Application for the use of nicotinamide riboside chloride as a permitted form of Vitamin B3 in food for special medical purposes in Australia and New Zealand

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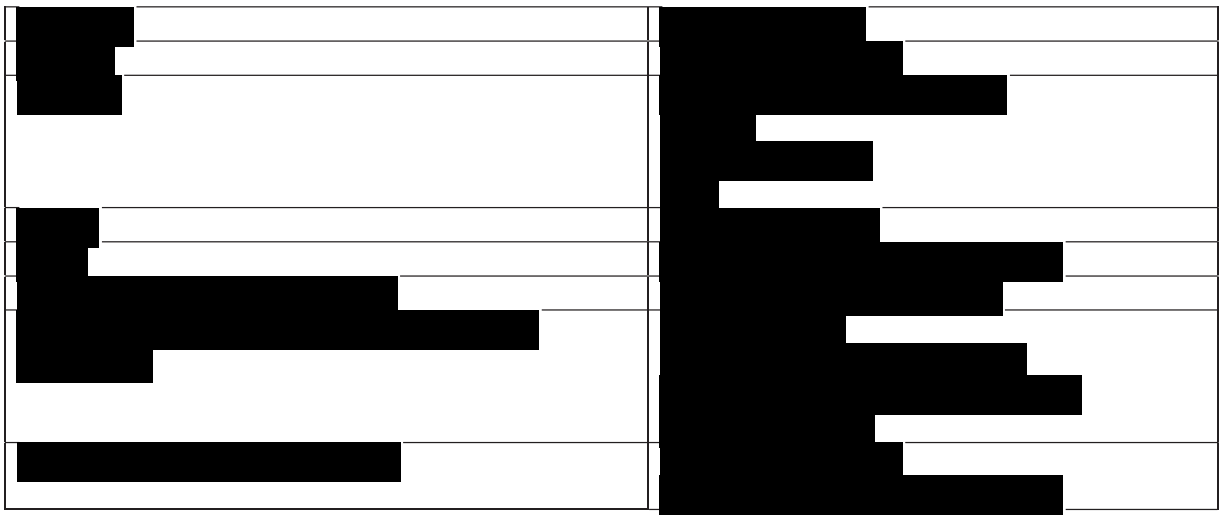
I - GENERAL REQUIREMENTS

1 Applicant Details

(addressing section 3.1.1.B of the FSANZ Application Handbook)



2 Purpose of the application

(addressing section 3.1.1.C of the FSANZ Application Handbook)

This application requests amendment to the Australia New Zealand Food Standards Code (**the Code**) to permit the use of nicotinamide riboside chloride as a permitted form of Vitamin B3 in foods for special medical purposes (hereafter referred to as **FSMPs**). ChromaDex manufactures nicotinamide riboside chloride under the brand name NIAGEN® (hereafter referred to as **NR**).

NIAGEN® is an innovative chemical substance that is a precursor for nicotinamide adenine dinucleotide (NAD+) in the human body, with fewer adverse effects or identified safety issues than is the case for other established and/or permitted NAD+ delivery vehicles. Evidence suggests that the use of NIAGEN® in FSMPs has a particularly strong potential as a method of delivering/increasing the anabolism of NAD+ to support human wellness during metabolic stress and aging.

Section 2.9.5—6 of the Code permits the addition of the following substances to food for special medical purposes (**FSMPs**):

(1)(a) a substance that is listed in Column 1 of the table to section S29—20 and that is in a corresponding form listed in Column 2 of that table;
(b) a substance that is listed in Column 1 of the table to section S29—7 and that is in a corresponding form listed in Column 2 of that table;
(c) any other substance, regardless of its form, that is permitted under this Code to be added to a food, if that substance is added in accordance with any applicable requirement of this Code.

Vitamin B3 is permitted to be added to FSMPs by sections S29—20 (in the form of nicotinic acid) and S29—7 (in the form of niacinamide (also known as nicotinamide)). The Code also

permits the addition of Vitamin B3 to certain foods other than FSMPs in the form of both nicotinic acid and nicotinamide.

Nicotinamide riboside chloride is **not** currently expressly listed in the Code as a permitted form of Vitamin B3. We note that Section 2.9.5-6(c) of the Code states that the following may be added to an FSMP:

*(c) any other substance, **regardless of its form,** that is permitted under this Code to be added to a food, if that substance is added in accordance with any applicable requirement of this Code.*

It is possible to interpret this section to read that *any* form of Vitamin B3 (including forms like NR that are not expressly listed in the Food Standards Code) can be added to a food for special medical purposes. Following this interpretation, it could be strongly argued that NR is permitted to be added to FSMPs without any need to amend the Food Standards Code. This position would align with the purpose of Standard 2.9.5, which stands apart from the rest of the Code from a regulatory perspective. Standard 2.9.5 was designed and introduced so as to facilitate the importation and domestic manufacture of products which nutritionally address a medical need and to remove regulatory barriers to consumer access. This purpose is reflected in the fact that for years food regulatory authorities permitted products to be on the Australian market if they could demonstrate compliance with Standard 2.9.5 when it was still in draft form, before it was gazetted.

Notwithstanding the above, the applicant wishes to not rely on a regulatory interpretation and instead pursue scientific review by FSANZ through the Application process to not only demonstrate the consumer need, efficacy and safety of NR but to also achieve regulatory certainty before entering the Australian market.

As a result, the Applicant is seeking amendment to the Code to list NR as a permitted form of Vitamin B3 for the selective purpose of adding NR to FSMPs.

Section S29—7 permits the use of Vitamin B3 (in the form of niacinamide) in infant formula products and food for infants in addition to FSMPs. On the other hand, Section S29—20 permits the use of Vitamin B3 (in the form of nicotinic acid) in FSMPs only. NR is intended to be consumed by adults and is not intended for use in infant formula or foods for infants. As such, ChromaDex considers amendment to section S29—20 to include NR as a permitted form of Vitamin B3 in FSMP is appropriate for the intended use. This application does **not** impact on the levels for Vitamin B3 set in Section 2.9.5—7.

NR is intended to be used as a source of Vitamin B3 in FSMPs that partially or totally replace the daily diet; products that are recommended to be used under medical supervision. It is therefore possible that NR will be added to FSMPs that are a sole source of nutrition. Section 2.9.5—7 includes compositional requirements for an FSMP that is represented as being suitable for use as a sole source of nutrition. In relation to Vitamin B3, such a product is required to contain the minimum amount specified in section S29—21; however, no maximum level for Vitamin B3 is listed. As you will read below, NR is a more effective form of Vitamin B3 while presenting less adverse effects and this Application is strictly limited to amending Schedule 29 and has no impact on Section 2.9.5-7.

3 Justification for the application

(addressing section 3.1.1.D of the FSANZ Application Handbook)

NR is safe for human consumption and provides a safer and more nutritionally efficient alternative source of Vitamin B3 to the currently permitted forms listed in the Code.
The Code does not currently expressly permit the use of NR as a permitted form of Vitamin B3 in FSMPs. Therefore, as outlined above, the Applicants seeks amendment to the Code to ensure regulatory certainty and regulator confidence in the addition of NR to FSMPs.

3.1 Regulatory impact information

3.1.1 Costs and benefits of the application

a) Consumers

Consumers of FMSP products containing NR will benefit from NR's properties as a permitted form of Vitamin B3. NR is a very effective form of Vitamin B3 that can deliver the beneficial effects of Vitamin B3 without potential adverse effects (such as flushing from use of nicotinic acid; an already permitted form of Vitamin B3).

b) Industry

Manufacturers of FSMPs that supply the Australian and New Zealand market can benefit from the availability of an alternative form of Vitamin B3 that has been the subject of numerous studies, including human clinical trials, that demonstrate NRs safety and efficacy (in relation to performing its function as a form of Vitamin B3).

c) Government

There should be minimal impact on government from the approval of NR as a permitted form of Vitamin B3 in FSMPs. FSMP is a category of foods that are intended to be used under medical supervision. NR has been demonstrated to be safe and an effective form of Vitamin B3. There is minimal reason for enforcement agencies to be concerned about the presence of NR in FSMP if NR is permitted to be added as a permitted form of Vitamin B3.

3.1.2 Impact on international trade

It is anticipated that this Application would have a net benefit on international trade as it would allow FSMPs containing NR to be sold in Australia. This would effectively allow such products to be accessed from international markets and facilitate trade. It might also encourage the development of Australian products containing NR which could be exported into other markets. As the market for FSMPs is considerably smaller than the market for foods in general, we do not expect the overall impact of this Application on international trade to be of a high magnitude.

4 Information to support the application

The application contains supporting information in accordance with the Application Handbook's requirements in Guideline 3.3.3 – Substances used for a nutritive purpose. The Application Handbook requirements in Guideline 3.3.3 are focussed on requests for the approval of a new nutritive substance or a change in the permissions for the use of a nutritive substance. This

application is requesting the addition of a new permitted form of Vitamin B3, rather than requesting approval of a new nutritive substance or an extension of use of a permitted nutritive substance. Therefore, some elements of Guideline 3.3.3 may not be applicable for this application. Any such instances are clearly highlighted and justified in sections A to G below.

5 Assessment procedure

(addressing section 3.1.1.F of the FSANZ Application Handbook)

The application is limited to requesting approval of NR a new permitted form of Vitamin B3 in FSMPs. The narrow focus of the application should see it assessed by FSANZ under the general procedure.

6 Confidential commercial information (CCI)

(addressing section 3.1.1.G of the FSANZ Application Handbook)

The application contains CCI as summarised in the table below. The detailed CCI has been provided separately to FSANZ and summarised in a general nature throughout the relevant sections of this Application. The information marked as CCI is proprietary to ChromaDex and is not in the public domain. The public release of this information would adversely affect ChromaDex's commercial interests.

Information requested to be considered as confidential	**Justification**
Annex 1	Proprietary manufacturing process information and/or information not in the public domain
Annex 2	Proprietary information not in the public domain
Annex 3	Proprietary information not in the public domain
Annex 4	Proprietary information not in the public domain

7 Other confidential information

(addressing section 3.1.1.H of the FSANZ Application Handbook)

The application does not contain any other confidential information that is not CCI.

8 Exclusive capturable commercial benefit (ECCB)

(addressing section 3.1.1.I of the FSANZ Application Handbook)

If approved, the application is expected to confer an ECCB on ChromaDex. NIAGEN® is a proprietary substance owned by ChromaDex. As such, ChromaDex expects to have exclusive control over the distribution of NR, the subject of this application, in Australia.

9 International and other national standards

(addressing section 3.1.1.J of the FSANZ Application Handbook)

9.1 International standards

Codex Alimentarius has established standards relating to FSMPs and products that may be subject to requirements for FSMPs, such as foods for use in weight control diets (CXS 181-1991) and very low energy diets for weight reduction (CXS 203-1995). These standards set compositional requirements for nutrients, including Vitamin B3, but do not specify permitted forms for these nutrients. Additionally, Codex standards relating to the labelling and claims for FSMPs (CXS 180-1991) and pre-packaged foods for special dietary uses (CXS 146-1985) do not specify compositional requirements for these foods. Therefore, amending the Code to add NR as a permitted form of Vitamin B3 to FSMP is unlikely to adversely impact on Codex standards or on countries that rely on these standards. The above Codex standards can be accessed via the list of Codex standards on the Codex Alimentarius website.[1].

9.2 Other national standards or regulations

9.2.1 Australia and New Zealand

NR has recently been granted permission to be used as an active ingredient in complementary medicines in Australia[2]. The Therapeutic Goods (Permissible Ingredients) Determination was updated in November 2019 to add NR. NR is permitted to be added to be added to complementary medicines at up to 300 mg/day.

In late 2019, NR was submitted for assessment by the Advisory Committee on Novel Foods (hereafter referred to as the **ACNF**). As reflected in the Record of Views, the ACNF – as a committee representational of the Australian and New Zealand food regulators – believes that NR is a nutritive substance and a form of Vitamin B3.

9.2.2 United States

NIAGEN® is Generally Recognized as Safe (GRAS) in the United States for use **in food products** (GRN 635 August 3, 2016) and the subject of two new dietary ingredient notifications (NDIN) (NDIN 882; NDIN 1062), which were filed with the United States Food and Drug Administration without objection. Copies of the GRAS Notification and NDIN no objection letters are provided in Annex 3.

GRN 635 determined the GRAS status of NIAGEN® added to selected foods and beverages to provide a source of Vitamin B3, particularly in vitamin waters, protein shakes, nutrition bars, gum and chews. The intended maximum use level is 0.027% by weight and will be in powdered beverages designed to be reconstituted with water or milk. The intended maximum use level in all other foods will be 0.0057% by weight. The mean intakes of NIAGEN® by all users aged 2+ years from all proposed food uses were estimated to be 51 mg/person/day or 0.8 mg/kg body weight/day. The heavy consumer (90th percentile all-user) intake of NIAGEN® from all proposed food-uses in persons aged 2+ years was estimated to be 145 mg/person/day.

[1] http://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/
[2] https://www.tga.gov.au/update-listed-medicine-ingredients-november-2019

NDIN 882 was filed without objection for the use NIAGEN® as a sole active ingredient in a dietary supplement capsule formulation. Each capsule (one serving) contains NIAGEN® at a level of not more than 180 mg. Consumers are recommended to take not more than one capsule or one serving a day for up to 90 days. This provides a daily intake of NIAGEN® of not more than 180 mg.

NDIN 1062 was subsequently filed without objection to increase the recommended intake of NIAGEN® as a sole active ingredient in a dietary supplement capsule formulation from 180 mg to not more than 300 mg/day.

ChromaDex has self-determined that NR is GRAS in uses up to 1,000mg/day. This self-determination was included as an addendum to the abovementioned GRAS notification in May 2019. The addendum itself is CCI but is included in Annex 3 to this Application.

9.2.3 European Union

ChromaDex Inc. was granted novel food approval for NR in January 2020. NR is permitted to be sold in food supplement capsules at up to 300 mg/day. The authorization for placing NR on the market by the European Commission in the Official Journal of the European Union is provided in Annex 3.

9.2.4 Canada

NR is listed on the Natural Health Products Ingredients Database (under nicotinamide riboside chloride) as a chemical substance in the following products:

- Natural Health Product (NHP) License for TRU NIAGEN finished product on October 30, 2018 (Natural Product Number 80088977 – Dose: 125 mg capsules, up to 375 mg/day);

- Natural Health Product (NHP) License for TRU NIAGEN finished product on May 29, 2019 (Natural Product Number 80092547 – Dose: 150 mg capsules, up to 900 mg/day); and

- Natural Health Product (NHP) License for TRU NIAGEN finished product on May 28, 2019 (Natural Product Number 80092534 – Dose: 300 mg capsules, up to 900 mg/day)

The product licenses issued by Health Canada are provided in Annex 3.

10 Statutory Declaration

Statutory Declaration – Australia

The information provided in Parts 1 to 3 must be attested to by a statutory declaration in some suitable form along the following lines:

STATUTORY DECLARATION

Statutory Declarations Act 1959 [1]

[redacted] Global Scientific & Regulatory Affairs, *located at 10900 Wilshire Blvd., Suite 600, Los Angeles, California* make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory [redacted] ction 11 of the *Statutory Declarations Act 1959*, [redacted] claration are true in every particular.

[redacted] 9-12-2020

A notary public or other officer completing this certificate verifies only the identity of the individual who signed the document to which this certificate is attached, and not the truthfulness, accuracy, or validity of that document.

State of California
County of Los Angeles

Subscribed and sworn to (or affirmed) before me on this 12th day of September 2020 by Andrew Shao, proved to me on the basis of satisfactory evidence to be the person who appeared before me.

[redacted]

(Seal)

PETER G HAROUN
COMM. #2208121
NOTARY PUBLIC - CALIFORNIA
LOS ANGELES COUNTY
My Commission Expires 07/30/2021

[1] http://www.comlaw.gov.au/Series/C1959A00052.
[2] A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*. The list of prescribed persons is available in the Statutory Declarations Regulations 1993 at http://www.comlaw.gov.au/Series/F1996B00198.

II - SUBSTANCES USED FOR A NUTRITIVE PURPOSE

A Information on the use of the nutritive substance

(addressing section 3.3.3.A of the FSANZ Application Handbook)

The information provided in support of this application relates to the use of NR as a permitted form of Vitamin B3. Vitamin B3 is already permitted by the Code to be added to a variety of foods, including FSMP.

A.1 Information on the purpose of the use of a nutritive substance in food

(addressing section 3.3.3.A.1 of the FSANZ Application Handbook)

NR is intended to be added to FSMPs as a form of Vitamin B3 and, as such, would perform an equivalent nutritional function to any other form of Vitamin B3 that is used in FSMPs. Safety data provided as part of this Application indicates that NR achieves the same nutritional benefits as other forms of Vitamin B3 in a more effective manner. This data demonstrates that NR is safe for human consumption and that NR consumption does not inhibit the absorption or modify the bioavailability of other nutrients.

A CCI summary of clinical results is included in Annex 1.

A.2 General data requirements for supporting evidence

(addressing section 3.3.3.A.2 of the FSANZ Application Handbook)

ChromaDex's NIAGEN® (NR) has predominately been used as the test material in studies provided as evidence of the safety and effectiveness (as a form of Vitamin B3) and is the commercial product for which approval is sought.

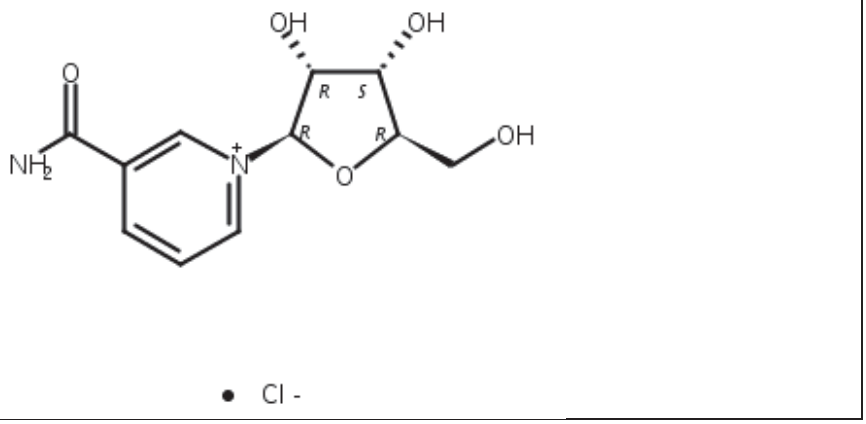
B Technical information on the use of the nutritive substance

(addressing section 3.3.3.B of the FSANZ Application Handbook)

B.1 Information to enable identification of the nutritive substance

(addressing section 3.3.3.B.1 of the FSANZ Application Handbook)

Table B.1-1 Identity of nicotinamide riboside chloride

Chemical name (IUPAC)	3-(Aminocarbonyl)-1-β-D-ribofuranosyl-pyridinium chloride (1:1)
CAS registry number	23111-00-4
Synonyms	Pyridinium, 3-(aminocarbonyl)-1-β-D-ribofuranosyl-, chloride (9Cl) Pyridinium, 3-carbamoyl-1-β-D-ribofuranosyl-, chloride (8Cl)
Molecular formula	$C_{11}H_{15}N_2O_5 \cdot Cl$
Molecular weight	290.7
Structural formula	

B.2 Information on the chemical and physical properties of the nutritive substance

(addressing section 3.3.3.B.2 of the FSANZ Application Handbook)

As NIAGEN® is yet to be incorporated into food products and the final formulation of foods containing NIAGEN® has not been determined, there is limited stability data with respect to the specific presence of NIAGEN® in food matrices. As a matter of course, all future food applications of NIAGEN® will be subject to rigorous stability testing. Additionally, we do maintain extensive stability data regarding the use of NIAGEN® in dietary/food supplement products. Although these products are not regarded as foods in Australia or New Zealand, the application of NIAGEN® in these products is comparable to its use in certain foods, and particularly in FSMPs which are the subject of this Application. As such, we believe stability data regarding the use of NIAGEN® in dietary/food supplements is highly relevant to its stability in these food matrices.

In particular, NIAGEN® and TRU NIAGEN® are part of an extensive stability program which covers the ingredient and all product formulations sold globally. The stability observed for NIAGEN® and TRU NIAGEN® (see report SS-2007-003-R-00, Annex 4) supports the inclusion for NIAGEN® in food products. The active ingredient NIAGEN®, at both real time and intermediate conditions, has demonstrated exceptional stability. The TRU NIAGEN® 300 mg, 60

count product is an encapsulated powder formulation with various components (see Annex 2, TN Product Composition_300mg). This dietary supplement product has been observed to meet specifications through 12-18 months at real time conditions thus far. Previous TRU NIAGEN® product formulations, ranging from 100 mg to 150 mg per capsule, have been observed to remain stable for 24+ months at real time conditions. We expect these and future formulations to be the same.

ChromaDex understands the mechanism of how NIAGEN® degrades over time. The major component that leads to the hydrolysis of NIAGEN® is water. In all product formulations, ChromaDex closely monitors the water content to ensure it does not exert a major influence in stability. Further, the degradation components of NIAGEN® are nicotinamide and ribose; both of which are not considered a safety concern. Although not required, it is also expected that refrigeration of raw ingredients and/or the final product would extend the shelf life of foods containing NIAGEN®.

Currently, NIAGEN® is considered Generally Recognized as Safe (GRAS) for inclusion in food products in the US and is authorized as a novel food by the European Commission in the Official Journal of the European Union. Given the stability for the various formulations presented and that any new food supplement and/or food product formulation will be part of the stability program, ChromaDex believes NIAGEN® would demonstrate comparable stability in food matrices such as powdered FSMP products.

In terms of particle size, NIAGEN®is not a nano-scale particle, and the size of the particle does not have an impact on its nutritional purpose. NIAGEN® is readily soluble in water regardless of the particle size. We therefore do not foresee particle size as creating any safety risk.

A CCI Stability Report is included in Annex 4.

B.3 Information on the impurity profile

(addressing section 3.3.3.B.3 of the FSANZ Application Handbook)

The chemical reactions that occur during manufacture means that there is potential for theoretical impurities to arise. However, the production process for NR has been optimised to maximise the purity of the finished product and the likelihood of impurities ending up in the finished product is remote.

A detailed discussion of theoretical impurities is CCI as it contains proprietary information about ChromaDex's manufacturing process. This information is included in Annex 1.

B.4 Manufacturing process

(addressing section 3.3.3.B.4 of the FSANZ Application Handbook)

A detailed CCI description of the manufacturing process for NR is provided in Annex 1. A general description of the manufacturing process is provided below.

Nicotinamide riboside chloride, NIAGEN®, is produced in a US facility that complies with cGMP for foods. It is synthesized in a two-step process. First, the starting material of D-ribofuranose tetra-acetate is reacted with hydrochloric acid to generate the D-ribofuranose triacetate chloride. After the charge, the solution is sampled for reaction completion by quantifying the amount of D-

ribofuranose triacetate chloride via NMR. When the reaction is complete, nicotinamide is charged to the mixture. The mixture is then stirred until the reaction to nicotinamide ribofuranose triacetate chloride is complete (Figure 1). The filter-cake is then analysed for nicotinamide-β-ribofuranose triacetate and held for use in Step 2 of the reaction.

In the second step, nicotinamide-β-ribofuranose triacetate chloride is deacetylated and washed to yield nicotinamide-β-riboside chloride, NIAGEN® (Figure 2).

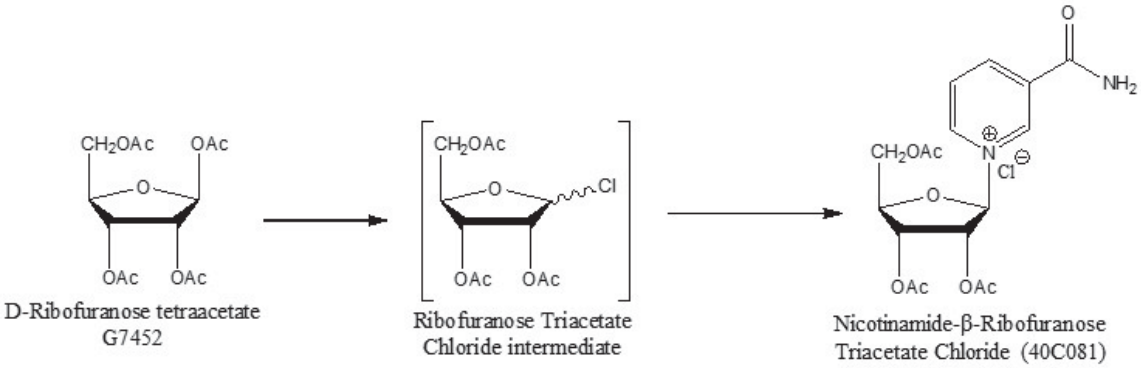


Figure 1. Step 1 Chemical Reactions

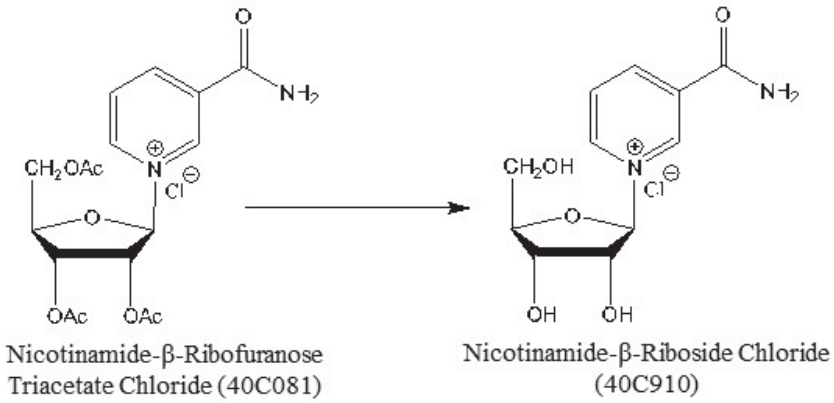


Figure 2. Step 2 Chemical Reaction

B.5 Specification for identity and purity

(addressing section 3.3.3.B.5 of the FSANZ Application Handbook)

The published sources identified in Schedule 3 – Identity and purity of the Code do not currently include a specification for NR. Prior to releasing each lot of NIAGEN® for subsequent use, a variety of physical, chemical, and microbiological tests are conducted to evaluate the quality of the finished product. Each lot must adhere to the specifications listed in Table B.5-1. ChromDex-developed methods listed in Table B.5.1 can be found in Annex 4.

Table B.5-1 Specifications for NR

Parameter	Specifications	Method
Color	White to Light Brown	Visual
Form	Powder	Visual
Identification	Conforms by NMR	0.700.12.4[%]
Nicotinamide Riboside Chloride	NLT 90 wt% NMT 103 wt%	0.700.10.2.METH3[**]
Water Content	NMT 2.0%	0.700.12.37[**]
Residual Solvents		
Acetone	NMT 5000 ppm	USP <467>[***]
Methanol	NMT 1000 ppm	USP <467>[***]
Acetonitrile	NMT 50 ppm	USP <467>[***]
Methyl Tert-Butyl Ether	NMT 500 ppm	USP <467>[***]
Reaction By-Products		
Methyl Acetate	NMT 1000 ppm	USP <467>[***]
Acetamide	NMT 27 ppm[*]	99-1-04-7.0-000616[**], [***]
Acetic Acid	NMT 5000 ppm	99.1-04-2.0-000665[**], [***]
Microbiological Limits		
Total Plate Count	NMT 1000 CFU/g	AOAC or equivalent[&]
Yeast and Mold	NMT 100 CFU/g	AOAC or equivalent[&]
Escherichia coli	Absent/10g	AOAC or equivalent[&]
Heavy Metals		
Arsenic	NMT 1ppm	USP <232>, <233>, <2232>[***]
Mercury	NMT 1ppm	USP <232>, <233>, <2232>[***]
Cadmium	NMT 1ppm	USP <232>, <233>, <2232>[***]
Lead	NMT 0.5 ppm	USP <232>, <233>, <2232>[***]

CFU – colony forming units; NLT – not less than; NMT – not more than; ppm - parts per million; USP = United States Pharmacopeia; AOAC = Association of Analytical Communities; CFU = colony forming units

[*]Based on California Proposition 65 (NSRL 10 µg/day)

[**]In-house validated analytical methods. All in-house analytical method and analytical method validation reports are provided in Annex 4. For the nicotinamide riboside chloride, nicotinamide, nicotinamide riboside monoacetate chloride, and furfural method and method validation reports see Meth-0.700.10.2.METH3, Val-CDXA-MVP-047-01, Val-CDXA-SV-0094D-01. For the acetamide method and method validation reports see Meth-99.1-04-7.0-000616 and Val-CDXA-MVP-054-00. For the water method see Meth-0.700.12.37. For acetic acid method and method validation reports see Meth-99.1-04-2.0-000665 and Val-CDXA-SV-0105B-00.

[***]Determined by Covance Laboratories. See Annex 2, Accred-Covance Laboratories-ISO17025 for the certificate of accreditation.

[&]Determined by Certified Laboratories. See Annex 2, Accred-Certified Laboratories (now Eurofins) -ISO17025 for the certificate of accreditation.

[%]NMR performed by Valparaiso University; analysis performed by ChromaDex. For method see Annex 4, Meth-0.700.12.4.pdf.

Detailed CCI reasoning for the parameters identified above is included in Annex 1.

B.6 Analytical method for detection

(addressing section 3.3.3.B.6 of the FSANZ Application Handbook)

ChromaDex has developed and validated a HPLC method for detection of NR (see Annex 4 0.700.10.2.METH3). This method may, however, be able to detect nicotinamide and nicotinic acid. Methods for detecting Vitamin B3 in foods exist.

B.7 Information on the proposed food label

(addressing section 3.3.3.B.7 of the FSANZ Application Handbook)

NR is intended to be added to FSMP as a permitted form of Vitamin B3. The focus of marketing and/or label statements for products will be on the nutritional profile and benefits of the overall product and/or components, such as vitamins. The focus will not be on permitted forms of vitamins. Therefore, there should be minimal impact on labelling statements for FSMP products containing NR as a permitted form of Vitamin B3.

C Information related to the safety of the nutritive substance

(addressing section 3.3.3.C of the FSANZ Application Handbook)

C.1 Information on the toxicokinetics and metabolism of the nutritive substance and, if necessary, its degradation products and major metabolites

(addressing section 3.3.3.C.1 of the FSANZ Application Handbook)

The safety of NR with respect to toxicokinetics and metabolism has been validated by the following studies:

- In vitro study evaluating the metabolism of nicotinamide riboside in blood, finding that NR is rapidly metabolized to nicotinamide in a cellular component of whole blood;
- 90-day sub-chronic toxicity study which included a toxicokinetic study of nicotinamide riboside metabolism, indicating that NR and nicotinamide were effectively metabolized within the 24-hr sampling period;
- Animal-based toxicity study, finding no adverse changes at various NR dosages;
- Animal-based study, finding that NR was rapidly absorbed and metabolized;
- Single-dose pharmacokinetic study in healthy adults, indicating that NR is metabolized in a manner similar to nicotinamide;
- In vitro hERG screening assay, finding that none of the NR concentrations tested affected hERG tail current density;
- 8-week repeat-dose study in healthy adults, which tested for urinary metabolites and metabolites in blood.

The specific content of the studies referred to above is CCI and is included in Annex 1. These studies reinforce that NR is safe and comparable to other forms of Vitamin B3.

C.2 Information from studies in animals or humans that is relevant to the toxicity of the nutritive substance and, if necessary, its degradation products and major metabolites

(addressing section 3.3.3.C.2 of the FSANZ Application Handbook)

C.2.1 Toxicological information

The toxicology package for NIAGEN® was developed to identify endpoints of toxicity and support the safety of long-term exposure in free living individuals. The toxicology studies that support the use of NIAGEN® include a bacterial reverse mutagenicity study (Study No. S15004), an in vitro chromosomal aberration assay (Study No. S15005), an in vivo micronucleus test (Study No. S15006), hERG screening assay (Study No. 20151223), an acute toxicology study in rats (Study No. S13101), a 14-day dose range-finder toxicology study in rats (Study No. S13120), animal-based toxicity studies, a 90-day repeated dose toxicity study in rats (Study No. 14022), a rat developmental toxicity study (Study No. G10957), and a one generation reproduction study in rats (Study No. G10959). The study reports for all these studies are provided in Annex 4. Additionally, the results from the bacterial reverse mutagenicity study, in vitro chromosomal aberration assay, in vivo micronucleus test an acute toxicology study in rats, a 14-day dose range-finder toxicology study in rats, and the 90-day repeated dose toxicity study in rats have also been published by Conze et al. (2016).

C.2.1.1 Genotoxicity

a. Bacterial Reverse Mutagenicity (Ames assay)

i. Methods

Bacterial reverse mutation assays were performed in compliance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practices (GLP) and Guideline No. 471. The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-Lot 40C910-15208-21). 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine, and 4-nitroquinoline-*N*-oxide were obtained from Sigma Aldrich Chemical Co., Inc. Aroclor 1254-induced rat liver S9 homogenate was obtained from Xenometrix AG. *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 were obtained from the National Collection of Type Cultures. *Escherichia coli* WP2 uvrA (pKM101) was obtained from Xenometrix. The study report, which contains the raw data, is provided in Annex 4 (see Study No. S15004). The study was also published by Conze et al. (2016).

The mutagenicity of NIAGEN® was determined using the plate incorporation and preincubation methods. In the plate incorporation method, 50, 159, 501, 1582, and 5000 µg NIAGEN® was mixed with selective top agar containing 0.6-0.8% agar, 0.5% NaCl, the tester strains *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *Escherichia coli* WP2 *uvr*A pKM101, histidine and biotin or tryptophan (depending on the type stain used), with and without of a metabolic activation system (S9 mix; 5-30% of the Aroclor 1254-induced rat liver S9 homogenate, NADP, glucose-6-phosphate, magnesium chloride, and potassium chloride) at 45-50°C. The mixture was overlaid onto solidified Vogel-Bonner minimal E basal agar (Vogel and Bonner, 1956), and after the selective top agar solidified, the plates were incubated at 37°C for 67 hr. The plates were examined for the presence of a background lawn and precipitate, and the number of revertant colonies were counted manually.

In a confirmatory assay, the tester strains *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *Escherichia coli* WP2 *uvr*A pKM101, and 99, 265, 699, 1869, or 5000 µg NIAGEN® were preincubated at 37°C for 30 min in the presence or absence of the S9 mix. Molten selective top agar containing 0.6-0.8% agar, 0.5% NaCl, and histidine and biotin or tryptophan (depending on the type stain used) was then added and the resulting mixture was plated and incubated at 37°C for 67 hr. The plates were examined for the presence of a background lawn and precipitate, and the number of revertant colonies were counted manually. Importantly, all experiments were performed in triplicate with vehicle and strain-specific positive controls. In the presence of the S9 mix, the positive controls for all strains was 2-aminoanthracene. In the absence of the S9 mix, the positive controls for strains TA98, TA100 and TA1535, TA1537, and WP2 *uvr*A pKM101 were 2-nitrofluorene, sodium azide, 9-aminoacridine, and 4-nitroquinoline-*N*-oxide, respectively.

NIAGEN® was considered cytotoxic if there was a 50% reduction in the mean number of revertants per plate compared to the mean vehicle control and/or at least a moderate reduction in the background lawn. NIAGEN® was considered mutagenic if there was a concentration-related increase in the number of revertants per plate in at least one tester strain over a minimum of two increasing concentrations of NIAGEN®. In the case of the strains TA98, TA100, and WP2 *uvr*A pKM101 the result was considered positive if the mean number of revertants was equal to or greater than two times the number of revertants obtained with the negative control. In the case of the strains TA1535 and TA1537 the result was considered positive if the mean number of revertants was equal to or greater than three times the number of revertants obtained with the negative control.

ii. Results

NIAGEN® was not cytotoxic at any of the doses used in this study (data not shown), and compared to the vehicle control, did not increase the number of revertant colonies in any of the frameshift or base-pair tester strains either when incubated in the presence or absence of the S9 mix, or using the plate incorporation (Table C.2.1.1-1) or preincubation methods (Table C.2.1.1-2). In contrast, all positive controls (2-Aminoanthracene 2-Nitrofluorene, 9-Aminoacridine, Sodium Azide, 4-Nitroquinoline-*N*-oxide) caused significant increases in the number of revertant colonies ($p < 0.05$), demonstrating both the sensitivity and validity of the assay. Therefore, NIAGEN® was not mutagenic under the conditions used in the studies.

Table C.2.1.1-1. Ames Assay Results, Plate Incorporation Method							
			Mean Colonies/Plate				
			Frameshift types		Base-pair types		Frameshift and base-pair types
	Test Item	Test Concentrations (µg/plate)	TA98	TA1537	TA1535	WP2uvrA pKM101	TA100
With S9 Mix	Water	-	22 ± 3	8 ± 2	10 ± 3	149 ± 3	101 ± 1
	NIAGEN®	50	27 ± 6	8 ± 2	12 ± 3	153 ± 3	108 ± 6
		159	23 ± 4	11 ± 3	8 ± 2	152 ± 3	103 ± 3
		501	22 ± 5	8 ± 3	10 ± 2	142 ± 4	104 ± 3
		1582	26 ± 1	9 ± 3	12 ± 2	144 ± 8	103 ± 1
		5000	24 ± 4	10 ± 8	11 ± 6	144 ± 6	104 ± 2
	2-Aminoanthracene	4	597 ± 14	83 ± 3	108 ± 4	-	883 ± 12
	2-Aminoanthracene	30	-	-	-	736 ± 6	-
Without S9 Mix	Water	-	21 ± 4	8 ± 4	12 ± 4	119 ± 6	104 ± 2
	NIAGEN®	50	27 ± 4	9 ± 2	14 ± 5	121 ± 16	106 ± 3
		159	23 ± 6	9 ± 5	14 ± 3	116 ± 6	108 ± 5
		501	27 ± 4	7 ± 2	17 ± 5	111 ± 10	111 ± 6
		1582	26 ± 3	7 ± 4	18 ± 7	122 ± 14	121 ± 15
		5000	27 ± 4	8 ± 3	18 ± 4	122 ± 17	121 ± 3
	2-Nitrofluorene	2	228 ± 10	-	-	-	-
	9-Aminoacridine	4	-	82 ± 3	-	-	-
	Sodium Azide	1	-	-	124 ± 3	-	526 ± 7
	4-Nitroquinoline-*N*-oxide	4	-	-	-	621 ± 3	-
"-" denotes not tested Values are mean +/- standard deviation. See Annex 4, Study No. S15004 for study report and raw data.							

Table C.2.1.1-2. Ames Assay Results, Preincubation Method							
			Mean Colonies/Plate				
			Frameshift types		Base-pair types		Frameshift and base-pair types
	Test Item	Test Concentrations (µg/plate)	TA98	TA1537	TA1535	WP2uvrA pKM101	TA100
With S9 Mix	Water	-	22 ± 6	8 ± 1	10 ± 4	105 ± 6	108 ± 5
	NIAGEN®	99	21 ± 5	6 ± 1	9 ± 1	96 ± 11	104 ± 6
		265	22 ± 4	6 ± 1	8 ± 1	107 ± 16	103 ± 6
		699	26 ± 5	9 ± 2	14 ± 1	86 ± 17	117 ± 4
		1869	22 ± 3	8 ± 2	15 ± 4	110 ± 10	105 ± 13
		5000	26 ± 6	5 ± 1	9 ± 1	99 ± 2	112 ± 4
	2-Aminoanthracene	4	571 ± 6	90 ± 7	101 ± 5	-	919 ± 8
	2-Aminoanthracene	30	-	-	-	562 ± 18	-
Without S9 Mix	Water	-	20 ± 8	9 ± 1	14 ± 2	96 ± 4	104 ± 6
	NIAGEN®	99	16 ± 1	9 ± 4	12 ± 2	98 ± 7	103 ± 9
		265	18 ± 5	8 ± 1	11 ± 2	95 ± 4	109 ± 3
		699	19 ± 4	8 ± 4	11 ± 3	91 ± 4	103 ± 8
		1869	17 ± 3	6 ± 4	9 ± 1	92 ± 7	113 ± 7
		5000	22 ± 2	6 ± 3	9 ± 1	94 ± 7	120 ± 4
	2-Nitrofluorene	2	216 ± 4	-	-	-	-
	9-Aminoacridine	4	-	92 ± 4	-	-	-
	Sodium Azide	1	-	-	126 ± 7	-	419 ± 9
	4-Nitroquinoline-*N*-oxide	4	-	-	-	505 ± 16	-
"-" denotes not tested Values are mean +/- standard deviation. See Annex 4, Study No. S15004 for study report and raw data.							

b. In vitro chromosomal aberration assay

i. Methods

In vitro chromosomal aberration assays were performed in compliance with the OECD Principles of GLP and Guideline No. 473. The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-Lot 40C910-14207-21). Cyclophosphamide monohydrate and ethyl methanesulphonate were obtained from Sigma Aldrich Chemical Co., Inc. Aroclor 1254-induced rat liver S9 homogenate was obtained from Xenometrix AG. Human peripheral blood lymphocytes (PBLs) were obtained from whole blood harvested from a healthy donor who was approximately 35 years old, had no history of smoking or alcoholism, and had not received medication one month prior to the blood draw. The whole blood was cultured in RPMI 1640 medium containing 10% fetal bovine serum, heparin, EDTA, amphotericin, penicillin, streptomycin, and phytohaemagglutinin (PHA) at 37°C and 5% CO_2 for 3 days per OECD Guideline 473 and ICH-harmonized guidances on genotoxicity testing of pharmaceuticals. The study report, which contains the raw data, is provided in Annex 4 (see Study No. S15005). The study was also published by Conze et al. (2016).

To determine the clastogenic activity of NIAGEN®, the PHA-stimulated whole blood cultures were centrifuged and the resulting PBLs were resuspended in RPMI 1640 containing 10% fetal bovine serum, amphotericin, penicillin, streptomycin, either vehicle (water), 1.25, 2.5, or 5 mg/ml of NIAGEN®, or the appropriate positive control (cyclophosphamide monohydrate and ethyl methanesulphonate), supplemented with either phosphate buffered saline or the metabolic activation system (S9 mix; 10% of the Aroclor 1254-induced rat liver S9 homogenate, 4mM NADP, 5 mM glucose-6-phosphate, 8 mM magnesium chloride, and 33 mM potassium chloride). The mixtures were then incubated at 37°C and 5% CO_2 for 3 and 19 hours, at which point colchicine was added to the cultures to a final concentration of 2 µg/ml. Three hours later, the cells were harvested by centrifugation at 800 to 1000 rpm for approximately 10 minutes, resuspended in 0.56 % pre-warmed potassium chloride, and incubated at room temperature for 25 to 30 minutes. The cell suspension was then centrifuged 800 to 1000 rpm for approximately 10 minutes, the resulting supernatant was discarded, and the cellular pellet was resuspended and incubated in cold fixative (acetic acid: methanol (1:3)) at room temperature for 10 to 15 minutes. This process was repeated 3 additional times with one incubation at 4°C for a minimum of 1 hour followed by two incubations at room temperature for 10 to 15 minutes.

After the final incubation, the cells suspension was dropped onto clean, cold slides, which were then gently dried over a flame, stained with 5% Giemsa, and scored for the presence of metaphase cells and the presence of aberrations. To determine the mitotic index, which was used as an indicator of cytotoxicity, a minimum of 1000 cells were scored for each group and the total number of metaphases was divided by the number of cells counted. The quotient was then multiplied by 100. To determine the types of aberrations (chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks, deletions, and fragments) a minimum of 300 metaphases containing 46 +/- 2 centromere regions were counted and the number of cells containing one or more different types of aberrations were recorded. The data was the subjected to a one-tailed Fisher Exact test. NIAGEN® was considered cytotoxic if there was a 45+/- 5% reduction in the mitotic index compared to the vehicle control. NIAGEN® was considered mutagenic if there was a concentration-related and statistically significant increase ($p<0.05$) in the number of chromosome aberrations.

ii. Results

NIAGEN® was not cytotoxic to ex vivo human peripheral blood lymphocytes at any of the concentrations used in the study as determined by the mitotic index (data not shown), and, compared to the vehicle control, did not increase the number of aberrant metaphases when incubated with or without S9 mix for 6 hours (Table C.2.1.1-3). Moreover, the types of aberrations (chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks) detected in the vehicle- and NIAGEN®-treated cells were similar. In contrast, the positive controls, cyclophosphamide and ethyl methansulphonate, significantly increased the number of aberrant metaphases ($p<0.05$), characterized as chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks, deletions, and fragments, thus confirming the sensitivity and validity of the assay. Similar results were also found when the lymphocytes were incubated with increasing amounts of NIAGEN® for 22 hours in the absence of the S9 mix (data not shown). NIAGEN® was therefore not clastogenic under the conditions used in the study.

Table C.2.1.1-3. Summary of Results – *In vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

	Dose Level per mL of Test Medium				
	Vehicle Control[a]	NIAGEN® 1.25 mg	NIAGEN® 2.5 mg	NIAGEN® 5 mg	Positive Control[b]
Without S9					
Total number of metaphases read	350	300	300	300	300
Number of aberrant metaphases					
Including gaps	5	3	1	2	30*
Excluding gaps	4	1	0	1	28*
With S9					
Total number of metaphases read	300	300	300	300	300
Number of aberrant metaphases					
Including gaps	1	3	3	5	25*
Excluding gaps	0	2	1	2	25*

[a]: Vehicle control= 150 µl sterile water
[b]: Positive controls= 650 µl ethyl methanesulphonate (EMS) in the absence of metabolic activation (without S9); or 55 µg cyclophosphamide in the presence of metabolic activation (with S9). See Annex 4, Study No. S15005 for study report and raw data.

c. In vivo micronucleus test

i. Methods

The in vivo micronucleus assay performed in compliance with the OECD Principles of GLP, OECD Guideline No. 474, and the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCESA), Government of India. The rats were obtained from Harlan Laboratories. Nutrilab Rodent Pellet feed was obtained from Provimi Animal Nutrition. Cyclophosphamide was obtained from Sigma Aldrich

Chemical Co., Inc. The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-Lot 40C910-14207-21). The study report, which contains the raw data, is provided in Annex 4 (see Study No. S15006). The study was also published by Conze et al. (2016).

All rats were housed at three rats per sex per cage, acclimatized for at least 5 days prior to treatment, and, except for the overnight fast prior to euthanasia on day 91, provided feed and water *ad libitum* throughout the study. Prior to dosing the rats were randomized by body weight to two groups (n=6/sex/group). At dosing, a single dose of vehicle (water), 500, 1000, and 2000 mg/kg of NIAGEN® or 40 mg/kg cyclophosphamide was administered by gavage at a rate of 10 ml/kg body weight. Twenty-four hours after dosing the vehicle, 500 mg/kg NIAGEN®-, 1000 mg/kg NIAGEN®-, 2000 mg/kg NIAGEN®-, and 40 mg/kg cyclophosphamide-treated groups were euthanized by carbon dioxide asphyxiation. Forty-eight hours after dosing, an additional vehicle and 2000 mg/kg NIAGEN®-treated group was also euthanized by carbon dioxide asphyxiation. The animals were observed for mortality at 1 and 2 hours and then twice daily after dosing for 2 days. Clinical signs were monitored 1 and 2 hours after dosing and then once daily for 2 days.

Immediately after euthanisation, bone marrow was harvested from the femurs of each animal, and centrifuged. The centrifuged cell suspension was smeared on 2 slides, which were then air-dried, fixed in methanol, and stained using a May-Gruenwald and Giemsa solution. The test item was considered toxic if polychromatic erythrocyte/total erythrocyte ratio was less than that what was observed in vehicle control group. NIAGEN® was considered mutagenic if at least one of the treatment groups exhibited a statistically significant ($p<0.05$) increase in the frequency of micronucleated immature erythrocytes when compared with the concurrent vehicle control.

ii. Results

No mortalities or clinical signs of toxicity were observed in any of the rats receiving NIAGEN®. In addition, bone marrow analyses showed that compared to the negative control, the administration of 500, 1000, and 2000 mg/kg of did not result in cytotoxicity or increase the percentage of polychromatic erythrocytes at either 24 or 48 hours of administration (Table C.2.1.1-4). In contrast, the positive control cyclophosphamide induced a statistically significant ($p < 0.05$) increase in the percentage of polychromatic erythrocytes at 24 hours demonstrating both the sensitivity and validity of the assay. Therefore, NIAGEN® was not genotoxic under the conditions used in this study.

Table C.2.1.1-4. Summary of Results - *In vivo* Micronucleus test						
	Dose (mg/kg bw)					
Parameters	Vehicle Control 0	NIAGEN® 500	NIAGEN® 1000	NIAGEN® 2000 (a)	NIAGEN® 2000 (b)	Positive Control 40
Males						
Sampling time (hours)	24	24	24	24	48	24
PCE's with micronuclei (%)	0.00	0.00	0.00	0.00	0.00	0.28*
Range	0-1	0	0-1	0	0	8-15
Mean P/E ratio	0.34	0.37	0.39	0.38	0.39	0.40
Females						
Sampling time (hours)	24	24	24	24	48	24
PCE's with micronuclei (%)	0.00	0.00	0.00	0.00	0.00	0.27*
Range	0	0	0	0	0	7-13
Mean P/E ratio	0.40	0.43	0.44	0.44	0.41	0.40
mg/kg b. wt.: milligram/kilogram body weight, Positive Control: Cyclophosphamide, % - per cent, PCE – Polychromatic erythrocytes, P/E: Polychromatic erythrocytes/ Total Erythrocytes * = P value Significant. See Annex 4, Study No. S15006 for the study report and raw data.						

C.2.3 Sub-chronic toxicity

a. Acute toxicity

i. Acute Oral Toxicity Study in Rats

An OECD- and GLP-compliant acute toxicity study was carried out on male and female rats (n = 5/sex/group) given a single dose of either vehicle (water) or 5000 mg/kg NIAGEN® via gavage. The animals were then observed and examined for 15 days. No mortalities, clinical signs, or gross pathological lesions were observed in any of the rats. Also, NIAGEN® did not significantly affect feed consumption in either sex. Cumulative body weight gain (days 1-15) was significantly lower in female rats given NIAGEN® compared to control group (Figure 13). Since change in day 15 body weight was minimal (-3%), the change was considered to be treatment-related but non-adverse. The summary table of treatment-related, dose-respondent, statistically significant observations noted in this study is provided in Table C.2.3-1. The study report, which contains the raw data, is provided in Annex 4 (see Study No. S13101). The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-LOT 00014315-192.) The study was also published by Conze et al. (2016).

Table C.2.3-1. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in Single Dose Oral Toxicity Study of Niagen in Sprague Dawley Rats

Conze, 2016, Safety Assessment of Nicotinamide Riboside, a Form of Vitamin B3

Annex 4 (Study No. S13101)

2.10.3 Sub-chronic toxicity

Parameters	Exposure	Sex	Niagen Dose group	
			Control	5000 mg/kg
Body weight gain (g)	1-15 days	M	103.76 ± 11.09	98.06 ± 5.38
		F	52.78 ± 5.94	45.85 ± 3.06*

*Significantly lower than control group (p-value < 0.05; student t-test)

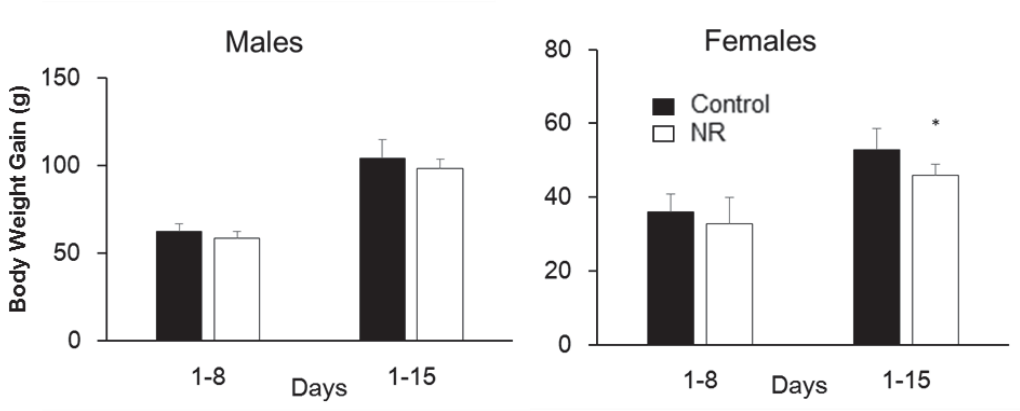


Figure 15. Body Weight Gain in Rats Administered a Single Dose of NIAGEN®
After a single oral dose (5000 mg/kg bw) of NIAGEN®, control and NIAGEN-treated male and female rats were monitored for clinical signs of toxicity including body weights for 15 days. A small, but significant reduction ($p < 0.05$), in cumulative body weight was observed in female rats at 15 days. See Annex 4, Study No. S13101 for study report and raw data.

ii. 14-day Repeat-Dose Range-Finding Oral Toxicity Study in Rats

In a 14-day range-finder toxicology study, rats (n = 5/sex/group) were gavaged daily with either vehicle (water) or 750, 1500, 2500, or 5000 mg/kg/day of NIAGEN®. Animals were monitored during the 14 days of treatment and euthanized on day 15 for gross pathological examination. A minimal reduction in mean body weight was observed in male rats in the 2500 mg/kg/day NIAGEN® (7-8% reduction) and 5000 mg/kg/day NIAGEN® (8-9% reduction) groups compared to the vehicle-treated group, on days 11, 14 and 15 (Figure 14). A test article-related decrease in overall (Day 1-14) feed consumption was observed at 5000 mg/kg/day (8%) in male rats (data not shown). No test item-related changes were observed in body weight and feed consumption in female rats. No gross pathological lesions were observed in male or female rats. Body weight reduction of approximately 10% was correlated with a feed intake reduction at 5000 mg/kg/day in males; therefore, this dose level was considered to be too high for further study. Based on the body weight reduction of 7-8% at 2500 mg/kg/day in the 14-day study, the doses of 300, 1000 and 3000 mg/kg/day were chosen for the 90-day sub-chronic toxicity study in rats.

The summary table of treatment-related, dose-respondent, statistically significant observations noted in this study is provided in Table C.2.3-2. The study report, which contains the raw data, is provided in Annex 4 (see Study No. S13120). The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-Lot 00014315-192). The study was also published by Conze et al. (2016).

Table C.2.3-2. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in 14-Day Dose Range Finding Oral Toxicity Study in Sprague Dawley Rats							
Conze, 2016, Safety Assessment of Nicotinamide Riboside, a Form of Vitamin B3							
Annex 4 (Study No. S13120)							
2.10.3 Sub-chronic toxicity							
			Niagen - Dose group				
Parameters	**Exposure[a]**	**Sex**	**0 mg/kg**	**750 mg/kg**	**1500 mg/kg**	**2500 mg/kg**	**5000 mg/kg**
Body weight (g)	Day 15	M	253.87 ± 10.41	246.74 ± 13.71	240.07 ± 7.63	233.42 ± 10.04*	229.94 ± 7.72*
		F	155.25 ± 3.22	158.83 ± 5.81	154.86 ± 7.41	165.65 ± 15.19	158.95 ± 10.24
Body weight gain (g)	Days 1-15	M	87.51 ± 6.51	81.07 ± 12.74	74.06 ± 8.18	65.86 ± 9.20*	63.08 ± 4.86*
		F	32.25 ± 6.12	34.49 ± 5.09	30.95 ± 2.87	42.11 ± 10.27	35.22 ± 4.16
* Significantly lower than control group ($p < 0.05$; Dunnett's test) [a] Day 15 data - treated for 14 days and euthanized on day 15							

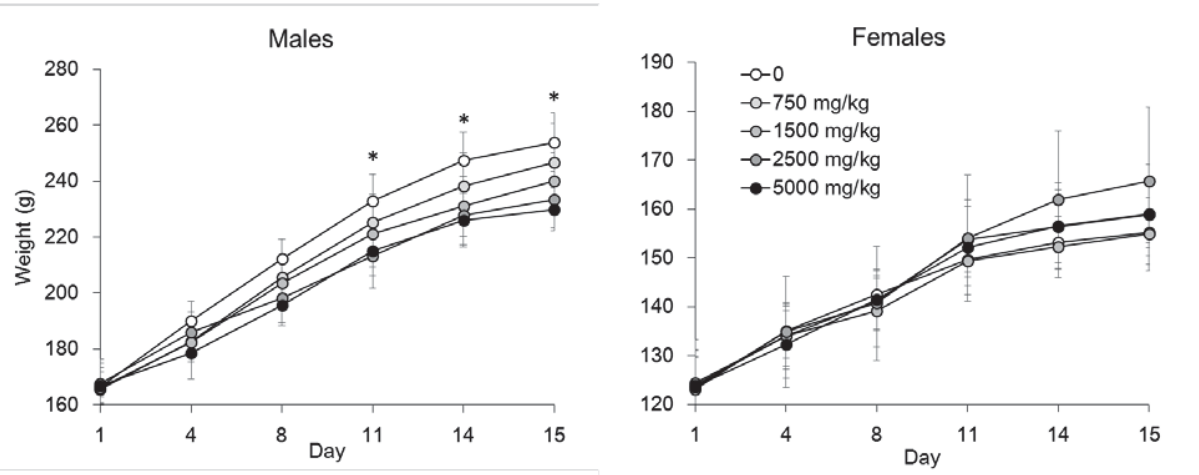


Figure 16. Body Weight Reduction in Rats Administered NIAGEN® for 14 Days
Rats were administered various doses of NIAGEN® daily over 14 days. Weights were monitored every few days over the course of the study. See Annex 4, Study No. S13120 for the study report. The graphs were obtained from Conze et al. (2016).

iii. Animal-based Toxicity Study with NIAGEN®

This study involved the administration of various doses of NIAGEN® to animal subjects up to 1,000 mg/kg/day. No abnormal clinical findings were observed at the dose levels tested.

Further CCI detail in relation to this study is included in Annex 1.

iv. Animal-based Toxicity Study

This study involved oral administration of NIAGEN® to animal subjects at various doses up to 1,000 mg/kg/day.

Further CCI detail in relation to this study is included in Annex 1.

b. 90-day Repeat-Dose Oral Toxicity Study in Rats

i. Methods

A 90-day oral sub-chronic toxicity study in Sprague Dawley rats was conducted in compliance with the OECD Principles of GLP, the OECD Guideline 408 for testing of chemicals, and the recommendations of the AAALAC and CPCESA, Government of India. The rats were obtained from Harlan Laboratories. Nutrilab Rodent Pellet feed was obtained from Provimi Animal Nutrition. Nicotinamide (≥ 99.5%; CAS No. 98-92-0) was obtained from Sigma Aldrich. The summary table of treatment-related, dose-respondent, statistically significant observations noted in this study is provided in Table C.2.3-7. The study report, which contains the raw data, is provided in Annex 4 (see Study No. S14022). The CoA for the lot of NIAGEN® used in this study is provided in Annex 2 (see CoA-Lot 40C910-13202-96). The study was also published by Conze et al. (2016).

All rats were housed two to three rats per sex per cage, acclimatized for at least 5 days prior to treatment, and, except for the overnight fast prior to euthanasia on day 91, provided feed and water *ad libitum* throughout the study. Prior to dosing, the rats were randomized by body weight to 5 groups (n=10/sex/group). During the 90-day treatment period, each group was gavaged daily with either vehicle (water), 300, 1000, 3000 mg/kg of NIAGEN® or 1260 mg/kg/day of nicotinamide, which is equivalent to 3000 mg/kg/day of NIAGEN® on a molar basis. Importantly, studies have shown that NR is metabolized similarly as nicotinamide, producing NAD+ and nicotinamide metabolites. Thus, a positive control group treated with nicotinamide was also included.

Dose formulation analyses showed that both NIAGEN® and nicotinamide were completely soluble in water and the dose formulations contained the targeted concentrations of nicotinamide riboside or nicotinamide. Stability analyses showed that when NIAGEN® and nicotinamide were dissolved in water, both nicotinamide riboside and nicotinamide were stable up to 6 hours at room temperature and 7 days at 2-8 °C. The parameters evaluated during the study were twice daily checks for mortality, daily evaluations for clinical signs, weekly detailed clinical examinations, and weekly body weight and food consumption measurements. Ophthalmological examinations were performed prior to treatment and prior to sacrifice. On day 91, after urine was collected individually from all animals after an overnight fast, the animals were anesthetized, and blood was collected from the sublingual vein for hematology, coagulation, and clinical chemistry evaluations. Then the animals were euthanized by exsanguination under deep anesthesia and subjected to necropsy and gross pathological examination. Hematological parameters included differential leukocyte count (DLC), reticulocyte, leukocyte, erythrocyte, eosinophil, neutrophil, lymphocyte platelets, basophils, monocytes, and large unstained cell (LUC) counts, hemoglobin (Hgb), hematocrit, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean hemoglobin concentration (MCHC), prothrombin time (PT), and activated partial thromboplastin time (APTT

Table C.2.3-7. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in 90-day Repeat-Dose Oral Toxicity Study in Sprague Dawley Rats							
Conze, 2016, Safety Assessment of Nicotinamide Riboside, a Form of Vitamin B3							
Annex 4 (Study No. S14022)							
2.10.3 Sub-chronic toxicity							
			Dose group				
Parameters	**Exposure (days)**	**Sex[2]**	**0 mg/kg/day**	**1260 (nicotinamide)[1] mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
Body weight (g)	1	M	121.44 ± 8.26	121.41 ± 9.04	121.60 ± 7.49	121.41 ± 8.24	122.76 ± 9.14
Body weight (g)	8	M	168.83 ± 9.92	150.67 ± 9.30*	162.97 ± 10.20	162.88 ± 10.71	154.23 ± 15.47*
Body weight (g)	15	M	208.96 ± 8.54	189.20 ± 9.79*	200.60 ± 11.15	202.04 ± 12.16	189.26 ± 11.37*
Body weight (g)	22	M	248.34 ± 9.13	219.35 ± 10.10*	238.32 ± 9.57	237.92 ± 14.70	219.89 ± 14.40*
Body weight (g)	29	M	281.84 ± 13.43	253.92 ± 16.35*	265.01 ± 9.88*	268.53 ± 16.73	248.97 ± 15.59*
Body weight (g)	36	M	307.92 ± 11.16	265.18 ± 18.90*	284.14 ± 11.46*	291.46 ± 16.33	266.54 ± 18.50*
Body weight (g)	43	M	332.45 ± 11.34	281.40 ± 20.99*	304.12 ± 12.69	311.90 ± 17.46	281.85 ± 18.99*
Body weight (g)	50	M	346.12 ± 11.74	292.25 ± 19.48*	317.06 ±1 3.51*	322.49 ± 19.51*	293.15 ± 20.87*
Body weight (g)	57	M	365.44 ± 13.33	303.01 ± 21.31*	330.90 ± 13.63*	327.53 ± 19.73*	303.83 ± 21.91*
Body weight (g)	64	M	373.09 ± 14.04	311.17 ± 23.52*	337.57 ± 14.43*	342.35 ± 22.71*	313.96 ± 21.24*
Body weight (g)	71	M	384.00 ± 14.96	313.23 ± 23.96*	347.01 ± 14.20*	348.62 ± 21.48*	322.20 ± 23.85*
Body weight (g)	78	M	394.21 ± 15.38	319.54 ± 24.38*	361.37 ± 18.68*	357.91 ± 22.70*	328.82 ± 22.79*
Body weight (g)	85	M	403.38 ± 17.38	326.84 ± 24.97*	372.51 ± 22.66	366.58 ± 23.10	336.97 ± 23.55*
Body weight (g)	90	M	413.46 ± 19.92	338.13 ± 29.62*	378.97 ± 22.97	376.20 ± 22.47	342.47 ± 23.60*
Body weight gain (g)	1-90	M	292.02 ± 18.91	216.72 ± 27.34*	257.37 ± 25.29*	254.79 ± 18.95*	219.71 ± 25.14*
* Significantly lower than control group ($p < 0.05$, Dunnett's test) [1]Nicotinamide dose, 1260 mg/kg/day, is equivalent to 3000 mg/kg/day of Niagen on equimolar basis [2]No statistically significant body weight change in females							

Table C.2.3-7. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in 90-day Repeat-Dose Oral Toxicity Study in Sprague Dawley Rats							
			Dose group				
Parameters	**Exposure (days)**	**Sex**	**0 mg/kg/day**	**1260 (nicotinamide) mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
Feed consumption (g/rat/day)	1-90	M	20.51 ± 0.70	18.67 ± 1.16*	19.22 ± 0.95	19.83 ± 0.91	18.36 ± 0.64*
* Significantly lower than control group (p < 0.05, Dunnett's test)							
			Dose group				
Parameters	**Exposure (days)**	**Sex**	**0 mg/kg/day**	**1260 (nicotinamide) mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
Urine pH	90	M	7.6 ± 0.64	6.8 ± 0.35	8.0 ± 0.53	6.9 ± 0.39	6.1 ± 0.16*
		F	7.2 ± 0.53	6.7 ± 0.54	7.1 ± 0.28	6.9 ± 0.47	6.3 ± 0.35*
* Significantly lower than control group (p < 0.05, Dunnett's test)							
Clinical Chemistries							
			Dose group				
Parameters	**Exposure (days)**	**Sex**	**0 mg/kg/day**	**1260 (nicotinamide) mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
ALT (U/L)	90	M	85.23 ± 26.11	152.11 ± 25.50*	75.94 ± 12.20	106.17 ± 45.34	159.35 ± 27.56*
		F	56.09 ± 15.54	125.22 ± 30.29*	56.34 ± 10.47	76.50 ± 20.42*	121.81 ± 22.35*
AST (U/L)	90	M	122.18 ± 27.34	139.47 ± 26.99	116.10 ± 13.94	138.23 ± 49.03	132.93 ± 23.63
		F	101.02 ± 18.67	126.31 ± 16.24*	106.1 ± 18.52	126.69 ± 35.51	126.27 ± 33.09
ALP (U/L)	90	M	99.85 ± 22.69	139.48 ± 24.88*	120.94 ± 16.68	110.96 ± 18.67	131.63 ± 19.73*
		F	60.79 ± 5.16	114.41 ± 30.91*	69.66 ± 11.23	85.96 ± 22.06	105.10 ± 26.99*
GGTP (U/L)	90	M	3.09 ± 0.90	3.81 ± 1.16	4.13 ± 1.22	3.47 ± 1.20	3.82 ± 1.22
		F	3.15 ± 1.17	5.46 ± 1.75*	3.9 ± 0.92	3.83 ± 1.42	5.01 ± 1.36*
Trig (mg/dL)	90	M	49.84 ± 18.96	98.57 ± 34.72*	59.30 ± 29.48	69.65 ± 29.91	128.34 ± 47.88*
		F	28.38 ± 6.9	87.00 ± 38.48*	28.24 ± 7.92	46.69 ± 12.97*	61.85 ± 23.31*
Sodium (mmol/L)	90	M	139.15 ± 3.37	135.88 ± 1.9	141.03 ± 6.05	137.99 ± 3.52	137.1 ± 4.7
		F	137.71 ± 1.28	135.83 ± 1.17*	137.86 ± 1.25	136.68 ± 1.46*	134.42 ± 1.22*

Table C.2.3-7. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in 90-day Repeat-Dose Oral Toxicity Study in Sprague Dawley Rats							
Chloride (mmol/L)	90	M	101.55 ± 2.66	97.52 ± 1.25*	102.13 ± 4.41	99.21 ± 1.3	97.34 ± 3.24*
		F	102.12 ± 0.96	98.93 ± 1.33*	101.43 ± 1.13	100.69 ± 1.52	98.12 ± 1.63*
*Significantly higher / lower than the control group ($p < 0.05$, Dunnett's test)							
Relative organ/body weight							
Parameters	**Exposure (days)**	**Sex**	**Dose group**				
			0 mg/kg/day	**1260 (nicotinamide) mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
Terminal Body Weight (g)	90	M	395.7 ± 18.36	312.87 ± 26.74 *	363.56 ± 23.22 *	354.23 ± 21.85 *	317.21 ± 25.8 *
		F	232.29 ± 8.1	215.61 ± 11.16 *	234.43 ± 23.28	219.51 ± 9.92	216.19 ± 14.75
Liver	90	M	2.958 ± 0.143	3.703 ± 0.116 *	3.013 ± 0.163	3.200 ± 0.18 *	3.600 ± 0.272 *
		F	2.902 ± 0.191	4.465 ± 0.239 *	3.003 ± 0.327	3.295 ± 0.181*	4.046 ± 0.174 *
Kidneys	90	M	0.715 ± 0.047	0.833 ± 0.086 *	0.701 ± 0.033	0.777 ± 0.02 *	0.876 ± 0.063 *
		F	0.676 ± 0.053	0.766 ± 0.019 *	0.645 ± 0.06	0.678 ± 0.058	0.822 ± 0.044 *
Brain	90	M	0.507 ± 0.027	0.583 ± 0.043 *	0.526 ± 0.038	0.550 ± 0.024 *	0.572 ± 0.042
		F	0.78 ± 0.034	0.781 ± 0.031	0.788 ± 0.055	0.801 ± 0.047	0.798 ± 0.041
Heart	90	M	0.368 ± 0.019	0.387 ± 0.015	0.356 ± 0.018	0.373 ± 0.015	0.384 ± 0.02
		F	0.392 ± 0.016	0.424 ± 0.029*	0.393 ± 0.015	0.398 ± 0.022	0.433 ± 0.032 *
Thymus	90	M	0.068 ± 0.011	0.068 ± 0.008	0.076 ± 0.015	0.086 ± 0.014 *	0.07 ± 0.009
		F	0.09 ± 0.011	0.084 ± 0.017	0.097 ± 0.024	0.093 ± 0.013	0.087 ± 0.014
Adrenals	90	M	0.012 ± 0.001	0.015 ± 0.001 *	0.013 ± 0.001	0.014 ± 0.001 *	0.014 ± 0.001 *
		F	0.027 ± 0.003	0.027 ± 0.003	0.028 ± 0.003	0.027 ± 0.002	0.029 ± 0.004
Ovaries	90	F	0.036 ± 0.005	0.049 ± 0.007 *	0.037 ± 0.005	0.036 ± 0.006	0.045 ± 0.008 *
* Significantly higher / lower than the control group ($p < 0.05$, Dunnett's test)							

Table C.2.3-7. Summary Table of Treatment-Related, Dose-Respondent, Statistically Significant Observations in 90-day Repeat-Dose Oral Toxicity Study in Sprague Dawley Rats							
Histopathological findings							
			Incidence by group				
Observations	**Exposure (days)**	**Sex**	**0 mg/kg/day**	**1260 (nicotinamide) mg/kg/day**	**300 (Niagen) mg/kg/day**	**1000 (Niagen) mg/kg/day**	**3000 (Niagen) mg/kg/day**
Liver, Number examined	90	M/F	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular		M	0	8	0	0	9
		F	0	10	0	0	10
Necrosis, single cell hepatocytes		M	0	0	0	0	0
		F	0	3	0	0	3
Testes, Number examined	90	M	10	10	10	10	10
Degeneration/atrophy, tubular		M	0	10	0	0	8
Epididmyides, Number examined	90	M	10	10	10	10	10
Reduced sperm luminal		M	0	10	0	0	7
Cell debris, luminal		M	0	10	0	0	9
Ovaries, Number examined	90	F	10	10	10	10	10
Hypertrophy, corpora lutea		F	0	6	0	0	4
Adrenals, Number Examined	90	M/F	10	10	10	10	10
Hypertrophy, cortical, zona glomerul glomerulosa		M	0	9	0	0	9
		F	0	6	0	0	4

Plasma clinical chemistry parameters included total protein, albumin, bile acids, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGTP), globulin, alkaline phosphatase (ALP), total cholesterol (TC), triglycerides, glucose, blood urea nitrogen (BUN), creatinine, inorganic phosphorous (Pi), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and chloride (Cl) levels. The organs that were collected, weighed, and preserved included the adrenals, aorta, bone marrow smear, brain including medulla/pons, cerebellum and cerebrum, caecum, colon, duodenum, epididymides, esophagus, eyes with optic nerve, biceps femoris muscles, femur bone with joint gross lesions, heart, ileum with Peyer's patches, jejunum, kidneys, liver, lungs with main bronchi and bronchioles, mandibular lymph nodes, mesenteric lymph nodes, mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, seminal vesicles and coagulating glands, rectum, salivary glands (mandibular, parotid and sublingual), sciatic nerve, skin (inguinal region), spinal cord at 3 levels - cervical, mid-thoracic and lumbar, spleen, sternum with marrow, stomach, testes, thymus, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus with cervix, and vagina. The preserved tissues were processed and embedded in paraffin, sectioned and stained with Haematoxylin and Eosin.

All samples were fixed in 10% neutral buffer formalin and stained with hematoxylin-eosin. The eye, optic nerve, and Harderian gland were pre-fixed in a 2.5% glutaraldehyde solution, and the nasal cavity, testis, and epididymis were pre-fixed in Bouin's solution.

All quantitative variables such as body weight, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, organ weights and organ weight ratios were tested for normality and homogeneity of variance within the group before performing a one-factor ANOVA. In cases, wherein, the data was found to be non-optimal (non-normal or heteroschedastic), ANOVA was done using a log transformation. Comparison of means between treatment groups and control group was done using a Dunnett's test. Even after transformation, when normality/homogeneity tests were significant, data was subjected to a Kruskal-Wallis test followed by a Dunn's test. All analyses and comparisons were evaluated at the 5% level.

ii. Results

a) Mortality, body weight and feed consumption

No treatment-related mortality or clinical signs were observed at any dose level in this study. Compared to vehicle-treated controls, a significant ($p < 0.05$) treatment-related decrease in body weight (17% reduction) was noted in male rats at the high dose (3000 mg/kg/day) of NIAGEN®; a similar decrease in body weight was observed at an equimolar dose (1260 mg/kg/day) of nicotinamide (Figure 18). Significant ($p < 0.05$) 8-9% reductions in body weight (<10%) were observed at the 300 and 1000 mg/kg/day dose of NIAGEN® in male rats. This decrease was <10% and therefore not considered to be adverse. No statistically significant differences in body weights were seen in NIAGEN®- or nicotinamide-treated females. In male rats, decreases in feed consumption were noted at 3000 mg/kg/day of NIAGEN® (9-14%) and 1260 mg/kg/day of nicotinamide (9-17%) throughout the treatment period. Decreases in feed consumption also occurred at 300 mg/kg/day on days 57-64 and at 1000 mg/kg/day on days 50-57. In female rats, decreases in feed consumption occurred at days 1-8 in the nicotinamide treated group and at days 15-22 in the 3000 mg/kg/day NIAGEN® group.

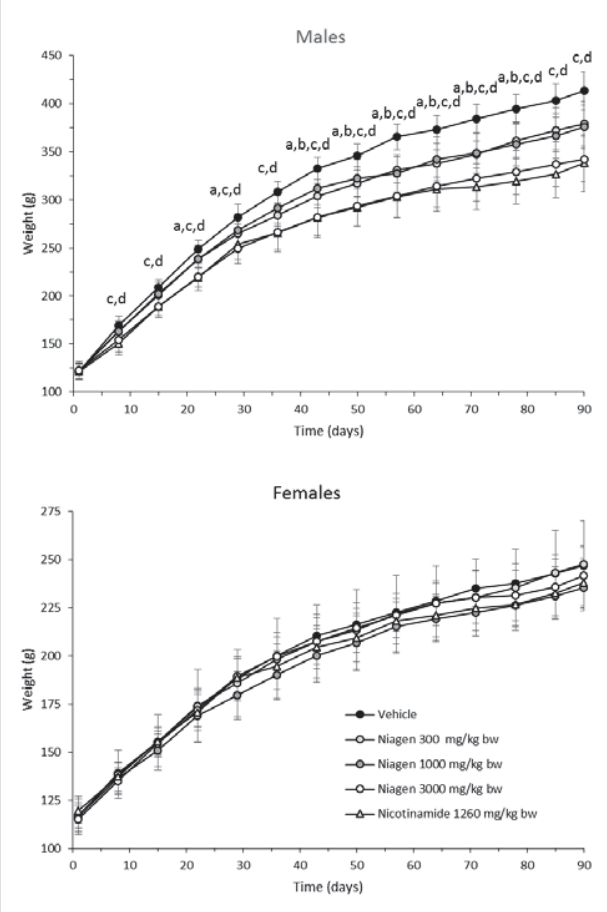


Figure 19. Body Weights of Male and Female Rats Treated with Vehicle Control, Nicotinamide Riboside or Nicotinamide For 90-Days

"a", "b", "c", and "d" denote significant ($p<0.05$) differences between rats treated with 300, 1000, and 3000 mg/kg/day of NIAGEN®, or 1260 mg/kg/day of nicotinamide, respectively, and rats treated with the vehicle control. The graphs were obtained from Conze et al. (2016). The study report and raw data are provided in Annex 4 (see Study No. S14022).

b) Hematological, clinical chemistry and urinalysis tests

Similar treatment-related changes in hematology parameters were observed at the high dose NIAGEN®- and nicotinamide-treated groups (Table C.2.3-8). Statistically significant ($p < 0.05$) treatment related increases in white blood cells (WBC) and neutrophils occurred in both males and females. Statistically significant ($p<0.05$) increases in monocytes were also noted in females treated with 3000 mg/kg/day of NIAGEN® and 1260 mg/kg/day of nicotinamide. At 1000 mg/kg/day of NIAGEN®, significant increases in WBC and neutrophils were observed in males and females, respectively. There were no significant changes in hematological parameters at male or female rats treated with 300 mg/kg/d of NIAGEN®. Importantly, the significant effects were not associated with any inflammatory changes in any of the organs examined. All other changes in hematology parameters, including those determined to be statistically significant, were considered to be due to normal biological variation, and not due to the administration of the test item.

NIAGEN® produced statistically significant ($p < 0.05$) increases at 3000 mg/kg/day in ALT, ALP, GGTP, triglycerides and bile acids; the effects on ALT and triglycerides were significant at 1000 mg/kg/day of NIAGEN® in females (Table C.2.3-9). Comparable effects on clinical chemistries were seen in the nicotinamide-treated group (Table C.2.3-9). A minimal but statistically significant decrease in sodium in females and chloride in males and females was noted at 3000 mg/kg/day of NIAGEN®. Similar results were seen in the nicotinamide-treated group. Significant reductions ($p < 0.05$) in sodium were also observed in males and females treated with 1000 mg/kg/day. All other changes in clinical chemistry parameters were considered incidental as the magnitude of change minimal and were considered to be due to normal biological variation.

Urinalysis showed increased urine volume in both nicotinamide and high dose NIAGEN®-treated males (data not shown). This effect was considered a treatment-related effect, which may have been correlated with microscopic changes in the adrenals. Urine pH was decreased in males (7.6 ± 0.64 in controls vs. 6.11 ± 0.16 in high dose) and females (7.2 ± 0.53 in controls vs. $6.3 \pm 6.3 \pm 0.35$ in high dose) at 3000 mg/kg/day of NIAGEN® but not the males and females treated with nicotinamide. The slightly acidic pH may have been due to the excretion of test item and an acidic metabolite.

Table C.2.3-8. Summary of Significant Changes-Hematological Parameters Day 91**					
	Doses (mg/kg/day)				
Parameters	Vehicle 0	NIAGEN® 300	NIAGEN® 1000	NIAGEN® 3000	Nicotinamide 1260^
Males					
White Blood Cells	8.47±1.97	9.16±1.54	11.11 ±1.77*	13.51± 2.64*	14.11±1.66*
Total Neutrophils	2.12±0.7	2.30±1.67	2.86±1.20	5.76± 1.47*	6.02±0.7*
Total Monocytes	0.28 ±0.09	0.33±0.30	0.40±0.19	0.42±0.09	0.45±0.12
Females					
White Blood Cells	6.25±1.20	6.23 ±1.84	7.40±1.01	10.38± 1.51*	9.99±2.64*
Total Neutrophils	0.93±0.31	1.11±0.57	1.63±0.29*	3.36± 1.19*	4.13±1.56*
Total Monocytes	0.16 ±0.07	0.15±0.05	0.15±0.04	0.32±0.08*	0.33±0.21*
Values represented as Mean ± SD *Significantly higher than the vehicle control group at $p < 0.05$. ^: equimolar ratio to NIAGEN® 3000 mg/kg/d **Data was obtained from Conze et al. (2016). The study report and raw data are provided in Annex 4 (see Study No. S14022).					

Table C.2.3-9. Summary of Significant Changes-Clinical Chemistries Day 91**					
	Doses (mg/kg/day)				
Parameters	Vehicle 0	NIAGEN® 300	NIAGEN® 1000	NIAGEN® 3000	Nicotinamide 1260^
Males					
ALT (U/L)	85.23 ± 26.11	75.94 ± 12.20	106.17 ± 45.34	159.35 ± 27.56*	152.11 ± 25.50*
AST (U/L)	122.18 ± 27.34	116.10 ± 13.94	138.23 ± 49.03	132.93 ± 23.63	139.47 ± 26.99
ALP (U/L)	99.85 ± 22.69	120.94 ± 16.68	110.96 ± 18.67	131.63 ± 19.73*	139.48 ± 24.88*
GGTP (U/L)	3.09 ± 0.90	4.13 ± 1.22	3.47 ± 1.20	3.82 ± 1.22	3.81 ± 1.16
Trig (mg/dL)	49.84 ± 18.96	59.30 ± 29.48	69.65 ± 29.91	128.34 ± 47.88*	98.57 ± 34.72*
Sodium (mmol/L)	139.15 ± 3.37	141.03 ± 6.05	137.99 ± 3.52	137.1 ± 4.7	135.88 ± 1.9
Chloride (mmol/L)	101.55 ± 2.66	102.13 ± 4.41	99.21 ± 1.3	97.34 ± 3.24*	97.52 ± 1.25*
Females					
ALT (U/L)	56.09 ± 15.54	56.34 ± 10.47	76.50 ± 20.42*	121.8 ± 22.35*	125.22 ± 30.29*
AST (U/L)	101.02 ± 18.67	106.1 ± 18.52	126.69 ± 35.51	126.27± 33.09	126.31 ± 16.24*
ALP (U/L)	60.79 ± 5.16	69.66 ± 11.23	85.96 ± 22.06	105.1 ± 26.99*	114.41 ± 30.91*
GGT (U/L)	3.15 ± 1.17	3.9 ± 0.92	3.83 ± 1.42	5.01 ± 1.36*	5.46 ± 1.75*
Trig (mg/dL)	28.38 ± 6.9	28.24 ± 7.92	46.69 ± 12.97*	61.85 ± 23.31*	87.00 ± 38.48*
Sodium (mmol/L)	137.71 ± 1.28	137.86 ± 1.25	136.68 ± 1.46*	134.42 ± 1.22*	135.83 ± 1.17*
Chloride (mmol/L)	102.12 ± 0.96	101.43 ± 1.13	100.69 ± 1.52	98.12 ± 1.63*	98.93 ± 1.33*

Values represented as Mean ± SD
*Significantly higher than the vehicle control group at $p < 0.05$.
^: equimolar ratio to NIAGEN® 3000 mg/kg/day
Significantly higher / lower than the vehicle control group at $p < 0.05$.
% Change: % change from vehicle control
^: equimolar ratio to NIAGEN® 3000 mg/kg/d
**Data was obtained from Conze et al. (2016). The study report and raw data are provided in Annex 4 (see Study No. S14022).

c) Gross Pathology

Bilateral small-size testes observed in 3000 mg/kg/day NIAGEN®- and nicotinamide-treated males were considered to be treatment-related and associated with degeneration/atrophy of the seminiferous tubules.

One single incidence of auxiliary region subcutaneous nodule microscopically associated with adenocarcinoma of the mammary gland was observed in one NIAGEN® high dose male. This neoplastic change was considered an incidental tumor of spontaneous origin as it is reported to occur naturally in young Sprague Dawley male rats (Ikezaki et al., 2011).

All other single or low incidences of gross pathologic findings observed in different groups were considered incidental and not related to test item as they were randomly distributed in different groups and were not dose dependent.

d) Organ Weights

At 3000 mg/kg/day, there were statistically significant reductions in absolute organ weights of brain, spleen, testes, epididymides, prostate, thyroid/parathyroid, pituitary and heart in males; brain and pituitary absolute organ weights were reduced and liver and kidney absolute organ weights were increased in females. In the nicotinamide-treated group, there were statistically significant reductions in absolute organ weights of brain, spleen, testes, epididymis, prostate, thyroid/parathyroid, pituitary, thymus and heart in males; brain and pituitary absolute organ weights were reduced and liver and ovary weights were increased in females. At 1000 mg/kg/day, there were statistically significant reductions in absolute organ weights of thyroid/parathyroid, pituitary and heart in males; no effects on absolute organ weights were seen in females. At 300 mg/kg/day, there were statistically significant reductions in absolute organ weights of brain and heart in males; no effects on absolute organ weights were seen in females. Relative organ weight changes in the 3000 mg/kg/day NIAGEN®-treated rats were similar to those of animals ingesting an equimolar dose of nicotinamide (Table C.2.3-10).

Treatment-related organ weight changes were observed in liver, kidneys, testes, epididymides and ovaries in 3000 mg/kg/day NIAGEN®- and nicotinamide-treated groups. At 1000 mg/kg/day of NIAGEN®, increases in liver and kidney weights were statistically significant. All other changes in organ weight relative to body weight which reached statistical significance were likely secondary to decrease in terminal body weight and/or random biological variation and not considered treatment related.

Relative to brain weight, at 3000 mg/kg/day, there were statistically significant reductions in heart, epididymides, prostate and thyroid/parathyroid in males; liver, heart, ovaries and kidney were increased in females. In the nicotinamide-treated group, there were statistically significant reductions in weights of spleen, epididymides, testes and heart in males; liver weight was increased. At 1000 mg/kg/day there were no changes in organ weights relative to brain weight in males and a statistically significant increase in liver weight in females. No changes in organ weights relative to brain weights were seen in either males or females treated with 300 mg/kg/day of NIAGEN®.

Table C.2.3-10. Summary of Significant Changes-Organ Weight Ratio Relative to Body Weight At Day 91**					
	Dose (mg/kg/day)				
Parameters	**Vehicle 0**	**NIAGEN® 300**	**NIAGEN® 1000**	**NIAGEN® 3000**	**Nicotinamide 1260^**
Males					
Terminal Body Wt.	395.7 ±18.36	363.56 ±23.22 *	354.23 ±21.85 *	317.21±25.8 *	312.87±26.74 *
Organ/body weight					
Liver	2.958 ±0.143	3.013 ±0.163	3.200 ±0.18 *	3.600±0.272 *	3.703 ±0.116 *
Kidneys	0.715 ±0.047	0.701 ±0.033	0.777 ±0.02 *	0.876±0.063 *	0.833 ±0.086 *
Brain	0.507 ±0.027	0.526 ±0.038	0.550 ±0.024 *	0.572 ±0.042	0.583 ±0.043 *
Heart	0.368 ±0.019	0.356 ±0.018	0.373 ±0.015	0.384 ±0.02	0.387 ±0.015
Thymus	0.068 ±0.011	0.076 ±0.015	0.086 ±0.014 *	0.07 ±0.009	0.068 ±0.008
Adrenals	0.012 ±0.001	0.013 ±0.001	0.014 ±0.001 *	0.014 ±0.001 *	0.015 ±0.001 *
Females					
Terminal Body Wt.	232.29 ±8.1	234.43 ±23.28	219.51 ±9.92	216.19 ±14.75	215.61 ±11.16 *
Organ/body weight					
Liver	2.902 ±0.191	3.003 ±0.327	3.295 ±0.181*	4.046 ±0.174 *	4.465 ±0.239 *
Kidneys	0.676 ±0.053	0.645 ±0.06	0.678 ±0.058	0.822 ±0.044 *	0.766 * ±0.019
Brain	0.78 ±0.034	0.788 ±0.055	0.801 ±0.047	0.798 ±0.041	0.781 ±0.031
Heart	0.392 ±0.016	0.393 ±0.015	0.398 ±0.022	0.433 ±0.032 *	0.424 * ±0.029
Thymus	0.09 ±0.011	0.097 ±0.024	0.093 ±0.013	0.087 ±0.014	0.084 ±0.017
Adrenals	0.027 ±0.003	0.028 ±0.003	0.027 ±0.002	0.029 ±0.004	0.027 ±0.003
Ovaries	0.036 ±0.005	0.037 ±0.005	0.036 ±0.006	0.045 ±0.008 *	0.049 ±0.007 *

Values represented as Mean ± SD
*: Significantly higher / lower than the vehicle control group at $p < 0.05$.
^: equimolar ratio to NIAGEN® 3000 mg/kg/day
**Data was obtained from Conze et al. (2016). The study report and raw data are provided in Annex 4 (see Study No. S14022).

e) Histopathological Findings

Treatment-related histopathological changes were observed in liver, thyroid, kidneys, testes, epididymides, ovaries and adrenals in both the 3000 mg/kg/day NIAGEN®-treated and nicotinamide-treated groups. All other single or low incidences of microscopic findings observed were considered incidental and not related to the test item as they were randomly distributed among groups. Importantly, the treatment-related histopathological changes noted in NIAGEN® at the high dose were similar to the findings observed in the equimolar nicotinamide group.

In the livers of 3000 mg/kg/day NIAGEN®-treated and nicotinamide-treated males and females, centrilobular hepatocellular hypertrophy was reported. This was characterized by enlarged hepatocytes containing granular eosinophilic cytoplasm, follicular cell hypertrophy, characterized by enlarged follicular epithelium which contained pale eosinophilic cytoplasm and small clear vacuoles, and hepatocyte single cell necrosis, which was considered a treatment-related adverse change. In the kidneys, chronic progressive nephropathy characterized by presence of foci or areas of basophilic tubules, with or without simple tubular hyperplasia, hyaline casts, atrophic tubules, dilated tubule, focal glomerular sclerosis/atrophy and mononuclear cell infiltration was seen.

In male rats, both 3000 mg/kg/day NIAGEN®-treated and nicotinamide-treated rats exhibited degeneration/atrophy of seminiferous tubules characterized by the presence of some tubules containing degenerating germ cells. Some tubules were also depleted of all germ cells and lined only by Sertoli cells and while others were partially depleted of germ cells. Degenerating tubules contained multinucleated germ cells, spermatid head retention, Sertoli cell cytoplasmic vacuolation and disorganization of germ cells. Reduced sperm and cell debris in epididymal lumen in nicotinamide and NIAGEN® high dose males were considered treatment-related effects.

In female rats of the 3000 mg/kg/day NIAGEN®-treated and nicotinamide-treated groups, hypertrophy of corpora lutea was seen. The affected ovaries contained large sized corpora lutea and lightly eosinophilic cytoplasm of the enlarged luteal cells.

In male and female rats of the 3000 mg/kg/day NIAGEN®-treated and nicotinamide-treated groups, hypertrophy of the zona glomerulosa of the adrenal cortex was considered a treatment-related non-adverse change. Hypertrophy of zona glomerulosa was characterized by increased thickness of zona glomerulosa layer and cytoplasm of hypertophic cells was lightly eosinophilic.

C.2.4 Reproductive and developmental toxicity

The following studies have been undertaken in relation to the reproductive and developmental toxicity of NR:

- Developmental toxicity study in rats at various doses up to 1,500 mg/kg/day. There were no morbidity/mortality, clinical signs, or abnormal physical examination findings reported in rat dams at any dose levels in this study. No dead fetuses were reported. The study included external visceral and skeletal fetal observations.
- One generation reproduction study in rats at various doses of up to 12,000 ppm in the diet. Based on the results, the study concluded that the NOAEL for fertility and reproductive performance is 12000 ppm (equal to 675.21 mg/kg /day in males and 1088.38 mg/kg /day in females) under the testing conditions and doses employed.

The specific content of the studies referred to above is CCI and is included in Annex 1.

C.2.5 Human data

The following studies have been conducted and support the safe consumption of NR by humans:

- A Randomized Placebo-Controlled Clinical Trial of Nicotinamide Riboside in Obese Men. The safety of ingestion of NR at 2,000 mg/day for a 12-week period was supported in this study by the lack of clinically relevant findings in the blood biochemistry and hematology parameters as well as a lack of difference in incidence, nature, and severity of test article adverse events as well as no reported serious adverse events. Cutaneous flushing, a well-known side effect of nicotinic acid, was not reported in relation to NR supplementation in this study.
- Single-dose, double blind, randomized, cross-over pharmacokinetic study in healthy adults. The results indicate that NR was absorbed and metabolized, and the metabolites that were product are similar to those produced following the ingestion of nicotinamide.
- Six-week, repeat-dose, randomized, placebo-controlled, double blind, crossover study in healthy midlife and older adults. The findings suggest that 6 weeks of 1000 mg/day oral NR supplementation is safe and tolerable and does not alter blood chemistry or markers of renal and liver function in middle-aged and older adults.
- 8-week repeat-dose study in healthy adults at various doses up to 1,000 mg/day. No clinically-relevant adverse effects or differences within or between dosage groups were found. There was no significant difference between-groups in mean systolic blood pressure, mean diastolic blood pressure, mean heart rate, weight, and BMI.
- 8-week repeat-dose crossover study in healthy adults at various doses up to 1,000 mg/day. The results suggest NR supplementation at 300 mg/d and 1000 mg/d for 8 weeks in healthy older adults increases whole blood NAD+ in a dose related manner without serious adverse effects or clinically significant changes in haematology or blood chemistry. Apart from a small but statistically significant difference for fasting serum glucose, there was no difference in heart rate, systolic and diastolic blood pressure, haematology values or serum chemistry values.

The specific content of the studies referred to above is CCI and is included in Annex 1.

C.2.6 Allergenicity

NIAGEN® is synthetic, having no animal or botanical origins. As such, the product is intrinsically non-GMO, gluten-free, pesticide-free, TSE-free and suitable for vegan consumption. Additionally, the product is not treated by irradiation, ETO, or any other means of sterilisation. The country of origin of NIAGEN® is the USA.

NIAGEN® is >90% pure nicotinamide riboside chloride impurities are well characterized. This synthetic product does not contain any protein contaminants. NIAGEN® is well tolerated in healthy adult males and females, with no adverse effects reported at doses up to 2000 mg/day in long term testing, see C.2.5. Because there is no protein in this synthetic product, there is no concern over residual allergenic protein that would be a food allergen.

NIAGEN® does not contain any of the following substances (From FALCPA (USFDA), Annex II to Regulation (EU) No. 1169/2011, Food Standards Australia New Zealand, and the Canadian Food Inspection Agency):

- Milk and products thereof

- Eggs and products thereof
- Fish and products thereof
- Crustaceans and products thereof
- Tree nuts and products thereof
- Peanuts and products thereof
- Wheat (and other gluten-containing cereals) and products thereof
- Soybeans and products thereof
- Celery and products thereof
- Mustard and products thereof
- Sesame seeds and products thereof
- Sulphur dioxide and sulphites
- Lupin and products thereof
- Molluscs and products thereof
- Bee products such as royal jelly, propolis, bee pollen, beeswax, and honey
- Other identified food allergens/sensitizing agents:
- Artificial colours and flavours
- Buckwheat, corn, and any other fruit, vegetable, or grain
- Mushrooms
- Meat
- Gelatine
- Seeds
- Spices
- Sources of glutamic acid such as MSG, hydrolysed wheat protein, and autolyzed yeast

C.2.7 Safety conclusion

ChromaDex's NIAGEN® NR is a form of Vitamin B3 that is well tolerated in animals and humans.

The UL for nicotinamide is 900 mg/day (NHMRC 2006). NIAGEN® at 1000 mg/day delivers 420 mg nicotinamide on a molar basis.

C.3 Safety assessment reports prepared by international agencies or other national government agencies, if available

(addressing section 3.3.3.C.3 of the FSANZ Application Handbook)

Following the submission of ChromaDex's novel food application in the European Union the European Commission requested an opinion from the European Food Safety Authority (EFSA) Panel on Nutrition, Novel Foods and Allergens (NDA). The NDA concluded that the use of NR in food supplements at up to 300 mg/day was safe and that nicotinamide from NR was bioavailable.

C.4 Safety conclusion

C.4.1 Safety conclusion

The safety of consumption of NIAGEN® NR at the intended levels of use is clearly demonstrated by the data package included in this application and described in section C:

- In vitro tests demonstrate that NIAGEN® NR is rapidly metabolised to nicotinamide (a permitted form of Vitamin B3) in blood. Animal and human studies also demonstrate that NIAGEN® NR is rapidly metabolised to nicotinamide and nicotinamide metabolites in plasma and urine (section C.2.1).

- NIAGEN® NR is not genotoxic, demonstrated by results of bacterial reverse mutagenicity study, in vitro chromosomal aberration assay and in vivo micronucleus test, described in section C.2.2.1.

- Reproductive and developmental rat studies have demonstrated a lack of reproductive and developmental toxicity at doses up to 325 mg/kg bw/d and 750 mg/kg bw/d respectively.

- Human studies have demonstrated that NR supplementation at doses up to 2000 mg/d increase whole blood NAD+ levels in a dose related manner. Supplementation with NR at up to 2000 mg/d has also been demonstrated to be safe in adult populations for up to 12 weeks.

- The Australian and New Zealand UL for nicotinamide is 900 mg/day (NHMRC 2006). NIAGEN® at 1000 mg/day delivers 420 mg nicotinamide on a molar basis, well below the UL.

C.4.2 Age range

Nicotinamide riboside chloride (NR) is a novel form of Vitamin B3 and has been recognized as such by the European Food Safety Authority (EFSA Official Journal_NR Auth), Health Canada (see Product Licenses with claims), and other resources (Erdman, 2012). Upon preliminary review of NR as a novel food, the Advisory Committee on Novel Foods (ACNF) determined the substance to be a nutritive substance (ACNF determination, 2019). As a nutritive substance, therefore, NR is acknowledged to have a wide margin of safety.

Published studies have established the safety of NR up to relatively high doses. The No-Observed-Adverse-Effect-Level (NOAEL) from a 90-day rodent toxicity study was determined to be 300mg/kg/day (see Annex 4, Study No. S14022 and Conze, 2016). Human studies have shown that a dose of up to 2000 mg/day of NR is safe, with no treatment related serious adverse events (Airhart et al., 2017 and Dollerup et al., 2018).

NR is safer than nicotinic acid (NA) and potentially nicotinamide (NAM), which are other forms of Vitamin B3 that have long been established as essential nutrients and are permitted in the Food Standards Code in fortification of food for all ages. NA in doses of 30-50 mg or more have been known to cause uncomfortable flushing in humans (NIH Niacin Fact Sheet). As noted above, high doses of NR have been administered with no reports of a flushing response (Airhart et al., 2017, Dollerup et al., 2018, and Conze 2019).

At high doses (>hundreds of mg/day) NAM is known to inhibit sirtuins, NAD+-dependent deacetylases that regulate many cellular processes, such as those involved in cellular health and repair, mitochondrial respiration, stress response, and cellular energy metabolism (Zhang and Sauve, 2018, Wang, 2019, and Hwang 2020). In contrast, a study in 2016 showed that NR does not inhibit sirtuins (Trammel, 2016). Adenine diphosphate ribose (ADP-ribose or ADPR) is formed as a byproduct of NAD+ consumption and serves as an indicator of sirtuin and other NAD+ consuming activities. NR significantly increased these levels more than NAM. This is consistent with earlier findings that showed NAM is an in vitro noncompetitive inhibitor of recombinant yeast Sir2 and human SIRT1 (Bitterman, 2002).

Tolerable upper intake levels (UL) have been established for NAM and NA (sometimes together as niacin) in many markets. ULs have been established for children and pregnant women through extrapolation of adult data (listed below), and all are significantly lower than the highest safe dose of NR used in clinical trials (2000 mg/day):

- 35 mg/day for niacin (Vitamin B3) in the US (NIH Niacin Fact Sheet) and AU (NRV for AU and NZ). This relatively low UL is based on the fact that NA (as a form of niacin), causes flushing. Neither NAM nor NR are known to cause flushing.
- 10 mg/day for NA and 900 mg/day for NAM in the EU (EFSA Opinion, 2002). The EU recognized the distinction between NA and NAM when setting the UL.

Forced degradation studies show nicotinamide riboside chloride degrades to approximately equimolar amounts of nicotinamide, ribose, a sugar, and chloride, an essential trace element (see Annex 4, SS-1805-004-R-00). Additionally, data from clinical studies indicate that NR is absorbed and metabolized, and the metabolites produced are the same as those produced following the ingestion of nicotinamide (14NBHC and 15NRHC)

The data provided as part of this Application therefore supports that NR is safe, and in some instances may even be considered to be safer than other forms of Vitamin B3 that are currently permitted for use in food.

FSANZ has queried whether – given that FSMPs are not limited to a particular age range – whether the safety data extends to children. This query of the safety for a single population demographic seems an unusual inquiry, given that any recipient of an FSMP is by definition a vulnerable consumer regardless of their age. Any FSMP product products must be safe for its intended consumer group, regardless of their age. Additionally, under Standard 2.9.5 of the Food Standards Code, FSMPs are to be used under medical supervision, ultimately allowing medical professionals to determine whether the FSMP is safe for their specific patient's needs.

Although it is intended that NR be used in FSMP products formulated for persons aged over the age of 18, the abovementioned data indicates that there is no basis to conclude that NR poses any risk to any particular segment of the general population, including children. NR is a recognized form of Vitamin B3, with a wider safety margin than NA and potentially NAM, both established essential nutrients permitted in foods for children, and has been proven to be safe at high doses in clinical studies with no serious adverse events.

Taking into consideration these facts, NR does not require an age range for inclusion in Schedule 29 for use in FSMPs.

D Information on dietary intake of the nutritive substance

(addressing section 3.3.3.D of the FSANZ Application Handbook)

FSMPs encompass food products which are recommended to be used under medical supervision and may represent a partial or total replacement of the daily diet of the targeted population. The daily intake of special dietary foods is typically determined by healthcare professionals, depending on the tolerance, nutritional requirements and overall health condition of the targeted population. These products are commonly formulated with a balanced ratio of protein, carbohydrates and fats, with the addition of sufficient vitamins and minerals to cover daily nutritional requirements.

The inclusion of NIAGEN® in these products will result in a maximum intake of 1000 mg NIAGEN®/day, whenever the products is used as a total diet replacement and 250-600 mg whenever the product is used as partial diet replacement.

Studies have identified very small amounts of NR to be naturally present in milk. However, this contributes only negligible amounts of NR to the diet. Further detail on the presence of NR in milk is CCI and is included in Annex 1.

D.1 A detailed list of the food groups or foods in which the use of a nutritive substance is proposed, or changes to currently permitted foods in which a nutritive substance is used

(addressing section 3.3.3.D.1 of the FSANZ Application Handbook)

NIAGEN® is intended to be used in FSMPs that are designed to manage an individual's medically determined nutritional requirements.

D.2 The maximum proposed level of the use of the nutritive substance for each food group or food, or the proposed changes to the currently permitted use levels

(addressing section 3.3.3.D.2 of the FSANZ Application Handbook)

The purpose of this Application is for NR to be listed in Section S29-20 as a permitted form of Vitamin B3 for use in FSMPs. This Application does not propose any amendment to the various maximum limits that apply to Vitamin B3 throughout the Code. Standard 2.9.5 of the Code does not set maximum limits for nutritive substances (including vitamins) used in general FSMPs with the purpose of allowing FSMPs to be specifically formulated to achieve the stated medical purpose.

As such, we expect NR to be used in FSMPs up to a level that is analogous with the current permitted use of other forms of Vitamin B3, taking into account the medical purpose of the product in which it is used. Due to the efficiencies associated with NR when compared with other forms of Vitamin B3, the amount of NR required in an FSMP might even be less than the corresponding amount of an alternative form of Vitamin B3.

D.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

(addressing section 3.3.3.D.3 of the FSANZ Application Handbook)

As indicated above, the likely level of consumption of NR via FSMPs is expected to be consistent with current consumption of other forms of Vitamin B3 in FSMPs. There is likely to be minimal, if any, data in the NNSs on consumption of FSMPs.

FSMPs containing NR have not yet been developed. At the time of submitting this Application, there is no other source of consumption data available within Australia or internationally.

D.4 The percentage of the food group to which the use of the nutritive substance is proposed or the percentage of the market likely to use the nutritive substance

(addressing section 3.3.3.D.4 of the FSANZ Application Handbook)

NR is only proposed to be added to FSMPs, which represent a relatively small category in the context of the market for food products generally. FSMPs face unique compositional permissions compared with other foods as they are designed specifically to assist in the dietary management of a medical outcome. They are intended to be consumed in the context of specialised medical supervision, rather than as a discretionary food.

Within the category of FSMPs, NR is only expected to be present at significant amounts in products where NR specifically or Vitamin B3 generally is required to achieve the stated medical purpose. Due to the efficiencies NR presents over other forms of Vitamin B3, some FSMP manufacturers may prefer to use NR over other forms of Vitamin B3. Nevertheless, as FSMPs are formulated based on specific functional outcomes rather than the general ideal of “supplementation”, we expect that only a moderate portion of FSMPs will contain NR in a significant amount.

D.5 Information relating to the use of the nutritive substance in other countries

(addressing section 3.3.3.D.5 of the FSANZ Application Handbook)

ChromaDex’s NIAGEN® NR has had GRAS status in the US since 2016 (GRN 635 and Addendums (2017)). In accordance with the GRAS determination, NIAGEN® is intended for use in nutrition/protein drinks consumed by the general population at a maximum use level of 600 mg/day (2x/day; 300 mg serving) and special dietary foods that are intended to partially or totally replace the daily diet; and are intended to be used under medical supervision. The use of NIAGEN® in special dietary foods delivers up to 1000 mg/day for total diet replacement (250 mg/serving; up to 4 servings/day).

The use of ChromaDex’s NIAGEN® NR in capsule form delivering 300 mg/day of NIAGEN® is also permitted in the EU (as a novel food in food supplements), Australia, and is the subject of new dietary ingredient notification in the US (NDIN 882 (2015, updated in 2017)); which the FDA accepted with no questions.

NR is also listed on the Natural Health Products Ingredients Database as a chemical substance.

D.6 For foods where consumption has changed in recent years, information on likely current food consumption

(addressing section 3.3.3.D.6 of the FSANZ Application Handbook)

Not applicable (see D.3).

D.7 Estimate of exposure to undesirable substances

All undesirable substances in NR are commonly found in food. Each substance is monitored and controlled in the production process so as not to be present in NR in levels that would exceed any relevant daily intake or upper limit levels. The quantification and control of such substances means that NR does not contain any undesirable substances in levels that would present a threat to public health.

The precise undesirable substances and control levels are CCI and are included in Annex 1.

E Information related to the nutritional impact of a vitamin or mineral

(addressing section 3.3.3.E of the FSANZ Application Handbook)

Information in section E (section E.2 in particular) is provided to demonstrate that NR has equivalent or improved functionality as a source of Vitamin B3 when compared to nicotinic acid and nicotinamide.

E.1 Information to demonstrate a need to permit the addition of a vitamin or mineral to food

(addressing section 3.3.3.E.1 of the FSANZ Application Handbook)

Vitamin B3 is already permitted to be added to food, including FSMP, as a vitamin. Therefore, the need for consumption of Vitamin B3 and the addition of Vitamin B3 to certain foods has been established by agencies such as the NHMRC and FSANZ.

E.2 Information to demonstrate the permitted addition of the vitamin or mineral has the potential to address the deficit or deliver a health benefit to the population or a population subgroup

(addressing section 3.3.3.E.2 of the FSANZ Application Handbook)

The status of NR as a form of Vitamin B3 is supported by studies of NIAGEN®, which is a synthetic source of NR. The two major forms of Vitamin B3 are nicotinic acid and nicotinamide. NIAGEN® has been demonstrated in human studies to increase urinary excretion of 1-meNAM and 2-PY, biomarkers of Vitamin B3 status (Trammel et al., 2016; Study No. 14NBHC; Study No. 15NRHC;). NIAGEN® has also been shown to be a precursor of NAD+ in short and long term clinical studies (Trammel et al., 2016; Study No. 15NRHC; Airhart et al., 2017) and is therefore considered to be a form of Vitamin B3 (reviewed in Chi and Sauve, 2013; IOM, 1998).

Vitamin B3 is a dietary precursor to nicotinamide adenine dinucleotide (NAD+) (Erdman et al., 2012). There is a history of safe consumption of other forms of Vitamin B3 such as nicotinamide. Vitamin B3 is essential for the formation of the pyridine nucleotide coenzymes NAD+ and nicotinamide adenine dinucleotide phosphate (NADP+). Both coenzymes function indispensably in oxidation-reduction reactions involved in glucose, fatty acid, ketone body,

and amino acid catabolism. Although the metabolism of nicotinic acid and nicotinamide to NAD+ is tissue and cell-type dependent, in general, intracellular nicotinic acid is converted to NAD+ by the biosynthetic (Preiss-Handler) pathway. Intracellular NAD+ glycosidases then release nicotinamide, which circulates to other tissues where it is absorbed and metabolized to NAD+ and NADP+ (Preiss and Handler, 1957a; Preiss and Handler, 1957b; Preiss and Handler, 1958a; Preiss and Handler, 1958b; Collins and Chaykin, 1972).

In contrast, nicotinamide and NR are converted to NAD+ by way of nicotinamide mononucleotide in the salvage pathway (Figure 21) (Rongvaux et al., 2002; Martin et al., 2001; Revollo et al., 2004; Bieganowski et al., 2004). Excess nicotinamide is methylated to 1-methsyl-nicotinamide (1-meNAM) in the liver, which is then excreted along with the two oxidation products 1-methyl-2-pyridone-5-carboxamide (2PY) and 1-methyl-4-pyridone-5-carboxamide (4PY) (Mrochek et al., 1976). Excess nicotinamide can also be excreted. Importantly, biochemical markers of Vitamin B3 status and cellular NAD+ levels are plasma and urinary levels of 1-meNAM and 2-PY (IOM, 1998).

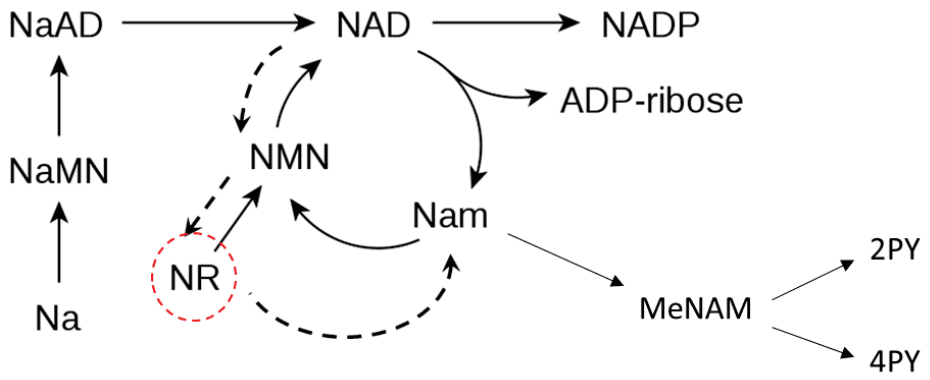


Figure 22. Metabolism of Nicotinamide Riboside
Intracellular nicotinamide riboside (NR; circled in red) is metabolized to NAD+ by way of nicotinamide mononucleotide (NMN) via the salvage pathway in a manner similar to the recycling of NAD+ via nicotinamide (Nam). NAD+ can also be metabolized to ADP-ribose and NADP+. In contrast, nicotinic acid (NA) is metabolized to NAD+ via the Preiss-Handler pathway by way of nicotinic acid mononucleotide (NaMN) and nicotinate adenine dinucleotide (NaAD). Excess nicotinamide is methylated to 1-methyl-nicotinamide (1-meNAM) and excreted in the urine along with its oxidation products 1-methyl-2-pyridone-5-carboxamide (2PY) and 1-methyl-4-pyridone-5-carboxamide (4PY).

ChromaDex has evaluated the metabolism of NR in an in vitro study using blood matrices, a toxicokinetic arm of a 90-day oral subchronic rat toxicity study, a single dose GCP-compliant, randomized, double-blind crossover pharmacokinetic study in healthy adults, and a GCP-compliant 8-week, randomized, double-blind, placebo-controlled, parallel group in healthy adults. Both the pharmacokinetic and 8-week clinical studies were designed to support the safety of use of NIAGEN® as well as document its status as a vitamin by measuring changes in NAD+, and key urinary metabolites recognized to be biomarkers of Vitamin B3 status.

In vivo nicotinic acid is converted to nicotinamide, which is a precursor for NAD+ and NADP+, Vitamin B3 circulates in the plasma as nicotinamide and nicotinic acid. Both forms are transported to cells and tissues, which they enter by diffusion to perform the intracellular functions of Vitamin B3. Vitamin B3 is trapped within the cell as NAD+ or NADP+. The major pathway of catabolism of nicotinic acid and nicotinamide is by methylation in the liver to 1-meNAM and subsequent oxidation to 2PY and 4PY. In humans, the two major excretion products are 1-meNAM and 2PY, which under normal conditions represent about 20-35 % and 45-60 % of Vitamin B3 metabolites, respectively (EFSA, 2014). Vitamin B3 metabolites are excreted in the urine even at low NE intakes. For NE intakes above about 11 mg/day, urinary excretion of Vitamin B3 metabolites increased sharply, which has been suggested to reflect saturation of body stores. The EFSA Panel notes that urinary excretion of Vitamin B3 metabolites is considered as a marker of Vitamin B3 status (EFSA, 2014).

NIAGEN® has been demonstrated to be another form of Vitamin B3 through the examination of the metabolism in a toxicokinetic arm of a 90-day oral subchronic rat toxicity study, a single dose GCP-compliant, randomized, double-blind crossover pharmacokinetic study in healthy adults, a GCP-compliant 8-week, randomized, double-blind, placebo-controlled, parallel group in healthy adults and a short-term escalating dose study. These studies document its status as a vitamin by demonstrating changes in NAD+, and key urinary metabolites recognized to be biomarkers of Vitamin B3 status.

Importantly, in the 8-week study conducted with 100, 300 and 1000 mg/day NIAGEN®, blood NAD+ content peaked at week 2 and plateaued thereafter for all doses (Study No. 15NRHC). This is consistent with rate of change data for blood NAD+ that shows a steady decline in magnitude following the first week of supplementation. This suggests that the activity of NR as a precursor to NAD+ biosynthesis quickly increases NAD+ content and then became saturated approximately 2 weeks after consistent intake and supports having no requirement for a defined duration of intake. Levels of urinary 1-meNAM, the primary endpoint of the study, were approximately 18 ng/µg creatinine in the subjects given 1000 mg/day NIAGEN® which is consistent with the order of magnitude that would be expected based on findings from the twelve-week study (Dollerup et al., 2017) at 2 grams/day resulting in urinary levels of 480 µmol/g creatinine (65 ng/µg creatinine).

F Information related to the nutrition impact of a nutritive substance other than vitamins and minerals

(addressing section 3.3.3.F of the FSANZ Application Handbook)

Not applicable

G Information related to potential impact on consumer understanding and behaviour

(addressing section 3.3.3.G of the FSANZ Application Handbook)

NR is intended to be added to FSMP as a permitted form of Vitamin B3. The focus of marketing of products will be on the nutritional profile and benefits of the overall product

and/or components, such as vitamins. The focus will not be on permitted forms of vitamins. Therefore, there should be minimal impact on consumer understanding and behaviour for FSMP products containing NR as a permitted form of Vitamin B3.

III – SPECIAL PURPOSE FOODS – OTHER FOODS

A.1 Information on the identity and physical and physiological need of the target population

(addressing section 3.6.3.A1 of the FSANZ Application Handbook)

This Application does not request a change to general compositional requirements across any food category. As described above, this Application requests that NR be listed as a permitted form of Vitamin B3 under Section S29-20 for use in FSMPs.

NR will be present in FMSPs in which Vitamin B3 is required to achieve the stated medical purpose. The target market for the inclusion of NR in Section S29-30 will therefore be individuals who, in the context of specialised medical supervision, require food that has been fortified with Vitamin B3 for the dietary management of a nutritional requirement or deficiency.

A.2 Purpose of the compositional change

(addressing section 3.6.3.A.2 of the FSANZ Application Handbook)

As discussed in detail above, NR is a safer and more nutritionally efficient form of Vitamin B3 than other forms currently listed in the Code, and will therefore benefit consumers who require FSMPs that have been fortified with Vitamin B3. We reiterate that this Application is not for a wide compositional change, but rather is for the addition of a new form of Vitamin B3 that manufacturers may choose to use in FSMPs where appropriate to achieve the stated medical purpose.

A.3 Information related to the safety of the proposed compositional change

(addressing section 3.6.3.A.3 of the FSANZ Application Handbook)

Please refer to Section C of Part II of this Application above for a detailed discussion of the safety of NR.

A.4 Information related to the nutritional impact or performance impact of the proposed compositional change

(addressing section 3.6.3.A.4 of the FSANZ Application Handbook)

NR is a safer, more nutritionally efficient form of Vitamin B3. Its proposed inclusion in Section S29-20 would give manufacturers of FSMPs access to an alternative form of Vitamin B3 for use in products where Vitamin B3 is required for the dietary management of a nutritional requirement or deficiency.

The nutritional impact of using NR would be substantially similar to that of using other forms of Vitamin B3, subject to the added benefits of NR identified above.

Please refer to Section B of Part II for further information about NR as a form of Vitamin B3 and to Section C of Part II for further information about the safety and efficacy of using NR as a form of Vitamin B3.

B.1 Data to enable the dietary exposure of the target population to be estimated

(addressing section 3.6.3.B.1 of the FSANZ Application Handbook)

This Application does not propose any amendment to the mandatory compositional

requirements for FSMPs, but rather requests permission to use a new form of an already-permitted vitamin. As outlined in Section D.2-D.3 of Part II above, there is minimal data available regarding the consumption of FSMPs containing NR. Nevertheless, we expect consumption to be analogous to consumption of existing FSMPs containing other forms of Vitamin B3.

B.2 Data on the recommended level of consumption of the special purpose food for the target population

(addressing section 3.6.3.B.2 of the FSANZ Application Handbook)

As discussed in Section D.4 of Part II, the unique usage case of FSMPs means that the level of consumption of NR through FSMPs will depend on the formulation that is required for the specific medical purpose that the product is intended to perform. As a matter of course, all FSMP products containing NR will undergo stringent testing during development. Any such products will also be consumed in the context of specialised medical supervision.

C.1 Information related to safety or nutritional impact of the proposed labelling change

(addressing section 3.6.3.C.1 of the FSANZ Application Handbook)

We do not believe that any labelling change is necessary. The addition of NR as a permitted form of Vitamin B3 would be adequately addressed through current labelling requirements for FSMPs.

C.2 Information to demonstrate that the proposed labelling change will be understood and will assist consumers, if applicable

(addressing section 3.6.3.C.2 of the FSANZ Application Handbook)

We do not believe that any labelling change is necessary. The addition of NR as a permitted form of Vitamin B3 would be adequately addressed through current labelling requirements for FSMPs.

D Information related to internationally recognised codes of practice and guidelines

(addressing section 3.6.3.D of the FSANZ Application Handbook)

As identified above, this Application is for the addition of NR as a permitted form of Vitamin B3 for use in FSMPs. As at the date of this Application, final products containing NR have not been formulated and developed. As such, there is no available information as to how final products adhere to internationally recognised codes of practice and guidelines.

We note that, any use of NR in FSMPs would be required to comply with Standard 2.9.5 and other relevant parts of the Code, which also reflect international standards such as *Codex Alimentarius*.

IV – REFERENCES (included in Annex 5)

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LIST OF STUDY REPORTS (in Annex 4):

Study Number 14NBHC

Study Number 15NRHC

Study Number 160312

Study Number 20151223

Study Number AV151003

Study Number G10957

Study Number G10959

Study Number S13101

Study Number S13120

Study Number S14022

Study Number S15004

Study Number S15005

Study Number S15006

Study Number SN17-921

Study Number SN17-940

V – CHECKLISTS

General requirements (3.1.1)		
Check	**Page No.**	**Mandatory requirements**
☑	Throughout	A Form of application ☑*Application in English* ☑ *Executive Summary (separated from main application electronically)* ☑ *Relevant sections of Part 3 clearly identified* ☑ *Pages sequentially numbered* ☑ *Electronic copy (searchable)* ☑ *All references provided*
☑	6	B Applicant details
☑	6	C Purpose of the application
☑	8	D Justification for the application ☑ *Regulatory impact information* ☑ *Impact on international trade*
☑	8	E Information to support the application ☑ *Data requirements*
☑	9	F Assessment procedure ☑ *General* ☐ *Major* ☐ *Minor* ☐ *High level health claim variation*
☑	9	G Confidential commercial information ☑ *CCI material separated from other application material* ☑ *Formal request including reasons* ☑ *Non-confidential summary provided*
☑	9	H Other confidential information *N/a Confidential material separated from other application material* *N/a Formal request including reasons*
☑	9	I Exclusive Capturable Commercial Benefit ☑ *Justification provided*
☑	10	J International and other national standards ☑ *International standards* ☑ *Other national standards*
☑	12	K Statutory Declaration
☑	60	L Checklist/s provided with application ☑ *3.1.1 Checklist* ☑ *All page number references from application included* ☑ *Any other relevant checklists for Chapters 3.2–3.7*

Substances used of a nutritive purpose (3.3.3)		
Check	**Page No.**	**Mandatory requirements**
☑	13	A.1 Purpose of the use of the substance
☑	13	A.2 General data requirements for supporting evidence

☑	14	B.1 Identification
☑	14	B.2 Chemical and physical properties
☑	15	B.3 Impurity profile
☑	15	B.4 manufacturing process
☑	16	B.5 Specification for identity and purity
☑	18	B.6 Analytical method for detection
☑	18	B.7 Proposed food label
☑	18	C.1 Toxicokinetics and metabolism, degradation products and major metabolites
☑	19	C.2 Animal or human studies
☑	45	C.3 International safety assessments
☑	47	D.1 List of food groups or foods likely to contain the nutritive substance
☑	47	D.2 Proposed maximum levels in food groups or foods
☑	48	D.3 Likely level of consumption
☑	48	D.4 Percentage of food group to use nutritive substance
☑	48	D.5 Use in other countries (if available)
☑	49	D.6 Where consumption has changed, information on likely consumption
☑	49	E.1 Need to permit addition of vitamin or mineral
☑	49	E.2 Demonstrated potential to address deficit or health benefit
☑	51	F.1 Nutritional purpose (other than vitamins and minerals)
☑	51	G.1 Consumer awareness and understanding
☑	51	G.2 Actual or potential behaviour of consumers
☑	51	H.3 Demonstration of no adverse effects on any population groups

Special purpose foods – Other foods (3.6.3)

Check	Page No.	Mandatory requirements
☑	53	A.1 Identity and need of target population
☑	53	A.2 Purpose of compositional change
☑	53	A.3 Safety of proposed compositional change
☑	53	A.4 Nutritional or performance impact
☑	53	B.1 Dietary exposure data
☑	54	B.2 Level of consumption
☑	54	C.1 Safety and nutritional impact of labelling change
☑	54	C.2 Demonstrated consumer understanding of labelling change
☑	54	D Internationally recognised codes of practice and guidelines