, reduces risk of or prevents diseases. Some probiotic claims may also relate to general health maintenance. Some examples of claims are shown in Table 4.

Table 4: Examples of Validated Claims for Probiotics on the Health Canada NHP Monograph

Probiotic Organisms	Health Claims
Lactobacillus johnsonii Strains La1, Lj1 and NCC 533	Management of *H. pylori* infections
All strains of *Saccharomyces boulardii/ Saccharomyces cerevisiae*	Risk reduction of antibiotic- associated diarrhea
Bifidobacterium adolescentis *Xanthophyllomyces dendrorhous* *Kluyveromyces lactis*	Helps support intestinal/ gastrointestinal health

Regulation of Probiotics in Canada

While the claims shown above relate mostly to disease prevention, the International Society of Sports Nutrition have referred to preliminary research data which has shown potential probiotic benefits relevant to an athletic population that include improved body composition, normalizing testosterone levels, reductions in cortisol levels indicating improved responses to a physical or mental stressor, reduction of exercise-induced lactate, and increased effects on cognition and mood [16]. These preliminary reports collectively provide opportunities for future health claims for probiotics.

In all cases, Health Canada requires evidence to support the claims. Evidence can range from *in vitro* studies to clinical trials that might have been conducted by the manufacturer or those published in the scientific literature. As described above for NDI and GRAS, the identity of the products, route of administration, frequency and duration of use, and target population must be the same as what is being provided as evidence. Health Canada assesses the evidence submitted with a Class III PLA based on risk classification of the claim and indication. High risk claims are for products that treat, prevent or cure serious health conditions; those that if left untreated are debilitating or life threatening. Medium risk claims are for those that treat, cure or prevent major health conditions; those that do not resolve naturally and require care. Low risk claims are for products that treat, cure or prevent minor conditions or symptoms; those which resolve naturally and in a timely manner or for which lack of efficacy does not pose a major risk. Low risk claims are most commonly made. The standard of evidence required increases with the risk of the claim and is well described in Health Canada's guidance documentation [19].

19. Pathway for Licensing Natural Health Products Making Modern Health Claims, Health Canada.

Assessment of Product Licence Applications (PLA)

There are three main steps in the assessment of PLA.

- **Administrative Verification:** PLA are verified for administrative completeness in a manner similar to NDI, to ensure that all the required documentation is included. Omission of any requirements or mis-classification of the application may result in a Notice of Refusal – Administrative Deficiency.

- **Regulatory Screening** for the relevant regulatory requirements. An application may be refused at this stage if the information is incomplete or inaccurate, or if the probiotic ingredient does not meet NHP definition, Monograph parameters, or lacks documentation relating to efficacy and safety.

- Once that PLA has been screened, a class III PLA will be assessed for requirements regarding **safety and efficacy**. At this stage, a PLA must meet all relevant NHP Regulatory requirements as well as the provisions of the Food and Drugs Act.

Some further clarifications on some aspects of the application may be required. These clarifications or deficiencies are conveyed to the submitter through an Information Request Notice (IRN) which typically must be responded to within 15 calendar days. A deficiency in any of the requirements that is not satisfactorily addressed will attract a Notice of Refusal. If all requirements are met, a Product Licence (with an NPN) is issued [17].

Probiotics in Food

Food products[20] is another path to market for probiotics in Canada. Food products containing probiotic organisms should comply with all relevant food legislations and all regulations relating to food, labelling requirement and advertisement of food products. In order for probiotics to be included in food, it must have a history of being safely included in food.

If the probiotic organism does not have a history of safe use in food, if it is genetically modified or is produced with a novel method, then the food in which it is included may be classed as a novel food [21]. If this is the case, an NSN will be required, however, and it is automatically included as part of the Novel Food premarket assessment. As indicated above with NSNs, the main focus of Novel Food applications is safety in humans and the impact on the environment [13]. Other considerations include the stability and viability of the organism.

To meet the definition of a probiotic and therefore be labelled in a truthful and non-misleading fashion, evidence of a health benefit must be provided. A Health Claim simply implies a relationship between the probiotic and a person's health. Function Health Claims may be considered for probiotic-containing food [15]. They describe the effect of the live microbial ingredient on normal functions of the human body. For instance, the food provides live microorganisms that naturally form part of the gut flora or that the food contributes to healthy gut flora. Therapeutic Claims are those that describe the treatment or mitigation of a disorder or abnormal state and are used in NHPs and Drugs. A therapeutic claim generally causes the food to be classified as an NHP; however, these claims may be considered for foods if the *Food and Drug Regulations* are amended to allow for the claim. This type of regulatory amendment is a significant undertaking.

20. The Food and Drug Act (Section 2) defines food as "any article manufactured, sold or represented for use as a food or drink, including chewing gum, and any ingredients that may be mixed with food for any purpose whatever".
21. Division 28 of Part B of the Canadian Food and Drug Regulations.

Labeling Requirement for Probiotics in US and Canada

The product containing the probiotic organism must be able to deliver the label amount of the organism throughout the shelf life of the product. The US FDA requires that probiotic quantities be disclosed in milligram quantities on the label of dietary supplements. Colony Forming Units (CFU) are a measure of the number of live microbes in the finished product that can also appear on the label of a dietary supplement but must be less conspicuous. In Canada, NHP-containing probiotics must be labelled with CFUs and do not require the weight of the probiotic ingredient to be disclosed. The accuracy of these modes labelling of probiotic active ingredients for the consumer is a topic that has been hotly debated.

In the US, there is no mandatory requirement for disclosing a "best before" date on the label; though most companies voluntarily do so in order to protect the consumer. In Canada, "expiry date" is required for NHPs and a "best before" date is required for foods that are perishable within 90 days. Storage conditions are similarly handled between the US and Canada. In the US, labelling storage conditions are not mandatory but should be disclosed if they affect the stability. In Canada, NHPs must disclose storage conditions if they differ from room temperature storage. Storage conditions are mandatory for foods requiring a "best before" date in Canada.

There are specific requirements for labelling of probiotic-containing products to ensure that all ingredients have been declared in the product to consumers (see Box 3) [18]. It is noteworthy that Health Canada requires manufacturers of probiotics to declare the identity of cryoprotectants, which are chemical reagents used to maintain the viability of the microorganism upon storage. If a cryoprotectant is detectable in the finished product, or if it is a priority allergen, it should be declared. This is similarly mandated for other processing aids used in foods in Canada. In the US, most processing aids are not required to be declared in the ingredients list on the food label because processing aids, by definition, have no technical or functional effect in the finished food or because they are not present or are present at insignificant levels in the finished food. Cryoprotectants and lyophilization ingredients do have a functional effect in a finished food because they serve a functional effect for the ingredient, which is considered a food in the US. In accordance with 21 FR 101.100(a)(4), added processing aids are considered present in an insignificant amount only if no detectable amount of the agent is present in the finished food. In other words, if you can detect it through chemical testing, or it is there as a technical or functional effect, then these processing aids should be declared. As an example, casein and whey can offer survival benefit to certain encapsulated strains in products. Therefore, if the ingredient casein, whey protein or both are present in the probiotic product, the product must contain the phrase "Contains milk" below the ingredients listing of the nutrition information.

One of the most pronounced differences in the labelling of probiotics between the US and Canada is the inclusion of health claims on the label. As dietary supplements are considered foods in the US, health claims are restricted to those authorized in the Code of Federal Regulations Title 21 Part 101. These are claims that have met the standard of significant scientific agreement and for which the regulations have been amended. Qualified health claims are also permitted, which do not meet the same rigorous scientific standard, but which are supported by scientific evidence and for which US FDA have issued a Letter of Enforcement Discretion. There are currently no authorized health claims or qualified health claims for probiotics. Health Canada on the other hand requires health claims and permits NHPs to make therapeutic health claims as described above. There is overlap between dietary supplement, foods and NHPs in structure/function claims that are permitted in both jurisdictions.

Masterfile

Masterfile preparation is one of the core services that are offered at KGK Science. Masterfiles for probiotics in US FDA and Health Canada differ. US FDA is resolving how it will allow companies to update NDI notifications with information on downstream customers who may use the NDI notification as the basis of their reasonable expectation of safety. This is the so-called NDI Masterfile system, but it will require future guidance to build out what is now preliminary. KGK offers a Health Canada Masterfile as a regulatory service. A Health Canada Masterfile is a reference that can be used to provide confidential information to Health Canada about an ingredient. This can be illustrated with the example of a company that cultures and distributes a specific probiotic strain to finished product manufacturers. In order for the companies that use these probiotic ingredients to make a successful application to Health Canada, there is a need to provide all the details about the ingredient including identity information, manufacturing information etc, which are all confidential commercial information (CCI). In order to protect this proprietary information, the producer of the probiotic strain would not need to reveal this directly to the third-party company using their ingredient. All that would be required is to provide this information to Health Canada in the form of a Masterfile. Therefore, the third-party companies will simply have to refer to the Masterfile, the basis of which the ingredient and the final product will be assessed as discussed above.

How to Achieve the Best Outcome in Getting a Probiotic Product to Market

KGK Science, with expertise in NDI notifications, GRAS conclusions, Masterfile, provides consultancy service for both the US and Canadian markets. KGK Science also conducts clinical trials for substantiation of efficacy and safety of probiotics and other products (Figure 5). The regulatory landscapes for probiotics in the USA and Canada are different and sometimes seem overly burdensome and complex. As outlined above, the science is complex and so are the regulations. The requirements differ in the two jurisdictions.

What if You Failed at a Previous Regulatory Filing?

If you have failed to achieve a particular regulatory outcome independently or through another CRO or regulatory service provider, the data suggests for you to keep trying. As Regulatory Consultants, we have an in-depth understanding of both landscapes and are capable of providing expert assistance with filing new applications and advise on any previous unfavorable decisions that might have been received by either Health Canada or FDA. If you have received an inadequate letter by using another regulatory service provider, our in-house experts can analyze and respond to an IAL letter with a new NDI submission to help increase the chance of gaining a successful notification (AKL). Our experts, who have in some cases worked alongside FDA employees, will set up meetings with federal regulators to discuss and address filing deficiencies outlined in your previously received response letters. In this context, the service of a Regulatory Consultant such us at KGK Science is invaluable.

Figure 5: Regulatory Capabilities at KGK Science

Substantiation

Possessing "competent and reliable scientific evidence" is mandatory for US FDA-regulated probiotic products. This is the embodiment of the FDA and FTC substantiation standard. There are 2 methods for demonstrating that a product does what it claims. You can perform a clinical trial on the marketed product, or you can compile a claims substantiation file on each ingredient contained in the marketed product. It is noteworthy that FDA encourages notifiers to meet with them to discuss their applications. In this context, the service of a Regulatory Consultant such us at KGK Science is invaluable. We will conduct a 4-step process to create a substantiation file for your regulatory team. We will analyze the entire body of literature on the ingredients in the product. We will grade the literature by its supporting level of evidence and extract the most relevant information as it pertains to your claims. We look for statistical significance, primary vs. secondary endpoints, effect size, study size, type of study performed, and quality of the written published report. Third, we will create a summary of substantiated scientific statement and assign grades to quantify the level of evidence supporting each statement. Fourth, we take the product claims and match them to the body of scientific evidence to look for gaps between claims made and the scientific statements that can be substantiated by the literature. A Claim Substantiation File is a common and practical way for companies to respond to FTC subpoena requests for substantiated evidence as the basis of your structure function claims.

Summary and Conclusion

The global probiotic market continues to grow and is projected to reach US$76 billion by 2024. Stakeholders should understand the unique regulatory landscape and requirements for introducing new products into the US and Canadian markets. The complex nature of probiotics requires an in-depth understanding of science, regulations, and guidance documents in order to achieve positive regulatory results to be used by marketing. KGK provides significant bench depth to support their clients to achieve favorable responses from US FDA and Health Canada. Product or ingredient safety is paramount for probiotic companies to both remain in compliance with regulations and avoid product liability/punitive damage laws. KGK Science offers pre-clinical and clinical protocol development support to design studies to achieve federal regulatory outcomes. If the study is not designed correctly from the outset, it will lead to significant shortcomings upon review by federal food authorities. Leverage KGK Science's experience from the start to avoid common, costly errors. Retailers are also looking for recognition of novel ingredients by federal authorities, including approval letters from FDA and Health Canada, as a measure of safety, regulatory compliance, and consumer confidence. Our regulatory consultants and strategies are critical in developing a successful path to market and reducing your regulatory risk.

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London - Head Office
255 Queens Avenue, Suite 1440
London, Ontario, Canada N6A 5R8
1-519-438-9374 | **sales@kgkscience.com**