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By FedEx

September 22, 2022

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

Re: GRAS Notice for High-Purity Rebaudioside I

Dear Sir or Madam:

We hereby submit the enclosed version of our GRAS notice for the use of high-purity rebaudioside I as a steviol glycoside preparation comprised of ⩾ 95% rebaudioside I, for use as a general-purpose sweetener in foods within the U.S., in accordance with current Good Manufacturing Practice (cGMP), excluding infant formulas. The statutory basis of the GRAS conclusion is scientific procedures.

Please note the current submission is the same GRAS notice we submitted on May 27, 2022, for the same intended use of rebaudioside I. The current submission is the same with the previous submission, other than the removal of an appendix that contains redacted information.

Rebaudioside I is not intended for use in products under the jurisdiction of the U.S. Department of Agriculture (USDA). The GRAS notice does not contain any designated confidential business information. In accordance with the Agency's guidelines, we have enclosed one original copy of the GRAS notice, and one complete electronic copy of the GRAS notice on a compact disk (CD).

We are committed to cooperating with the Agency and believe an open dialog is one of the most effective ways to accomplish that objective. If any questions arise in the course of your review, please contact us, preferably by telephone or e-mail, so that we can provide a prompt response.

Sincerely,

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To:
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Subject: GRAS Notice for High-Purity Rebaudioside I

To Whom it may Concern:

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Manus Bio Inc. hereby informs the United States Food and Drug Administration of the conclusion that High-Purity Rebaudioside I, manufactured by Manus Bio Inc., as defined in the enclosed documents, is GRAS under the specified conditions of use as a food ingredient on the basis of scientific procedures, and therefore, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.
Information supporting the GRAS status of the High-Purity Rebaudioside I, which includes detailed information on the notified substance and a summary of the basis of the safety of High-Purity Rebaudioside I, under the intended conditions of use, also are enclosed for review by the Agency.

I hereby certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus free using Symantec Endpoint Protection

Sincerely,

Christine Santos, PhD
Chief Technology Officer
Manus Bio Inc.

External use permitted

GRAS Notification for high-purity Rebaudioside I

Prepared for:
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 USA

Date:

May 27, 2022

GRAS Notification for High-Purity Rebaudioside I

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GRAS Notification for High-Purity Rebaudioside I

Part 1 Certification, Common name, Conditions for use, Basis for GRAS

1.1 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Manus Bio Inc. hereby informs the United Stat es (U.S.) Food and Drug Administration (FDA) that the high-purity rebaudioside I (≥ 95% rebaudioside I), manufactured by Manus Bio Inc., and identified as Nutrasweet Icon™ is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below . As such, its intended use is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FD&C Act). In addition, as a responsible official of Manus Bio, the undersigned hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and considered all unfavorable as well as favorable information known to Manus Bio. Included is all pertinent information to the evaluation of the safety and GRAS status of high purity rebaudioside I (≥95% rebaudioside I) as a general-purpose sweetener, as described herein.

Signed,

May 3rd, 2022

Christine Santos PhD.
Manus Bio Inc.
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Cambridge, MA 02138
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csantos@manusbio.com

1.2 Common Name of Notified Substance

Stevia Extract, Stevia Leaf Extract, Stevia Sweetener, Stevia Leaf Sweetener, Steviol glycosides, Rebaudioside I, Reb I, NutraSweetI™

1.3 Conditions of Use

Manus Bio intends to market high purity rebaudioside I, as a steviol glycoside preparation comprised of ≥ 95% rebaudioside I, for use as a general-purpose sweetener in foods within the U.S., in accordance with current Good Manufacturing Practice (cGMP), excluding infant formulas, and meat and poultry products. The U.S. FDA raised "no questions" on the use of another high purity rebaudioside I product following the submission of GRAS Notice No. 911 as well as numerous other steviol glycoside preparations, as general-purpose sweetening agents. These previous FDA "no questions" responses provided no restrictions on the specific food uses or their use-levels. The use-levels of high-intensity sweeteners are restricted based on the technological properties of the sweetening agent (i.e., sweetness potency). As a result, because the sweetness profile of high-purity rebaudioside I (≥95% rebaudioside I) is comparable to the sweetness profiles of other steviol glycoside preparations, the food uses and use-levels of high-purity rebaudioside I (≥95% rebaudioside I) are likely to reflect those currently available in the U.S.

1.4 Basis for GRAS

Pursuant to Title 21, Section 170.30 of the Code of Federal Regulations (CFR), Manus Bio Inc.'s Nutrasweet I™ high-purity rebaudioside I (≥95% rebaudioside I) has been determined to be GRAS by Manus Bio for use as a general-purpose sweetener in foods, on the basis of scientific procedures. As such, its intended use is not subject to premarket approval requirements of the FD&C Act.

This GRAS determination is based on information generally available in the public domain pertaining to the safety of steviol glycosides and the production process, as discussed herein. This GRAS notice is a complete, representative, and balanced assessment that includes favorable and unfavorable relevant information available to the evaluation of the safety and GRAS status of Rebaudioside I. It is also based on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of high purity rebaudioside I (≥95% rebaudioside I) as a general-purpose sweetener. [**See Appendix A. titled** "**GRAS Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of high-purity Rebaudioside I (≥95% Rebaudioside I) produced by enzymatic conversion of stevia leaf extract."**

The U.S. FDA has reviewed the safety of over 50 different steviol glycoside preparations and have consistently raised no objections regarding the GRAS status of steviol glycosides for use as general-purpose sweeteners in food and beverage products. Of note, the U.S. FDA did not raise any objections regarding GRN 911, in relation to the GRAS status of rebaudioside I for use as a general-purpose sweetener in foods. The rebaudioside I described in GRN 911 is similar to Manus Bio's high purity rebaudioside I (≥95% rebaudioside I) produced by enzymatic conversion of steviol glycosides. Likewise, the Manus Bio process for manufacturing the food ingredient is by conversion of stevia leaf extract using UDP-glucosyltransferase enzymes similar to GRN 911.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the U.S. FDA for review and copying upon request during business hours at the offices of:

Manus Bio Inc.
1762 Lovers Lane
Augusta, GA. 30901

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Manus Bio's view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act.

Part 2. Identity, Method of Manufacture, Specifications,and Physical or Technical Effect

2.1 Identity

Rebaudioside I is commonly known as Stevia Extract, Stevia Leaf Extract, Stevia Sweetener, Stevia Leaf Sweetener, Steviol glycosides, Rebaudioside I and Reb I. The Preparation is also marketed as NutraSweet Icon™. Rebaudioside I is derived from an extract of *Stevia rebaudiana* Bertoni using genetically modified *E. Coli* K-12. The general chemistry of steviol glycosides and enzyme modified steviol glycosides have been previously reviewed in a number of GRAS notices including GRN 667, GRN 715, GRN 745 and GRN 1010.

2.2 Chemical and Physical Characteristics

Rebaudioside I is a naturally occurring steviol glycoside first reported to beobtained from the leaves of *Stevia rebaudiana* by Ohta et. al. (2010). High purity rebaudioside I (≥95% rebaudioside I) is a white-to-off-white powder that has a clean taste with no abnormal or off odor and is freely soluble in water. High purity rebaudioside I (≥95% rebaudioside I) is approximately 167 times sweeter than sucrose consistent with GRN 911, which is consistent with the sweetness profile of steviol glycosides (FAO, 2016). Besides rebaudioside I, the preparation contains ≤5% other steviol glycosides, moisture and salts.

All steviol glycosides including rebaudioside I share a common glycosylated *ent*-Kaurene steviol backbone and differ only with respect to the type and number of glycoside units they do or do not contain (*i.e.,* glucose, xylose, rhamnose, fructose, deoxyglucose, galactose, and/or arabinose) conjugated at positions R_1 and R_2. Due to the common steviol backbone, all steviol glycosides have been proven to share a similar metabolic fate. The general structure for steviol glycosides is shown in Figure 2.2-1.

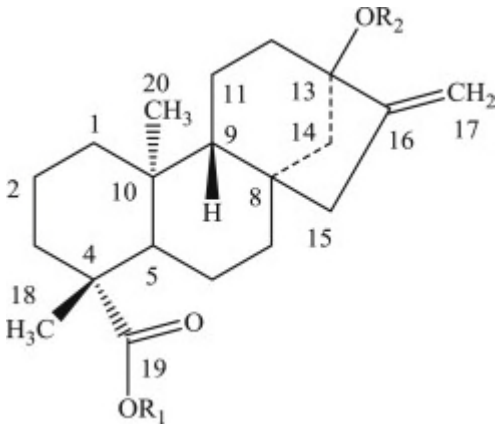


Figure 2.2-1 Chemical Structure of Steviol Glycosides

R_1 and R_2 may be a single or multiple glycoside unit, including glucose, xylose, rhamnose, fructose, deoxyglucose, galactose, and/or arabinose.

Rebaudioside I contains 2 glucose subunits linked at position R_1 and 3 glucose subunits at R_2 (Figure 2.2-2). It should be noted that Manus Bio's high-purity rebaudioside I (≥95% rebaudioside I) is a highly purified product that contains ≥95% Rebaudioside I, which is consistent with the purity criteria for steviol glycosides as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2017a).

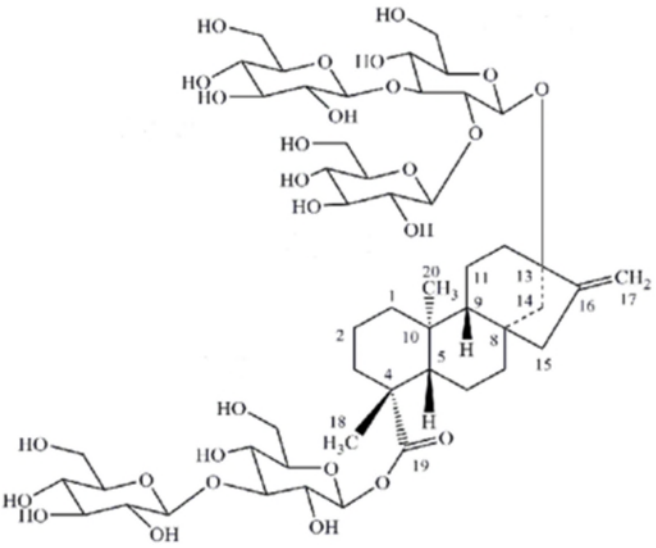


Figure 2.2-2 Chemical Structure for Rebaudioside I

Chemical Name: (4α13-[(O-β-D-glucopyranosyl-(1→ 2)-O-[β-D-glucopyranosyl- (1→ 3)]-β -D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester

Synonyms: Rebaudioside I, Reb I

Molecular Weight (MW, Da): 1129.2

CAS Number: 1220616-34-1

2.3 Method of Manufacturing

Manus Bio manufactures high purity Rebaudioside I in a process similar to that described in GRN 1010, GRN 667, GRN 715, and GRN 823. The multi-step method utilizes UGT enzymes produced in *E. coli* K-12 to enhance the rebaudioside I content in stevia leaf extract, followed by physical separation methods to purify rebaudioside I to high purity (≥95%).

2.3.1 Materials:

All raw materials, processing aids, additives and purification equipment used to manufacture high purity rebaudioside I (rebaudioside I ≥95%) are food-grade ingredients, permitted by U.S. food additive regulations, have GRAS status, or otherwise determined to be appropriate for use in food for their respective uses. The food-grade ingredients are compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United States Pharmacopeia, andEuropean Pharmacopoeia).

2.3.2 UGT Enzyme Production Organism:

The parental strain *E. coli* K-12 sub-strain MG1655 Fnr- was obtained from the *E. coli* Genetic Stock Center (CGSC) and is currently listed under the designation CGSC 6300. *E. coli* K-12 is a non-pathogenic and non-toxigenic organism belonging to Biosafety Level 1 according to the National Institutes of Health (NIH, 2016). Additional supporting evidence for the safety of *E. coli* K-12 is cited in a number of other GRAS notices including GRN 624, GRN 659, GRN 735, GRN 745, and GRN 1010. *E. coli* K-12 has a long history of safe use in the industrial production of specialty chemicals and human drugs (U.S. EPA, 1997). For example, a food enzyme preparation (chymosin) obtained from a genetically modified *E. coli* K-12 strain was affirmed as GRAS by the FDA in 1990 (Flamm, 1991; Olempska-Beer *et al.*, 2006) and has been used safely for cheese production worldwide. *E. coli* also serves as a host for the production of enzymes currently used in a GRAS-approved process for the enzymatic conversion of steviol glycosides (GRN 745, 2018).

The parental strain *E. coli* K-12 was engineered to express enzymes (UDP-glucosyl transferases) for the glycosylation of steviol glycosides and to improve the overall production efficiency of rebaudioside I. In addition, the strain was engineered to increase the supply of uridine diphosphate glucose (UDP-Glu), a precursor required for glycosylation of steviol glycosides through a series of gene deletions and overexpressions. All heterologously overexpressed genes originated from biosafety level 1 organisms that are not associated with any known allergens or toxins, including *Stevia rebaudiana, Oryza sativa, Glycine max*, and *Bifidobacterium bifidum*.

Overexpressed genes were synthesized, codon-optimized for *E. coli,* and introduced into stable, non-

essential regions of the genome *via* standard techniques utilizing homologous recombination with positive selection andcounter selectable markers (Datsenko and Wanner, 2000). Gene deletions were generated using standard techniques utilizing homologous recombination with positive selection and counter selectable markers (Datsenko and Wanner, 2000). These regions include but are not limited to *endA*, *recA*, and *araA*. All selection markers used during the engineering process were removed, and no antibiotic resistance markers are present in the final production strain. The identity of the final production strain was confirmed by Sanger sequencing of modified regions and by whole genome sequencing.

2.3.3 Manufacturing Process:

Manus Bio's high-purity rebaudioside I (≥95% rebaudioside I) is produced by enzymatic conversion of steviol glycosides using an *E. coli* strain derived from *E. coli* K-12. (see Appendix B) The production strain is grown in media containing steviol glycoside extracts prepared from the leaves of *S. rebaudiana* Bertoni in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides (FAO, 2016). In brief, steviol glycosides are extracted from the stevia leaf by a series of crushing, dissolution, solvent extraction, and precipitation steps. The manufacturing process for this starting material is described in detail in GRAS Notice (GRN 275) (U.S. FDA, 2008). Specifications for the starting material used in the production of rebaudioside I are provided in Table 2.3-1. Within the growth medium containing stevia leaf extract, UGT enzymes produced by the *E. coli* K-12 cells mediate the glycosylation of steviol glycosides to rebaudioside I. After sufficient rebaudioside I has been produced, the media and *E. coli* K-12 biomass are heat inactivated and centrifugation and/or filtration is used to remove the inactivated biomass and precipitated enzymes. Rebaudioside I is then purified using physical processing steps including filtration, aqueous crystallization, centrifugation, rinsing and drying using typical food processing equipment and steps. Dried rebaudioside I may be in a crystalline or amorphous solid form and may be further milled, spray-dried, freeze-dried, agglomerated, compacted, and granulated or undergo other physical form modifications to achieve a desirable particle size of the final product, ≥95% rebaudioside I. The purification processes described are consistent with the methodologies for the manufacture of steviol glycosides as described in the CTA published by FAO/JECFA (FAO, 2016).

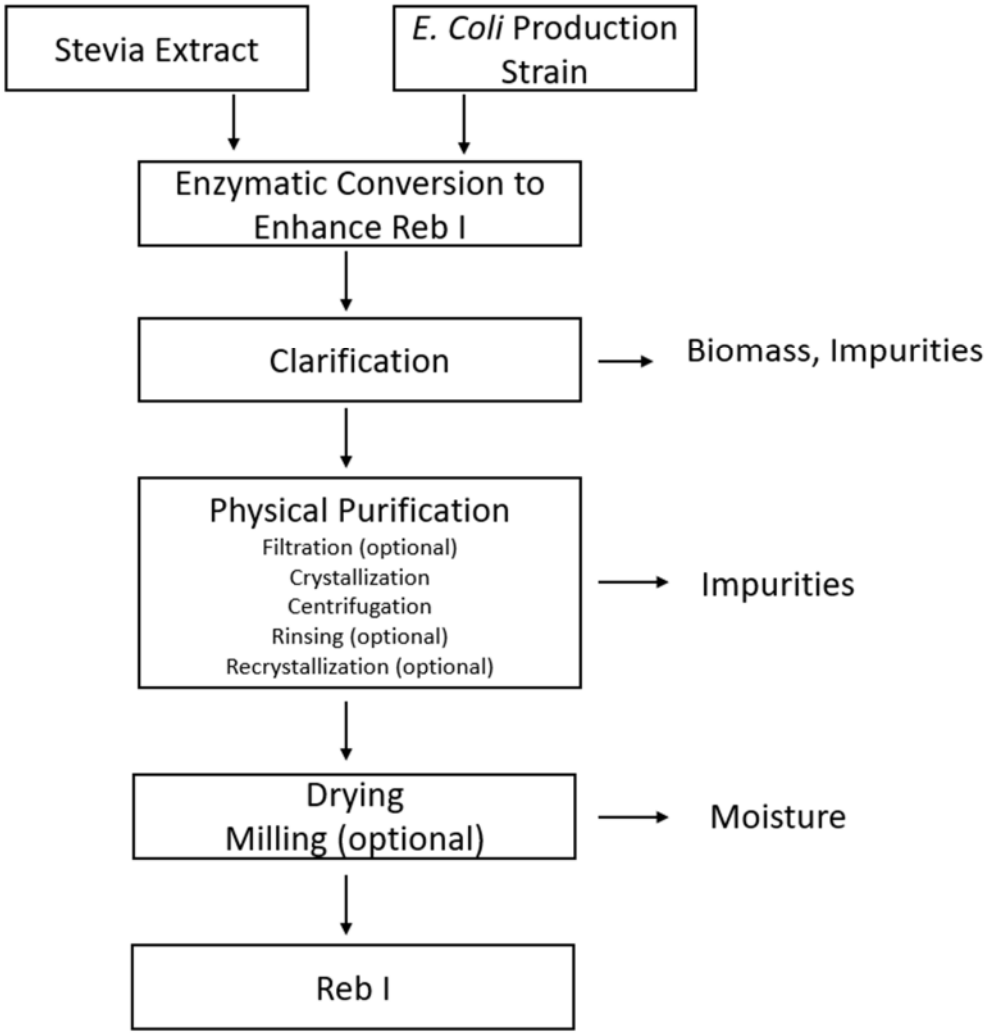
Manus Bio's ≥95% rebaudioside I is manufactured in a facility registered as an FDA Food Facility. The plant operates under cGMPs (Good Manufacturing Practices) outlined in the Food Safety Modernization Act 21 CFR 117, including required HACCP and Food Defense Plans, and is subject to audit from regulatory authorities including the U.S. FDA and The State of Georgia Department of Agriculture. Manufacturing shall be certified to a GFSI (Global Food Safety Initiative) compliant audit scheme.

An overview of a typical manufacturing process flow is provided in Figure 2.3-1 below although order and type of physical purification steps can be varied to achieve the same product specifications.

Table 2.3-1: Specifications for Steviol Leaf Extract Starting Material

Items	Standard	Test Method
Appearance	White Powder	Visual Check
Steviol Glycosides	NLT 90.0 %	HPLC
Specific Optical Rotation	-30° ~ -38°	Polarimeter
Specific Absorbance	NMT 0.2	Polarimeter
Loss on Drying	NMT 3.0%	JECFA VOL.4
Residue on Ignition	NMT 0.20%	AOAC 945.46
Heavy Metal	NMT 10 ppm	ICP MS AOAC
Arsenic	NMT 1 ppm	ICP MS AOAC
Mercury	NMT 0.1 ppm	ICP MS AOAC
Lead	NMT 3.0 ppm	ICP MS AOAC
Cadmium	NMT 1.0 ppm	ICP MS AOAC
Total Plate Count	NMT 1000 cfu/g	AOAC 966.23
Yeast and Mold	NMT 100 cfu/g	AS 1766.2.2
E.Coli	Negative	ISO7251
Pathogenic Bacteria	Negative	ISO7251
Salmonella	Negative	ISO6579
Staphylococcus	Negative	FDA/BAM Online Chap.12
Residual of Solvent Ethanol	300 ppm max	USP31 <467>
Residual of Solvent Methanol	100 ppm max	USP31 <467>

Figure 2.3-1 Manufacturing process of Manus Bio' s Rebauadioside I



2.4 Product Specifications and Batch Analyses

2.4.1 Product Specifications

Appropriate food-grade specifications have been established for high-purity rebaudioside I (≥95% rebaudioside I) which meet or exceed the specifications for steviol glycosides established by JECFA (2017a) (Table 2.4-1). All analytical methods used to measure each specification parameter are internationally- recognized methods (e.g., United States Pharmacopeia [USP), Association of Official Analytical Chemists [AOAC], or JECFA). Total steviol glycoside content is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from *S. rebaudiana* Bertoni (JECFA, 2017a,b).

Table 2.4-1 Product Specifications for High-Purity Rebaudioside I (≥95% Rebaudioside I)

Specification Parameter	Specification
Appearance	White-to-off-white powder
Total steviol glycosides[A] (anhydrous basis)	≥95%
Rebaudioside I[A]	≥95%
Loss on drying[B]	≤6%
pH (1% solution)[C]	4.5 to 7.0
Residual ethanol[D]	≤5,000 ppm
Residual methanol[D]	≤200 ppm
Total ash[E]	≤1%
Lead[F]	≤1 ppm
Arsenic[F]	≤1 ppm
Cadmium[F]	≤1 ppm
Mercury[F]	≤1 ppm
Total plate count[G]	<1,000 CFU/g
Mold[H]	<100 CFU/g
Yeast[H]	<100 CFU/g
Coliforms[I]	<3 MPN/g
Escherichia coli[I]	Not detected
Salmonella[J]	Negative/25 g

A. JECFA 2017 a,b B. FCC Appendix IIC C. USDS PHM D. EN14110 E. USP <281>
F. USP<233> (ICP-MS) G. AOAC 2015.13 H FDA/BAM Chap 18 I. AOAC 2018.13 J. RapidChek/AOAC RI 030301;
CFU = colony-forming units, MPN = Most Probable Number, PPM = parts per million

2.4.2 Batch Analyses

Analysis of 3 non-consecutive lots (A003, 002A, 002C) of high-purity rebaudioside I (≥95 % rRebaudioside I) demonstrates that the manufacturing process produces a consistent product which meets the established product specifications. A summary of the batch analyses is presented in Table 2.4.2-1 and microbial analysis in Table 2.4.2-2. Product analysis has confirmed that Manus Bio's steviol glycoside product is comprised of rebaudioside I, other steviol glycosides and water (see appendix C for certificates of analysis).

Table 2.4.2-1 Summary of the Product Analysis for the Physical Parameters of 3 Non-Consecutive Lots of High-Purity Rebaudioside I (≥95% Rebaudio side I)

Specification Parameter	Specification	Manufacturing Lot No.		
		Batch A003 (MAI2110281)	Batch 002A (MAI2110261)	Batch 002C (MAI2110282)
Appearance	White-to-off-white powder	Pass	Pass	Pass
Total steviol glycosides (anhydrous basis)	≥95%	Pass	Pass	Pass
Rebaudioside I	≥95%	97.5%	97.8%	97.6%
Loss on drying	≤6%	2.6 %	3.8 %	3.0 %
pH (1% solution)	4.5 to 7.0	Pass	Pass	Pass
Residual ethanol	≤5,000 ppm	100 ppm	200 ppm	1100 ppm
Residual methanol	≤200 ppm	≤100 ppm	≤100 ppm	≤100 ppm
Total ash	≤1%	0.36%	<0.001%	<0.001%
Lead	≤1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Arsenic	≤1 ppm	<0.1 ppm	<0.1 ppm	<0.1 ppm
Cadmium	≤1 ppm	<0.02 ppm	<0.02 ppm	<0.02 ppm
Mercury	≤1 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm

Table 2.4.2-2 Summary of the Product Analysis for the Microbial Parameters of 3 Non-Consecutive Lots of High-PurityRebaudioside I (≥95 % Rebaudio side I)

Specification Parameter	Specification	Manufacturing Lot No. A003	002A	002C
Total plate count	<1,000 CFU/ g	970 CFU/ g	<1 CFU/ g	210 CFU/ g
Mold	<100 CFU/ g	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g
Yeast	<100 CFU/ g	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g
Coliforms	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g
Escherichia coli	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g	<10 CFU/ g
Salmonella	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g	Negative/ 25 g

CFU/g = colony-forming units per gram

2.4.3 Residual Protein, Residual DNA

To confirm the removal of residual protein from the final product, 3 non-consecutive batches of the high-purity rebaudioside I (≥95% rebaudioside I) (Lot Numbers A003, 002A, and 002C) were analyzed using the bicinchoninic acid (BCA) assay method. The results of the analysis indicated that the levels of protein within the high- purity rebaudioside I material were below the limit of detection. The limit of detection for the assay was 22.5 ppm. (For residual protein analysis, see Appendix D.)

To confirm the absence of residual DNA in rebaudioside I, 3 non-consecutive lots of final product (Lot Numbers A003, 002A, and 002C) were assayed by polymerase chain reaction (PCR). The results confirm that genomic DNA in the rebaudioside I final product is below the analytical limit of detection of 320 fg/µL. (For residual DNA analysis, see Appendix E.)

2.4.4 Agricultural Residues

A combined LCMS/GCMS screen using QuEChERs method for pesticide residues was conducted on the starting stevia leaf material for assessing for the absence of residues of commonly used pesticides in the product. The results confirm that the levels of residues of commonly used pesticides are below the limits of detection (see Appendix F for pesticide residue data).

2.5 Technical Effect

Rebaudioside I is intended to be used as a sweetening ingredient or as part of a sweetening mixture. Rebaudioside I is approximately 167 times sweeter than sucrose at typical food and beverage use levels.

2.6 Stability Data

A number of scientific and authoritative bodies, including JECFA, the European Food Safety Authority (EFSA), and Food Standards Australia/New Zealand (FSANZ), have reviewed the stability of steviol glycosides. The stability of steviol glycosides also are discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999; Oehme *et al.,* 2017). At the 68th meeting, JECFA evaluated the stability of steviol glycosides under conditions mimicking their use in foods and noted that steviol glycosides do not undergo browning or caramelization when heated and are stable under elevated temperatures (JECFA, 2007). In addition, steviol glycosides (approximately 90 to 94% purity) are stable for at least 180 days when stored at temperatures up to 24°C and pH 2.0 to 4.0. However, at elevated temperatures (80°C), steviol glycoside solutions maintained in water and pH 4.0 and 3.0 for 8 hours showed 4 and 8% decomposition, respectively. At temperatures of 100°C over the same time period, higher rates of decomposition were observed, with 10 and 40% being decomposed at pH 4.0 and 3.0, respectively. These results indicate that the stability of steviol glycosides is pH and temperature dependent. Based on the available evidence, JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions (JECFA, 2007). Steviol glycosides have additionally been shown to be stable during baking (Prakash et al., 2008) and in one study approximately 95% recoverable after a 15-minute baking time at 185 °C (Jooken et al., 2012). Decomposition of steviol glycoside powder at elevated temperatures occurred slowly with more than 80% remaining after extended incubation (2 h) at 180 °C with most of the

products identifiable as hydrolysis products to other steviol glycosides such as Rebaudioside B (Jooken et al., 2012).

The U.S. FDA has reviewed the stability of high-purity rebaudioside I preparations in a previous GRAS notice (GRN 911). There exists a number of studies on the stability of steviol glycosides, including stevioside, rebaudioside A, rebaudioside M and rebaudioside I under different storage conditions (*e.g.,* in different forms, such as powder and solution, in acidic conditions, and various temperatures) in the publicly-available scientific literature (Wood *et al.,* 1955; Chang and Cook, 1983; Kinghorn, 2002; GRN 252; GRN 253; Chaturvedula et al., 2013; Prakash et al., 2014). These studies are discussed in detail in GRN 512, GRN 667, GRN 780, and GRN 846 and are incorporated by reference in this notice. Ultimately, the results of these stability studies suggest that the stability of steviol glycosides is pH- and temperature-dependent, which are consistent with the conclusions of JECFA (2007). More recently, a study evaluating the structural stability of 3 commercial batches each of dried stevia leaves, the first aqueous infusion of the ground stevia, and a high-purity stevia leaf extract (~95% steviol glycosides) confirmed that the processing steps do not chemically alter or modify the steviol glycoside content (Oehme et al., 2017).

In addition to the stability studies within the scientific literature, a storage stability study on rebaudioside I was discussed in GRN 911 which reported that the rebaudioside I content did not change over a 6-month period and remained ≥95% rebaudioside I. The results of these storage stability studies are consistent with the results reviewed by JECFA (2007) in that the stability of steviol glycosides, including rebaudioside I, are thermally stable under normal storage conditions.

Rebaudioside I is expected to exhibit similar chemical stability to other closely related steviol glycosides *(e.g.,* stevioside & rebaudioside A) based on their chemical structure similarity. Therefore, it is anticipated that the results of the stability studies in the preparations described in GRN 512, GRN 667, GRN 745, GRN 759, GRN 780, GRN 799, GRN 812, GRN 846 GRN 911 & GRN 882 and the results of the stability studies available in the publicly available scientific literature, can be extended to support the stability of Manus Bio's high-purity rebaudioside I (≥ 95% rebaudioside I).

Further to the information outlined within the literature, Manus Bio has conducted a stability study on 2 batchs of high purity rebaudioside I (≥95% rebaudioside I) produced by enzymatic conversion of steviol glycosides (Lot No. A003 and 002A). In this study, samples of approximately 25 g of high purity rebaudioside I (≥95% rebaudioside I) are stored at 25 °C in polypropylene containers mimicing commercial packaging. Total steviol glycosides and rebaudioside I content have been measured by HPLC at Time = 0, 3, and 6 months. Results demonstrate no significant changes in rebaudioside I content (Table 2.6-1).

Table 2.6-1 Results: Stability Study on 2 Batchs of High-Purity Rebaudioside I (≥95% Rebaudioside I)

Lot No A003 (MAI2110281)			
Parameter	T= 0	Month 3	Month 6
Loss on Drying (%)	2.6	4.5	5.7
Rebaudioside I (%) (HPLC)	97.5	98.9	98.6

Lot No 002A (MAI2110261)			
Parameter	T= 0	Month 3	Month 6
Loss on Drying (%)	3.8	5.4	5.8
Rebaudioside I (%) (HPLC)	97.8	98.1	97.9

Part 3. Dietary Exposure

3.1 Intended Use of High-Purity Rebaudioside I (≥95% Rebaudioside I) and Levels of Use in Foods

High purity rebaudioside I (≥95% rebaudioside I) is intended for use as a general-purpose sweetener in accordance with cGMP, excluding infant formulas and meat and poultry products. High purity rebaudioside I (≥95% rebaudioside I) has a sweetness intensity of approximately 167 times that of sucrose. To date, the U.S. FDA raised no questions regarding the use of other high-intensity sweeteners, including other steviol glycoside preparations, as general-purpose sweeteners that have no restrictions on their specific food uses and use-levels. In addition, the use-levels of high-intensity sweeteners are restricted based on the technological properties of the sweetening agent (i.e., sweetness potency). Therefore, considering that steviol glycosides, including the ingredient that is the subject of this GRAS notice, are characterized by a sweetness profile that is, for the most part, comparable to other high-intensity sweeteners, the uses and use-levels of high-purity rebaudioside I (≥95% rebaudioside I) produced by enzymatic conversion of steviol glycosides reflects those currently permitted for other high-intensity sweeteners in the U.S.

3.2 Estimated Consumption of High-Purity Rebaudioside I (≥95% Rebaudioside I) Based upon Intended Food Uses

3.2.1 History of Consumption of Steviol Glycosides

Stevia rebaudiana ***Bertoni*** and the individual steviol glycosides derived from the plant have been consumed as sweeteners in various foods and beverages since the late 1800s (Geuns, 2003). According to Blumenthal (1995) and Geuns (2003), the native peoples of Brazil and Paraguay have consumed the *S. rebaudiana* plant for hundreds of years as a food ingredient and as a tea. Similarly, *S. rebaudiana* became a popular herbal tea ingredient in the U.S. in the 1980s (Blumenthal, 1995; Ferlow, 2005). Stevioside, the first isolated steviol glycoside from the *S. rebaudiana* leaf, has been consumed in Japan for more than 30 years (Geuns, 2003; Ferlow, 2005). Approximately 160,000 metric tons of stevioside, as sucrose equivalents, were reportedly consumed in Asia in 1995; in 1999, this level increased to approximately 200,000 metric tons as sucrose equivalents (International Sugar Organization, 2001).

3.2.2 Estimated Consumption of High-Purity Rebaudioside I (≥95% Rebaudioside I) from Proposed Food Uses

The dietary consumption of various steviol glycoside preparations has been estimated using a post-market surveillance approach as outlined in a number of GRAS notices for steviol glycosides submitted to the U.S. FDA (e.g., GRN 667, 715, 733, 745, 759, 780, 799, 812, 846, and 882). Generally, this approach uses the data from Renwick (2008) in which dietary exposure to rebaudioside A was estimated based on the available post-market surveillance data for other high-intensity sweeteners, and by assuming full replacement of the currently approved high-intensity sweeteners with the new sweetener [i.e., high-purity rebaudioside I (≥95% rebaudioside I)]. While conservative, this approach yields intake estimates that are realistic as they reflect actual post-market intakes of high-intensity sweeteners. Renwick (2008) estimated the average and high-end dietary intakes of rebaudioside A as sucrose equivalents in various population groups, such as non-diabetic and diabetic adults and children, and adjusted the values accordingly using the sweetness intensity of rebaudioside A relative to sucrose (approximately 200-fold sweeter than sucrose).

This post-market surveillance approach can be used to estimate the dietary intakes of high purity rebaudioside I (≥95% rebaudioside I) (Table 3.2.2-1) in similar population groups. High purity rebaudioside I (Nutrasweet I™) (≥95% rebaudioside I) is approximately 167 times sweeter than sucrose. The estimated intake values for high purity rebaudioside I (≥95% rebaudioside I) were calculated based on the sweetness potency and the molecular weight of rebaudioside I.

Table 3.2.2-1 Estimated Consumption High-Purity Rebaudioside I (≥95% Rebaudioside I) Using the Intense Sweetener Intake Assessment Methodology described by Renwick (2008)

			Consumption Estimates			
	Intakes of Intense Sweeteners (expressed as sucrose equivalents) (mg/kg bw/day)		High-Purity Rebaudioside I (≥95% Rebaudioside I)[a] (mg/kg bw/day)		High-Purity Rebaudioside I (≥95% Rebaudioside I)[a,b] (mg/kg bw/day) as Steviol	
Population Group	**Average Consumer**	**High Consumer**	**Average Consumer**	**High Consumer**	**Average Consumer**	**High Consumer**
Non-diabetic adults	255	675	1.53	4.04	0.43	1.14
Diabetic adults	280	897	1.68	5.37	0.47	1.51
Non-diabetic children	425	990	2.54	5.93	0.72	1.67
Diabetic children	672	908	4.02	5.44	1.13	1.53

bw = body weight.
[a] Approximately 167 times as sweet as sucrose.
[b] Calculated based on the molecular weights of steviol (318.45 g/mol) and Rebaudiside I (1,129 g/mol) [steviol conversion factorof 0.28].

For non-diabetic adults, average and high-end intakes of high purity rebaudioside I (≥95% rebaudioside I) of up to 0.43 and 1.14 mg/kg body weight/day expressed as steviol equivalents, respectively, were calculated. For diabetic adults, average and high-end intakes were slightly higher at up to 0.47 and 1.51 mg/kg body weight/day. Average and high-end exposures to high purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents, in non-diabetic children were calculated to be up to 0.72 and 1.67 mg/kg body weight/day, respectively. Although average intakes of high purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents, were estimated to be higher at up to 1.13 mg/kg body weight/day in diabetic children compared to values for non-diabetic children, high-end values in diabetic children (1.53 mg/kg body weight/day) were lower than high-end values in non-diabetic children. The estimated intakes of high purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents, are all below the current acceptable daily intake (ADI) defined by the JECFA for steviol glycosides (FAO, 2016) of 0 to 4 mg/kg body weight/day as steviol.

In 2008, JECFA considered various intake models for the estimation of dietary exposure to steviol glycosides, including the intake analysis conducted by Renwick (2008) as part of their evaluation of the safety of steviol glycosides. Although higher intake estimates than those presented by Renwick (2008) were identified using other methodologies, including ones considering replacement of all sweeteners used in or as food (up to

approximately 6 mg/kg body weight/day, expressed as steviol equivalents), JECFA noted that such replacement estimates were highly conservative and that actual exposures to steviol glycosides (expressed as steviol equivalents) would be 20 to 30% of these values (1 to 2 mg/kg body weight/day, expressed as steviol equivalents). JECFA also noted that the post-market surveillance approach further confirmed the lower intake estimate range.

Part 4. Self-Limiting Levels of Use

The use of high purity rebaudioside I (≥95% rebaudioside I) is largely limited by the desired sweetness intended for a particular food or beverage product. Therefore, the use of high purity rebaudioside I (≥95% rebaudioside I) as a general-purpose sweetener in foods is self-limiting based on its organoleptic properties.

Part 5. Experience Based on Common Use in Food Before 1958

General recognition of safety of the use of rebaudioside I has been established through scientific procedures. Therefore, information regarding experience based on common use of the notified substance in food prior 1958 is not applicable.

Part 6. Narrative and Safety Information

The safety of steviol glycosides has been extensively reviewed by the U.S. FDA in a number of GRAS notices including GRN 780 and GRN 1010 (which was submitted by Manus Bio).The Agency has raised no objections to 58 GRAS notices describing the GRAS status of major individual steviol glycosides, including stevioside, rebaudiosides A, C, D, E, I, and M, mixtures of steviol glycosides, and glucosylated and enzyme-modified steviol glycosides (GRNs 252, 253, 275, 278, 282, 287, 303, 304, 318,
323, 329, 337, 348, 349, 354, 365, 367, 369, 375, 380, 388, 389, 393, 395, 418, 448, 452, 456, 461, 467, 473, 493, 512, 516, 536, 548, 555, 607, 619, 626, 632, 638, 656, 662, 667, 702, 715, 733, 744, 745, 759, 780, 799, 812, 823, 846, 867, 878, 882, 911 and 1010).

In addition to the U.S. FDA, the safety of steviol glycosides has been reviewed by several scientific bodies and regulatory agencies, including JECFA, European Commission's Scientific Committee on Food (SCF), the European Food Safety Authority (EFSA), Food Safety Australia/New Zeand (FSANZ), and Health Canada. The existing safety database on steviol glycosides includes an extensive evaluation of the metabolism and pharmacokinetics of steviol glycosides in rodents and humans, and a standard battery of toxicological tests, including acute toxicity, short- and long-term toxicity and carcinogenicity, reproductive and developmental toxicity, *in vitro* and *in vivo* mutagenicity and genotoxicity,as well as several human studies.

Much of the early studies investigating the safety of steviol glycosides were conducted with stevioside, the predominant steviol glycoside in *S. rebaudiana* leaves (Aze *et al.*, 1991; Toyoda *et al.*, 1997). Since then, additional toxicity testing has been conducted on rebaudioside A and D (Curry and Roberts, 2008; Curry *et al*., 2008; Nikiforov and Eapen, 2008; Williams and Burdock, 2009). Due to the common metabolic fate of steviol glycosides, the scientific bodies and regulatory agencies described above have extended their safety opinions to include all steviol glycosides, rather than individual steviol glycosides (JECFA, 2017a,b).

Thus, considering that the existing safety database on steviol glycosides has been extensively reviewed by

the U.S. FDA, the pertinent generally available data and information used to support the safety of steviol glycosides, including major individual steviol glycosides and other steviol glycoside mixtures/preparations, is incorporated by reference to information cited within prior GRAS notifications.

Updated searches of the scientific literature were conducted through January 2022 to identify new data and information relevant to the safety of steviol glycosides that have been published since the U.S. FDA's last review. Given the shared metabolic fate of steviol glycosides, the safety of high-purity rebaudioside I (≥95% rebaudioside I) can be supported based on the existing safety database for steviol glycosides, the safety conclusions for steviol glycosides by JECFA and other scientific and regulatory authorities/bodies, and the safety of the production strains. At the time of preparation of this GRAS notice, GRN 1010 was the most recent steviol glycoside GRAS notice to receive a "no questions" letter from the U.S. FDA which summarized literature prior to November 2020.

6.1 Absorption, Distribution, Metabolism, and Elimination

An extensive database exists outlining the metabolic fate (absorption, distribution, metabolism, and elimination) of steviol glycosides. The available data and information on the metabolic fate of individual steviol glycosides as discussed in detail in several GRAS notices (e.g., GRN 619, 626, 667) is incorporated by reference in this dossier. *In vitro* and *ex vivo* studies have demonstrated that steviol glycosides are not hydrolysed by digestive enzymes of the upper gastrointestinal tract due to the presence of β-glycosidic bonds and are not absorbed through the upper portion of the gastrointestinal tract (Hutapea et al., 1997; Koyama et al., 2003a; Geuns et al., 2003, 2007). Therefore, steviol glycosides enter the colon intact, where they are subject to microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Gardana et al., 2003; Renwick and Tarka, 2008). Several *in vitro* studies mimicking the anaerobic conditions of the colon, reviewed extensively by Renwick and Tarka (2008), have confirmed the ability of gut microflora from mice, rats, hamsters, and humans to hydrolyse steviol glycosides completely to steviol (Wingard et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Koyama et al., 2003a,b; Nikiforov et al., 2013; Purkayastha et al., 2016). Specifically, Purkayastha et al. (2016) conducted incubation studies with pooled human fecal homogenates collected from adult males and females using rebaudiosides A, B, C, D, E, F, M, steviolbioside and dulcoside A, and showed that all glycosides were converted to steviol over a 24–48 h incubation period. These studies demonstrated that the number and location of sugar units attached to the steviol backbone do not significantly affect the microbial hydrolysis. Likewise, this study demonstrated that steviol glycosides, irrespective of the type of sugar moiety (e.g., glucose, rhamnose, xylose) attached to the steviol backbone, were metabolized to steviol at generally similar hydrolysis rates. Incubation tests were also conducted in fecal homogenates collected from adult and pediatric populations with steviol glycosides from stevia leaf extracts and steviol glycosides produced by enzymatic conversion of rebaudioside A to larger molecules by attaching glucose units via β- or α-glycosidic bonds. The steviol glycosides produced by extraction from stevia leaf or enzymatic conversion of stevia leaf extract were also reported to share a similar metabolic fate in the fecal homogenates from adults and children supporting a similar metabolic fate of steviol glycosides in all age groups (Purkayastha 2020). Steviol glycosides are hydrolyzed sequentially, in which one sugar moiety is removed at a time, with the degradation rates dependent on the structural complexity of each steviol glycoside (Wingard et al., 1980; Koyama et al., 2003b). Despite the differences in chemical structure, however, the rates of hydrolysis of different steviol glycosides to steviol are relatively similar, especially during the first 24 hours of incubation in *in vitro* metabolic studies with human fecal homogenates (Purkayastha et al., 2014, 2015, and 2016). Following microbial degradation, the steviol metabolite is absorbed systemically into the portal vein and distributed to the liver, spleen, adrenal glands, fat, and

blood (Nakayama et al., 1986; Sung, 2002 [unpublished]; Koyama et al., 2003b; Wang et al., 2004; Roberts and Renwick, 2008).

Steviol is conjugated to glucuronic acid to form steviol glucuronide in the liver. The steviol glucuronide metabolite and any unconjugated steviol or unhydrolyzed fraction of the administered glycosides are excreted primarily in the urine, and, to a lesser extent within the feces in humans (Wingard et al., 1980; Nakayama et al., 1986; Kraemer and Maurer, 1994; Sung, 2002 [unpublished]; Geuns and Pietta, 2004 [unpublished]; Simonetti et al., 2004; Geuns et al., 2006, 2007; Roberts and Renwick, 2008; Wheeler et al., 2008).

In summary, due to the common molecular structure for steviol glycosides, consisting of a steviol backbone conjugated to different numbers and types of sugar moieties, all individual steviol glycosides share a common metabolic fate, as described above. Therefore, the safety database that has been established for individual steviol glycosides (*e.g.,* stevioside, rebaudioside A, rebaudioside D) can be extrapolated to support the safe use of purified steviol glycosides in general, regardless of the steviol glycoside distribution of the preparation, including high purity rebaudioside I (≥95% rebaudioside I).

6.2 Summary of Safety Evaluations on Steviol Glycosides by Scientific and Regulatory Authorities/Bodies

The safety of steviol glycosides was reviewed by JECFA at their 51st, 63rd, 68th, 69th, 82nd 86th, 87th and 91st meetings in 1998, 2004, 2007, 2008, and 2016, 2018, 2019 and 2021 respectively. As outlined above, the safety of steviol glycosides has also been reviewed by FSANZ, the European Commission's SCF, the EFSA, and Health Canada (SCF, 1985, 1999; FSANZ, 2008; EFSA, 2010, 2015; Health Canada, 2012). These scientific bodies and regulatory agencies have unanimously concluded that consumption of steviol glycosides is not a human safety concern and have established an ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents. Subsequent to these evaluations, EFSA concluded that "extending the current specifications to include [two additional steviol glycosides], rebaudiosides D and M, as alternatives to rebaudioside A in the predominant components of steviol glycosides would not be of safety concern" (EFSA, 2015), while EFSA, JECFA, FSANZ, and Health Canada recently expanded the definition of steviol glycosides to include all individual steviol glycosides present in the *S. rebaudiana* Bertoni leaf, including rebaudioside I (EFSA, 2020; FSANZ, 2017; Health Canada, 2017; JECFA, 2017a,b). In addition to these safety evaluations, the U.S. FDA has reviewed the safety of 58 different steviol glycoside preparations and has consistently raised no objections regarding the GRAS status of steviol glycosides.

The most recent FAO JECFA Monograph 26 prepared at the JECFA 91st meeting defines enzymatic modified steviol glycosides as a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana* Bertoni undergo enzymatic conversion of major steviol glycosides that occur in the leaf to minor ones with preferable sensory characteristics. Annex 3 JECFA (2021) monograph 26 defines enzyme-modified steviol glycosides as a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni. The product is obtained from the enzymatic treatment of purified steviol glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni. The purified leaf extract is treated with enzymes produced by non-toxigenic nonpathogenic strains of *Pichia pastoris* (also known as *Komagataella phaffii*) and *Escherichia coli* that have been genetically modified with genes from multiple

donor organisms to produce glucosyltransferase (EC 2.4.1.17) and sucrose synthase (EC 2.4.1.13). The resulting material is heated and filtered to denature and remove the enzymes. The raw product is concentrated using resin adsorption/desorption or solid/liquid filtration, followed by purification and preparation of the product of commerce using processes that may include decolorization, crystallization, and spray drying. This manufacturing technique maximizes the production of specific steviol glycosides that are not naturally present in high concentrations in the leaf extract (91st JECFA (2021) and published in FAO Monographs 26 (2021)). The process outlined within the JECFA monograph defines the methodology used by Manus Bio to produce rebaudioside I.

In these evaluations, the safety data and information that were reviewed by these scientific bodies and regulatory agencies were generally available in the published scientific literature. In a 2-year study in rats, a no-observed-adverse- effect level (NOAEL) of 970 mg/kg body weight/day, equivalent to 383 mg/kg body weight/day as steviol, was determined (Toyoda *et al.,* 1997). The results of the study by Toyoda *et al.* (1997) was the basis for the established ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents, for steviol glycosides following application of a safety factor of 100 (JECFA, 2006; FSANZ, 2008; EFSA, 2010; Health Canada, 2012).

6.3 Additional Safety Data for Steviol Glycosides

The safety of steviol glycosides has been extensively reviewed in a number of GRAS notifications submitted to the U.S. FDA, as outlined above, which are incorporated by reference in this notice. The safety of steviol glycosides was most recently evaluated by the U.S. FDA in its evaluation of GRN 1010 for purified steviol glycosides, which included a comprehensive search of the scientific literature to capture publications relevant to the safety of steviol glycosides up to November 2020. In order to identify new data related to the safety of steviol glycosides following the U.S. FDA review of GRN 1010, a comprehensive search of the scientific literature was conducted from August 2020 to January 2022. The search was limited to articles with full texts within peer-reviewed scientific journals. The literature search was completed using ProQuest and included searches of the following databases for pertinent literature on the safety of steviol glycosides: BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Lancet Titles, MEDLINE®, New England Journal of Medicine, NTIS: National Technical Information Service, Registry of Toxic Effects of Chemical Substances (RTECS®), ToxFile®, TOXLINE.

Following review of those steviol glycoside manuscripts published between August 2020 and January 2022, which were not captured in GRN 1010, there were a total of 3 articles which were deemed to potentially provide information relevant to the safety evaluation of steviol glycosides in general and therefore more specifically rebaudioside I.

The first, a study conducted by Pasqualli et al (2020) evaluated the effects of the steviol glycoside metabolite steviol, which forms the backbone of the molecule, on the immunological system using *in vitro* methodology. The objective of the study was to evaluate the effects of steviol on human lymphocyte cells and their sub populations related to the immune system by assessing cytotoxicity, genotoxicity and mutagenic effects. The authors indicated that the study was carried out at average concentrations of consumption. However, it appears that the authors mistakenly considered that steviol itself is a sweetener rather than being a metabolite of the various steviol glycosides and therefore it is unclear how the dosages used in the analysis were determined. Unlike steviol glycosides, steviol is not directly added to foods for sweetening purposes.

The authors found significant decreases in both CD4+ and CD8+ T lymphocytes populations. In contrast, no

effects were reported for CD3+ T lymphocytes. The authors also conducted an alkaline comet assay and a chromosomal aberration test and mitotic index. These studies showed cytotoxicity and DNA damage including numerical and structural chromosome changes. The authors went on to conclude that "Although steviol is used globally as a sweetener in thousands of foods and beverages, its use should be cautious as a study points out that steviol has cytotoxic, genotoxic and mutagenic effects in the concentrations used in the culture of human lymphocytes cells". However, a close evaluation of the study indicates that the authors have failed to appreciate that steviol is not sweet and is not used for sweetening purposes.

While it is clearly understood and outlined in the published literature that it is the aglycone steviol which is formed in the GI tract and subsequently absorbed, this compound then undergoes phase II metabolism and detoxification to form glucuronides, which are subsequently eliminated. Since the various analyses conducted by Pasqualli et al (2020) did not include any metabolic activation potential, the tests conducted by the authors do not represent the systemic handling and metabolism of steviol that follows absorption. In addition, sub-chronic toxicological studies conducted with various purified steviol glycosides (Curry and Roberts 2008; Nikiforov and Eapen 2008; Nikiforov et al 2013) as well as a carcinogenicity study conducted with stevioside (Toyoda et al 1997) have shown that systemic plasma levels of steviol resulting from the metabolism of steviol glycosides and subsequent absorption, results in no adverse effects. The results reported by Pasqualli et al (2020) should therefore be viewed with caution.

The second study conducted by Mahalak et al (2020) assessed the impact of steviol glycosides and erythritol on human and *Cebus apella* gut microbiome. Steviol glycosides and erythritol were assessed on the microbiome as these ingredients are typically sold together in commercial products. As part of their analysis, the authors measured a) the ability of selected steviol glycosides to impact growth of representative bacterial strains, b) the commercial stevia product to impact human gut microbes in terms of short chain fatty acids production (*in vitro*) and c) an adult female monkey model was used to understand how steviol glycosides and erythritol impact the microbiome *in vivo*.

The results showed that for all strains of microorganisms tested, there were no changes in growth other than for *ϐ thetaiotaomicron* over a 24 hr period. Therefore, steviol glycosides were reported not to alter the diversity or components of the human gut microbiome in an *in vitro* model. Furthermore, the alpha diversity of the gut microbiota using 16s rRNA sequencing showed no significant difference with respect to species richness following interaction with the various stevia compounds. Similarly, steviol glycosides had no effect on beta diversity of the human gut microbiome taking into account phylogenetic information as well as taxonomic information. Steviol glycosides were therefore determined to have no measurable impact on the human gut microbial community structure *in vitro*. In contrast, "stevia" was found to increase butyric acid production but does not alter bile acid concentrations in the human gut microbiota *in vitro* model.

Following collection of faecal samples after administration of stevia to a female adult *Cebus apella* for a period of 2 weeks in the drinking water, these samples were analysed using 16s rRNA sequencing. Overall, the results showed that while there were many fluctuations in the microbial community composition, there were no statistically significant differences. However, when the faecal samples were analysed for alpha and beta diversity there was found to be a significant and consistent increase in alpha diversity. The authors put the differences between the *in vitro* and *in vivo* analysis results related to alpha and beta diversity, down to the differences in the dosage levels tested, with the female *Cebus apella* being administered a dose of 62 mg/kg/day of the Splenda Naturals plus stevia material. This level of administration would appear to be significantly higher that the ADI of 0-4 mg/kg/day that has been derived for steviol glycosides based upon steviol equivalents. While Mahalak et al (2020) reported differences especially *in vivo* following high

dosages in the adult monkey, they concluded that the study did not find a negative impact of steviol glycosides on the gut microbiol community.

In addition, the potential carcinogenicity of steviol glycosides was evaluated by Chappell et al (2021) who used a systematic evaluation approach incorporating mechanistic data with the totality of evidence. The authors incorporated the results from over 900 key characteristic of carcinogen (KCC'S) relevant assays, using a structured, quantitative framework to review mechanistic evidence. This included data from individual steviol glycosides and derivatives, metabolites and whole leaf extracts. These studies were weighted for quality and relevance. Following integration of all the data the totality of the evidence was reported to demonstrate that exposure to steviol glycosides is unlikely to pose a carcinogenic risk to humans. This was determined to agree with previous regulatory decisions regarding steviol glycosides.

The remainder of those studies that were obtained within the literature search were considered to be of limited relevance with respect to the overall safety assessment of steviol glycosides as determined by International regulatory Authorities, including the U.S. FDA. Overall, the additional studies identified in the updated search of the scientific literature did not call into question the safety of steviol glycosides.

6.4 Safety of the Production Strain

The production strains are derived from *E. coli* K-12. The genome of *E. coli* K-12 has been sequenced and confirms the absence of antibiotic resistance genes and other sequences of concern (Blattner *et al.*, 1997; Hayashi *et al.*, 2006; NCBI, 2018). Furthermore, the *E. coli* parental strain is a member of the well- defined family *Enterobacteriaceae* and JECFA monograph 26 has indicated that *E. coli* is an acceptable microorganism to produce the enzymes used in the enzymatic conversion process JECFA 2021).

In addition, all overexpressed genes within the genome of *E. coli* K-12 originate from biosafety level 1 organisms that are not associated with any known allergens or toxins. The manufacturing process also includes sterilization and purification procedures that have been shown to remove any proteins and recombinant DNA. Analysis of 3 non-consecutive batches of high purity rebaudioside I (≥95% rebaudioside I) has demonstrated the successful removal of recombinant DNA, and any proteins from the final product.

6.4.1 History of Use and the Production Strain

E. coli K-12 has been in use as a laboratory organism for over 50 years and it constitutes one of the most extensively characterized microorganisms (Bachmann, 1972; Jensen, 1993). Along with its use in laboratory research, *E. coli* K-12 has a long history of safe use in the food and pharmaceutical industries. Chymosin, a food enzyme preparation used in the production of cheese, derived from a genetically modified *E. coli* K-12 strain was affirmed as GRAS by the U.S. FDA in 1990 (Flamm, 1991; Olempska-Beer *et al.*, 2006). In addition, FDA review of GRN 745 concerning the production of rebaudioside M using a strain of *E. coli* K-12 received a "no questions" response from the Agency regarding its GRAS status for use in the production of steviol glycosides with a high rebaudioside M content. (U.S. FDA, 2018). Likewise, Manus Bio recently obtained a "no questions" response from the Agency following the submission of GRN 1010, which used *E. coli* K-12 in the production of rebaudioside M.

6.4.2 Pathogenicity/Toxicogenicity of the Parental Strain

As discussed in GRN 745 and GRN 1010, *E. coli* K-12 is not considered a human or animal pathogen and has

been classified as Biosafety Level 1 according to the NIH Guidelines (NIH, 2016). *E. coli* K-12 is often used as a reference organism when investigating the virulence factors of pathogenic *E. coli* strains as it is non-pathogenic (Blanc-Potard *et al.*, 2002; Kaper *et al.*, 2004). This species and its derivatives are unable to colonize the mammalian gastrointestinal tract, and do not produce toxins such as Shiga toxin, and are unable to persist in soil and water (Bogosian *et al.*, 1996; U.S. EPA, 1997). As previously described, the parental strain does not carry any introduced antibiotic resistance genes and the complete genome of this strain has been sequenced, confirming the absence of any toxigenic potential (Blattner *et al.*, 1997; Hayashi *et al.*, 2006).

6.4.3 Potential Allergenicity of the Enzymes

While no protein or rDNA has been identified within the rebaudioside I product, Manus Bio still undertook a potential allergenicity evaluation of those enzymes which could potentially escape the purification process. The potential allergenicity of all non-native enzymes was investigated using an *in-silico* approach. A sequence homology search was conducted according to the approach outlined by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (2001) and the WHO/FAO (2009) using the Allergen Online Database Version 20 (available at http://www.allergenonline.org; updated February 2020) maintained by the Food Allergy Research and Resource Program (FARRP) of the University of Nebraska (FARRP, 2020). This was done to confirm that the enzymes do not contain amino acid sequences similar to other known allergens that might produce an allergenic response. The database contains a comprehensive list of putative allergenic proteins developed via a peer reviewed process for the purpose of evaluating food safety.

No matches were identified from searching with the full amino acid sequence for each enzyme. According to the FARRP guidelines, an identity threshold of greater than 50% or an E-score lower than $1x10^{-7}$ suggest cross-reactivity with the known allergen to be a possibility.

A second homology search was conducted according to the approach outlined by the FAO/WHO (2001) and the WHO/FAO (2009). In accordance with this guideline, the Allergen Online database was searched using a sliding window of 80-amino acid sequences (segments 1-80, 2-81, 3-82, etc.) derived from the full-length amino acid sequence for each enzyme. The 80-amino acid alignment search was conducted using default settings (E value cutoff = 1 and maximum alignments of 20). Significant homology is defined as an identity match of greater than 35% (Codex Alimentarius, 2009). Using this search strategy, again no matches were identified and the level of protein in the final product NutraSweet I™ is reported as below the limit of detection.

6.1 GRAS Panel Evaluation

Manus Bio has concluded that high-purity rebaudioside I (≥95% rebaudioside I), meeting appropriate food-grade specifications and manufactured in a manner consistent with cGMP, is GRAS for use as an ingredient in various food products, as described in Part 1.3, on the basis of scientific procedures. Manus Bio's high purity rebaudioside I (≥95% rebaudioside I) is substantially equivalent to other steviol glycoside products that have received a "no questions" response from the U.S. FDA including rebaudioside I manufactured via enzymatic conversion and currently on the U.S. market. The FDA "no questions" responses also include those specific rebaudioside ingredients produced by enzymatic conversion using *E. coli* K-12 (GRN 745 and 1010) as the host micro-organism for producing the enzymes used in the enzymatic conversion process as well as those extracted from the leaves of *S. rebaudiana.*

The GRAS status of high-purity rebaudioside I (≥95% rebaudioside I) is based on conclusions of scientific bodies and regulatory authorities regarding steviol glycoside safety, data generally available in the public

domain pertaining to the safety of steviol glycosides, and a unanimous opinion among a panel of experts ('the GRAS Panel'), who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Ashley Roberts Ph.D. President AR Toxicology Inc., Jose -Avalos, Ph.D. Princeton University Princeton, NJ., and Stanley M. Tarka, Jr., Ph.D. (The Tarka Group Inc., and The Pennsylvania State University, College of Medicine).

The GRAS panel, convened by Manus Bio, independently and critically evaluated all data and information presented herein, and concluded that high purity rebaudioside I (≥95% rebaudioside I) produced by enzymatic conversion of steviol glycosides is GRAS for use as a general-purpose sweetener, as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel and evaluation of such data as it pertains to the proposed GRAS uses of high purity rebaudioside I (≥95% rebaudioside I), are presented in Appendix A.

6.2 Conclusions

Based on the data and information presented herein, Manus Bio has concluded high-purity rebaudioside I (≥95% rebaudioside I), meeting appropriate food-grade specifications, and manufactured according to cGMP, is safe for use as a general-purpose sweetener as presented in Section 1.3. Manus Bio also has further concluded that pivotal data and information relevant to the safety of high-purity rebaudioside I (≥95% rebaudioside I) are publicly available and therefore the intended uses of high-purity rebaudioside I (≥95% rebaudioside I) can be concluded to be GRAS on the basis of scientific procedures.

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APPENDIX A:

GRAS Panel Report Concerning the Generally Recognized as Safe (GRAS} Status of high-purity Rebaudioside I (≥95% Rebaudioside I).

INTRODUCTION

Manus Bio intends to market high purity rebaudioside I (≥ 95% rebaudioside I), identified as Nutrasweet I™ and produced via enzymatic conversion for use as a general-purpose sweetener in the United States (U.S.). Steviol glycosides have historically been obtained following hot water extraction from the leaves of *Stevia rebaudiana* Bertoni and subsequent solvent purification. More than 40 different steviol glycosides have been identified in the leaf extracts to date using this manufacturing approach some of which are present in low quantities but possess improved sensory charcateristics over those present at larger concentrations. Manus Bio has therefore developed an alternative manufacturing process to produce Rebaudioside I, which is only present at low levels within the leaf. Manus Bio's high-purity rebaudioside I (≥95 % rebaudioside I) is produced through enzymatic conversion of steviol glycosides (>90% total steviol glycosides) extracted from *S. rebaudiana Bertoni* using UDP- glucosyltransferase enzymes obtained from genetically modified *Escherichia coli* strains derived from *E. coli* K-12. High purity rebaudioside I is comprised of ≥95% rebaudioside I and ≥95% total steviol glycosides, which meets the ≥95% steviol glycoside purity criteria established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex.

At the request of Manus Bio, an Expert Panel of independent scientists (the GRAS Panel), qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether, under the conditions of intended use as a sweetening agent, high- purity rebaudioside I (≥95% rebaudioside I) would be Generally Recognized as Safe (GRAS), based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Ashley Roberts Ph.D., President AR Toxicology Inc.; Jose Avalos, Ph.D. Princeton University, Princeton, NJ., and Stanley M. Tarka Jr., Ph.D. (The Tarka Group Inc., and The Pennsylvania State University, College of Medicine). For purposes of the GRAS Panel 's evaluation, "safe" or "safety " means there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use, as defined by the U.S. Food and Drug Administration (FDA) in 21 CFR 170 .3 (i) (U.S. FDA, 2017).

The GRAS Panel independently and collectively evaluated a dossier titled "Documentation Supporting Nutrasweet I as Generally Recognized as Safe (GRAS) for Use as a General-Purpose Sweetener", which included a comprehensive summary of scientific information on high purity rebaudioside I (≥95% rebaudioside I). This dossier was prepared with information available in the public domain and also included details pertaining to the manufacturing method, product specifications and supporting batch analyses, its intended use as a general-purpose sweetener in food and beverages, consumption estimates for the intended uses, and a summary of the scientific literature pertaining to the safety of steviol glycosides, in general. The GRAS Panel also evaluated other information deemed appropriate or necessary.

Following their independent and critical evaluation of such data and information, the GRAS Panel convened on April 12, 2022 via teleconference and unanimously concluded that the intended use described herein for high-purity rebaudioside I (≥95% rebaudioside I), meeting appropriate food-grade specifications as described in the supporting dossier and manufactured according to current Good Manufacturing Practice (cGMP), is safe, suitable, and GRAS based on scientific procedures. A summary of the basis of the GRAS Panel's conclusion is presented below.

CHEMISTRY AND MANUFACTURING

The subject of this GRAS evaluation is high-purity rebaudioside I (≥95% rebaudioside I), which is a highly purified steviol glycoside ingredient comprised of ≥ 95% Rebaudioside I and ≥ 95% total steviol glycosides, consistent with the purity criteria for steviol glycosidesas established by JECFA (JECFA, 2017 a,b). The remaining 5% of high- purity rebaudioside I (≥95% rebaudioside I) primarily includes additional steviol glycosides and water. Due to the common steviol backbone, all steviol glycosides share a common metabolic fate in which they are hydrolyzed to steviol in the lower gastrointestinal tract, which is then absorbed into the body, conjugated with glucuronic acid, and eliminated through the urine in humans.

High-purity rebaudioside I (≥95% rebaudioside I) is manufactured using raw materials and processing aids that are food-grade ingredients, permitted by U.S. regulation, have GRAS status, or have been self-affirmed as safe for use in food for their respective uses. The high-purity rebaudioside I (≥95% rebaudioside I) product is manufactured by an enzymatic conversion process using strains derived from *E. coli* K-12. High purity rebaudioside I (≥95% rebaudioside I) is manufactured in a multi-stage process; in the first step steviol glycosidesare extracted from the stevia leaf by a series of crushing, dissolution, extraction, and precipitation steps that are consistent with the methodology outlined in the JECFA Chemical and Technical Assessment (CTA) for steviol glycosides outlined at the 82nd meeting (FAO, 2016). The steviol glycoside mixture used in step 1 contains >90% total steviol glycosides. In the second stage, the production *E. coli* strains are introduced to the media containing the nutrients and glycosides which then undergo enzymatic conversion. The bioconversion process is the same as that outlined in Annex 3 of JECFA monograph 26 that was prepared at the 91st meeting (2021). In the final stage the resulting steviol glycoside mixture is purified in a series of crystallization, filtration, and washing steps to yield a final product containing ≥95 % rebaudioside I and ≥95 % total steviol glycosides.

Appropriate food-grade product specifications have been established for high-purity rebaudioside I (≥95% rebaudioside I) based on the steviol glycosides specification established by JECFA (JECFA, 2017a,b). The steviol glycoside content of high-purity rebaudioside I (≥95% rebaudioside I) is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from Stevia rebaudiana Bertoni (JECFA, 2017a,b). Review by the GRAS Panel of the analytical data for 3 non-consecutive lots of high purity rebaudioside I (≥95% rebaudioside I) confirms that the final product is produced in compliance with the established product specifications. In addition, residual protein in the final product was confirmed to be below the detection level in 3 non-consecutive lots of high purity rebaudioside I (≥95 % rebaudioside I) *via* the bicinchoninic acid (BCA) assay. The absence of residual DNA in the final product for these same lots was likewise confirmed to below the detection limit *via* polymerase chain reaction (PCR), compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United

States Pharmacopeia, European Pharmacopoeia).

Absence of residues of commonly used pesticides was also confirmed in the starting steviol glycoside material (>90% total steviol glycosides).

JECFA has concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions (JECFA, 2007). The stability study on high-purity Manus Bio rebaudioside I (≥95% rebaudioside I) is currently on-going; however, the 3-month results indicate that the total steviol glycoside and Rebaudioside I contents are stable when kept in commercial packaging under normal storage conditions.

INTENDED FOOD USES AND ESTIMATED INTAKE

High-purity rebaudioside I (≥95 % rebaudioside I) is intended for use as a general-purpose sweetener that will be added to various food and beverage products that are consistent with the current uses of other high-intensity sweeteners on the U.S. market. The estimated intakes of high purity rebaudioside I (≥95 % rebaudioside I) were calculated using a post-market surveillance approach as described by Renwick (2008). The estimated intakes were calculated by adjusting the post-market surveillance data for other high-intensity sweeteners using the sweetness intensity of high-purity Rebaudioside I (≥95 % Rebaudioside I) relative to sucrose (i.e., approximately 167 X sweeter than sucrose by weight). The results are shown in Table 1. For non-diabetic adults, average and high-end intakes of high purity rebaudioside I (≥ 95% rebaudioside I) of up to 0.43 and 1.14 mg/kg body weight/day expressed as steviol equivalents, respectively, were calculated. For diabetic adults, average and high-end intakes were slightly higher at up to 0.47 and 1.51 mg/ kg body weight / day. Average and high-end exposures to high purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents, in non-diabetic children were calculated to be up to 0.72 and 1.67 mg/kg body weight/ day, respectively. Although average intakes of high-purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents were estimated to be higher at up to 1.13 mg/ kg body weight/day in diabetic children compared to values for non -diabetic children, high-end values in diabetic children (1.53 mg/kg body weight/ day) were lower than high-endvalues in non-diabetic children. The predicted intakes of high-purity rebaudioside I (≥95% rebaudioside I), expressed as steviol equivalents, are all below the current acceptable daily intake (ADI) defined by the JECFA for steviol glycosides (FAO, 2016) of 0 to 4 mg/kg body weight/day as steviol. JECFA re-assessed the dietary exposure to steviol glycosides using different intake models, including the approach described by Renwick (2008), and noted that the replacement estimates were highly conservative and that actual exposures to steviol glycosides, expressed as steviol equivalents, would range from 0.4 to 7.2 mg/kg body weight/day (FAO, 2016). JECFA made note that this method overestimates dietary exposure (FAO, 2016).

Table 1 Estimated Consumption High-Purity Rebaudioside I (≥95% Rebaudioside I) Produced by Enzymatic Conversion of Steviol Glycosides Using the Intense Sweetener Intake Assessment Methodology described by Renwick (2008)

			Consumption Estimates			
	Intakes of Intense Sweeteners (expressed as sucrose equivalents) (mg/kg bw/day)		High-Purity Rebaudioside I (≥95% Rebaudioside I)[a] (mg/kg bw/day)		High-Purity Rebaudioside I (≥95% Rebaudioside I)[a,b] (mg/kg bw/day) as Steviol	
Population Group	**Average Consumer**	**High Consumer**	**Average Consumer**	**High Consumer**	**Average Consumer**	**High Consumer**
Non-diabetic adults	255	675	1.53	4.04	0.43	1.14
Diabetic adults	280	897	1.68	5.37	0.47	1.51
Non-diabetic children	425	990	2.54	5.93	0.72	1.67
Diabetic children	672	908	4.02	5.44	1.13	1.53

INFORMATION TO ESTABLISH SAFETY

The GRAS Panel reviewed the available data supporting the safety of individual steviol glycosides to evaluate the safety of high purity rebaudioside I (≥ 95% rebaudioside I). The available data included a discussion on the metabolic fate of steviol glycosides, a summary of the extensive conclusions on the safety of steviol glycosides by global scientific and regulatory authorities/bodies, including the U.S. FDA, and other data that was deemed pivotal in determining the safety of high purity rebaudioside I (≥ 95% rebaudioside I) produced by Manus Bio.

In vitro and ex vivo studies have demonstrated that steviol glycosides are not hydrolysed by digestive enzymes of the upper gastrointestinal tract due to the presence of β-glycosidic bonds and are not absorbed through the upper portion of the gastrointestinal tract (Hutapea et al., 1997; Koyama et al., 2003a; Geuns et al., 2003, 2007). Therefore, steviol glycosides enter the colon intact, where they are subject to microbial degradation by members of the *Bacteroidaceae* family, resulting in the release of the aglycone steviol (Gardana et al., 2003; Renwick and Tarka, 2008). Several in vitro studies mimicking the anaerobic conditions of the colon, reviewed extensively by Renwick and Tarka (2008), have confirmed the ability of gut microflora from mice, rats, hamsters, and humans to hydrolyse steviol glycosides completely to steviol (Wingard et al., 1980; Hutapea et al., 1997; Gardana et al., 2003; Koyama et al., 2003a,b; Nikiforov et al., 2013; Purkayastha et al., 2016). Specifically, Purkayastha et al. (2016) conducted incubation studies with pooled human fecal homogenates collected from adult males and females using rebaudiosides A, B, C, D, E, F, M, steviolbioside and dulcoside A, and showed that all glycosides were converted to steviol over a 24- 48 h incubation period. These studies demonstrated that the number and location of sugar units attached to the steviol backbone do not significantly affect the microbial hydrolysis. Likewise, this study demonstrated that steviol glycosides, irrespective of the type of sugar moiety (e.g., glucose, rhamnose, xylose) attached to the steviol backbone, were metabolized to steviol at generally similar hydrolysis rates. Incubation tests were also conducted in fecal homogenates collected from adult and pediatric populations with steviol glycosides from stevia leaf extracts and steviol glycosides produced by enzymatic conversion of Rebaudioside A to larger molecules by attaching glucose units *via* beta or alpha glycosidic bonds. The steviol glycosides produced by extraction from stevia leaf or enzymatic conversion of stevia leaf extract were reported to share a similar metabolic fate in the fecal homogenates from adults and children thereby supporting a similar metabolic fate of steviol glycosides in all age groups (Purkayast ha 2020). Steviol glycosides have been reported to be hydrolyzed sequentially, in which 1 sugar moiety is removed at a time, with the degradation rates dependent on the structural complexity of each steviol glycoside (Wingard et al., 1980; Koyama et al., 2003b). Despite the differences in chemical structure, however, the rate of hydrolysis of different steviol glycosides to steviol are relatively similar, especially during the first 24 hours of incubation of in vitro metabolic studies with human fecal homogenates (Purkayastha et al., 2014, 2015, 2016). Following microbial degradation, the steviol metabolite is absorbed systemically into the portal vein and distributed to the liver, spleen, adrenal glands, fat, and blood (Nakayama et al., 1986; Sung, 2002 [unpublished]; Koyama et al., 2003b; Wang et al., 2004; Roberts and Renwick, 2008). Steviol is conjugated to glucuronic acid to form steviol glucuronide in the liver. The steviol glucuronide metabolite and any unconjugated steviol or unhydrolyzed fraction of the administered glycosides are excreted primarily in the urine, and, to a lesser extent, feces in humans (Wingard et al., 1980; Nakayama et al., 1986; Kraemer and Maurer, 1994; Sung, 2002 [unpublished]; Geuns and Pietta, 2004 [unpublished]; Simonetti et al., 2004; Geuns et al., 2006, 2007; Roberts and Renwick, 2008; Wheeler et al., 2008). Thus, due to the similar metabolic fate of steviol glycosides, the safety database that has been established for individual steviol glycosides (e.g., stevioside, rebaudioside A, rebaudioside D), can be extrapolated to support the safety of other purified steviol glycosides in general, regardless of the glycosidic distribution of the preparation, including high purity rebaudioside I (≥95%

rebaudioside I) produced by enzymatic conversion of steviol glycosides.

The safety of steviol glycosides have been extensively reviewed by various scientific and regulatory authorities/bodies, such as JECFA, U.S. FDA, Food Standards Australia New Zealand (FSANZ), the European Commission's Scientific Committee on Food {SCF), the European Food Safety Authority (EFSA), and Health Canada (SCF, 1985, 1999; FSANZ, 2008; EFSA, 2010, 2015; Health Canada, 2012b; JECFA, 2006, 2017a,b). Based on safety data and information that were generally available in the published scientific literature, these scientific bodies and regulatory agencies have universally concluded that steviol glycosides are of no safety concern and have established an ADI of 0 to 4 mg/kg body weight, expressed as steviol equivalents, based on the results of a 2-year study in rats (Toyoda et al., 1997). The no-observed-adverse-effect level (NOAEL) of 970 mg/ kg body weight/day, equivalent to 383 mg/kg body weight/day as steviol equivalents, determined from the results of the study by Toyoda et al. (1997) became the basis for the established ADI following application of a safety factor of 100 (JECFA, 2009; FSANZ, 2008; EFSA, 2010; Health Canada, 2012b). Subsequent to these safety evaluations, the EFSA concluded that "extending the current specifications to include [two additional steviol glycosides], rebaudiosides D and M, as alternatives to rebaudioside A in the predominant components of steviol glycosides would not be of a safety concern" (EFSA, 2015). The following regulatory authorities including EFSA, JECFA, FSANZ, and Health Canada have also recently expanded the definition of steviol glycosides to include all individual steviol glycosides present in the *S. rebaudiana* Bertoni leaf, including rebaudioside I (EFSA, 2020; FSANZ, 2017b; Health Canada, 2017; JECFA, 2017). The FAO JECFA Monographs 26 defines enzymatic conversion as a process in which steviol glycosides that have been extracted from the leaves of *Stevia rebaudiana Bertoni* undergo enzymatic conversion of major steviol glycosides to minor ones. Annex 3 JECFA (2021) monograph 26 defines Enzyme-modified steviol glycosides as a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni. The product is obtained from the enzymatic treatment of purified steviol glycosides extracted from the leaves of *Stevia rebaudiana* Bertoni. The purified leaf extract is treated with enzymes produced by non-toxigenic nonpathogenic strains of *Pichia pastoris* (also known as *Komagataella Phaffii*) and *Escherichia coli* that have been genetically modified with genes from multiple donor organisms to produce glucosyltransferase (EC 2.4.1.17) and sucrose synthase (EC 2.4.1.13). The resulting material is heated and filtered to denature and remove the enzymes. The raw product is concentrated using resin adsorption/desorption or solid/liquid filtration, followed by purification and preparation of the product of commerce using processes that may include decolorization, crystallization, and spray drying. This manufacturing technique maximizes the production of specific steviol glycosides that are not naturally present in high concentrations in the leaf extract. (91st JECFA (2021) and published in FAO Monograph 26 (2021)).

The U.S. FDA has reviewed the safety of over 50 different steviol glycoside preparations and have consistently raised no objections regarding the GRAS status of steviol glycosides for use as general-purpose sweeteners in food and beverage products. Of note, the U.S. FDA did not raise any objections regarding GRN 911, which describes the GRAS status of Rebaudioside I produced by an enzymatic bioconversion process for use as a general-purpose sweetener in foods (U.S. FDA, 2021). The rebaudioside I described in GRN 911 is similar to Manus Bio' s high purity rebaudioside I (≥95% rebaudioside I) produced by enzymatic conversion of steviol glycosides. In the Manus Bio process the food ingredient is produced by conversion of stevia leaf extract using UDP-glucosyltransferase enzymes produced by *E. coli* K-12 similar to GRN 745.

A comprehensive search of the scientific literature was conducted through January 2022 to capture publications relevant to the safety of steviol glycosides that became available following the U.S. FDA review of GRN 1010.

The parental strain, *E. coli* K-12, from which the production strains are derived, has an extensive history of use as a laboratory organism and is considered one of the most extensively characterized microorganisms (Bachmann, 1972; Jensen, 1993). *E. coli* K-12 has a long history of safe use in the food and pharmaceutical industries. For example, a genetically modified strain of *E. coli* K-12 producing chymosin was affirmed as GRAS by the U.S. FDA in 1990 (Flamm, 1991; Olempska-Beer et al., 2006). *E. coli* also serves as a host for the production of enzymes currently used in a GRAS-approved process for the enzymatic conversion of steviol glycosides. (GRN745). Further, *E. coli* K-12 and its derivatives are unable to colonize the mammalian gastrointestinal tract, and do not produce toxins and are unable to persist in the soil and water (Bogosian et al., 1996; U.S. EPA, 1997). The parental strain does not carry any introduced antibiotic resistance genes and the complete genome of this strain has been sequenced, confirming the absence of any toxigenic potential (Blattner et al., 1997; Hayashi et al., 2006). The identity of the final production strain was also confirmed by both Sanger sequencing of engineered regions and whole genome sequencing.

The scientific evidence reviewed by the GRAS Panel demonstrates that under the conditions of intended use, Manus Bio's high purity rebaudioside I (≥95% rebaudioside M) would not produce any adverse health effects.

CONCLUSION

We, the undersigned independent qualified members of the GRAS Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that Manus Bio's high-purity rebaudioside I (≥95% rebaudioside I), produced by enzymatic conversion of stevia leaf extract, using UDP-glucosyltransferases from *E. coli* K12, meeting appropriate food-grade specifications and produced in accordance with current Good Manufacturing Practice (cGMP), is safe for use as a general-purpose sweetener in conventional foods and beverages.

We further unanimously conclude that the proposed use of Manus Bio's high-purity rebaudioside I (≥95% rebaudioside I) meeting appropriate food-grade specifications, as presented in the supporting dossier and produced consistent with cGMP is Generally Recognized as Safe (GRAS) under its intended conditions of use as a general-purpose sweetener in conventional foods and beverages based on scientific procedures.

It is our professional opinion that other qualified experts critically evaluating the same information, would concur with these conclusions.

4/28/2022

Jose -Avalos, Ph.D.
Princeton University
Princeton, NJ

Date

2 May 2022

Ashley Roberts, Ph.D.
President
AR Toxicology, Inc.

Date

21 April 2022

Stanley M. Tarka, Jr., Ph.D.
Fellow, ATS
The Tarka Group, Inc.,
The Pennsylvania State University, College of Medicine

Date

APPENDIX B: Manufacturing Process Details

Manus Bio's high-purity rebaudioside I (≥95% rebaudioside I) is produced by enzymatic conversion of steviol glycosides using an *E. coli* strain derived from *E. coli* K-12. (see Appendix B) The production strains are grown in media containing steviol glycoside extracts prepared from the leaves of *S. rebaudiana* Bertoni in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides (FAO, 2016). In brief, steviol glycosides are extracted from the stevia leaf by a series of crushing, dissolution, solvent extraction, and precipitation steps. The manufacturing process for this starting material is described in detail in GRAS Notice (GRN 275) (U.S. FDA, 2008). Specifications for the starting material used in the production of rebaudioside I are provided in Table 2.3-1. Within the growth medium containing stevia leaf extract, UGT enzymes produced by the *E. coli* K-12 cells mediate the glycosylation of steviol glycosides to rebaudioside I. After sufficient rebaudioside I has been produced, the media and *E. coli* K-12 biomass is heat inactivated and centrifugation and/or filtration is used to remove the inactivated biomass and precipitated enzymes. Rebaudioside I is then purified using physical processing steps including filtration, aqueous crystallization, centrifugation, rinsing and drying using typical food processing equipment and steps. Dried Rebaudioside I may be in a crystalline or amorphous solid form and may be further milled, spray dried, freeze dried, agglomerated, compacted, and granulated or undergo other physical form modifications to achieve a desirable particle size of the final product, ≥95% Rebaudioside I. The purification processes described are consistent with the methodologies for the manufacture of steviol glycosides as described in the CTA published by FAO/JECFA (FAO, 2016).

Manus Bio's ≥95% Rebaudioside I is manufactured in a facility registered as an FDA Food Facility. The plant operates under cGMPs (Good Manufacturing Practices) outlined in the Food Safety Modernization Act 21 CFR 117, including required HACCP and Food Defense Plans, and is subject to audit from regulatory authorities including the U.S. FDA and The State of Georgia Department of Agriculture. Manufacturing shall be certified to a GFSI (Global Food Safety Initiative) compliant audit scheme.

An overview of a typical manufacturing process flow is provided in Figure 2.3-1 below although order and type of physical purification steps can be varied to achieve the same product specifications.

APPENDIX C: Certificate of Analysis for three non-consecutive lots

NUTRASWEET I™ NATURAL

1762 Lovers Lane,
Augusta GA
Ph.: (1) 706-303-5600

Certificate of Analysis

NutraSweetI™ Natural

Stevia Leaf Extract (Rebaudioside I)

Manufacturing Date 10/28/2021 ***Lot No. MAI2110281***

Retest Date 10/28/2023

Specification Parameter *Physical and Chemical Parameters*	High-Purity Rebaudioside I (≥95% Rebaudioside I)	Manufacturing Lot No Result	Method of Analysis
Appearance	White to off-white powder	Pass	VISUAL
Total steviol glycosides (anhydrous basis)	≥95%	>95	TN34236 [Monograph 23 (87th JECFA Meeting 2019)]
Rebaudioside I	≥95%	97.54	HPLC
Loss on drying	≤8%	2.6	FCC Appendix IIC
pH (1% solution)	4.5 to 7.0	6.8	TN60730 (AOAC 981.12)
Residual ethanol	≤5,000 ppm	Not Detected	EN 14110 (mod)
Residual methanol	≤200 ppm	Not Detected	EN 14110 (mod)
Total ash	≤1%	<1%	USP 281
Lead	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Arsenic	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Cadmium	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Mercury	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Specification Parameter *Microbial Parameters*	**High-Purity Rebaudioside I (≥95% Rebaudioside I)**	**Manufacturing Lot No Result**	**Method of Analysis**
Total plate count	<1,000 CFU/g	970 CFU/g	AOAC 2015.13
Mold	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Yeast	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Coliforms	<3 MPN/g	<3 MPN/g	AOAC 2018.13
Escherichia coli	Not detected	Not detected	AOAC 2018.13
Salmonella	Negative/25 g	Negative	TN10512 (CRA Microbiological Methods IV-B)

MANUSBIO

NUTRASWEET I™ NATURAL

1762 Lovers Lane,
Augusta GA
Ph.: (1) 706-303-5600

Certificate of Analysis

NutraSweetI™ Natural

Stevia Leaf Extract (Rebaudioside I)

Manufacturing Date 10/26/2021 ***Lot No. MAI2110262***

Retest Date 10/26/2023

Specification Parameter *Physical and Chemical Parameters*	High-Purity Rebaudioside I (≥95% Rebaudioside I)	Manufacturing Lot No Result	Method of Analysis
Appearance	White to off-white powder	Pass	VISUAL
Total steviol glycosides (anhydrous basis)	≥95%	>95	TN34236 [Monograph 23 (87th JECFA Meeting 2019)]
Rebaudioside I	≥95%	97.58	HPLC
Loss on drying	≤6%	3.0	FCC Appendix IIC
pH (1% solution)	4.5 to 7.0	5.43	TN60730 (AOAC 981.12)
Residual ethanol	≤5,000 ppm	<200 ppm	EN 14110 (mod)
Residual methanol	≤200 ppm	Not Detected	EN 14110 (mod)
Total ash	≤1%	<1%	USP 281
Lead	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Arsenic	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Cadmium	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Mercury	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Specification Parameter *Microbial Parameters*	**High-Purity Rebaudioside I (≥95% Rebaudioside I)**	**Manufacturing Lot No Result**	**Method of Analysis**
Total plate count	<1,000 CFU/g	210 CFU/g	AOAC 2015.13
Mold	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Yeast	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Coliforms	<3 MPN/g	<3 MPN/g	AOAC 2018.13
Escherichia coli	Not detected	Not detected	AOAC 2018.13
Salmonella	Negative/25 g	Negative	TN10512 (CRA Microbiological Methods IV-B)

MANUSBIO

NUTRASWEET I™ NATURAL

1762 Lovers Lane,
Augusta GA
Ph.: (1) 706-303-5600

Certificate of Analysis

NutraSweetI™ Natural

Stevia Leaf Extract (Rebaudioside I)

Manufacturing Date 10/26/2021 ***Lot No. MAI2110261***

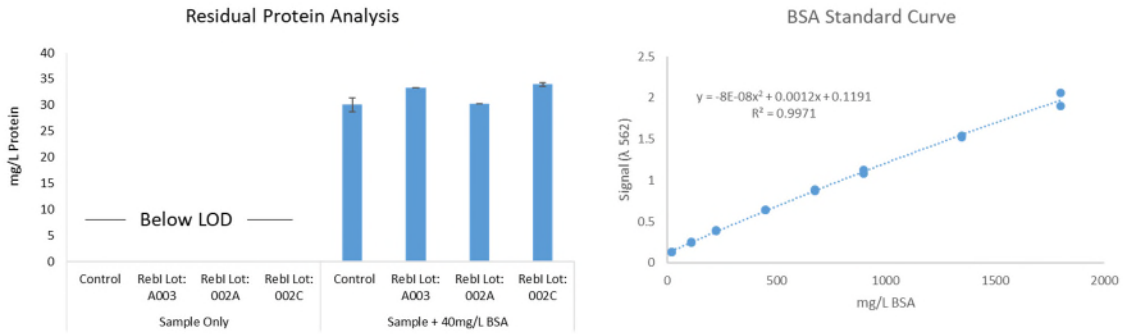
Retest Date 10/26/2023

Specification Parameter *Physical and Chemical Parameters*	High-Purity Rebaudioside I (≥95% Rebaudioside I)	Manufacturing Lot No Result	Method of Analysis
Appearance	White to off-white powder	Pass	VISUAL
Total steviol glycosides (anhydrous basis)	≥95%	>95	TN34236 [Monograph 23 (87th JECFA Meeting 2019)]
Rebaudioside I	≥95%	97.84	HPLC
Loss on drying	≤6%	3.8	FCC Appendix IIC
pH (1% solution)	4.5 to 7.0	6.85	TN60730 (AOAC 981.12)
Residual ethanol	≤5,000 ppm	<200 ppm	EN 14110 (mod)
Residual methanol	≤200 ppm	Not Detected	EN 14110 (mod)
Total ash	≤1%	<1%	USP 281
Lead	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Arsenic	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Cadmium	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Mercury	≤1 ppm	<1 ppm	USP 233(ICP-MS)
Specification Parameter *Microbial Parameters*	**High-Purity Rebaudioside I (≥95% Rebaudioside I)**	**Manufacturing Lot No Result**	**Method of Analysis**
Total plate count	<1,000 CFU/g	<10 CFU/g	AOAC 2015.13
Mold	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Yeast	<100 CFU/g	<10 CFU/g	FDA/BAM Chapt. 18
Coliforms	<3 MPN/g	<3 MPN/g	AOAC 2018.13
Escherichia coli	Not detected	Not detected	AOAC 2018.13
Salmonella	Negative/25 g	Negative	TN10512 (CRA Microbiological Methods IV-B)

MANUSBIO

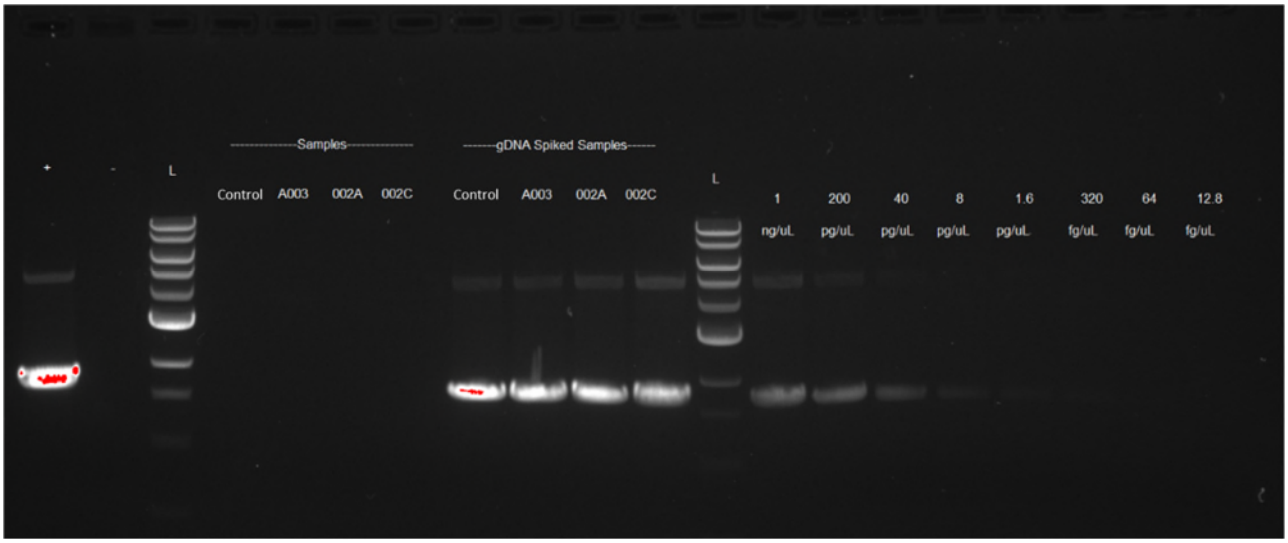
APPENDIX D: Residual Protein analysis

Samples were reconstituted to a concentration of 1 g/L and measured using a 96 well plate format BCA assay on a Tecan Infinite M1000 Pro plate reader. The limit of detection was 22.5 ppm on a w/v basis. The results of the analysis were below the limit of detection, which provides further evidence that downstream processing successfully removed residual proteins from the final product.



APPENDIX E: Residual DNA Analysis

To confirm the absence of residual DNA in Rebaudioside I, 3 non-consecutive lots of final product were assayed by polymerase chain reaction (PCR). Primer design and PCR conditions were designed to amplify a specific fragment of one of the genes inserted in the production strain. Extracted genomic DNA containing the selected gene was used as a positive control. Reb I samples were prepared in aqueous solution and assayed by PCR at a final concentration of 100 mg/L. Samples were assayed with or without positive control DNA at a final concentration of 1 ng/µL. A standard curve was prepared from 1 ng/µL to 0.1 fg/µL to determine the limit of detection of the assay (320 fg/µL). All samples with a positive control spike showed an expected PCR product, whereas all the Reb I samples without a genomic DNA spike showed no product. The gel images were analyzed using ImageJ for quantitative analysis. Background subtracted mean grey values from each of the samples were fit to values from the standard curve and found to be below the limit of detection of the assay. These results confirm the absence of residual genomic DNA in the Reb I final product above the analytical limit of detection of 320 fg/µL.



Sample	Background Subtracted Mean Grey Value	Calculated Concentration (ng/µL)
A003	-2.219	below LOD*
002A	1.043	below LOD*
002C	1.246	below LOD*

*LOD = limit of detection

APPENDIX F: Pesticide Residue

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REPORT OF ANALYSIS
For: (30263) MANUS BIO INC

Analysis	Level Found As Received	Units	Reporting Limit	Method	Analyst-Date	Verified-Date
Sample ID: **Lot: SG9020201015** Lab Number: **8950423** Date Sampled: **2021-08-06 1200**						
Methamidophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Acephate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Omethoate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Monocrotophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Trichlorfon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dicrotophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethoate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mevinphos Isomer 1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mevinphos Isomer 2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Vamidothion/Vamidoate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Carbaryl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethomorph Isomer 1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethomorph Isomer 2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Aldicarb Sulfone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Butoxycarboxim (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Aldicarb sulfoxide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Oxamyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methomyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Aldicarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Thidazuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Thiophanate-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
ethiofencarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tebuthiuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
methabenzthiazuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methiocarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Alanycarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Furathiocarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Benfuracarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Butocarboxim (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
3-hydroxycarbofuran (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
dioxacarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
bendiocarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
propoxur (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
carbofuran (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
primicarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
fluometuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
isoprocarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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monolinuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
forchlorfenuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
metobromuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
isoproturon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
fenobucarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
diuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Siduron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
diethofencarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
cycluron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
promecarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
iprovalicarb isomer 1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
iprovalicarb isomer 2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
chloroxuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
diflubenzuron-s (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
fenoxycarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
pyraclostrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
indoxacarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
hexaflumuron-h (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
novaluron-h (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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teflubenzuron-h (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
lufenuron-h (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
triflumuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
thiobencarb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Linuron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
chlorotoluron (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metobromuron-S (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
propham-s (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
flufenoxuron-h (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Forchlorfenuron-S (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
bifenazate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Neburon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bupirimate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Buprofezin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Carboxin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Clethodim (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Clothianidin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyazofamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ethiprole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Ethofumesate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenamidone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flubendiamide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flufenacet (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Hexythiazox (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mefenacet (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methoprtryne (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prometryne (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyridaben (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Simetryn (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Terbutryn (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Thiabendazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Thiacloprid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Thiamethoxam (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Thiafanox (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tricyclazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metribuzin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propargite (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Baycor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Bromuconazole-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyproconazole-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Diclobutrazol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Difenoconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Diniconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Epoxiconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ethirimol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Etoxazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenarimol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenbuconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluquinconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flusilazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flutriafol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fuberidazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Hexaconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ipconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Nuarimol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Paclobutrazol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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REPORT OF ANALYSIS
For: (30263) MANUS BIO INC

Analysis	Level Found As Received	Units	Reporting Limit	Method	Analyst-Date	Verified-Date
Sample ID: Lot: SG9020201015	Lab Number: **8950423** (con't)					
Penconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propiconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tebuconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetraconazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triadimenol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triflumizole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triticonazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromuconazole-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyproconazole-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Formetanate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mexacarbate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Monceren (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Aminocarb E (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyromazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Nitenpyram (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pymetrozine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Imidacloprid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cymoxanil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Acetamiprid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Carbetamide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Oxadixyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyracarbolid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Imazalil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metalaxyl (Mefenoxam) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prometon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Secbumeton (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Terbumeton (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ametryn (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Halofenozide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Furalaxyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spiroxamine-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spiroxamine-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Azoxystrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flutolanil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mandipropamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mepronil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyrimethanil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methoxyfenozide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Fenhexamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Myclobutanil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Butafenacil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluoxastrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mepanipyrim (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tebufenozide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Picoxystrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Carfentrazone-ethyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimoxystrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Rotenone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyprodinil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Zoxamide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Famoxadone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Benzoximate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prochloraz (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Clofentezine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Trifloxystrobin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Piperonyl butoxide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tebufenpyrad (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Pyriproxyfen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Quinoxyfen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenproximate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenazaquin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Doramectin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metaflumizone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triadimefon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fludioxonil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Boscalid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Kresoxim-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Benalaxyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Amitraz (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Carbendazim (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Isocarbophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flonicamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorantraniliprole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Moxidectin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluazinam (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ivermectin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Eprinomectin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
emamectin-benzoate a (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
emamectin-benzoate b (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
fenpropimorph (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spirodiclofen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
spirotetramat (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
spinetoram (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spiromesifen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spinosad (Spinosyn A) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Spinosad (Spinosyn D) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Desmedipham (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phenmedipham (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mevinphos-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mevinphos-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Demeton-S (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Naled (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Sulfotep (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethoate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phorate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Demeton-O (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Diazinon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Disulfoton (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methyl parathion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Malathion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenthion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
TEPP (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Trichloronat (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetrachlorvinphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tribufos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fensulfothion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Sulprofos (Bolstar) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
EPN (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Azinphos-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Coumaphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
alpha-BHC (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
beta-BHC (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
gamma-BHC (Lindane) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
delta-BHC (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Heptachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Aldrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Endrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dieldrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Endosulfan I (alpha) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Endosulfan sulfate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Endrin ketone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methoxychlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
EPTC (Eptam) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Butylate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Deisopropylatrazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ethalfluralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Trifluralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Simazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Atrazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Terbufos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fonofos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Sample ID: Lot: SG9020201015	Lab Number: **8950423** (con't)					
Triallate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethenamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Acetochlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Alachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prometryn (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromacil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metolachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorpyrifos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyanazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pendimethalin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Butachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Oxadiazon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Hexazinone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Azinphos-ethyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Benfluralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
beta-Endosulfan (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bifenthrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Biphenyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromfenvinphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Bromfenvinphos-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromophos-ethyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Bromopropylate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Captafol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Captan (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Carbophenothion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorbenside (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorfenapyr (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorfenson (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chloroneb (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chloropropylate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorothalonil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorpyrifos-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorthiophos-3 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
cis-Chlordane (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
cis-Nonachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Clomazone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cycloate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Cyfluthrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyfluthrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyfluthrin-3 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyfluthrin-4 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyhalothrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cyhalothrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cypermethrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cypermethrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cypermethrin-3 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Cypermethrin-4 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Deltamethrin-1 (Tralomethrin deg.-1) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Deltamethrin-2 (Tralomethrin deg.-2) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Desethylatrazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Di-allate-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Di-allate-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dichlobenil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dichlofluanid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dichlofluanid metabolite (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dichloroaniline, 3,4'- (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Dichlorvos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dicofol deg. (DCBP) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Dimethachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Diphenamid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Diphenylamine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Edifenphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Endosulfan ether (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ethion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Ethoprophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Etofenprox (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Etridiazole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenamiphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenchlorphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenitrothion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenson (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fipronil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluchloralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluridone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Folpet (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Heptachlor-endo-epoxide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Heptachlor-exo-epoxide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Hexachlorobenzene (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Iodofenphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Iprodione (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Isazofos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Isodrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Isofenphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Isopropalin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Lenacil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Leptophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Metazachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Methacrifos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
MGK-264 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Mirex (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
N-(2,4-Dimethylphenyl)formamide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Nitralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Nitrofen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Norflurazon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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o,p'-DDD (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
o,p'-DDE (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
o,p'-DDT (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Oxyfluorfen (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
p,p'-DDD (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
p,p'-DDE (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
p,p'-DDT (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pebulate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pentachloroaniline (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pentachloroanisole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pentachlorobenzene (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pentachlorobenzonitrile (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pentachlorothioanisole (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Permethrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Permethrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phenothrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phenothrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phosalone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Phosmet (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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MANUS BIO INC
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1762 LOVERS LANE
AUGUSTA GA 30903-1869

REPORT OF ANALYSIS
For: (30263) MANUS BIO INC

Analysis	Level Found As Received	Units	Reporting Limit	Method	Analyst-Date	Verified-Date
Sample ID: Lot: SG9020201015 Lab Number: **8950423** (con't)						
Pirimiphos-ethyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pretilachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Procymidone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prodiamine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Profenofos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Profluralin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propanil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propargite-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propargite-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propisochlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Propyzamide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Prothiofos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyraclofos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyrazophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Pyridaphenthion (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Quinalphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Quintozene (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Resmethrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Resmethrin-2 (Bioresmethrin) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

The result(s) issued on this report only reflect the analysis of the sample(s) submitted.

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Tecnazene (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tefluthrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Terbacil (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Terbuthylazine (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetrachloroaniline, 2,3,5,6- (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetradifon (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetrahydrophthalimide (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetramethrin-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tetramethrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Tolylfluanid (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
trans-Chlordane (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
trans-Nonachlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Transfluthrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triadimenol-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triadimenol-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Triazophos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Vinclozolin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
2-Phenylphenol (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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4,4'-Methoxychlor olefin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
2,4'-Methoxychlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
alpha-Endosulfan (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Allidochlor (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Anthraquinone (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Allethrin-3,4 (Bioallethrin) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Acrinathrin-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
lambda-Cyhalothrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flucythrinate-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Flucythrinate-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenvalerate-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
tau-Fluvalinate-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluvalinate-1 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fenvalerate-2 (Esfenvalerate) (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Fluvalinate-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
tau-Fluvalinate-2 (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorpropham (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlorthal-dimethyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23
Chlozolinate (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	rwp6-2021/08/23	akj2-2021/08/23

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Fluazifop-P-butyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
Acequinocyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
Tolclofos-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
(E)-Chlorfenvinphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
(Z)-Chlorfenvinphos (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
Fenpropathrin (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23
Pirimiphos-methyl (QuEChERS)	n.d.	ppm	0.01	AOAC 2007.01 (mod)	mp6-2021/08/23	akl2-2021/08/23

All results are reported on an AS RECEIVED basis., n.d. = not detected , ppm = parts per million, ppm = mg/kg

For questions please contact:
[redacted]
Kailey Parr
Account Manager
kparr@midwestlabs.com (402)829-9863

From: Hahn, Martin J.
To: Kampmeyer, Christopher
Subject: [EXTERNAL] GRAS Notice for High-Purity Rebaudioside I
Date: Tuesday, February 7, 2023 4:59:35 PM

Chris:

Thank you for your call today and request for clarification on the relationship between Hogan Lovells and ManusBio Inc. In September 2022 we submitted a GRAS notice on behalf of our client ManusBio. We submitted the cover letter on Hogan Lovells letterhead without explaining that ManusBio is our client. We then submitted the GRAS notification that had been prepared and signed by ManusBio. Please use this e-mail to confirm ManusBio is a client of Hogan Lovells. We also ask that FDA direct any correspondence regarding the GRAS notification to my attention.

Xin Tao appeared on the earlier filings and e-mail correspondence. Xin left Hogan Lovells in January to join another law firm. I will be the sole contact for this submission.

If you have any questions or require any additional correspondence to clarify the relationship, please let me know.

Many thanks again for your call today. We look forward to receiving correspondence from the agency.

Martin Hahn
Partner

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From: Hahn, Martin J.
To: Zhang, Janet
Subject: [EXTERNAL] RE: GRN 001106
Date: Thursday, March 30, 2023 3:53:20 PM
Attachments: image001.png

Dr. Zhang
Thank you for your request. We are writing to confirm the engineering does indeed result in the organism expressing enzymes related to the supply of UDP-glucose. The dossier noted on page 8 that "the strain was engineered to increase the supply of uridine diphosphate glucose (UDP-Glu)." We could have explained with more specificity the supply of the UDP-Glu is increased by expressing enzymes that allow for the formation of the UDP-Glu.
We look forward to the agency's review of the GRAS notification and are available to answer any additional questions that may arise during the review.
Best regards.

Martin Hahn
Partner

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From: Zhang, Janet <Janet.Zhang@fda.hhs.gov>
Sent: Friday, March 17, 2023 10:09 AM
To: Hahn, Martin J. <martin.hahn@hoganlovells.com>
Subject: GRN 001106

[EXTERNAL]
Good morning Mr. Hahn,

We have a question regarding GRN 001106 you submitted on behalf of Manus Bio, Inc. Please provide your response within 10 business days.

Question:

The notifier states on page 8 that the production organism used in the manufacture of rebaudioside I was engineered to express enzymes (UDP-glucosyl transferases) for the glycosylation of steviol glycosides and to increase the supply of uridine diphosphate glucose (UDP-glucose). Does the engineering of this organism result in the expression of an enzyme that is related to the supply of UDP-glucose? For example, we note that on page 23, the notifier discusses the JECFA evaluation of enzymatically modified steviol glycosides that includes the use of glucosyltransferase (EC 2.4.1.17) and sucrose synthase (EC 2.4.1.13), and the notifier states that the process outlined within the JECFA monograph defines the methodology used by Manus Bio to produce rebaudioside I.

Best regards,

Jianrong (Janet) Zhang, Ph.D.
FDA/OFVM/CFSAN/OFAS/DST
College Park, MD 20740
Phone: 240-402-1327
janet.zhang@fda.hhs.gov