EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF

INOSITOL

AS A FOOD INGREDIENT

Submitted To:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
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May, 2024

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1. PART I- SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR § 170 Subpart E consisting of sections § 170.203 through § 170.285, Sichuan Bohaoda Biological Technology Co., Ltd. (Sichuan) hereby informs the FDA that Inositol (myo-inositol) produced by fermentation with a recombinant *Escherichia coli* BL21(DE3) strain, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Sichuan's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described below.

1.1. Basis of Conclusion:

This GRAS conclusion for the use of Inositol has been reached in accordance with the requirements in 21 CFR 170.220.

1.2. Name and address of organization:

Sichuan Bohaoda Biological Technology Co., Ltd.
No. 9 Longxiang Avenue, Yantan District High-tech Industrial Park,
Zigong city, Sichuan Province，
CHINA

1.3. Name of substance:

The name of the substance of this GRAS assessment is Inositol (myo-inositol). The substance is also known as cyclohexane-1,2,3,4,5,6-hexol.

1.4. Intended conditions of use:

Inositol is intended to be used as a food ingredient (Nutrient supplement[1]) in: Beverage and beverage bases; Milk products; Processed fruits and fruit juices; and Processed vegetables and vegetable juices at levels up to 250 mg/kg. It is recognized that there are Standard of Identity requirements for some of these specified foods and these foods will not be referred by their commonly recognized names. Inositol will not be used in infant formula.

1.5. Statutory Basis for GRAS conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6. Exemption from Premarket approval requirements:

Sichuan has concluded that Inositol is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that Inositol, meeting the

[1] 21 CFR §170.3 (o) (20) - Nutrient supplements: Substances which are necessary for the body's nutritional and metabolic processes.

specifications cited herein, and when used as a food ingredient in selected conventional food products, is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Inositol, when used as described in this dossier, is GRAS based on scientific procedures.

1.7. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting

Sichuan Bohaoda Biological Technology Co., Ltd.
No. 9 Longxiang Avenue, Yantan District High-tech Industrial Park,
Zigong city, Sichuan Province，
CHINA

Phone: +86-0813-2610661
Email: sichuanbohaoda2021@163.com

The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.8. Data exempt from Disclosure:

Parts II through VII of this GRAS notification do not contain any data or information that is exempt from disclosure under the Freedom of Information Act. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document. Therefore, the information contained in this dossier can be made publicly available.

1.9. Certification:

Sichuan certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Sichuan, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of Inositol. Sichuan accepts responsibility for the GRAS determination that has been made for Inositol as described in this dossier.

1.10. Name, position/title of responsible person who signs dossier and signature:

Name: Huabin Gan
Position/Title- Corporate legal person
Sichuan Bohaoda Biological Technology Co., Ltd.
No. 9 Longxiang Avenue, Yantan District High-tech Industrial Park,
Zigong city, Sichuan Province，
CHINA

Phone: +86-0813-2610661
Email: sichuanbohaoda2021@163.com

Signature: 

1.11. FSIS/USDA – Use in Meat and/or Poultry:

Sichuan does not intend to add Inositol to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

2. PART II- IDENTITY AND TECHNICAL INFORMATION

2.1. Description and Characterization

The subject of this GRAS assessment, inositol (myo-inositol) is a standardized preparation produced by fermentation using a recombinant *Escherichia coli* BL21(DE3) strain, and containing not less than 97% and not more than 102% inositol, on anhydrous basis. It is a white crystalline powder that primarily consist of inositol. General descriptive characteristics of inositol are summarized in Table 1. Inositol is a carbocyclic sugar that is endogenously produced in the body and occurs naturally in meats, plants, and dairy products. Generally, it is prepared from an aqueous (0.2% sulfur dioxide) extract of corn kernels by precipitation and hydrolysis of crude phytate. Inositols are polyols consisting of a six carbon ring structure with a hydroxyl group at each carbon position. The chemical structure of inositol is provided in Figure 1.

Table 1. General Descriptive Characteristics of Inositol

Parameter	Description*
Source	By fermentation of *Escherichia coli* BL21(DE3) strain
Common name	Inositol; Myo-Inositol
Alternative names	Scyllo-inositol; epi-Inositol; Muco-Inositol
IUPAC name	Cyclohexane-1,2,3,4,5,6-hexol
CAS No.	87-89-8
Chemical formula	$C_6H_{12}O_6$
Molecular weight	180.16 g/mol
Appearance	Powder
Color	White
Odor	Neutral
Taste	Sweet taste
Solubility	Freely soluble in water
Storage	Store sealed in a cool, dry place
Shelf life	2 years

*Based on information provided by Sichuan and other publicly available

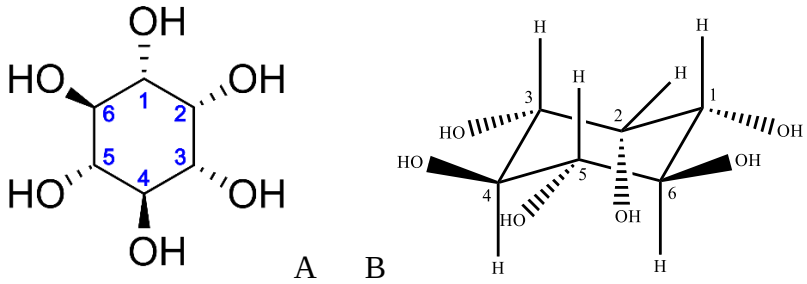

Figure 1. Chemical structure of Inositol (myo-inositol) (sterochemical presentation).

2.2. Manufacturing Process

Inositol (myo-inositol) from Sichuan Bohaoda Biological Technology Co., Ltd. (Sichuan) is manufactured according to current good manufacturing practices (cGMP) for food ingredients. As described below, inositol is produced by fermentation using a genetically engineered *E. coli* BL21(DE3) strain.

The manufacturing process for inositol produces a consistent product that meets the specifications and quality/purity criteria listed in the *Food Chemicals Codex* (FCC) monograph

for inositol (FCC, 2022). Inositol is routinely analyzed to ensure conformance to FCC specifications.

Production Process:

For the production of inositol, starch or maltodextrin are used as raw materials. A multi-enzyme reaction system is established to convert raw materials into inositol. To improve the inositol production, five specific genetic manipulations were performed in the genome of recipient *E. coli* BL21 (DE3) strain by Sichuan (Table 2).

Inositol is produced by non-fermentative enzymatic catalysis of starch or maltodextrin. The preparation method is described in detail in a US patent[2]. Inositol is synthesized in one step that involves an *in vitro* multi-enzyme reaction system. It directly uses whole-cell intracellular enzymes as enzyme preparations. The different conversion steps (Figure 2) are as follows:

1. Isoamylase (IA) converts maltodextrin containing branched chains into straight-chain dextrin,
2. Glucan phosphorylase (GP) converts the straight-chain dextrin into glucose-1-phosphate,
3. Phosphoglucomutase (PGM) converts the glucose 1-phosphate into glucose 6-phosphate,
4. Myo inositol-1-phosphate synthase (IPS) converts the glucose 6-phosphate into inositol 1-phosphate,
5. Inositol monophosphatase (IMP) decomposes inositol 1-phosphate into inositol and phosphoric acid.

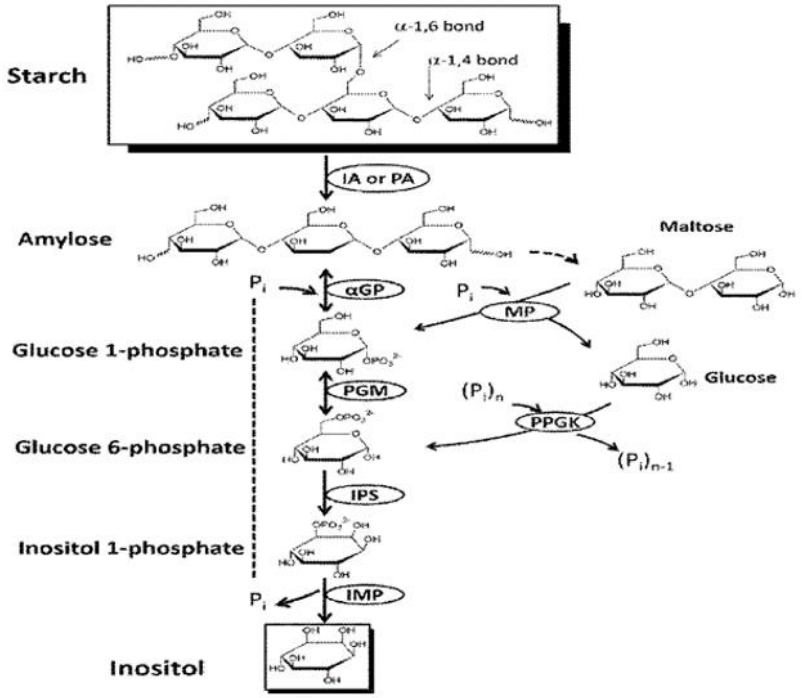

Figure 2. Enzymatic Steps Involved in the Conversion of Starch to Inositol

[2]Zhang et al. 2020. Inositol Preparation Method. US Patent: US 10,597,682 B2.

In the production of inositol, isoamylase converts maltodextrin containing branched chains into straight-chain dextrin, glucan phosphorylase converts the straight-chain dextrin into glucose-1-phosphate, Phosphoglucomutase converts the glucose 1-phosphate into glucose 6-phosphate, myo inositol-1-phosphate synthase converts the glucose 6-phosphate into inositol 1-phosphate, inositol monophosphatase decomposes inositol 1-phosphate into inositol and phosphoric acid.

The enzyme-catalyzed reaction is carried out at 70℃ in a phosphate buffer of 50 mM. The phosphate in the buffer can meet the requirements of the enzyme reaction. The multi-enzyme reaction system further comprises magnesium sulfate. None of the raw materials used in the production process are major allergens or are derived from major allergens. When the conversion rate is ≥80%, ceramic membranes are used to filtrate the bacterial residues. Then, the reaction solution is decolorized by activated carbon and concentrated. The final product is obtained through the following processes: cooling, crystallization, centrifugation, washing, drying, screening, and packaging. The raw materials and processing aids used in the manufacture of inositol are all food-grade substances, typically used in enzyme-catalyzed reactions and for food ingredient production.

Microorganisms and Enzymes Used in Production:

As described earlier, the production strain used is *Escherichia coli* BL21(DE3), which is non-pathogenic and non-toxigenic. Stability of target gene integration and expression is established. Table 2 summarizes the name, the donor organism and the biological functions of the 5 targets genes introduced in *Escherichia coli* BL21(DE3).

Table 2. Details of Genetic Modifications and Functions of Enzymes Involved in Production of Inositol

Target gene 1	Name	Isoamylase	**Donor organism**	***Sulfolobus tokodaii***
	Biological functions	Isoamylase is a type of starch debranching enzyme. It cuts the alpha-1, 6-glucoside bond at the branch of polysaccharides. Isoamylase has high hydrolytic activity for macromolecular substrates such as amylopectin and glycogen, while it has low hydrolytic activity for subgroups of dextrin and cannot hydrolyze pullulan.		
Target gene 2	Name	Glucan phosphorylase	**Donor organism**	***Thermotoga maritima***
	Biological functions	Glucan phosphorylase catalyzes the production of glucose-1-phosphate (G1P) from glucans in the presence of inorganic phosphate. This reaction is reversible.		
Target gene 3	Name	Phosphoglucomutase	**Donor organism**	***Thermococcus Kodakarensis***
	Biological functions	Phosphoglucomutase (PGM) catalyzes the interconversion between glucose 1-phosphate and glucose 6- phosphate, and plays an important role in sugar metabolism.		
Target gene 4	Name	Inositol-1-phosphate synthase	**Donor organism**	***Archaeoglobus fulgidus***
	Biological functions	Inositol-1-phosphate synthase converts glucose 6-phosphate (G6P) to inositol 1-phosphate (I1P), which is an irreversible reaction.		
Target gene 5	Name	Inositol monophosphatase	**Donor organism**	***Thermotoga maritima***
	Biological functions	Inositol monophosphatase catalyzes the hydrolysis of inositol 1- phosphate to produce inositol and inorganic phosphate, which is an irreversible reaction.		

E. coli are bacteria commonly found in intestines of humans and warm-blooded animals. Most strains of *E. coli* are harmless. *E. coli* can be killed at about 70°C. The enzymatic reaction is carried out at 70°C for up to 2 days. *E. coli* BL21(DE3) is a microorganism generally considered non-pathogenic and unlikely to survive in host tissues or to cause disease (Chart et

al., 2000). The genome sequence of *E. coli* BL21 (DE3) showed the absence of genes encoding invasion factors, adhesion molecules and enterotoxins associated with virulence. Three batches of inositol produced by Sichuan were tested by a third party, and the residual DNA, residual protein and endotoxin of the host cells were below the detection limit.

The available information shows that genetically modified *E. coli* has been used in the production of human-identical milk oligosaccharides (HiMO) including several GRAS ingredients such as 2'-Fucosyllactose; lacto-N-neotetraose (LNnT); 2'-fucosyllactose/difucosyllactose (2'-FL/DFL); lacto-N-tetraose (LNT); 6'-sialyllactose (6'-SL) sodium salt; 3'-sialyllactose (3'-SL) sodium salt; 3-fucosyllactose (3-FL); and lacto-N-fucopentaose I/2'-fucosyllactose (LNFP-I/2'-FL).

As a product of genetically engineered microorganisms, inositol is of high purity and does not contain viable production strains, DNA or protein fragments from the production strain. The absence of cells, DNA or protein fragments supports the safety of the final product. The *E. coli* strain used in the production of inositol is considered as non-pathogenic and non-toxigenic.

2.3. Specifications and Identity

In order to ensure a consistent and safe product, Sichuan has established food grade specifications for Inositol (Table 3). The analytical methods used for the qualitative and quantitative analysis of the individual specification parameters are validated for their intended purpose. Analytical results from five lots of inositol demonstrate that it is consistently manufactured and meets the standard specifications. The purity of inositol is at least 97% of the product and is based on High-Performance Liquid Chromatography – Fourier-transform infrared spectroscopy (HPLC-FTIR) analysis.

To ensure the purity of the final product, upper limits have been established for the raw materials and processing aids used in the manufacturing, heavy metals, and microbiological parameters. Analytical data from five non-consecutive representative batches of inositol (Table 4) demonstrate compliance with the product's physical, chemical and microbiological specifications and the ability of the method of manufacture to produce a consistent product.

2.3.2. Potential Impurities

In order to control the levels of residual impurities, as well as heavy metals, microbes, and production organism-derived DNA and endotoxin, process controls and product specifications are in place to ensure a consistent, food-grade finished ingredient. The microbial residues generated during the production of inositol were removed by ceramic membrane filtration, centrifugation, and washing and are strictly controlled in the final product through testing.

The total proteins generated during the production of inositol were removed by filtration in process control, strictly controlled, and levels of residual host cell protein (HCP) in the final product are tested by Enzyme-linked Immunosorbent Assay (ELISA). The ELISA kit used is designed for quantitative detection of host cell proteins from *E. coli* BL21 expression strains, suitable for process intermediates and bulk samples of recombinant protein products such as interleukins (IL), recombinant human interferons (rhIFN), recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF), recombinant human tumor necrosis factor (rhTNF), and growth factors (EGF/FGF/PDGF). The assay has greater sensitivity, especially as the subject of GRAS is of high purity and if there are no bacterial proteins, there is no need to

consider other proteins. The test results from three batches (Table 5) showed that no residual *E. coli* host cell protein values were detected in the samples. The residual *E. coli* host cell protein calculated by using the minimum concentration were less than the lowest concentration used in the standard curve (1 ng/mL).

The nucleic acids (DNA) generated during the production of inositol were removed by filtration and are strictly controlled, monitored by PCR - Fluorescence Probe in the final product testing. The test results showed that residual nucleic acids were negative (Table 5). To ensure that exposures to endotoxins do not exceed the usual levels that are expected, batch analyses from three non-consecutive batches was analyzed using Gel-Clot endotoxin test. The findings did not reveal presence of endotoxin in any of the samples tested (Table 5). For the detection of bacterial endotoxins, Gel-Clot Method, as described in the Chinese Pharmacopoeia 2020 is used. This is a non-quantitative detection method. Hence in Table 5, the term conforms to specifications is used.

Table 3. Food Grade Specifications of Inositol

Parameters	Specification	Analytical Methods
Identification	The retention time of the major peak of the sample solution corresponds to that of the standard solution	USP43-NF38 - 5829
Assay (anhydrous basis)	Not less than 97% and not more than 102%	USP43-NF38 - 5829
Water	Not more than 0.5%	USP43-NF38 - 5829
Organic impurities	Not more than 0.3% of any individual impurities Not more than 1.0% of total impurities	USP43-NF38 - 5829
Clarity of solution	The same clarity as that of water	USP43-NF38 - 5829
Conductivity	Not more than 20 uS/cm	USP43-NF38 - 5829
Color of solution	The test solution is not more intensely colored than Standard solution A, Standard solution B, Standard solution C or water.	USP43-NF38 - 5829
Heavy metals		
Arsenic	≤0.5 ppm	Chinese Pharmacopoeia 2020, Part 4, page 234
Lead	≤0.5 ppm	Chinese Pharmacopoeia 2020, Part 4, page 234
Mercury	≤0.5 ppm	Chinese Pharmacopoeia 2020, Part 4, page 234
Cadmium	≤0.5 ppm	Chinese Pharmacopoeia 2020, Part 4, page 234
Microbiological limits		
Total Aerobic Bacteria	$\leq 10^3$ cfu/g	Chinese Pharmacopoeia 2020, Part 4, page 160
Molds & yeasts	$\leq 10^2$ cfu/g	Chinese Pharmacopoeia 2020, Part 4, page 160
Escherichia coli	Negative	Chinese Pharmacopoeia 2020, Part 4, page 165
Salmonella	Negative	Chinese Pharmacopoeia 2020, Part 4, page 165
Endotoxins*	Conforms (negative)	Gel-Clot Test
Residual Protein*	≤100 mg/kg	ELISA
Residual DNA*	Negative (≤ 0.03 pg/μL)	PCR - Fluorescence Probe

USP43-NF38 (2020). *Not every batch tested. CFU = colony-forming units; NF = National Formulary; ppm = parts per million; USP = United States Pharmacopeia.

Table 4. Summary of the Chemical Product Analysis for Five Non-Consecutive Lots of Inositol

Parameter	Specification Limit	Manufacturing Lot No.				
		0101230 21003	0101230 21101	0101230 21403	0101230 21501	0101230 21202
Identification	The retention time of the major peak of the sample solution corresponds to that of the standard solution	Passed test	Passed test	Passed test	Passed test	Passed test
Assay (anhydrous basis)	Not less than 97% and not more than 102%	99.10%	99.35%	99.18%	99.18%	99.29%
Water	Not more than 0.5%	0.07%	0.06%	0.07%	0.06%	0.05%
Organic impurities	Not more than 0.3% of any individual impurities Not more than 1.0% of total impurities	Passed test	Passed test	Passed test	Passed test	Passed test
Clarity of solution	The same clarity as that of water	Passed test	Passed test	Passed test	Passed test	Passed test
Conductivity	Not more than 20 uS/cm	11.41 uS/cm	7.13 uS/cm	7.19 uS/cm	7.28 uS/cm	9.36 uS/cm
Color of solution	Not more intensely colored than Standard solution A, B, C or water.	Passed test	Passed test	Passed test	Passed test	Passed test
Heavy Metals						
Arsenic	≤0.5 ppm	Not detected	Not detected	Not detected	Not detected	Not detected
Lead	≤0.5 ppm	Not detected	Not detected	Not detected	Not detected	Not detected
Mercury	≤0.5 ppm	Not detected	Not detected	Not detected	Not detected	Not detected
Cadmium	≤0.5 ppm	Not detected	Not detected	Not detected	Not detected	Not detected
Microbial Parameters						
Total Aerobic Bacteria	$\leq 10^3$ cfu/g	<10 cfu/g	<10 cfu/g	20 cfu/g	<10 cfu/g	50 cfu/g
Molds & yeasts	$\leq 10^2$ cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Escherichia coli	Negative	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella	Negative	Not detected	Not detected	Not detected	Not detected	Not detected

CFU = colony forming units; ppm = parts per million; Detection limits: Arsenic- 0.05 mg/kg; Lead- 0.005 mg/kg; Mercury- 0.002 mg/kg; Cadmium- 0.002 mg/kg.

Table 5. Summary of the Endotoxins, Residual Protein and Residual DNA for Three Non-Consecutive Lots of Inositol.

Parameter	**Specification Limit**	**Manufacturing Lot No.**		
		010121112804	**010121112901**	**010121113002**
Endotoxins	Conforms (Gel-Clot Test, Chinese Pharmacopoeia 2020)	Conforms	Conforms	Conforms
Residual Protein	≤100 mg/kg	＜5	＜5	＜5
Residual DNA	ND (≤ 0.03 pg/μL)	ND (≤ 0.03 pg/μL)	ND (≤ 0.03 pg/μL)	ND (≤ 0.03 pg/μL)

ND= Not Detected as ≤ 0.03 pg/μL. The detection limits of Residual DNA is 0.06 ppm.

3. PART III- DIETARY EXPOSURE

3.1. Background Exposure from Natural Presence in Food

Inositol is found in all living cells at measurable quantities. It is endogenously produced *via* the enzyme-mediated steps of cyclization of glucose-6-phosphate, followed by dephosphorylation (SCOGS, 1975). Inositol occurs naturally as free inositol or as a component of phospholipids in animal tissues, specifically the brain, heart, stomach, kidney, spleen, and liver tissues (SCOGS, 1975; EFSA, 2014). Inositol is a key component of the lipid bilayer of cellular membranes and is a precursor for the phosphatidylinositol cycle. Inositol is source for several secondary messengers, such as diacylglycerol, inositol-1,4,5-triphosphate, and phosphatidylinositol-3,4,5-biphosphate, that play different roles (Carlomagno and Unfer, 2011). In cereals, inositol is present in high amounts as polyphosphoric acid esters, called phytic acids. Inositol is also naturally present in cow's milk and human milk at average levels of 80 mg/L and 450 mg/L, respectively (SCOGS, 1975; Holub, 1986).

Clements and Darnell (1980) measured the amount of inositol present in 487 foods. The greatest amounts of inositol were found in fruits, beans, grains, and nuts. Inositol contents of some foods are provided in Table 6. Fresh vegetables and fruits were found to contain more inositol than did frozen, canned, or salt-free products. The inositol intake that could be provided by such diets ranged from 225 to 1500 mg/day per 1800 kcal and within this range the agreement between the calculated and measured amounts of this material was excellent. The daily intake of inositol in adults consuming balanced diets has been estimated to be 1,000 mg/day, while the estimated daily intake of inositol from its addition to infant formula ranges from 1 to 65 mg/day (SCOGS, 1975; Clements and Darnell, 1980). In contrast to these values, the endogenous production of inositol by the kidneys in humans has been estimated to be 4,000 mg/day. The production of inositol in kidney far exceeds the daily dietary intake, indicating that the kidney is likely an important regulator of inositol levels in the body (Clements and Diethelm, 1979). In addition to its natural presence in food, inositol is found in a wide range of dietary supplements, with recommendations of daily intakes of inositol of up to 2,050 mg/day (DSLD, 2023).

Table 6. Inositol Content of Selected Foods*

Food Source	Products with Highest Inositol Levels	Inositol (mg/g)
Beverages	Coffee, instant powder	6.46
	Fruit punch canned	0.17
Breads/Cereals	Stone ground wheat	11.5
	Hamburger bun	4.78
	Pumpernickel	1.60
	Bran, 40% flakes, dry	2.74
	Bran raisin	1.07
Fruits	Cantaloupes, oranges, prunes dried, grapefruits, limes	1.94 to 4.70
Meat	Beef	0.05 to 0.64
	Pork	0.06 to 0.42
	Poultry	0.08 to 1.31
Nuts	Various	0.12 to 2.78
Vegetables	Beans (green shelled fresh), peas, rutabaga	1.10 to 4.40

Table 6. Inositol Content of Selected Foods*

Food Source	Products with Highest Inositol Levels	Inositol (mg/g)

*Adapted from Clements and Darnell (1980)

3.2. Intended Uses and Food Categories

Sichuan intends to use inositol as a nutrient and food ingredient in selected conventional foods, such as Beverage and beverage bases, Milk products, Processed fruits and fruit juices, and Processed vegetables and vegetable juices at use levels up to 250 mg/kg. Use of inositol as a nutrient in foods is GRAS as per 21 CFR §184.1370. The intended uses of inositol are substitutional to the existing or approved uses. A summary of the food categories and use levels in which inositol is intended for use is provided in Table 7. The proposed uses and use levels are same with the allowed scopes of inositol in China, according to GB 14880.

The subject of this GRAS notice, inositol, will not be used in any foods for which food standards would preclude its use. Foods such as meat and poultry products that come under USDA jurisdiction are excluded from the list of intended food uses of inositol. Additionally, inositol is not proposed for use in infant formula and is not targeted to be marketed to toddlers and children.

Table 7. Intended Uses of inositol and Maximum Use Levels

Food category	Intended use	Use level (mg/kg)
Beverage and beverage bases	Flavored water	120
	Enhanced water	120
	Soft drinks	120
Milk products	Dry milks	250
Processed fruits and fruit juices	Fruit juices	120
	Fruit drinks	120
Processed vegetables and vegetable juices	Vegetable juices	120

*These food-uses represent non-standardized food products; however, in order to obtain a conservative intake estimate, surrogate codes for the standardized food products were chosen.

3.3. Estimated Daily Intake from the Proposed Uses

An assessment of the anticipated intake of inositol as an ingredient under the intended conditions of use (Table 6) was conducted using data available from the 2017-2020 cycles of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES). The NHANES data were used to estimate the mean and 90th percentile intake of inositol for each of the following population groups: Toddlers, Children, Teenagers, Adults, Elderly and All Population. Intake estimates are based only on those segments of the population that consumed one or more of the target foods on a survey day.

Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018) which is a web-based software tool that allows estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) and food consumption data. Datasets are combined in the software to provide accurate and efficient exposure assessments. A summary of the estimated daily intake of inositol from the proposed use in selected food categories (Table 7)

is provided in Table 8 on an absolute basis (mg/person/day) and in Table 9 on a body weight basis (mg/kg bw/day).

Among the total population, the mean and 90th percentile per consumer intakes of inositol from its addition to foods were determined to be 55.1 and 119.0 mg/person/day, respectively. Adult males presented the greatest mean and 90th percentile intakes of inositol, at 74.0 and 158.5 mg/person/day, respectively. On a body weight basis, the total population mean and 90th percentile intakes of inositol were determined to be 0.85 and 1.83 mg/kg bw/day, respectively. Among the individual population groups, toddlers were identified as having the highest mean and 90th percentile (on body weight basis) intakes of any population group, at 2.17 and 4.24 mg/kg bw/day, respectively.

Table 8. Summary of the Estimated Daily Intake of Inositol from Proposed Food Uses (mg/day)

Population Group	Age Group (Years)	Per Capita Intake (mg/day)		Per Consumer Intake (mg/day)			
		Mean	90th percentile	%	n	Mean	90th percentile
Toddlers	1 to 3	22.0	52.1	73.4	544	29.9	59.5
Children	4 to 10	28.8	64.1	80.3	1,152	35.9	68.8
Male Teenagers	11 to 18	44.0	95.8	79.0	611	55.6	99.2
Female Teenagers	11 to 18	31.2	67.0	76.9	619	40.5	72.9
Male Adults	19 to 64	52.4	135.3	70.8	1,809	74.0	158.5
Female Adults	19 to 64	34.5	92.1	65.7	1,951	52.5	112.5
Elderly	65 and up	33.0	85.0	67.4	1,136	48.9	108.4
All Population	All ages	38.3	97.1	69.6	7,890	55.1	119.0

n = sample size; *Calculated by using DaDiet, a Dietary Intake Evaluation Tool by Dazult.

Table 9. Summary of the Estimated Daily Intake of Inositol from Proposed Food Uses (mg/kg bw/day)

Population Group	Age Group (Years)	Per Capita Intake (mg/kg bw/day)		Per Consumer Intake (mg/kg bw/day)			
		Mean	90th percentile	%	n	Mean	90th percentile
Toddlers	1 to 3	1.59	3.77	73.4	544	2.17	4.24
Children	4 to 10	1.03	2.36	80.3	1,152	1.29	2.58
Male Teenagers	11 to 18	0.73	1.72	79.0	611	0.92	1.86
Female Teenagers	11 to 18	0.54	1.14	76.9	619	0.71	1.28
Male Adults	19 to 64	0.59	1.56	70.8	1,809	0.83	1.78
Female Adults	19 to 64	0.45	1.13	65.7	1,951	0.69	1.36
Elderly	65 and up	0.42	1.08	67.4	1,136	0.62	1.28
All Population	All ages	0.59	1.53	69.6	7,890	0.85	1.83

bw = body weight; n = sample size; *Calculated by using DaDiet, a Dietary Intake Evaluation Tool by Dazult.

3.4. Cumulative Intake from Background and Proposed Uses

The addition of the inositol background intake of about 1,000 mg/day from an omnivorous diet to the estimated intake from proposed uses would result in mean and 90th percentile intakes of 1,055 and 1,119.0 mg/day, respectively. The cumulative estimated daily

intakes of Sichuan's inositol at the intended use level in selected food categories and through background exposure in the diet are below the level deemed safe for human consumption with no adverse effects (*i.e.*, up to 4,000 mg inositol/day). Inositol intakes of up to 12 g/day have been reported to only result in gastrointestinal symptoms (Carlomagno and Unfer, 2011). The proposed uses of inositol in the selected food categories such as beverage, milk products, processed fruits, vegetables and juices are substitutional. Hence, a detailed cumulative intake analysis from all existing and other uses of inositol has not been undertaken. In any event, the addition of Sichuan's inositol to that naturally occurring in the diet results in a negligible increase.

4. PART IV- SELF LIMITING LEVELS OF USE

Inositol does not have any self-limiting intake levels of use under the conditions of use described in this GRAS notification.

5. PART V- EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of inositol in this document is not based on common use in food before 1958. However, inositol occurs naturally in animal tissues, in milk and several fruits and vegetables such as in fruits, beans, grains, and nuts that have been consumed prior to 1958. Notwithstanding this, the present GRAS assessment for the use of inositol as a food ingredient is based on scientific procedures.

6. PART VI- NARRATIVE

6.1. Historical and Current Uses

Inositol was first isolated by Scherer in 1850, and called “inosite” because of its sweet taste. It was fully purified in 1887 by Maquenne and its inert (compared with glucose) chemical behavior, the molecular weight of its acetyl and benzoyl esters, and other chemical properties led to establish its cyclohexanol structure (Irving, 2016). Inositol is a physiological compound belonging to the sugar family. Inositols are sugar-like compounds that are widely distributed in nature and are a part of membrane molecules, participating as second messengers in several cell-signaling processes. Myo-inositol is the precursor of inositol triphosphate, a second messenger regulating many hormones such as thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH), and insulin. Inositol is found in plants, mammals and bacteria. Human beings are exposed to inositol from the diet.

As per 21 CFR §184.1370, inositol is GRAS as a direct human food ingredient when used under the following conditions and in accordance with cGMP (FDA, 2023): (1) as a nutrient supplement as defined in 21 CFR §170.3(o)(20), (2) in special dietary foods, as well as in infant formula. Inositol is included in the Center for Responsible Nutrition (CRN, 1998) List of Dietary Ingredients “Grandfathered” Under DSHEA (Dietary Supplement Health and Education Act of 1994). Based on the information from the New Dietary Ingredient Notification (NDIN) website, D-chiro-inositol, a stereoisomer of inositol, was the subject of a NDIN submitted to the FDA in 2018. The agency filed the notification (NDIN 1081) without comment for use in dietary supplements at use levels up to 600 mg/day (FDA, 2018). A search of USDA database ‘FoodData Central’ with the term inositol resulted in 564 hits as “Branded Foods,” demonstrating that inositol is commonly consumed as a component of food (USDA, 2023).

In the European Union (EU), inositol was added to the EU list of novel foods. It is authorized for use in processed cereal-based food and baby food, foods for particular nutritional uses, foods intended for total diet replacement for weight control, and in infant formula and follow-on formula (4 to 40 mg/100 kcal) (EU, 2016). As per Food Standard Australia and New Zealand (FSANZ), inositol is permitted for use in infant formula at levels of 1.0 to 9.5 mg/100 kJ (FSANZ, 2022) and in formulated caffeinated beverages at a maximum level of 100 mg/day (Australia New Zealand Food Standards Code, Standard 2.6.4) (FSANZ, 2015). In Canada, a Natural Health Product (NHP) monograph is available for inositol, with permitted levels up to 650 mg/day (Health Canada, 2023).

As described earlier, inositol is endogenously produced and also occurs naturally as free inositol or as a component of phospholipids in animal tissues. In cereals, inositol is present in high amounts as polyphosphoric acid esters. In human milk, inositol is found at an average level of 450 mg/L. Inositol has been measured in 487 foods, with high amounts in fruits, beans, grains, and nuts (Clements and Darnell, 1980). The inositol intake from such diets could be up to 1500 mg/day. The daily intake of inositol in adults consuming a balanced diet has been estimated to be 1000 mg/day. The endogenous production of inositol by the kidneys in humans has been estimated to be 4000 mg/day. A search of the Dietary Supplement Label Database reveals currently on market listing of 13242 labels found (7.2% of database) containing inositol, the majority of which have a manufacturer's suggested dose of up to 2000 mg/day (DSLD, 2024).

In summary, inositol is found in human milk, animal tissues, fruits, lentils, and nuts and is synthesized endogenously, particularly in kidneys. Inositol is commonly marketed as a dietary

supplement and is also added to foods. The available information suggests that humans are exposed to inositol from diet or from use of dietary supplements. This also suggests that at naturally found levels, inositol is unlikely to cause any adverse effects.

6.2. Data Pertaining to Safety

Given the natural occurrence of inositol in foods such as animal tissues, fruits, lentils, nuts and its endogenous synthesis, the need for systematic toxicity studies of inositol has been diminished. However, given its role in important in cellular signal transduction and phospholipid assembly, DNA methylation, and the production of nucleoproteins and membranes, in recent years there has been considerable effort to elucidate its importance in nutrition. Published literature contains several studies on inositol investigating its role in several biochemical pathways, most of them controlling vital cellular mechanisms such as cell development, signaling and nuclear processes, metabolic and endocrine modulation, cell growth, signal transduction, etc.

For the present GRAS assessment, the safety determination of inositol is based on the totality of the available evidence, including human clinical observations/trials, animal experimental studies, and *in vitro* studies. Efforts have been made to present the data supporting inositol safety as well as any data on potential adverse effects. An attempt has been made to interpret these findings from relevant studies as it relates to the present GRAS assessment. The assessment of efficacy studies is limited to a review of the results related to safety and tolerability. In the following sections, relevant biological and toxicological studies on inositol and structurally related substances are described that provide support for the conclusions reached in this determination.

6.2.1. Absorption, Metabolism, Distribution and Elimination

In an early study, intake of 30 g inositol over the course of one day by a single human subject resulted in recovery of approximately 9% of the dose in urine and was found to be unchanged over the course of 3 days (Anderson and Bosworth, 1916). In this study, none of the inositol was found in the feces. These investigators also reported that, following administration of 15 g inositol on the first day, followed by 30 g/day for an additional 3 days to an individual, the overall recovery of inositol was 8.6% in the urine, while daily urinary excretion of inositol was 2.5 to 10.6%. In a separate publication, Anderson (1916) reported that feeding of inositol to rabbits revealed low levels of inositol recovery in the urine (2 to 5%). However, subcutaneously or intravenously administered inositol to rabbits resulted in urinary recovery of much higher levels (26 to 50%). Anderson (2016) also reported recovery of higher levels of inositol in the feces of dogs (77%) fed 10 g inositol/day for 6 days. However, this was likely related to the increased incidence of diarrhea, limiting gastrointestinal absorption.

The available information indicates that following oral administration to humans and animals, inositol is slowly absorbed from the gastrointestinal tract and only a minor amount is eliminated in the urine (SCOGS, 1975; Clements and Darnell, 1980). As reported in SCOGS (1975), following oral administration of inositol to fasted rats (1,250 mg/kg bw), inositol was very slowly absorbed, requiring 24 to 48 hours for near-complete absorption, with only 1% excreted in the urine.

In a series of studies, Daughaday and Larner (1954) investigated renal excretion of inositol in normal and diabetic rats. These investigators reported that gavage administration of

inositol to normal and diabetic Sprague-Dawley rats resulted in absorption of 25% and 46% of the dose after 6 hours, respectively. Diabetic rats absorbed inositol faster from the gut than did normal rats. As regards urinary elimination, normal rats excreted low levels of inositol (<3%) in the urine, while diabetic and depancreatized rats excreted up to 30% of the ingested inositol over a period of 24 hours. The increased excretion of inositol in the urine of diabetic rats was suggested to be related to the inhibition of tubular reabsorption of inositol by the kidneys (Daughaday and Larner, 1954). Lewin et al. (1976) reported that in healthy and nephrectomized rats, approximately 16% of the injected dose of radio-labeled inositol was recovered as respired carbon dioxide over 5 hours in normal rats. However, nephrectomized rats were unable to excrete any of the inositol as carbon dioxide.

De Grazia et al. (2012) evaluated the effect of coffee on myo-inositol absorption. Following a 2-week inositol-poor diet, 12 healthy subjects (females- 18 to 24 years) consumed either powdered inositol (4 g inositol) in water or in gel capsule (3.6 g inositol) form, with and without coffee intake. The maximum plasma concentration (C_{max}) for inositol was 45.69 µmol/L at baseline in the powder group as compared to 33.45 µmol/L after coffee intake. In the gel capsule form, the C_{max} was not significantly different from baseline as compared to post-coffee intake (88.2 µmol/L vs. 83.64 µmol/L). Coffee administration did not affect time to reach maximum plasma inositol concentrations in either group (120.6 to 133.1 minutes). The area under the curve (AUC) values from 0 to 1,440 minutes were 47,394 and 36,774 µmol.min/L in the powder group, without and with coffee consumption, respectively, and 79,475 and 70,684 µmol.min/L in the gel capsule group, without and with coffee consumption, respectively. Benjamin et al. (1997) reported that following a single intake of 20 g inositol by 9 subjects, the average plasma inositol concentration was 40.8±23 mg/ml compared to 13.5±7 mg/ml following placebo (glucose) administration.

The available evidence indicates that inositol is actively transported and that uptake occurs in a sodium- and energy-dependent manner (Holub, 1986). The primary site of metabolism of inositol was first proposed to be the kidney, where the ring structure of inositol is cleaved to yield D-glucuronic acid and subsequently D-xylulose 5-phosphate, which enters the pentose phosphate cycle (Howard and Anderson, 1967). Indeed, rat's kidneys can catabolize all of the inositol ingested in an average daily diet. Studies conducted in nephrectomized and normal rats support the evidence that the kidneys are the primary site of inositol metabolism (Howard and Anderson, 1967; Lewin et al., 1976). The inositol not excreted is likely to undergo catabolism and be incorporated into phospholipids and utilized by microsomes (Yagi and Kotaki, 1969; Holub, 1986). Contrary to these observations, Yagi and Kotaki (1969) reported that the liver may also be an important site of exogenous inositol metabolism in the body.

In summary, the available information from several studies indicates that inositol is slowly absorbed from the gastrointestinal tract and a small amount is excreted in the urine. Inositol is actively transported and uptake occurs in a sodium- and energy-dependent manner. One of the main site of inositol metabolism is the kidney. The inositol not excreted is likely to undergo catabolism and be incorporated into phospholipids and utilized by microsomes. The available metabolism-related information suggests that inositol is unlikely to cause any adverse effects at the proposed use levels.

6.2.2. Human Clinical Studies of Inositol

In multiple clinical studies in adults, the safety and tolerability of inositol supplementation, either alone or in combination with other ingredients, has been investigated. Some of these studies in different population groups have been reviewed and described in reviews and meta-analysis. The available studies of inositol and its isomers (primarily myo-inositol) in adults are summarized in Table 10, while some of the more recent studies are further described below. In studies in adults, the daily supplementation of up to 20 g inositol is found to be safe and well-tolerated.

In a recent systematic review and meta-analysis of randomized controlled trials, Greff et al. (2023) evaluated the efficacy and safety of inositols in treating polycystic ovary syndrome (PCOS). For this review and meta-analysis, 26 randomized controlled trials (RCTs) were identified, including data of 1691 patients (806 inositol, 311 with placebo, and 509 metformin groups). In patients treated with inositols, the risk (CI: 1.13; 2.85) of having a regular menstrual cycle was found by 1.79 higher than in the case of placebo. Moreover, the inositols showed non-inferiority compared to metformin in this outcome. No adverse effects were reported. The investigators concluded that inositol is an effective and safe treatment in PCOS.

In another review and meta-analysis, Crawford et al. (2023) assessed whether antenatal dietary supplementation with myo-inositol is safe and effective, for the mother and fetus, in preventing gestational diabetes. For this analysis, four randomized controlled trials (all conducted in Italy) reporting on 567 women who were less than 11 weeks to 24 weeks pregnant at the start of the trials were selected. In three trials, participants were supplement with 4000 mg myo-inositol plus 400 µg folic acid daily in divided doses, while in one trial subjects received 2000 mg myo-inositol, 400 mg d-chiro-inositol, 400 µg folic acid and 10 mg manganese per day. Myo-inositol was associated with a reduction in the rate of gestational diabetes (low quality evidence), reducing the incidence from 28% in women who did not take the supplement, to between 8% and 18% in the women who took it. There were no maternal adverse effects of inositol supplementation in the two trials that reported on this outcome (the other two trials did not mention this).

In a randomized, double-blind, controlled trial, Esmaeilzahed et al. (2023) investigated the effects of myo-inositol in 60 eligible overweight, pregnant women at 12-14 weeks of gestation. The participants were divided into two groups and the treatment group received myo-inositol 2000 mg plus 400 µg folic acid daily and the control group received 400 µg of folic acid daily from 14-24 gestational weeks. The occurrence of gestational diabetes was determined based on 75-g 2-hour oral glucose tolerance test (OGTT) at 24-28 gestational weeks. Additionally, insulin therapy, insulin resistance and lipid profile, gestational weight gain, and fetal and maternal outcomes were studied. The incidence of gestational diabetes in the myo-inositol group was noticeably reduced compared to that in the control group. There were no differences between the two groups in terms of fasting blood sugar, fasting insulin, homeostasis model assessment-estimated insulin resistance (HOMA-IR), insulin therapy, and triglyceride. There was no report of severe adverse reactions. The investigators did not provide any additional information about any adverse effects.

In a randomized, double-blind, controlled clinical trial, Wozniak et al. (2023) investigated the efficacy and tolerability of omega-3 fatty acids (FAs) and inositol alone and in combination for the treatment of pediatric bipolar spectrum disorder in young children. In this study, male and female children ages 5-12 meeting DSM-IV diagnostic criteria for a pediatric bipolar spectrum disorder and displaying mixed, manic, or hypomanic symptoms without

psychotic features at the time of evaluation were recruited. In this double-blind randomized study, participants (n=61) were randomized into one of three treatment arms: inositol+placebo (n=19), omega-3 FAs+placebo (n=20), or inositol+omega-3 FAs (n=22). Inositol capsules contained 500 mg inositol powder each and placebo capsules contained 500 mg lactose powder. Participants weighing ≥25 kg were dosed at 2,000 mg (4 capsules containing 500 mg) of inositol or placebo daily. Participants weighing <25 kg were dosed at 80 mg per kg rounded down to the nearest 500 mg capsule. These doses were maintained for the duration of the trial (12 weeks). Omega-3 fatty acids were provided in the form of high EPA omega-3 fatty acid soft gels (325 mg EPA and 225 mg DHA per 2 capsules).

In the study by Wozniak et al. (2023), safety was assessed at each visit using spontaneous reports of treatment-emergent adverse events. Changes in vital signs (blood pressure, temperature, height, and weight) were recorded at every office visit. An electrocardiogram and complete blood count with differential, electrolytes, liver function tests, and serum glucose and prolactin levels were obtained at the beginning and end point of the study. All participants who were randomized were included in the safety and tolerability analyses. Overall, 10 (50%) participants from the omega-3 fatty acids group, 12 (63%) participants from the inositol group, and 12 (55%) participants from the combination group dropped out of the study, but none were due to adverse events. These dropout rates did not statistically differ. Treatments with omega-3 fatty acids and inositol were very well tolerated. One serious adverse event was reported and determined to be unrelated to the study treatment. In the inositol group, 7 adverse effects were reported (Cold/infection/allergy; Headache; Gastrointestinal; Insomnia; Sedation; Agitated/irritable). The investigators concluded that combination may offer a safe alternative for youth with bipolar spectrum disorder.

In a double-blinded placebo-controlled randomized clinical trial, Arefhosseini et al. (2023) investigated the effects of inositol supplementation on cardiometabolic factors, anthropometric measures, and liver function in obese patients with NAFLD. In this trial, 48 obese patients (completed the study) with NAFLD were randomly assigned to either inositol (4 g/day) or placebo (maltodextrin 4 g/day) along with dietary recommendations for 8 weeks. Glycemic indices, lipid profile, liver enzymes anthropometric measures, and blood pressure were evaluated pre- and post-intervention. In both groups, anthropometric measures decreased significantly, while the reduction in body weight and systolic blood pressure in the group receiving inositol was significantly greater than in the placebo group after adjusting for baseline values and energy intake. Although energy and macronutrient intakes decreased significantly in both groups, between-group differences were not significant after adjusting for the potential confounders. Inositol supplementation resulted in a significant reduction in serum fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). There were significant improvements in lipid profile, liver enzymes, and AST/ALT ratio, as well as serum ferritin level in the inositol group, compared to the placebo group at the endpoint. Of 51 patients, 48 subjects (24 patients in each group) completed the trial while two patients in the placebo group and one patient in the MI group dropped out for reasons unrelated to the interventions. No adverse effects of inositol supplementation at dose of 4 g/day for 8 weeks were reported.

He et al. (2021) investigated the effects of different stereoisomers of inositol on insulin sensitivity of gestational diabetes mellitus (GDM) patients. In this study, pregnant women (n=20/group) were divided into different groups to receive oral supplements containing 400 μg/day folic acid (placebo) (Group A), 1500 mg myo-inositol (Group B), 250 mg D-chiro-

inositol and 400 μg folic acid (Group C), or 250 mg D-chiro-inositol and 1500 mg myo-inositol (Group D) consumed twice daily for 8-week. Subjects were evaluated for adverse maternal and infant outcomes, including biochemical parameters. No significant differences were reported in body mass index (BMI), age, gestational weeks, and blood pressure during pregnancy. Biochemical parameters, HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) and HOMA-ISI (insulin sensitivity index) values were not significantly different at baseline. Following the treatment period, 2-hour postprandial glucose, glycosylated hemoglobin, fasting insulin, total cholesterol, and triglyceride levels were significantly decreased in the treatment groups. In all treatment groups, HOMA-IR was significantly decreased and HOMA-ISI was significantly increased, with a significantly higher degree of change in Group C and D than subjects in Group B when compared to placebo. The occurrence of adverse effects was significantly decreased in Group C and D, relative to placebo. No significant differences was observed for the occurrence rate of adverse effects between placebo and group B (receiving 1500 mg myo-inositol alone).

In a prospective, randomized, placebo-controlled clinical trial, Celentano et al. (2020) investigated the effects of oral supplementation of different inositol stereoisomers (myo-inositol, d-chiro-inositol, combined myo- and d-chiro-inositol) on maternal gestational diabetes mellitus and fetal outcomes in high-risk patients. In this study, non-obese women (n=180) with elevated fasting blood glucose were provided 400 μg folic acid (control), 4000 mg myo-inositol and 400 μg folic acid, 500 mg D-chiro-inositol and 400 μg folic acid, or 27.6 mg D-chiro-inositol and 1100 mg myo-inositol during their first trimester until delivery. Subjects were primarily assessed for oral glucose tolerance with secondary outcomes including insulin therapy, fetal abdominal circumference, polyhydramnios, route of delivery, pre-eclampsia or pregnancy-induced hypertension, pre-term birth, neonatal hypoglycemia, and neonatal intensive care unit stay. During the course of the study, no adverse events were reported and no significant differences were reported in baseline maternal characteristics. No significant differences were reported in route of delivery, birth weight, or fetal gender. Participants given 4000 mg myo-inositol had a significant decrease in incidences of abnormal oral glucose tolerance, as well as a lower mean abdominal circumference percentile. Similarly, subjects receiving 4000 mg myo-inositol were also reported to have significantly increased gestational age at delivery. At baseline, as compared to control, significant decreases in glycemic controls at 1 and 2 hours were reported in all treatment groups. No adverse effects of inositol treatment were reported.

In a randomized controlled trial, Shokrpour et al. (2019) compared the effects of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome (PCOS). In this 12-week study, 53 patients (18 to 40 years) with diagnosed PCOS were administered 500 mg metformin 3 times daily (n=27) or 2000 mg myo-inositol and 200 μg folic acid 2 times a day (n=26). Subjects were evaluated for body weight, BMI, glycemic control, and lipid profile. No significant differences between the groups at baseline and end of trial in anthropometric measurements such as height, weight, and BMI were noted. Supplementation with myo-inositol significantly reduced fasting plasma glucose (FPG), HOMA-IR, serum insulin levels, serum triglycerides, and very low-density lipoprotein-cholesterol (VLDL-C) levels compared to the metformin group. A significant increase in quantitative insulin sensitivity check index in the myo-inositol group in comparison to the metformin group was noted. No adverse events were reported.

Table 10. Summary of Available Human Clinical Studies of Inositol Following Oral Administration in Subjects with Various Health Conditions

Reference	Health Condition; Study Design; Number of Subjects	Dose; Duration	Parameters Investigated and Relevant Safety Findings
Esmaeilzahed et al. (2023)	Overweight, pregnant women; Randomized, double-blind, controlled trial; 60	Myo-inositol 2000 mg plus 400 µg folic acid daily; Control group 400 µg folic acid daily During 14-24 gestational weeks	Oral glucose tolerance test (OGTT) at 24-28 gestational weeks. Additionally, insulin therapy, insulin resistance and lipid profile, gestational weight gain, and fetal and maternal outcomes. Gestational diabetes in myo-inositol group was noticeably minimized No differences in fasting blood sugar, fasting insulin, homeostasis model assessment-estimated insulin resistance (HOMA-IR), insulin therapy, and triglyceride
Arefhosseini et al. (2023)	Obese patients with NAFLD; randomized, double-blinded placebo-controlled; 51 participated 48 completed (n=24/group)	Myo-inositol 4000 mg/day; Placebo: maltodextrin 4 g/day 8 weeks	Anthropometric measures, blood pressure. Serum FBS, TC, HDL-c, LDL-c, ALT, AST, HbA1c. Two patients in the placebo group and one patient in the inositol group dropped out for reasons unrelated to the interventions. No adverse effects of inositol supplementation, at dose of 4 g/day for 8 weeks, were reported.
Greff et al. (2023)	Polycystic ovary syndrome (PCOS); Meta-analysis; 26 Randomized controlled trials 1691 patients (806 inositol, 311 with placebo, and 509 metformin groups)	600 to 4000 mg/day; 7 to 24 weeks;	Efficacy and safety of inositols in treating polycystic ovary syndrome (PCOS); No adverse effects were reported. The investigators concluded that inositol is an effective and safe treatment in PCOS.
He et al. (2021)	Gestational diabetes mellitus; (GDM); Cohort ; 80 pregnant F; 20/group	Control: 400 µg folic acid; MI: 1500 mg 2x/day; DCI: 250 mg 2x/day; MI/DCI: 1500 MI mg/ 250 DCI 2x/day; 8 weeks All treatments: 2x/day	Triglyceride, total cholesterol, FPG, OGTT postprandial glucose (2-hour postprandial blood glucose), FI, and glycosylated hemoglobin levels and HOMA-IR and HOMA-ISI, adverse maternal and infant outcomes (*i.e.*, hypoglycemia, excessive amniotic fluid, premature infants, macrosomia, fetal distress); NSD in age, gestational weeks, or BP; No difference in BMI; NSD in baseline data; Significant decrease in 2-hour postprandial glucose, glycosylated hemoglobin, FI level, total cholesterol, and triglyceride in the treatment groups compared to the controls; NSD between HOMA-IR and HOMA-ISI before treatment; Significant decrease HOMA-ISI; No significant occurrence rate of adverse maternal and infant outcomes in all groups

Celentano et al. (2020)	GDM; Prospective, randomized, placebo-controlled clinical trial; 180 non-obese F in first trimester of pregnancy with elevated fasting glucose MI (myo-inositol): 40; DCI (di-chloro-inositol): 40; MI/DCI: 40	Control: 400 µg folic acid Myo-inositol (MI): 4000 mg and 400 µg folic acid DCI: 500 mg and 400 µg folic acid MI/DCI: 27.6 mg DCI and 1100 mg MI; First trimester to delivery	Primary outcome: Abnormal maternal OGTT; No adverse events reported NSD in baseline maternal characteristics; Significant decrease in incidence of abnormal OGTT in myo-inositol group; Significant decrease in glycemic controls in all treatments compared to the control; Significant decrease in mean abdominal circumference percentile in MI group; NSD in delivery, birth weight, or fetal gender; Significant increase in gestational age at delivery in myo-inositol group. No adverse effects of inositol treatment were reported.
Genazzani et al. (2019)	PCOS Retrospective; 254 overweight and obese F	MI: 1000 mg Alpha-lipoic acid (ALA): 400 mg; MI + ALA: 1000 mg MI and 400 mg ALA; 12 weeks	LH, FSH, estradiol, androstenedione, total testosterone, insulin, HOMA, BMI, OGTT, Significant decrease in insulin maximal response to glucose load in all groups; Significant decrease in insulin, BMI and HOMA index in ALA group. No adverse effects of inositol treatment were reported.
Shokrpour et al. (2019)	PCOS Randomized, controlled; 53 F Control: 27; Treatment: 26	Control: 500 mg metformin (3x/day) Treatment: 2000 mg and 200 µg folic acid (2x/day); 12 weeks	Body weight, BMI, glycemic control, lipid concentration; Significant reduction in FPG, HOMA-IR, serum insulin levels, serum triglycerides, and VLDL-C levels; Significant increase in quantitative insulin sensitivity check index. . No adverse effects were reported.
Santamaria et al. (2018)	GDM Three- Randomized controlled trials; 595 F	MI: 2000 mg MI/day + 200 µg folic acid; Placebo: 200 µg folic acid; During pregnancy	Body weight, gestational age, glucose value, GDM diagnosis, OGTT Significant decrease in preterm birth, macrosomia, and large-for-gestational-age babies; Significant decrease in prevalence of GDM; Significant difference in HOMA index. No adverse effects of inositol were reported.
Deepti et al. (2017)	PCOS and chronic periodontitis Randomized controlled clinical trial ; 60 F; 30/group	Control: 25 systemically and periodontally healthy females; myo-inositol: 1000 mg; 6 months myo-inositol: 2x/day	Waist circumferences, WHR, BMI, MFG, hair growth, FSH/LH, serum testosterone, serum prolactin, lipid profile, serum hs-CRP, FBS, FI, serum, periodontal probe, plaque index, gingival index, bleeding on probing, probe depth, clinical attachment level. Significant difference in WC, WHR, and MFG at the 6-month follow-up; Significant difference in all metabolic parameters in all groups in the 3- and 6-month follow-up; Significant difference in BMI after the 6-month follow-up; Significant difference in improvement of hs-CRP. No adverse effects of inositol reported.

Fruzzetti et al. (2017)	PCOS Randomized control clinical trial; 50 F; 25/group Control: 30 healthy individuals	Control: Not receiving any treatment; Metformin: 1500 mg; Myo-inositol: 4000 mg + 400 µg folic acid; 6 months	Insulin secretion, BMI, menstrual cycle length, acne, hirsutism Significant difference in baseline insulin sensitivity and androgens Significant decrease in BMI in treatment groups; NSD in androgen levels in any group; After 6 month the HOMA-IR and AUC-insulin decreased in the metformin and inositol groups compared to the control; After 6 months a normal menstrual cycle was observed in 53% of people in the metformin group and 44% in the inositol group. No adverse effects of inositol were reported.
Banuls *et al.* (2016a)	Healthy subjects; Randomized double-blind clinical trial; 40; 20 M and 20 F (n=20/group)	Control: Sucrose-enriched beverage; Inositol-enriched beverage: 2.33 g; 12 weeks	Anthropometric measurements, fasting glucose levels, insulin and HOMA-IR index, lipoprotein profile, and postprandial glucose concentrations Significant decrease in insulin HOMA-IR and Apo B levels Significant increase in LDL particle size, in BMI and fasting glucose concentration in control group; NSD between control and treatment in anthropometric or biochemical parameters; Significant decrease in insulin and HOMA-IR index in the inositol-enriched beverage group at 6 weeks; Significant difference between the sucrose-enriched beverage and inositol-enriched beverage groups in bw and BMI. No adverse effects of inositol were reported.
Banuls et al. (2016b)	Prediabetes; Double-blind, randomized, controlled trial; 44 impaired fasting glucose M and F; 15 M and 29 F; Control: 20; Treatment: 24	Control: Sucrose-enriched beverage- 1x/day; Inositol-enriched beverage: 2.33 g- 2x/day; 12 weeks	Anthropometric and biochemical parameters, post-prandial and fasting nocturnal glycemia, bw, BMI, BP, waist circumference, glucose concentration, HOMA-IR, cholesterol, triglycerides, HDL-C, LDL-C. Significant decrease in insulin, and HOMA-IR was seen in non-obese patients; Significant increase in fasting glucose levels in non-obese control groups; Significant increase in nocturnal glucose levels in sucrose-enriched beverage in obese patients; NSD between control and treatment in anthropometric or biochemical parameters; No adverse reactions observed in individuals consuming inositol-enriched beverage
Moretti et al. (2016)	Hirsute PCOS; Randomized clinical trial; Patients with PCOS hirsute (n=70) received oral contraception or 50 mg bicalutamide daily. Patients with PCOS (n=60) treated with myo-inositol (n=30) and myo-inositol and metformin (n=30).	NR; 12 months and 6 months; post-treatment observation	Clinical and biochemical safety index, liver ultrasound, severity of hirsutism; Insulin sensitizing compounds are ineffective in lowering hirsutism or other androgen excess symptoms; Insulin sensitizing compounds can correct metabolic disorders; In patients that consumed myo-inositol (with or without metformin), significantly reduced BMI, abdominal waist, insulin plasma levels, and triglycerides, as well as significantly increased plasma HDL-C, were reported. No further details of clinical and biochemical safety parameters. No adverse effects of inositol were reported.

D'Anna et al. (2014)	Metabolic syndrome; Randomized, open-label; 60 post-menopausal F; 30/group	Control: Placebo; Treatment: 30 mg cocoa polyphenols, 80 mg soy isoflavones, 2000 mg myo-inositol. 12-month and 6-month treatment and 6-month follow-up	Adverse events and standard clinical evaluations and routine laboratory analyses were done every 6 months. BMI, SBP, DBP, waist circumference, glucose, HDL-C, triglycerides, B-ALP, adiponectin, visfatin, resistin; NSD in HDL-C concentrations or adiponectin levels at 6 months. A significant difference in glucose, triglycerides, B-ALP, visfatin, and resistin; Significant change in adiponectin, resistin, visfatin, B-ALP, glucose, and triglycerides Significant in change basal values of triglycerides and adiponectin. No changes in BMI. No adverse effects of treatment were reported.
Giordano et al. (2011)	Metabolic syndrome; Randomized, placebo-controlled; 80 post-menopausal F with metabolic syndrome	Dietary intervention plus placebo or 4,000 mg/day; 6 months	Clinical chemistries; Investigators did not report the incidence of side effects Significant reductions in inositol-treated subjects in relation to blood glucose, insulin, total cholesterol, triglycerides, and BP *vs*. placebo group
Minozzi et al. (2008)	Hirsutism; Prospective clinical trial; 46 F (mean age: 24 years)	4,000 mg/day; 6 months	Serum total cholesterol, HDL, LDL, triglycerides, apolipoprotein B, lipoprotein(a), serum adrenal and ovarian androgens, fasting glucose and insulin concentrations were evaluated. Fasting glucose concentrations were normal. No adverse effects reported.
Papaleo et al. (2007)	PCOS; Study design not reported; 25 F (28 to 38 years)	4,000 mg/day; 6 months	Incidence of side effects was not reported. The investigators concluded that inositol is safe treatment.
Allan et al. (2004)	Psoriasis patient; Randomized, double-blind, placebo-controlled, cross-over; 15 subjects on lithium; 11 subjects not on lithium	Lithium + inositol No lithium + inositol 6,000 mg/day; 10 weeks	Changes in the severity of psoriasis were measured. No adverse effects to inositol intake reported in subjects on lithium, while 1 subject withdrew due to nausea and 1 withdrew due to worsening psoriasis in the non-lithium group. No additional information was available.
Gelber et al. (2001)	Bulimia nervosa and binge eating disorder; Double-blind, placebo-controlled, cross-over; 12 patients for 6 weeks in each arm	0 or 18,000 mg/day; 6 weeks	Global Clinical Impression, Visual Analogue Scale, and Eating Disorders Inventory; 12 subjects withdrew (8 during placebo, and 4 during inositol); not related to side effects or treatment; 5 subjects reported mild abdominal pain, flatulence, or soft stools (resolved after dose reduced to 12,000 mg/day)

Chengappa et al. (2000)	Bipolar disorder; Randomized, double-blind (+ open phase), placebo-controlled; 24 subjects (mean age: 43 years)	Placebo (d-glucose) or 12,000 mg inositol/day (adjunctive to valproate, carbamazepine, or lithium treatment); 6 to 13 weeks	Safety ratings were done weekly; 2 subjects receiving placebo withdrew 2 subjects receiving inositol reported loose stool/diarrhea (1 resolved with treatment, 1 withdrew due to this side effect during extended intake period) Reports of other side effects were similar in inositol and placebo groups No effect on clinical chemistry analysis during double-blind or open phases
Benjamin et al. (1997)	Panic disorder; Double-blind, placebo-controlled, cross-over; 9 subjects	0 or 20,000 mg; Single dose	One subject withdrew after inositol intake due to diarrhea, insomnia, and energy; 3 additional subjects reported insomnia, and 3 reported diarrhea; Overall, 3 placebo subjects rated (on a scale) moderate or severe insomnia compared to 4 subjects in the inositol group
Benjamin et al. (1995)	Panic disorder with and without agoraphobia; Double-blind, placebo-controlled, cross-over; 25 subjects; 21 completed	0 or 12,000 mg/day Placebo was mannitol (n=10) or glucose (N=11) 4 weeks	Of the 25 patients enrolled in the study, 21 completed it. Two withdrew before beginning treatment. One patient completed 4 weeks of inositol and 1 week of placebo and then withdrew without a clear explanation. Side effects were minimal; no serious adverse effects reported; two subjects complained about sleepiness while taking inositol

AUC = area under the curve; B-ALP = bone-specific alkaline phosphatase; BMI = body mass index; bw = body weight; DBP = diastolic blood pressure; DCI = D-chiro-inositol; F = female; FI = fasting insulin; FPG = fasting plasma glucose; FSH = follicle stimulating hormone; HDL-C = high-density lipoprotein cholesterol; HOMA = homeostatic model assessment; HOMA-IR = homeostatic model assessment of insulin resistance; HOMA-ISI = homeostatic model assessment-insulin sensitivity index; hs-CRP = high sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; MFG = Modified Ferriman-Gallwey score; MI = myo-inositol; NR = not reported; NSD = no significant difference; OGTT = oral glucose tolerance test; PCOS = polycystic ovarian syndrome; SBP = systolic blood pressure; VLDL-C = very low-density lipoprotein cholesterol; WC = waist circumference; WHR = weight–height ratio.

In a review article, Formoso et al. (2019) reviewed the available evidence on the effects of inositol and antioxidant-based supplement administration during pregnancy complicated by insulin resistance and/or by diabetes. In addition to animal model studies, several clinical trials have been conducted in order to evaluate the safety of inositol supplementation. These reviewers noted that in clinical trials in which inositol dosages ranged from 4 to 60 g/day and the exposure time ranged from 1 to 12 months, the only adverse events reported were mild gastrointestinal symptoms (nausea, flatus, and diarrhea) but only at doses greater of 12 g per day. As regards fetal safety, the transplacental passage of inositol to the fetus seems not to be clinically relevant. Myo-inositol has been used in preterm infants (<29 weeks of gestational age) with respiratory distress syndrome in order to assess safety and pharmacokinetics of different daily inositol doses (10, 40, or 80 mg/kg bw/day). In this study, treatment with inositol at levels up to 80 mg/kg bw/day for over 10 weeks did not result in increased incidence of any adverse event as compared with control babies (Phelps et al., 2016; Formoso et al., 2019).

In a secondary analysis on three randomized, placebo-controlled clinical trials, Santamaria et al. (2018) assessed clinical and metabolic outcomes in pregnant women at risk for gestational diabetes mellitus. The three clinical trials included a total of 595 women that were provided with daily oral supplements containing 200 μg folic acids (placebo) or 2000 mg myo-inositol plus 200 μg folic acid. Main measures were the rate of adverse clinical outcomes: macrosomia (birthweight, ≥4000 g), large-for-gestational-age babies (fetal growth, ≥90 percentile), fetal growth restriction (fetal growth, ≤3 percentile), preterm birth (delivery before week 37 since the last menstruation), gestational hypertension, and gestational diabetes mellitus. In the supplements group, significantly decreased occurrence of preterm birth, macrosomia, and large-for-gestational-age babies were reported. A significantly reduced prevalence of gestational diabetes mellitus was noted in the treatment group. As compared to placebo, subjects in the supplements group revealed significantly increased HOMA index value. No adverse effects of inositol were reported in any subjects and such effects were not noted to be the reason for withdrawal from the study.

In a randomized clinical trial, Fruzzetti et al. (2017) compared the effects of myo-inositol and metformin in women with PCOS. In this study, women diagnosed with PCOS (n=25/group) were daily orally supplemented with 4000 mg myo-inositol and 400 μg folic acid or 1500 mg metformin for 6 months. A control group of healthy women (n=30) not receiving any treatment was included. Participants were studied for insulin secretion, BMI, menstrual cycle length, acne, and hirsutism. At the end of 6 months, subjects receiving myo-inositol revealed significantly decreased BMI relative to baseline. As compared to controls, significantly decreased HOMA-IR and insulin AUC and significantly increased Matsuda index were reported in subjects receiving myo-inositol. Supplementation with myo-inositol did not show any significant changes in androgen levels following 6 months of treatment. No adverse effects of inositol were reported.

In a randomized, double-blind, controlled trial in healthy subjects, Bañuls et al. (2016a) investigated the effects of chronic consumption of carob pod inositol-enriched beverage on postprandial glycemia and insulin sensitivity. In this study, 40 subjects (20/sex) were instructed to follow a normocaloric diet and assigned to receive an inositol-enriched beverage (2.23 g pinitol) or a sucrose-enriched beverage (control) twice daily over a period of 12 weeks. Anthropometric parameters such as body weight, blood pressure, and waist circumference, and biochemical parameters such as glucose levels, HOMA-IR, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were measured. Significantly

decreased insulin, HOMA-IR, and apolipoprotein B levels and significantly increased low-density lipoprotein particle size were reported in the treatment group. A significant reduction in mean postprandial glucose levels taken at breakfast, lunch, and dinner and in the AUC 24 hours following consumption of the inositol-enriched beverage was also noted. No adverse effects were reported.

In an attempt to conduct risk assessment of inositol, Carlomagno and Unfer (2011) reviewed available safety related literature on inositol. Twelve clinical studies of inositol in populations with significant underlying health conditions were assessed. It was concluded that doses of up to 4000 mg inositol/day are safe for human consumption with no adverse effects and intakes up to 12 g/day result in only gastrointestinal symptoms. These investigators also noted that the adverse effects did not change from 12 to 30 g inositol/day. In addition to human studies, these reviewers reported that findings from sub-acute animal studies indicated inositol had no toxic effects in rats at levels up to 2 g/100 g diet.

In summary, the safety and tolerance of inositol intake has been extensively evaluated in a number of clinical studies. In few clinical studies conducted in healthy subjects, no significant effects on safety parameters or any major adverse effects were noted. In multiple studies in individuals with conditions such as panic disorder, eating disorders, metabolic syndrome, and PCOS, effects of inositol were evaluated, with doses of inositol up to 20 g and for durations up to 6 months. In all of these studies, inositol supplementation was well received, with minor adverse effects such as diarrhea, abdominal pain, and insomnia. In general, inositol was well tolerated without any significant adverse effects in doses up to 4 g/day and for durations up to 6 months.

6.2.2. Pre-Clinical Studies of Inositol

6.2.2.1. Short-term and Subchronic Studies of Inositol

No acute toxicity studies of inositol were found in the published literature. The available evidence from short-term feeding studies indicates that inositol at levels up to 5000 mg/kg bw/day for one month is generally well tolerated in rats, although diarrhea was noted in dogs following administration of very high doses (10 g/dog/day for 6 days) (Anderson, 1916; SCOGS, 1975).

In a study in mice, Croze et al. (2013) investigated the metabolic effects of myo-inositol. For these investigations, CD-1 Swiss female mice were given daily intraperitoneal injections containing saline (control) or myo-inositol at a dose of 1.2 mg/g bw for 15 days. In another set of experiments, mice were provided myo-inositol in drinking water at 6 g/L (equivalent to 0.9 mg/g bw daily based on water intake) for 15 days. Animals were studied for body weight, glucose and insulin tolerance, insulin secretion, histopathology, and lipogenesis. Additionally, biochemical parameters such as plasma total cholesterol, triacylglycerols, nonesterified fatty acid (NEFA) levels, adiponectin, leptin, and total lipid content were measured. Regardless of route of administration, no significant differences in body weight, feed intake, or water intake were noted. Mice treated intraperitoneally with myo-inositol showed significantly reduced plasma glucose, leptin, and NEFA. Glucose tolerance tests revealed significantly reduced plasma glucose, AUC of glycemia, and insulin levels. As such no adverse effects of myo-inositol were noted.

In a weanling piglet study, Ogunribido et al. (2022) investigated the effects of reduced protein high-phytate diets supplemented with myo-inositol on growth performance and apparent total tract digestibility. In this randomized block study design, 128 piglets were divided into 8 replicates, with 4 piglets per replicate, and were fed either: a diet that met the nutrient requirements of phosphorus in weanling pigs (positive control); a diet that contained a 3% reduction in the required phosphorus content (negative control); the negative control diet supplemented with 2 g/kg myo-inositol; or the negative diet supplemented with 3000 phytase units (FTU)/kg phytase. Body weight, growth, and feed intake for a period of 28 days were measured. As compared to the positive control, piglets treated with inositol had significantly reduced average daily weight gain and final body weight. However, as compared to the negative control, this effect was not significant. No adverse effects of myo-inositol administration were reported.

In a subchronic study, Croze et al. (2015) investigated the effects of myo-inositol on metabolism in type 2 diabetic mice given a high-fat diet. In this study, male C57BL/6 mice (n=10/group) were provided a standard diet (control), a high-fat diet with myo-inositol (HFD-MI) in drinking water, or a high-fat diet without myo-inositol (HFD) in drinking water, providing a dose of 0.58 mg/g bw inositol daily for 4 months. The dose of myo-inositol was selected such that it reflects the maximum tolerable dose of 20 g/day established in human clinical trials. Body weight, feed and myo-inositol intake, insulin tolerance, urinalysis, organ weights (*i.e.*, liver, heart, kidneys, gastrocnemius muscles, and epididymal, retroperitoneal and subcutaneous inguinal white adipose tissue), and histopathology of the liver and retroperitoneal white adipose tissue were evaluated. Additionally, biochemical parameters including plasma total cholesterol, triacylglycerides, NEFA, and urea levels were measured.

As compared to mice given HDF, no significant differences in body weight or energy intake were reported in HFD-MI mice (Croze et al., 2015). In HFD-MI mice, kidney and gastrocnemius muscle weights were significantly higher compared to HFD mice. A sharply increased fat storage throughout the study was noted in HFD mice, while HFD-MI mice showed significant suppression in increased fat storage. No significant differences were noted in total white adipose tissue weight between HFD-MI mice and HFD mice. The cell weight in the retroperitoneal fat pad was significantly reduced in HFD-MI mice as compared to HFD mice. HFD-MI mice showed significantly reduced fatty acid synthase activity relative. The investigators suggested that this contributed to the decreased accumulation of the white adipose tissue in HFD-MI mice. As compared to HDF mice, no significant differences were noted in plasma metabolites or ectopic redistribution of lipids to liver or muscle in HFD-MI mice. Plasma leptin was significantly decreased in HFD-MI mice compared to HFD mice. This was consistent with the reduced total white adipose tissue mass. In HFD-MI mice, plasma adiponectin was similar to control mice. No significant differences were noted between the groups as regards expression of lipogenic enzymes or adipokines in white adipose tissue (Croze et al., 2015). Although the primary objective of this study was to investigate the efficacy of inositol supplementation, no adverse effects of inositol were noted.

6.2.2.2. Reproductive and Developmental Effects of Inositol

In some studies, reproductive and developmental toxicity of inositol alone or along with other chemicals has been investigated. The findings from these studies suggest that inositol is unlikely to cause reproductive developmental effects. The available studies related to inositol effects on maternal, reproductive and offspring parameters are summarized in Table 11.

Valproic acid (VPA), an anticonvulsant drug, is known for its teratogenic potential in both humans and experimental animal models. A relationship exists between valproic acid exposure and the cellular depletion of myo-inositol (INO). Inositol has been shown to rescue neural tube defects (NTDS) in the curly tail mouse. Massa et al. (2006) investigated the interactions of valproic acid and inositol in the developing embryo. For this study, 2 strains of mice were used: SWV/Fnn (known to be sensitive to valproic acid) and LM/Bc (known to be resistant to valproic acid-induced NTDs). In this study, pregnant female mice were randomly assigned to 4 experimental groups: control, VPA (600 mg/kg bw), INO (400 mg/kg bw), and VPA plus INO. VPA was injected intraperitoneally at 8.5 days postcoitum (dpc). INO was administered orally twice a day from 6.5 to 10.5 dpc. At term the dams were euthanized, the uteri were removed, and all of the general toxicological parameters (number of implants, resorptions, dam weight, and fetus weight) were recorded and analyzed. The findings from this study revealed that postimplantation loss in the SWV/Fnn strain and NTDs in the LM/Bc strain were significantly increased after the coadministration of VPA and INO. The investigators concluded that INO enhances VPA-induced teratogenicity in the mouse. Although the findings from this study reveals that inositol in combination with VPA causes teratogenicity in the mice, the group receiving inositol alone did not reveal adverse effects.

Cogram et al. (2020) investigated the effectiveness and safety during pregnancy of two isomers, myo- and D-chiro-inositol, in preventing mouse NTDs. In this study, inositol was administered either directly to embryos *in vitro*, or to pregnant females by subcutaneous or oral routes. Pregnant mice (curly-tail neural tube defect model) were orally administered during gestation days 8 to 10 with myo-inositol or D-chiro-inositol at dose level of 800 µg/kg bw/day. Although D-chiro- and myo-inositol both reduced the frequency of spina bifida in curly tail mice by all routes of administration, D-chiro-inositol consistently exhibited the more potent effect, reducing spina bifida by 73-86% in utero compared with a 53-56% reduction with myo-inositol. Pathological analysis revealed no association of either myo- or D-chiro-inositol with reduced litter size or fetal malformation.

Table 11. Reproductive and Developmental Toxicity Studies on Inositol

Reference	Species; Sex; Number	Route; Exposure Period; Dose	Safety Related Findings
Massa et al. (2006)	Mice [SWV/Fnn (sensitive to VPA), and LM/Bc (resistant to VPA-induced neural tube defects)] Female; pregnant 12 dams/group	Oral (gavage); GD 6 to 10; Total dose 800 mg/kg bw/day Inositol, with and without injection of VPA	No significant change in fetal body weight or incidence of embryonic mortality observed in either strain; No increase in neural tube defects; Co-administration of inositol and valproic acid significantly increased embryo lethality *vs.* all other groups in the SWV/Fnn strain; Co-administration also significantly reduced fetal bw and significantly increased the incidence of exencephalic fetuses *vs.* all other groups in SWV/Fnn strain. No adverse effects of inositol treatment alone were reported.
Cogram et al. (2002)	Mice (curly-tail neural tube defect model) Female; pregnant	Oral (gavage); GD 8 to 10; 800 µg/kg bw/day Inositol or D-chiro-inositol	No adverse effects on resorption rates, litter size or fetal crown-rump length No significant increase in incidences of fetal malformations or anomalies

Khandelwal et al. (1998)	Rats (Sprague-Dawley) Female; pregnant and prediabetic (65/group)	Oral (gavage); GD 6 to 12; 0.08 to 0.5 mg/mL/day	Significantly reduced incidence of diabetes-related neural tube defects compared to diabetic controls but not enough to non-diabetic controls
Reece et al. (1997)	Rats (Sprague-Dawley) Female; pregnant and diabetic (8 to 10/group)	Oral (gavage); GD 6 to 12 ; 0.08 to 0.5 mg/ml/day	Significantly reduced incidence of diabetes-related neural tube defects compared to diabetic controls Significant increase in absorptions in 0.16 mg/day group
Burton and Wells (1976)	Rat pups (Holtzman) Male/Female	Oral (diet); GD 7 to weaning (3 months); 0.5% in diet (250 mg/kg bw/day)	*Maternal effects:* Inositol levels were significantly increased in plasma, liver, kidney, and intestines of pups, and in the milk and mammary tissues No adverse effects on bw or clinical signs reported; *Reproductive and offspring effects:* Supplementation did not significantly alter male pup sperm counts or fertility indices in male and female rats
Ershoff (1946)	Rats (Long-Evans) Female	Oral (diet); 60 days prior to mating; 1% in diet (500 mg/kg bw/day)	No effect on the ability for dams to reproduce No effect on fetal body weight No other parameters studied

bw = body weight; F = female; GD = Gestational Day; M = male; NSD = no significant difference; VPA = valproic acid.

6.2.2.3. Genotoxicity Studies of Inositol

In one study, described in SCOGS (1975) report, genotoxicity of inositol was evaluated using *in vitro* reverse mutation assays conducted in 3 strains of *Salmonella typhimurium* and 2 strains of *Saccharomyces cerevisiae* with and without metabolic activation at concentrations of up to 5% inositol. The study was not made publicly available. However, the results of the *in vitro* assays indicate that inositol showed no evidence of mutagenicity. The available evidence related to the presence of inositol in diet and its uses indicate that inositol is unlikely to be genotoxic.

6.2.6. Safety of Production Strain

The available information shows that *Escherichia coli* are commensal residents of the gut microbiota of humans and several other animal species. Based on the sequence similarity of housekeeping genes, different strains of *E. coli* are taxonomically grouped into five different phylogroups, such as A, B1, B2, D, and E (Archer et al., 2011). Commensal strains present in humans are typically found in Group A or B1, with non-related pathogenic strains classified under Group B2, D, and E. According to their relative pathogenicity for healthy adult humans, 3 group A laboratory strains, as well as strains K-12, B, C, and their derivatives are designated as Risk Group 1 organisms (Archer et al., 2011; Daegelen et al., 2009). As per recent National Institutes for Health (NIH) guidelines for research involving recombinant or synthetic nucleic acid molecules, Risk Group 1 organisms "are not associated with disease in healthy adult humans" (NIH, 2023). Among these strains, *E. coli* K-12 and the B derivatives (e.g., BL21) are commonly used for production of industrial, pharmaceutical, and food biotechnology preparations.

The safety of *E. coli* BL21 (DE3), the strain used in the production of inositol, has been demonstrated in several comprehensive studies. This *E. coli* strain does not carry the well-recognized pathogenic components required for *E. coli* strains to cause enteric infections. Hence, *E. coli* BL21 (DE3) is considered as nonpathogenic (non-virulent) and unlikely to survive in host tissues or to cause disease (Chart et al., 2000). The whole genome sequence for *E. coli* BL21 (DE3) strain has been assembled. The genome of this strain only marginally differs from that of widely used in production strain, i.e., *E. coli* K-12 (Studier et al., 2009). The whole genome sequencing of *E. coli* BL21 (DE3) revealed the absence of genes encoding invasion factors, adhesion molecules, and enterotoxins associated with virulence (Jeong et al., 2009). The findings from an acute oral toxicity study in mice revealed that the *E. coli* BL21 (DE3) endotoxin produced no toxicity, even at the highest dose of 1,000,000 endotoxin units (3.3 mg/kg bw) (Harper et al., 2011).

It should be noted that the production strain *E. coli* BL21 (DE3) was engineered with genes with known functions, which do not confer toxicogenicity, virulence, or DNA, using site-specific homologous recombination; it is commonly accepted that *E. coli* BL21 (DE3) is non-toxigenic and is not capable of DNA transfer to other organisms. The stability of the target gene integration and stability of target gene expression in the production strain has been confirmed. As mentioned earlier, the safety of the host organism, *E. coli* BL21 (DE3), is summarized in several GRAS notices that received "no questions" letters from the FDA. This information suggests that *E. coli* is a suitable and safe strain in the production of food ingredients.

Based on the comprehensive characterization of the strain used in inositol production and its widespread uses, the use of *E. coli* BL21 (DE3) as the host strain and production strain are not expected to result in any safety concerns.

6.3. GRAS Panel Evaluation, Summary and Discussion

At the request of Sichuan Bohaoda Biological Technology Co., Ltd. (Sichuan), an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[3], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to assess the Generally Recognized As Safe (GRAS) status of inositol produced by fermentation with a recombinant *Escherichia coli* BL21(DE3) strain, when used as a food ingredient and as a Nutrient supplement[4] in beverage and beverage bases, milk products, processed fruits and fruit juices, and processed vegetables and vegetable juices at levels up to 250 mg/kg. A comprehensive search of the scientific literature for safety and toxicity information on inositol and the production strain was conducted through March 2024 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Sichuan and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on May 06, 2024 and unanimously agreed to the decision described herein.

Sichuan ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[5]. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel members received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel members were not contingent upon the outcome of their deliberations.

The subject of this GRAS assessment, inositol (myo-inositol) is a standardized fine, white crystals or white crystalline powder preparation produced by fermentation. Inositol is a carbocyclic sugar that is endogenously produced in the body and occurs naturally in meats, plants, and dairy products. Sichuan's inositol is produced as per current Good Manufacturing Processes (cGMP) by fermentation with recombinant *E. coli* BL21 (DE3) strain. Appropriate food grade specifications for inositol have been established. The final product is highly purified and contains not less than 97% and not more than 102% inositol, on anhydrous basis. Analytical data from five nonconsecutive lots of inositol demonstrate that the manufacturing process produces a consistent product that meets the defined specifications that include parameters on its identity/composition, and established limits for heavy metal and microbiological contaminants. All raw materials, processing aids, and food contact substances employed in the manufacturing of inositol are GRAS and/or conform to the specifications stated in 21 CFR and/or the FCC. The genetically engineered strain of *E. coli* BL21 (DE3) used by Sichuan in the production of inositol, is non-toxigenic, not capable of DNA transfer to other organisms, and has the same virulence profile as host strain *E. coli* BL21 (DE3). Process controls and product specifications are in place

[3]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.
[4]21 CFR §170.3 (o) (20) - Nutrient supplements: Substances which are necessary for the body's nutritional and metabolic processes.
[5]Available at:
https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

to control the levels of residual impurities, as well as heavy metals, micro-organisms, and production organism-derived DNA and endotoxin, ensuring a consistent, food-grade finished ingredient.

Sichuan intends to use inositol in beverage and beverage bases, milk products, processed fruits and fruit juices, and processed vegetables and vegetable juices at levels up to 250 mg/kg. The intended use levels and food categories are same as those approved by the Ministry of Health of the People's Republic of China under GB 14880-2012 National Food Safety Standard for the Use of Nutritional Fortification Substances in Foods. In the US, as per 21 CFR §184.1370, inositol is GRAS as a direct human food ingredient, when used under the following conditions and in accordance with cGMP. As inositol is permitted for use in food, the proposed uses will be substitutional and are unlikely to affect the current existing use or the cumulative intake. The intended use of inositol is considered as substitutional to those currently on the US market.

The available information indicates that in adults consuming a balanced diet, the daily intake of inositol is approximately 1000 mg/day, while endogenous production has been estimated as 4000 mg/day. As per 21 CFR §184.1370, inositol is GRAS as a direct human food ingredient when used under the following conditions and according to cGMP: as a nutrient supplement as defined in 21 CFR §170.3(o)(20), in special dietary foods, as well as in infant formula. Inositol is commonly marketed as a dietary supplement in the US. In the European Union, inositol is authorized as a novel food for use in food.

Findings from numerous clinical studies, many of which were conducted in subjects with various underlying health conditions, support the safety of the intended use of inositol. In clinical studies in healthy subjects, no significant effects on safety parameters or any major adverse effects were noted. In several studies in individuals with conditions such as panic disorder, eating disorders, metabolic syndrome, and PCOS, participants were provided doses of inositol up to 20 g and for durations up to 6 months. In all of these studies, inositol supplementation was well received, with minor adverse effects such as diarrhea, abdominal pain, and insomnia. No significant adverse effects were reported, and inositol was well tolerated at doses up to 4 g/day and for durations up to 6 months. Inositol at a level of up to 4 g/day is safe for human consumption with no adverse effects and intakes up to 12 g/day result in only gastrointestinal symptoms with similar symptoms up to 30 g/day.

In the absence of typical acute and subchronic toxicity studies, safety of inositol is supported by several short-term feeding studies. These studies indicate that large doses of inositol (up to 5000 mg/kg bw/day for 1 month) in rats are generally well tolerated. In dogs, administration of very large doses (10 g/dog/day for 6 days) of inositol resulted in diarrhea. In additional studies, mice were provided 6 g/L in drinking water for 15 days and were compared to mice administered 1.2 mg/bw intraperitoneally and piglets were fed for 28-days. No adverse effects or toxicological effects were identified in either study. In a 4-month subchronic feeding study mice were fed a high-fat diet plus 0.58 mg myo-inositol/g bw. Supplementation with inositol and a high-fat diet resulted in a reduction of white adipose tissue without any significant differences in safety-related parameters. The findings from reproductive and developmental studies suggest that inositol is unlikely to cause reproductive developmental toxicity. Additionally, unpublished genotoxicity studies have demonstrated that inositol has shown no evidence of mutagenicity.

The GRAS assessment for the use of inositol in selected food categories is based on the totality of available evidence. All pivotal data and information described in this dossier and used to establish the safety of Sichuan's inositol under its intended conditions of use are "generally available" (i.e., in the public domain). From the data and information presented herein, Sichuan concludes that inositol produced with a genetically engineered strain of *E. coli* BL21 (DE3) is GRAS for its intended uses in selected foods, based on scientific procedures.

In summary, there is sufficient qualitative and quantitative scientific evidence, including *in vitro,* animal and human data, to assess the safety-in-use of inositol produced by fermentation with a recombinant *Escherichia coli* BL21(DE3) strain, the subject of this present GRAS assessment. The safety assessment of the inositol is based on the totality of the available evidence, including human clinical studies. The totality of the evidence supports the safety of inositol for its uses in beverage and beverage bases, milk products, processed fruits and fruit juices, and processed vegetables and vegetable juices at levels up to 250 mg/kg. On the basis of scientific procedures[6], the consumption of inositol produced by fermentation as an added food ingredient is considered safe. The intended uses are compatible with current regulations, *i.e.*, inositol is used in specified foods (described in this document) and is produced according to current good manufacturing practices (cGMP).

[6] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

6.4. GRAS Panel Conclusion

Based on a critical evaluation of the publicly available data, summarized herein, the Expert Panel members whose signatures appear below have individually and collectively concluded that inositol produced by fermentation with a recombinant *Escherichia coli* BL21(DE3) strain, meeting the specifications cited herein, and when used as a food ingredient and as a nutrient in selected conventional food products, such as beverage and beverage bases, milk products, processed fruits and fruit juices, and processed vegetables and vegetable juices at levels up to 250 mg/kg, is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that inositol produced by fermentation, when used as described, is Generally Recognized As Safe (GRAS) based on scientific procedures.

Signatures

James T. Heimbach, Ph.D., F.A.C.N. — May 6, 2024 — Date

David Ribet, Ph.D. — May 6, 2024 — Date

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.
Advisor to Expert Panel — May 6, 2024 — Date

7. PART VII- LIST OF SUPPORTING DATA AND INFORMATION

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Form Approved: OMB No. 0910-0342; Expiration Date: 08/31/2025
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001198	May 21, 2024
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
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Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Huabin Gan	Corporate legal person

Organization *(if applicable)*
Sichuan Bohaoda Biological Technology Co., Ltd.

Mailing Address *(number and street)*
No. 9 Longxiang Avenue, Yantan District High-tech Industrial Park, Zigong city, Sichuan Province, China

City	State or Province	Zip Code/Postal Code	Country
Zigong city	Sichan Province	643031	China

Telephone Number	Fax Number	E-Mail Address
+86-0813-2610661		sichuanbohaoda2021@163.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Wing Yu	Technical manager of food division, CIRS GROUP

Organization *(if applicable)*
CIRS GROUP USA INC

Mailing Address *(number and street)*
4250 Fairfax Drive, Suite 600, Arlington, VA 22203

City	State or Province	Zip Code/Postal Code	Country
Arlington	Virginia	22203	United States of America

Telephone Number	Fax Number	E-Mail Address
+1 703 520 1420		wing.yu@cirs-group.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Inositol

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Inositol is intended to be used as a food ingredient (Nutrient supplement) in: Beverage and beverage bases; Milk products; Processed fruits and fruit juices; and Processed vegetables and vegetable juices at levels up to 250 mg/kg. It is recognized that there are Standard of Identity requirements for some of these specified foods and these foods will not be referred by their commonly recognized names. Inositol will not be used in infant formula.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Sichuan Bohaoda Biological Technology Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Inositol
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Sichuan Bohaoda Biological Technology Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

No. 9 Longxiang Avenue, Yantan District High-tech Industrial Park, Zigong city, Sichuan Province， China
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Wing Yu Wing Yu 2024.05.21 15:04:02 +08'00'	Wing Yu, Technical manager of food division， CIRS GROUP	05/20/2024

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	COSM_3667_19743_SichuanBohaodaBiolog.pdf	Administrative
	InositolGRASdossierfinalforFDA-done.pdf	Administrative

Dear Dr. Deng,

RE: GRN 1198- Inositol

This responds to your email of October 16, 2024, regarding FDA queries that need to be addressed for Sichuan Bohaoda Biological Technology Co., Ltd. (Sichuan Bohaoda)'s GRAS notice GRN 001198 for the intended use of inositol as a food ingredient. We are providing a point-by-point response to all your queries along with some relevant clarifications/discussion.

1. On page 7, the notifier states that inositol meets the specifications and quality/purity criteria listed in the Food Chemicals Codex (FCC) monograph. The FCC specifies the limits for inorganic impurities including calcium, chloride, and sulfate. We note that GRN 001198 does not specify limits for calcium, chloride, and sulfate. In addition, the FCC provides specific tests including melting range or temperature determination, and residue on ignition that are not included in the specifications in GRN 001198. Please discuss why these specification parameters are not relevant to your inositol and/or the safety of its use.

Response:

Sichuan Bohaoda has conducted tests according to the FCC, and the results comply with the FCC. COAs of three batches of products are attached (Appendix I). It appears that the FCC specifications for inositol is for the product derived from an aqueous (0.2 percent sulfur dioxide) extract of corn kernels by precipitation and hydrolysis of crude phytate. These processes are responsible for inorganic impurities such as calcium, chloride, and sulfate. We did not include the inorganic impurities, as the product is manufactured by fermentation using recombinant *Escherichia coli* BL21 (DE3) strain.

2. In Table 3 (page 11), the notifier lists a specification limit for organic impurities. Please identify what these organic impurities are.

Response:

The organic impurities refer to glucose and maltose.

3. In Table 4 (page 12), we note the results from the batch analyses for heavy metals are consistently below the corresponding limits of detection (LOD) of the analytical method (0.002 mg/kg-0.05 mg/kg) and are at least one order of magnitude lower than the proposed specification limits (≤ 0.5 mg/kg). In view of FDA's Closer to Zero initiative, we recommend lowering the specification limits for heavy metals to better reflect the results of the batch analyses. We typically see limits for heavy metals established at no more than 0.1 mg/kg based on similar results from batch analyses.

Response:

We accept to establish the limits for heavy metals at no more than 0.1 mg/kg.

4. On page 15, the notifier states that the intended use of inositol is as a nutrient and food ingredient. Further, the notifier states that the use of inositol as a nutrient in foods is GRAS per 21 CFR 184.1370 and that "the intended uses of inositol are substitutional to the existing or approved uses". We note that 21 CFR 184.1370 covers only the use of inositol as a nutrient supplement, not as a food ingredient in general. Please confirm that you intend to use inositol as a nutrient supplement only. In addition, please confirm that the intended uses are substitutional to those in 21 CFR 184.1370 and therefore there will be no increase in cumulative dietary exposure to inositol.

Response:

We agree with FDA recommendations. Sichuan Bohaoda intends to use inositol as a nutrient supplement only, and we confirm that the intended uses are substitutional to those in 21 CFR 184.1370 and therefore there will be no increase in cumulative dietary exposure to inositol.

5. In Tables 8 and 9 (page 16), please clarify whether "All population" refers to the population aged 1 year and older.

Response:

For intake analysis we used Dazult's Dietary Intake Evaluation Tool, DaDiet. Based on the intake analysis performed, all population refers to population aged 1 year (minimum age) and older.

6. On page 37, the notifier states that "All raw materials, processing aids, and food contact substances employed in the manufacturing of inositol are GRAS and/or conform to the specifications stated in 21 CFR and/or the FCC." Please confirm that all raw materials, processing aids, and food contact substances used in the manufacturing process are used in accordance with applicable U.S. regulations, are GRAS for their intended uses, or are the subject of an effective food contact notification.

Response:

Sichuan Bohaoda confirms that all raw materials, processing aids, and food contact substances used in the manufacturing process of inositol are used in accordance with applicable U.S. regulations, are GRAS for their intended uses, or are the subject of an effective food contact notification.

7. For the administrative record, please provide the following information of the production strain:

a. A strain name for its identity.

b. whether the production strain has been deposited in a recognized culture collection. If so, please provide the deposit designation.

c. whether genome data of the genetically engineered *E. coli* strain is

available in the public domain, e.g., a NCBI accession number.

Response:

We are sorry for lack of clarity on the use of microorganism in the production of inositol. Please note that five different production strains are developed and used in the manufacturing of inositol. Each of these strains is derived from the parent strain of *E.coli* BL 21 (DE3), with each strain having a separate gene sequence inserted, expressing one specific enzyme, rather than inserting all five gene sequences into a single parent strain. As described in the GRAS notice inositol is synthesized in one step that involves an *in vitro* multi-enzyme reaction system. The production strains have not been deposited in a recognized culture collection. The strains name and the gene expressed are as follows:

1) *E.coli* BHD-STIA, which expresses Isoamylase- gene from *Sulfolobus tokodaii, and* the gene number on KEGG (genes database) is STK_09280.

2) *E. coli* BHD-TMαGP, which expresses Glucan phosphorylase, and the gene was from *Thermotoga maritima* and the gene number on KEGG is Tmari_1175.

3) *E. coli* BHD-TKPGM, which expresses Phosphoglucomutase- the gene was from *Thermococcus kodakarensis* and the gene number on KEGG is TK1108.

4) *E. coli* BHD-AFIPS, which expresses Inositol-1-phosphate synthase- the gene is from *Archaeoglobus fulgidus* and the gene number on KEGG is AF_1794.

5) *E. coli* BHD-TMIMP, which expresses Inositol monophosphatase and the gene was from *Thermotoga maritima* and the gene number on KEGG is TM1415.

At the genes database, each GENES entry is identified by the combination of organism code and gene identifier.

8. In the section "Safety of Production Strain" in Part 6.2.6 (Pages 35-36), the discussion is mainly focused on *E. coli* species and the host strain *E. coli* BL21 (DE3). The production strain is genetically engineered *E. coli* which is genomically different from the host stain BL21 (DE3). To confirm the safety of this production strain, please provide a summary of genotypic and phenotypic characteristics of the strain, including verification methods, such as bioinformatic searches used to screen for virulence factors and cytotoxicity assays to verify the strain is non-toxigenic.

Response:

The five production strains are derived from parental strain *E. coli* BL21 (DE3) by genetic modification. They are Gram-negative, short rod-shaped bacteria with blunt, rounded ends. Except for the specific genetic modifications, the production strains are unlikely to have significant differences in growth, reproductive stability, viability, or pathogenicity compared to the parent strain. The parent strain *E. coli* BL21 (DE3) is considered non-pathogenic and unlikely to survive in host tissues and cause disease(Chart & Smith, 2000). Considering the comprehensive characterization of *E. coli* BL21 (DE3) and its widespread use in human milk oligosaccharides and protein production, the production strains are not expected to result in any safety concerns.

The defined function of the introduced exogenous genes is to express key enzymes involved in the biosynthesis of inositol. These exogenous genes, synthesized *in vitro*, are introduced into the parent strain without transferring any other characteristics of the donor organisms, thus they are unlikely to be related to any potential toxicity, anti-nutritional factors, allergenicity, or pathogenicity of the donor, and do not pose any risk to the final product, inositol.

Allergenicity analysis was conducted using the Allergen Online Database and the Structural Database of Allergenic Proteins, which indicated that the exogenous proteins do not exhibit high sequence homology with known allergens, suggesting a lower potential for allergenicity. In addition to allergenicity, bioinformatic analysis of all exogenous proteins has been carried out to verify the similarity among these proteins, known toxic proteins and anti-nutritional factors. This analysis involved sequence alignment of the test exogenous proteins against sequences in the NCBI and UniProt databases. The results showed that the exogenous proteins do not have a high degree of sequence similarity with known toxic proteins or anti-nutritional factors. The bioinformatic analysis did not reveal any safety concerns.

Additionally, inositol is of high purity and does not contain viable production strains, DNA or protein fragments from the production strain. The absence of cells, DNA or protein fragments supports the safety of the final product. In conclusion, the strains used in inositol production are considered as non-pathogenic and non-toxigenic.

9. Please include a statement to confirm whether the production strain carries any antibiotic resistant genes.

Response:

The ampicillin resistance gene was introduced into the production strains by exogenous plasmids and serves as a marker gene. Ampicillin is a β-lactam antibiotic, and β-lactam antibiotics are unstable in protonic solvents due to their β-lactam structure. The β-lactam ring hydrolyzes to form free carboxyl and amino groups, and subsequent intermolecular polymerization forms amide bonds, resulting in the loss of antimicrobial activity.

Additionally, inositol is of high purity and does not contain viable production strains or DNA or protein fragments from the production strain. The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Consultation on Biotechnology and Food Safety noted that the presence of antibiotic resistant genes is not a concern if it is not present in foods or released intentionally into the environment (Joint FAO/WHO, 1996).

10. In Part 2.2 (page 8), the notifier states that “five specific genetic manipulations were performed in the genome of recipient *E. coli* BL21 (DE3) strain by Sichuan”. In Table 2 on page 9, the notifier provides the information of gene functions and donor

microorganisms. Please clarify whether the DNA samples of the five genes are *de novo* synthesized or cloned from the donor microorganisms.

Response:

The DNA samples of the five genes are *de novo* synthesized.

11. Please briefly specify how the purity of the production strain inoculum for the manufacturing process is ensured.

Response:

The purity of the production strain inoculum is ensured as follows:

1. Aseptic operations: All operations involving contact with the production strains are conducted under aseptic conditions using sterilized equipment and tools.

2. Sterilization of media and containers: All media, containers, and auxiliary tools are sterilized using autoclaving (121°C, 30 minutes) before use.

3. Environmental control: The laboratory and production areas are regularly disinfected, irradiated with ultraviolet light, and the number of people entering these areas is controlled to restrict access to non-essential personnel.

12. Please confirm that fermentation is performed under a controlled and sterile condition.

Response:

Fermentation and sterile conditions are controlled as follows:

1. Fermentation tank and medium sterilization: Steam sterilization *in situ* is applied to fermentation tanks, media, pipes, valves, etc., to ensure that the fermentation process is carried out under sterile conditions.

2. Air filtration system: During fermentation, sterile compressed air purified by a high-efficiency air filtration system is introduced into the fermentation tanks to prevent contamination from airborne microbes.

3. Monitoring and testing: Regular microbial testing of the production environment, cultures, and final products is conducted to promptly identify any potential sources of contamination.

13. Please confirm whether ethanol is involved in the manufacturing process and, if so, please elaborate on its fate.

Response:

Ethanol is not used in the manufacturing process.

14. In Table 3 on Page 11, the notifier lists the analytical methods for both *E. coli*

and *Salmonella* are “Chinese Pharmacopoeia 2020, Part 4, page 165”. Since it is unusual that the testing methods of both pathogens are described on the same page, please confirm whether this information is correct.

Response:

Sorry for the confusion. The misunderstanding was caused by the page number. Sichuan Bohaoda is using 'Method 1106 of Part 4, Chinese Pharmacopoeia 2020.' This is a Microbial Limits Test for Non-Sterile Products, which includes the detection methods for microorganisms such as *E. coli* and *Salmonella*. Although the method starts on page 165, it is a comprehensive part of the pharmacopoeia. We request to revise the description of this method to 'Method 1106 of Part 4, Chinese Pharmacopoeia 2020.'

15. Human clinical data discussed in Section 6.2.2. report the ability of inositol, at doses much higher than the 90th percentile estimated dietary exposure from the intended uses, to shift the menstrual cycle in women with polycystic ovary syndrome (PCOS). Please provide a rationale to contextualize the ability of oral inositol from the intended uses to shift the menstrual cycle, or not, in consumers, healthy or otherwise, to support your GRAS conclusion.

Response:

Thank you for your comment and recommendation. We agree that the inositol doses used in several clinical studies are substantially high (up to 20,000 mg/day) as compared to the 90th percentile estimated daily intake (EDI, 158.5 mg/person/day) of inositol from the proposed uses in selected food. The commonly recommended or clinically relevant dose of inositol in women with PCOS is 4000 mg/day. This dose of inositol is about 25-fold higher as compared to the EDI.

Women with PCOS can have irregular periods or no periods at all. Inositol has been claimed to regulate menstrual cycles by interacting with hormones and neurotransmitters. Inositol has been widely used as a treatment for pathologies associated with insulin resistance, such as PCOS and diabetes. The available clinical data suggest that inositols (stereoisomers), because of their insulin-sensitizing effect, successfully improved the metabolic and reproductive aspects of PCOS (Wojciechowska et al., 2019). Inositol has been reported to be effective in normalizing ovarian function, improving oocyte and embryo quality in PCOS (Merviel et al., 2021). Inositol increases insulin sensitivity, decreases hyperandrogenism and improves the menstrual cycle.

Lagana et al. (2018) reported that myo-inositol and D-chiro-inositol are insulin second messengers, and myo-inositol is involved in follicular gonadotropin pathways which orchestrate ovulation. The tissue-specific ratio of myo-inositol and D-chiro-inositol is modulated by insulin through aromatase and is altered in insulin resistance, with reduced epimerization of myo-inositol to D-chiro-inositol in insulin-sensitive tissues. In ovaries, the ratio of these two inositols is 100:1. However, it is dramatically reduced by insulin-stimulated epimerase in hyperinsulinemic women with PCOS. Patients with PCOS tend to have a more estrogenic profile relative to healthy individuals. Inositols have proved to be effective in PCOS, improving metabolic and hormonal state, and

restoring spontaneous ovulation. In a review article, DiNicolantonio and O'Keefe (2022) extensively discussed mechanism of action of inositol in several health conditions, including its use in patients with PCOS. At lower doses, inositol is unlikely to affect the menstrual cycle.

As mentioned above and also extensively described in GRN 1198, majority of available clinical studies in women with PCOS are conducted to investigate therapeutic effects of inositol. The doses used in these clinical studies are quite high (about 4000 mg/day). The effects of inositol in these studies at high doses are likely to be physiological effects. As compared to the therapeutic dose used in clinical trials, the intended uses of inositol in food resulting in maximum daily intake of 158.5 mg/day is quite low and unlikely to shift the menstrual cycle in consumers, healthy or otherwise. The intended use of inositol in food is unlikely to have any medicinal or non-nutritive physiological benefits. In summary, the available information suggest that proposed low level uses of inositol in limited food categories is unlikely to be of any safety concern.

16. We note that the majority of the publications discussed in Section 6.2 are not traditional toxicology studies but were instead designed to assess non-nutritive physiological "benefits", designed as clinical drug trials to improve the severity of a disease, and/or use test subjects or animal models with multiple, varied chronic conditions like PCOS and diabetes. Because of this, their utility in the risk assessment of relatively pure inositol as a food ingredient for use in the general population is not immediately evident, as we assess only the safety of, and not potential benefits of, a food ingredient. Please succinctly identify what data and information are considered pivotal to the GRAS conclusion; this may be best represented in the form of a single table.

a. If this table includes data from any clinical or pre-clinical studies highlighted in Tables 10 and 11, or other traditional animal toxicology data discussed within Section 6.2, please identify the NOAEL or LOAEL from each of these pivotal data sets.

b. Further, please note if the NOAEL was the highest dose tested and contextualize these values to the estimated dietary intake from the intended uses.

Response:

We agree that there is lack of conventional toxicological studies of inositol in the literature. However, the findings from these studies in rodent models of chronic diseases, including diabetes and cancer, suggest that the toxicity of inositol is low. The relevant important (pivotal) safety related studies of inositol in human and animals are summarized in Table 1. Additionally, some of the human and animal studies are further described below.

As mentioned in GRN 1198, safety and tolerance of inositol has been extensively evaluated in several clinical studies. In clinical studies in healthy subjects, no significant effects on safety parameters or any major adverse effects were noted. In several studies, effects of inositol were investigated in subjects with panic disorder, eating disorders, metabolic syndrome, and PCOS, at doses up to 20 g and for

durations up to 6 months. In these studies, inositol supplementation was well received, with minor adverse effects such as diarrhea, abdominal pain, and insomnia at high doses. Inositol was well tolerated without any significant adverse effects at doses up to 4 g/day and for durations up to 6 months. Some pivotal safety studies of inositol in human subjects are summarized in Table 1.

In an open-label, multiple dose, dose escalation clinical 2-stage study, Lam et al. (2006) investigated the effects of inositol in smokers with bronchial dysplasia. In this study, 40-74 years old subject, n=16 (both sexes) in stage I (safety tolerability) and 10 (both sexes) in stage II participated. The stage I study lasted for one month and the dose used was 12-30 g/day. Based on findings from this study, the maximum tolerated dose (MTD) was selected as 18 g/day. In stage II study, subjects (n=10) were exposed to MTD (18 g/day) for 3 months. The NOAEL was determined as 18 g/day. The margin of exposure (MOE) i.e., ratio of the NOAEL value to the estimated daily intake (EDI) was determined for different population to range from 61 to 201. For all population, the MOE was 140. Generally, an acceptable MOE value for a NOAEL-based assessment of inositol based on a human study is ≥10, taking into account a factor 10 for the interindividual variation between humans in toxicokinetics and toxicodynamics. As the study was conducted in subjects with bronchial dysplasia and its relevance to the general healthy population, an additional safety factor of 3 can be applied. Therefore, an acceptable MOE value can be considered as 30. The MOE of 140 for all population is over 4.5 fold higher compared to acceptable MOE.

Pugliese et al. (1990) investigated the effects of inositol at dietary levels of 0.5, 1 and 2% in diabetic and non-diabetic rats. In this study, inositol at dietary levels of 2% inositol (equivalent to 1000 mg/kg bw/day) reduced some of the metabolic, neurological and circulatory changes of diabetes, but dietary exposure at 1 and 2% had no effect (minimal, if any) on these parameters in non-diabetic rats. The investigators noted that myo-inositol supplemented diets had no effect on body weight gain, renal hypertrophy, or urine volume, indicating that these changes were not linked to imbalances in myo-inositol metabolism. The highest dose used in this study was 546-fold higher as compared to the 90th percentile EDI from the proposed uses of inositol.

Tilton et al. (1993) investigated the effects of dietary myo-inositol supplementation on diabetes-induced vascular structural lesions in male Sprague-Dawley rats. In this study, the only adverse effects observed were thickening of basement membranes of capillaries of the retina and glomeruli of non-diabetic rats treated for nine months with 2% myo-inositol in the diet (equivalent to 1000 mg/kg bw/day, as per PAFA, 1993) and worsening of the capillary thickening in glomeruli along with an increase in the amount of pericyte-containing capillaries in the retina of diabetic and non-diabetic rats treated for five or nine months with 2% myo-inositol in the diet (Tilton et al., 1993). As only one dose was used, a NOAEL could not be determined. The highest dose used in this study was 546-fold higher as compared to the 90th percentile EDI from the proposed uses of inositol.

Liao et al. (2007) investigated the effect of inositol on chemically induced cancers in mice. In order to induce cancer, female C57BL/6 mice were exposed for long-term to cyclic dextran sulphate sodium (DSS) in combination with an iron-enriched diet to induce ulcerative colitis (which is associated with colon cancer). Myo-Inositol or hexaphosphate inositol (HI) was given at 1% (equivalent to 2500 mg/kg bw/day) in

the drinking water of groups of colitis-induced or non-induced mice for 255 days. In mice not treated with DSS, myo-inositol or HI did not induce any change in body weight, food consumption or mortality, as compared to negative controls receiving water. No colorectal tumors were found in mice receiving myo-inositol of HI. The colons of these mice were morphologically normal. None of the treatments affected mortality, body weight gain or feed consumption. Both forms of inositol decreased the number and size of colorectal tumors in the colitis-induced groups. No colorectal tumors were found in untreated controls or non-induced groups given myo-inositol or hexaphosphate inositol. The inositol dose used was 1351-fold higher as compared to the EDI from the proposed uses of inositol.

In summary, the available human and animal studies suggest that at the intended uses of inositol as described in GRN 1198, and the resulting maximum exposure of inositol is unlikely to cause adverse effects and is considered safe.

Table 1. Safety Studies of Inositol in Human and Animals

Reference	Health Condition; Study Design; Number of Subjects	Dose; Duration	Parameters Investigated and Relevant Safety Findings
Safety data from Human Clinical Trials			
Lam et al. (2006)*	Smokers with bronchial dysplasia; Open-label, multiple dose, dose escalation clinical study (2-stage); 40-74 years old; n=16 (both sexes) in Stage I (safety tolerability); 10 (both sexes) in Stage II (maximum tolerable dose)	12-30 g/day (Stage I) 18 g/day (Stage II); Stage I: 1 month dose escalation study; Stage II: 3 months treatment at maximum tolerated dose (NOAEL level) established in Stage I: 18 g/day	Flatulence, loose stool or diarrhea and mild gastrointestinal symptoms (Stage I and II); a significant decrease in blood pressure and slight increase in hemoglobin levels after more than one month with 18 g/day (Stage II), not regarded as adverse effects by the authors. NOAEL = 18 g/day
Carlomagno and Unfer (2011)	12 trials; Depression, Alzheimer disease, panic disorder and PCOS; Over 250 subjects exposed to inositol	Ranged from 4 to 30 g/day; Exposure time ranged from 1 to 12 months	Risk assessment. Gastrointestinal symptoms (nausea, flatus, loose stools, diarrhea) at 12 g/day or higher. Severity of adverse events stays the same also at 30 g/day. Notably, the dosage of 4 g/day of inositol commonly used in clinics is completely free of side effects. NOAEL = 4 g/day (57 mg/kg bw/day for 70 kg individual) As compared to 90th percentile estimated daily intake (EDI) of 1.83 mg/kg bw/day from the proposed uses (all population), the NOAEL 31-fold higher.

Formoso et al. (2019)	Pregnancy complicated by insulin resistance and/or diabetes; over 700 subjects exposed to myo-inositol; total over 1400 received different treatments	Ranged from 4 to 60 g/day; Exposure time ranged from 1 to 12 months; primarily first trimester to delivery	Only adverse events reported were mild gastrointestinal symptoms (nausea, flatus, and diarrhea) but only at doses greater of 12 g per day. NOAEL = 4 g/day (57 mg/kg bw/day for 70 kg individual) and is 31-fold higher.
Phelps et al. (2016)	Preterm infants with respiratory distress syndrome. Infants ≤ 29 week GA (n = 122, 14 centers) randomized	Placebo or inositol at 10, 40, or 80 mg/kg bw/day for 10 weeks, 34 weeks postmenstrual age or discharge.	Inositol at doses up to 80 mg/kg/day for 7-10 weeks is well tolerated and does not increase adverse events. Adverse events and comorbidities were fewer in the inositol groups, but not significantly so. NOAEL = 80 mg/kg bw/day and is 44-fold higher.
Safety Data from Animal Studies			
Reference	**Species; Sex; Number**	**Route; Exposure Period; Dose**	**Safety Related Findings**
Pugliese et al. (1990). Effect of myo-inositol on diabetes-induced vascular functional changes.	Sprague-Dawley rats; male; Seven groups, of which groups I, II and III were nondiabetic controls	Feeding diet containing 0.5, 1 and 2% of inositol (equivalent to 250, 500 and 1000 mg/kg bw/day). In control (non-diabetic) inositol tested at 1 and 2% only.	Inositol at 1 and 2% had no effect (minimal, if any) on metabolic, neurological and circulatory changes of diabetes in non-diabetic rats. No effect on body weight gain, renal hypertrophy, or urine volume, indicating that these changes were not linked to imbalances in myo-inositol metabolism. Food consumption was slightly decreased, significant in highest dose. The highest dose tested was 546-fold higher compared to EDI from the proposed uses of inositol.
Tilton et al. (1993)	Sprague-Dawley rats; male. Diabetic and non-diabetic rats treated for five or nine months. Three groups: (I) Rats were fed with 2% myo-inositol diet for 9 months; (II) Untreated for 5 months then treated with myo-inositol for the 4 months; (III) Untreated for 9 months. Controls included untreated and myo-inositol-treated groups.	Oral, 2% myo-inositol in the diet (equivalent to 1000 mg/kg bw/day) Effects of dietary myo-inositol supplementation on diabetes-induced vascular structural lesions was studied	Inositol did not affect weight gain, plasma glucose, glycosylated hemoglobin, food consumption, urine volume, and albuminuria in control and diabetic rats. Thickening of basement membranes of capillaries of the retina and glomeruli of non-diabetic rats was noted. NOAEL could not be determined (only one dose used). Dose used is 546-fold higher as compared to EDI from the proposed uses of inositol.

Liao et al. (2007). Effect of inositol on chemically induced cancers	C57BL/6 mice; female; n=5/group	Inositol or hexaphosphate inositol (HI) at 1% in drinking water (equivalent to 2500 mg/kg bw/day) to colitis-induced or non-induced mice for 255 days.	No changes in body weight, feed consumption or mortality (control rats). No colorectal tumors in mice receiving inositol; colons morphologically normal.
Massa et al. (2006)	Mice [SWV/Fnn - sensitive to VPA), and LM/Bc (resistant to VPA-induced neural tube defects)] Female; pregnant 12 dams/group	Oral (gavage); GD 6 to 10; Total dose 800 mg/kg bw/day Inositol, with and without injection of VPA	No significant change in fetal body weight or incidence of embryonic mortality observed in either strain; No increase in neural tube defects; Co-administration of inositol and valproic acid significantly increased embryo lethality *vs.* all other groups in the SWV/Fnn strain; Co-administration also significantly reduced fetal bw and significantly increased the incidence of exencephalic fetuses *vs.* all other groups in SWV/Fnn strain. No adverse effects of inositol treatment alone were reported. The dose used, 800 mg/kg bw/day is 432-fold higher compared to EDI
Cogram et al. (2002)	Mice (curly-tail neural tube defect model) Female; pregnant	Oral (gavage); GD 8 to 10; 800 mg/kg bw/day Inositol or D-chiro-inositol	No adverse effects on resorption rates, litter size or fetal crown-rump length No significant increase in incidences of fetal malformations or anomalies. NOAEL = 800 mg/kg bw/day; 432-fold higher compared to EDI
Burton and Wells (1976)	Rat pups (Holtzman) Male/Female	Oral (diet); during gestation and lactation; pups were fed after weaning until 3 months of age. GD 7 to weaning (3 months); 0.5% in diet (250 mg/kg bw/day- maternal; 500 mg/kg bw/day-offspring)	*Maternal effects:* Inositol levels were significantly increased in plasma, liver, kidney, and intestines of pups, and in the milk and mammary tissues No adverse effects on bw or clinical signs reported. NOAEL = 250 mg/kg bw/day. *Reproductive and offspring effects:* Supplementation did not significantly alter male pup sperm counts or fertility indices in male and female rats NOAEL = 500 mg/kg bw/day.

*The margin of exposure (MOE) is the ratio of the NOAEL value to the exposure. Generally, an acceptable MOE value for a NOAEL-based assessment of inositol based on a human study is ≥10, taking into account a factor 10 for the interindividual variation between humans in toxicokinetics and toxicodynamics. As the study was conducted in subjects with bronchial dysplasia and its relevance to the general healthy population, an additional safety factor of 3 can be applied. Therefore, an acceptable MOE value can be considered as 30. The MOE for different population groups at 90th percentile ranged from 61 to 201; for all population, the MOE was 140.

17. The notifier discusses that a comprehensive search of the scientific literature for safety data and toxicity information for inositol was performed through March 2024.

    a. Please report the search terms used, and data bases searched.

    Response:

    For the GRAS assessment of inositol, pertinent scientific literature searches regarding the metabolism and pre-clinical and clinical safety of inositol was performed. For preclinical data search, following approach was undertaken: Substance term (inositol); Animal study term; Route of administration term; Safety terms; Acute/repeat dose study term; Safety factors term; Carcinogenicity term; Reproductive toxicity terms; Genotoxicity terms were used. Clinical safety literature searched using keywords to identify human studies; keywords used to identify route of administration; and safety terms were used. The data bases searched included: PubMed; PubMed Central; AGRICOLA; AGRIS; NTIS: National Technical Information Service, etc.

    b. If needed, please perform an updated literature search from March 2024 to present, and report if any new data or information were found that would contradict the current GRAS conclusion.

    Response:

    An updated search of database from March 1, 2024 to October 25, 2024 did not reveal any new safety related publication of inositol.

We hope the above information and clarifications addresses your queries. If you have any further questions or need additional explanation, please let us know.

Thank you for the opportunity to provide our responses to your questions.

Best regards

Wing Yu

Agent for: Sichuan Bohaoda Biological Technology Co., Ltd.

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Appendix I

Certificate of Analysis from three Batches Demonstrating Compliance with Food Chemical Codex Inositol Specifications

(Attached separately)

四川博浩达生物科技有限公司
SICHUAN BOHAODA BIOTECHNOLOGY CO., LTD.
检验报告书
CERTIFICATE OF ANALYSIS

Commodity 品名	Inositol 肌醇	Batch NO. 批号	01012409070100	Batch 批量	6300 kg
Packing 包装	25 Kg/barrel 25kg/桶	Production Date 生产日期	September 07,2024 2024.09.07	Expiry Date 有效期至	September 06,2028 2028.09.06
Standard 标准	Food Chemicals Codex Thirteenth Edition (FCC13)				

Inspection

Inspect Item 检测项目	Specifications 标准要求	Test Results 结果
Description 感官	This product is white crystalline powder, odorless, sweet, stable in air; soluble in water, insoluble in ether and chloroform. 本品为白色结晶状粉末，无臭，味甜，在空气中稳定；溶于水，不溶于乙醚和三氯甲烷	Passed Test 符合规定
Identification (A,B) 鉴别	The retention time of the major peak of the Sample solution corresponds to that of the Standard solution. 样品溶液的主峰保留时间和标准溶液相对应	Passed test 符合规定
Assay，on the anhydrous basis 含量，以干基计	Not less than 97.0 % and not more than 102.0% 不少于 97.0%和不超过 102.0%	99.36%
Calcium 钙（Ca）	Through experiments 通过实验	Passed test 符合规定
Sulfate 硫酸盐（SO_4^{2-}）	≤ 0.006% Not more than 0.006%	＜0.006%
Chloride 氯化物（Cl^-）	≤ 0.005% Not more than 0.005%	＜0.005%
Limit of lead 铅限量	Not more than 4mg/kg 不超过 4mg/kg	Not detected 未检出(＜0.05mg/kg)
Loss on Drying 干燥失重	Not more than 0.5% 不超过 0.5%	0.06%
Melting Point 熔点	224.0～ 227.0℃	224.0～225.7℃
Ignition Residue 灼烧残渣	≤0.1% Not more than 0.1%	0.01%
Total Aerobic Bacteria 总需氧细菌	≤10^3cfu/g	＜10cfu/g
Molds & yeasts 霉菌和酵母菌	≤10^2cfu/g	＜10cfu/g
Escherichia coli 大肠杆菌	Not detected	Not detected 未检出
Salmonella 沙门氏菌	Not detected	Not detected 未检出
Conclusion 结论	The goods are complied with FCC13. 产品符合标准 FCC13.	

Analyser（分析）：刘利华 2024.09.23　Checker（复核）：王茂宇 2024.09.23　Chief Inspector（批准）：宋佼佼 2024.09.23

四川博浩达生物科技有限公司
SICHUAN BOHAODA BIOTECHNOLOGY CO., LTD.
检验报告书
CERTIFICATE OF ANALYSIS

Commodity 品名	Inositol 肌醇	Batch NO. 批号	01012409300100	Batch 批量	5600 kg
Packing 包装	25 Kg/barrel 25kg/桶	Production Date 生产日期	September 30,2024 2024.09.30	Expiry Date 有效期至	September 29,2028 2028.09.29
Standard 标准	Food Chemicals Codex Thirteenth Edition (FCC13)				

Inspection		
Inspect Item 检测项目	Specifications 标准要求	Test Results 结果
Description 感官	This product is white crystalline powder, odorless, sweet, stable in air; soluble in water, insoluble in ether and chloroform. 本品为白色结晶状粉末，无臭，味甜，在空气中稳定；溶于水，不溶于乙醚和三氯甲烷	Passed Test 符合规定
Identification (A,B) 鉴别	The retention time of the major peak of the Sample solution corresponds to that of the Standard solution. 样品溶液的主峰保留时间和标准溶液相对应	Passed test 符合规定
Assay，on the anhydrous basis 含量，以干基计	Not less than 97.0 % and not more than 102.0% 不少于 97.0%和不超过 102.0%	99.35%
Calcium 钙（Ca）	Through experiments 通过实验	Passed test 符合规定
Sulfate 硫酸盐（SO_4^{2-}）	≤ 0.006% Not more than 0.006%	＜0.006%
Chloride 氯化物（Cl^-）	≤ 0.005% Not more than 0.005%	＜0.005%
Limit of lead 铅限量	Not more than 4mg/kg 不超过 4mg/kg	Not detected 未检出(＜0.05mg/kg)
Loss on Drying 干燥失重	Not more than 0.5% 不超过 0.5%	0.06%
Melting Point 熔点	224.0～ 227.0℃	224.0～225.7℃
Ignition Residue 灼烧残渣	≤0.1% Not more than 0.1%	0.01%
Total Aerobic Bacteria 总需氧细菌	≤10^3cfu/g	15cfu/g
Molds & yeasts 霉菌和酵母菌	≤10^2cfu/g	＜10cfu/g
Escherichia coli 大肠杆菌	Not detected	Not detected 未检出
Salmonella 沙门氏菌	Not detected	Not detected 未检出
Conclusion 结论	The goods are complied with FCC13. 产品符合标准 FCC13.	

Analyser（分析）：刘利华 2024.10.09　Checker（复核）：王茂宇 2024.10.09　Chief Inspector（批准）：宋佼佼 2024.10.09

四川博浩达生物科技有限公司
SICHUAN BOHAODA BIOTECHNOLOGY CO., LTD.
检验报告书
CERTIFICATE OF ANALYSIS

Commodity 品名	Inositol 肌醇	Batch NO. 批号	01012408250100	Batch 批量	2700 kg
Packing 包装	25 Kg/barrel 25kg/桶	Production Date 生产日期	August 25,2024 2024.08.25	Expiry Date 有效期至	August 24,2028 2028.08.24
Standard 标准	Food Chemicals Codex Thirteenth Edition (FCC13)				

Inspection		
Inspect Item 检测项目	Specifications 标准要求	Test Results 结果
Description 感官	This product is white crystalline powder, odorless, sweet, stable in air; soluble in water, insoluble in ether and chloroform. 本品为白色结晶状粉末，无臭，味甜，在空气中稳定；溶于水，不溶于乙醚和三氯甲烷	Passed Test 符合规定
Identification (A,B) 鉴别	The retention time of the major peak of the Sample solution corresponds to that of the Standard solution. 样品溶液的主峰保留时间和标准溶液相对应	Passed test 符合规定
Assay，on the anhydrous basis 含量，以干基计	Not less than 97.0 % and not more than 102.0% 不少于 97.0%和不超过 102.0%	99.34%
Calcium 钙（Ca）	Through experiments 通过实验	Passed test 符合规定
Sulfate 硫酸盐（SO_4^{2-}）	≤ 0.006% Not more than 0.006%	＜0.006%
Chloride 氯化物（Cl^-）	≤ 0.005% Not more than 0.005%	＜0.005%
Limit of lead 铅限量	Not more than 4mg/kg 不超过 4mg/kg	Not detected 未检出(＜0.05mg/kg)
Loss on Drying 干燥失重	Not more than 0.5% 不超过 0.5%	0.07%
Melting Point 熔点	224.0～ 227.0℃	224.0～225.4℃
Ignition Residue 灼烧残渣	≤0.1% Not more than 0.1%	0.01%
Total Aerobic Bacteria 总需氧细菌	≤10^3cfu/g	＜10cfu/g
Molds & yeasts 霉菌和酵母菌	≤10^2cfu/g	＜10cfu/g
Escherichia coli 大肠杆菌	Not detected	Not detected 未检出
Salmonella 沙门氏菌	Not detected	Not detected 未检出
Conclusion 结论	The goods are complied with FCC13. 产品符合标准 FCC13.	

Analyser（分析）：刘利华 2024.09.03　Checker（复核）：王茂宇 2024.09.03　Chief Inspector（批准）：宋佼佼 2024.09.03

From: 于艳艳
To: Deng, Kaiping
Cc: 黄玥琦
Subject: [EXTERNAL] Re:Specification_GRN 001198- Inositol produced by E. coli
Date: Wednesday, December 11, 2024 3:09:06 AM
Attachments: image001.png

Dear Dr. Deng,

Sorry for our oversight. The sampling size for ***E. coli***, ***Salmonella*** and endotoxin testing is as follows,

- ***E. coli:*** 1g
- ***Salmonella***: 10g
- Endotoxin: 1g

Thank you.

All my best regards,

Wing Yu
Food Division | Technical Manager

Hangzhou REACH Technology Group Co, Ltd

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发件人："Deng, Kaiping" <Kaiping.Deng@fda.hhs.gov>
发送日期：2024-12-10 22:34:34
收件人："于艳艳" <wing.yu@cirs-group.com>

抄送人："黄玥琦" <hyq@cirs-group.com>
主题：Specification_GRN 001198- Inositol produced by E. coli

Dear Ms. Yu,

We note that the specification of *Salmonella* is “absent” but you did not specify the sample weight, such as 10 or 25 g. Could you please clarify the sampling size for *E. coli, Salmonella* and endotoxin testing?

Thank you, and I look forward to hearing from you!

Kaiping

Kaiping Deng
Regulatory Review Scientist / Microbioogy Reviewer

Regulatory Management Branch

Division of Food Ingredients
Office of Pre-Market Additive Safety
Office of Food Chemical Safety, Dietary Supplements, and Innovation
Human Foods Program
U.S. Food and Drug Administration

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FDA U.S. FOOD & DRUG ADMINISTRATION