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Supporting document 1

Risk Assessment: Nutrition, safety, food technology and dietary intake assessment report

Application A1214 – Nicotinamide riboside chloride as Vitamin B3 in FSMP

Executive summary

The purpose of this application is to amend the *Australia New Zealand Food Standards Code* (the Code) to permit the use of nicotinamide riboside chloride (NRC) as a permitted form of vitamin B3 (niacin) in food for special medical purposes (FSMPs).

To determine the bioavailability of NRC, FSANZ considered studies in humans and in laboratory animals on the effect of NRC supplementation on the concentration of nicotinamide adenine dinucleotide (NAD⁺) and metabolites in blood and/or urine. In human studies, NRC supplementation (100 to 2000 mg/day) in volunteers was associated with increases in blood concentrations of NAD⁺ and several NAD⁺ metabolites, relative to baseline values or placebo treatments showing that it is a bioavailable form of niacin. However, none of the studies included a comparator treatment group receiving nicotinic acid (NA) or nicotinamide (Nam) so it was not possible to establish bioequivalence to already permitted forms of niacin in the Code.

NRC has been shown to increase hepatic NAD⁺ levels in mice and increase plasma MeNam concentrations in other animal studies. A 90-day rat study showed that similar doses of NRC or Nam caused an elevation of plasma Nam and MeNam. Similar plasma concentrations of MeNam were observed in the two treatment groups, however the maximum plasma concentration (C_{max}) and total systemic exposure (AUC) to Nam was higher (approximately 1.6- to 1.8-fold) in the Nam group compared to the NRC group. Plasma MeNam levels peaked earlier in the NRC group than in the Nam group.

FSANZ concludes that based on the available evidence in laboratory animals and humans NRC is a bioavailable form of niacin which at intakes ranging from 100 to 2000 mg/day in humans would be expected to support normal physiological function. In the absence of human studies to establish the bioavailability of NRC compared to already permitted forms of niacin, FSANZ cannot judge with certainty the extent to which lower NRC intakes which match adult Recommended Dietary Intakes for niacin (14 mg/day in women, 16 mg/day in men) would support essential requirements, when it is the only form of vitamin B3 in the diet.

No evidence was identified to indicate that NRC would inhibit the absorption of other nutrients.

The acute oral toxicity of NRC is low. No adverse effects were observed in rats gavaged with NRC at 300 mg/kg bw/day for 90 days, but statistically significant changes in some clinical pathology parameters were observed in rats dosed with 1000 mg/kg bw/day. In the same study, a number of adverse effects were observed in rats dosed with 3000 mg/kg bw/day, but the same adverse changes occurred in a positive control group of rats treated with an equimolar dose of Nam (1260 mg/kg bw/day). Additional animal studies also supported a no observed adverse effect level (NOAEL) of 300 mg/kg bw/day.

No chronic toxicity/carcinogenicity studies of NRC were submitted or located from other sources, but NRC was not genotoxic and no pre-neoplastic lesions were observed in the 90 day rat study. In a developmental study in rats, the fetal NOAEL was identified as 750 mg/kg bw/day on the basis of decreased fetal bodyweights at 1500 mg/kg bw/day, together with increases in the incidence of abnormalities commonly observed in association with maternal toxicity. In a one-generation reproductive study in rats, the NOAEL for fertility and reproductive performance was 12 000 ppm in the parental diet, the highest dose tested, equivalent to 675.2 mg/kg bw/day NRC in P generation males and 1088.4 mg/kg bw/day NRC in P generation females.

In human tolerance studies of up to 12 weeks in duration, NRC was well tolerated at doses up to 2000 mg/day.

NRC would not be expected to be an allergen, on the basis of its rapid metabolism to Nam and low molecular weight.

Since NRC is metabolised to Nam it is important to consider NRC intakes relative to the upper level of intake (UL) for Nam. The maximum daily intake of NRC proposed in the Application was 1000 mg/day, which assuming equimolar conversion, is equivalent to 420 mg Nam. This is less than half the UL for Nam in non-pregnant, non-lactating adults (900 mg), and is also below the UL for children aged 9-13 years (500 mg/day) and adolescents aged 14-18 years (750 mg/day). It is above the UL for children aged 1-3 years (150 mg/day) and 4-8 years (250 mg/day), however it is expected that FSMPs are prescribed by, and will be used under the supervision of medical practitioners, at intakes that would not exceed the UL.

The margin of exposure (MOE) between the NOAEL of 750 mg/kg/day for fetal toxicity in a rat developmental study, and the maximum intake of 1000 mg/day NRC for a pregnant woman weighing 60 kg was 45. The corresponding MOE between the NOAEL in the one-generation reproductive toxicity in rats and proposed maximum human intake was approximately 66. On the basis of the low MOEs (<100) use at the maximum proposed NRC intake level in pregnant or lactating women is not supported. However, as for paediatric use, lower intakes approximating Recommended Daily Intakes of niacin, prescribed and supervised by a medical practitioner, do not represent a safety concern in pregnant or lactating women.

The technological assessment concluded that the NRC manufactured by the Applicant is of appropriate chemical composition, purity and stability to fulfil the technological function. FSANZ has developed specifications based on information provided by the Applicant.

In conclusion, FSANZ considers that based on the best available evidence in laboratory animals and humans, NRC is a bioavailable form of niacin which at intakes ranging from 100 to 2000 mg/day would be expected to support normal physiological function. However none of the studies included a comparator treatment group receiving NA or Nam, therefore it was not possible to establish bioequivalence to forms of niacin already permitted in the Code. On that basis it is not possible for FSANZ to establish with certainty whether lower NRC intakes

which matched adult Recommended Dietary Intakes for niacin would support essential requirements, when it is the only form of vitamin B3 in the diet.

NRC is not expected to represent a safety concern when prescribed and used under medical supervision at intakes below the UL for Nam, and for pregnant or lactating women, at levels of intake consistent with Recommended Dietary Intakes for niacin.

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Abbreviations relevant to NAD⁺ metabolism

| | |
|-------------------|---|
| 2-PY | N1-methyl-2-pyridone 5-carboxamide |
| 4-PY | N1-methyl-4-pyridone 5-carboxamide |
| ADPR | Adenosine diphosphate ribose |
| MeNam | N1-methyl-nicotinamide |
| NA | Nicotinic acid |
| NAAD | Nicotinic acid adenine dinucleotide |
| NAD | Nicotinamide adenine dinucleotide |
| NAD ⁺ | Nicotinamide adenine dinucleotide – oxidised form |
| NADH | Nicotinamide adenine dinucleotide – reduced form |
| Nam | Nicotinamide |
| Nam oxide | Nicotinamide oxide |
| NAMN | Nicotinic acid mononucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| NADP ⁺ | Nicotinamide adenine dinucleotide phosphate – oxidised form |
| NADPH | Nicotinamide adenine dinucleotide phosphate – reduced form |
| NAR | Nicotinic acid riboside |
| NR | Nicotinamide riboside |
| NE | Niacin equivalents |
| NMN | Nicotinamide mononucleotide |

1 Introduction

FSANZ received an application from ChromaDex Inc. to amend the Code to permit the use of nicotinamide riboside chloride (NRC) as a permitted form of vitamin B3 in food for special medical purposes (FSMPs) that partially or totally replace the daily diet. These products are recommended to be used under medical supervision.

The application refers to 'vitamin B3', however the Code refers to 'niacin' in its permissions, and from herein the latter term will be referenced. Niacin is the generic descriptor commonly used for the closely related compounds nicotinic acid (pyridine-3-carboxylic acid) and nicotinamide (niacinamide or pyridine-3-carboxamide). Niacin functions as a source of NAD⁺ in the body which is required for a range of cellular functions. These compounds are water soluble and naturally present in many foods. Niacin is an essential nutrient which must be obtained through dietary sources as the body cannot produce it on its own.

This application requests permission to use a new form of an already permitted vitamin. This application does not propose any amendment to the mandatory compositional, labelling or other requirements for FSMPs.

2 Nutrition Risk Assessment

2.1 Objectives for the nutrition risk assessment

Objectives for the nutrition assessment include the following:

- To determine whether NRC is an equivalent source of NAD⁺ to currently permitted forms of niacin
- To determine the ability of NRC to inhibit or modify the absorption of other nutrients.

2.2 Introduction

Niacin (vitamin B3) is the generic term that is commonly used to refer to two water-soluble organic compounds – nicotinic acid (pyridine-3-carboxylic acid) and nicotinamide (pyridine-3-carboxamide). Absorbed niacin is converted into the metabolically active coenzyme nicotinamide adenine dinucleotide (NAD; NADH and NAD⁺ when reduced and oxidised respectively) and nicotinamide adenine dinucleotide phosphate (NADP; NADPH and NADP⁺ when reduced and oxidised respectively) (NHMRC 2006, NIH 2021). NAD and NADP are required in most metabolic redox processes. NAD is also needed for non-redox adenosine diphosphate-ribose transfer reactions involved in DNA repair and calcium mobilisation. Because of its role in energy metabolism, niacin requirements are, to some extent, related to energy requirements.

NAD⁺ was previously considered to be formed via only three distinct pathways (Figure 1): (i) nicotinic acid (NA) is converted to NAD⁺ via the Preiss-Handler Pathway, with precursors nicotinic acid mononucleotide (NAMN) and nicotinic acid adenine dinucleotide (NAAD); (ii) nicotinamide (Nam) is formed as a by-product of NAD⁺ consuming enzymes, and recycled via the salvage pathway to nicotinamide mononucleotide (NMN) and then to NAD⁺. Excess Nam is converted in the liver to methyl-nicotinamide (MeNam) and metabolised to N1-methyl-2-pyridone 5-carboxamide (2-PY) and N1-methyl-4-pyridone 5-carboxamide (4-PY); and (iii) the amino acid tryptophan is converted to NAD⁺ via the de novo/kinurenine pathway.

2.3 Niacin dietary sources and bioavailability

Niacin is found in organ and muscle meats and meat products, fish, peanuts and wholegrains. Niacin from animal-based products is mainly in the form of NAD and NADP. Niacin in plant products, is mainly in the form of nicotinic acid. As mentioned previously, NAD can be made in the body from the amino acid tryptophan, at an average conversion efficiency of 60:1 (Wahlqvist 2002) and therefore foods that are good sources of tryptophan, including eggs, milk and cheese are considered to be good sources of niacin. The niacin content of food is usually expressed as niacin equivalents (NE), which is calculated as the sum of niacin content and 1/60 tryptophan content (Rucker et al. 2001). The efficiency of tryptophan conversion is increased when niacin intake is low (Carpenter 1981). Inadequate iron, riboflavin or vitamin B6 status decreases the conversion of tryptophan to niacin (McCormick 1988).

Table A1.4 in **Appendix 1** shows the contributors to dietary intakes of niacin equivalents for Australia and New Zealand from the latest national nutrition surveys. The main contributing food groups across both countries are meat and meat products and dishes, and cereals and cereal products and dishes.

The mean absorption of niacin varies from 23-70%, depending on the food. The lowest absorption of niacin is from cereals and highest from animal products (Institute of Medicine

1998). In mature grains, most of the niacin is bound to protein although alkali treatment of grain increases availability (Carter & Carpenter 1982; Carpenter & Lewin 1985). Niacin added during enrichment or fortification is in the free form and is highly bioavailable (Institute of Medicine 1998). An aqueous solution of nicotinic acid has been shown to be almost completely absorbed in the stomach and upper small intestine at doses of 3 to 4 grams (Bechgaard and Jespersen 1977).

Once niacin is absorbed and metabolised, niacin metabolites are excreted in the urine. Two major excretion products of niacin metabolism are MeNam and 2-PY (Institute of Medicine 1998). Small amounts of 4-PY are also excreted (Bender 2003; EFSA 2014). The amount of niacin metabolites that are excreted is determined by dietary intake of niacin and tryptophan (Goldsmith et al. 1952; 1955; Jacob et al. 1989). However, 2-PY excretion decreases to a greater extent in response to a low dietary intake, and a 2-PY to 4-PY ratio of less than 1.0 is indicative of niacin deficiency.

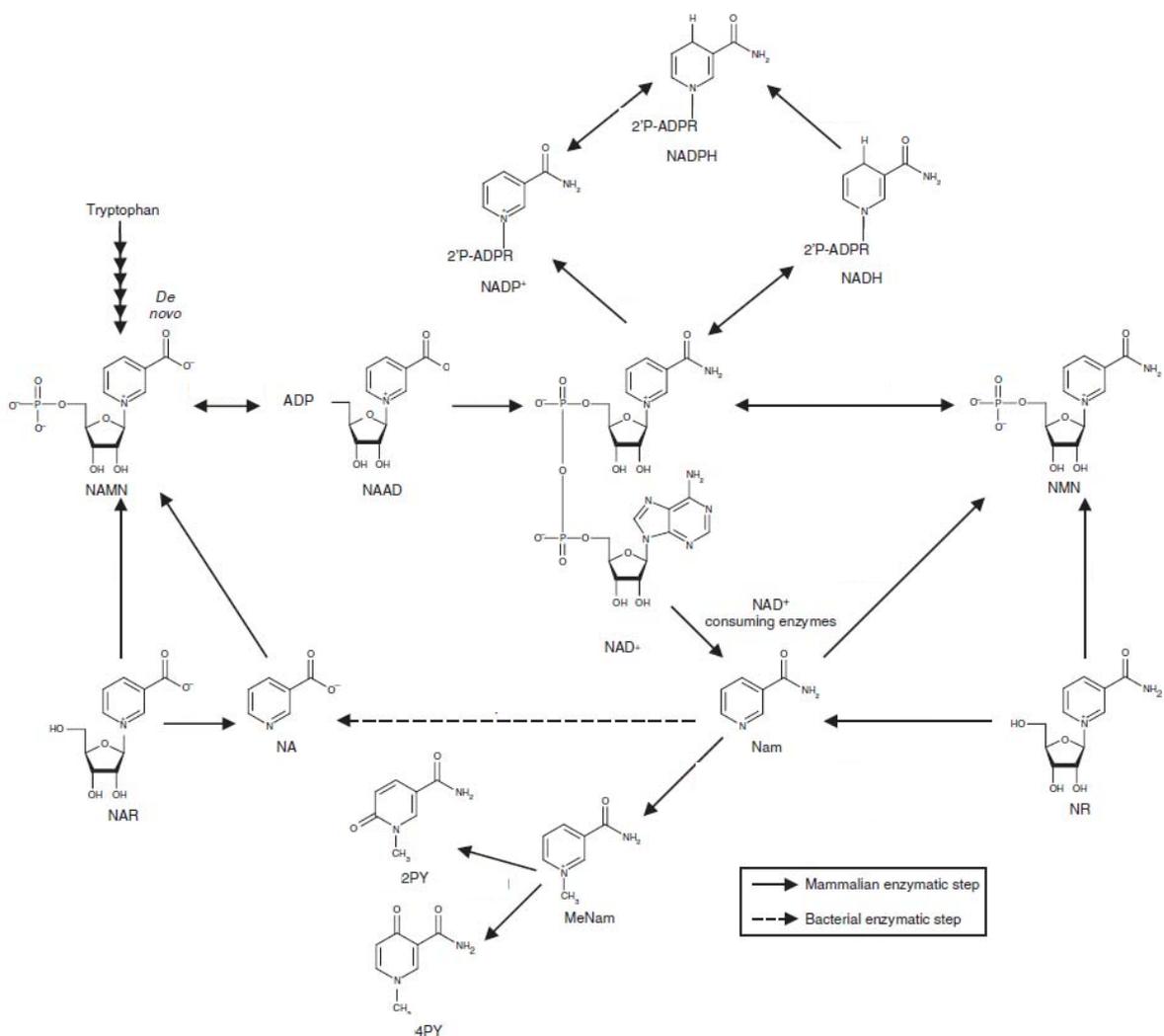


Figure 1. NAD⁺ metabolism. NAD⁺ is synthesized by salvage of the vitamin precursors nicotinic acid (NA), nicotinamide (Nam) and nicotinamide riboside (NR), or from tryptophan in the *de novo* pathway (reproduced from Trammell et al. 2016 under the Creative Commons Attribution 4.0 International Licence <http://creativecommons.org/licenses/by/4.0/>)

2.4 Bioavailability of Nicotinamide Riboside

Nicotinamide riboside (NR) is a more recently discovered precursor of NAD⁺ that is found in cow's milk and contributes to intracellular NAD⁺ concentration (Bieganowski and Brenner 2004, Tempel et al. 2007, Bogan and Brenner 2008, Chi and Sauve 2013). NR is converted to NAD⁺ via a separate three-step salvage pathway following cleavage of the ribose group to form Nam (Belenky et al. 2007) or via a two-step pathway, with the intermediate nicotinamide mononucleotide (NMN) (Bieganowski and Brenner 2004; **Figure 1**).

The Applicant provided eight studies in support of NRC as a form of niacin, including published and unpublished studies; in some cases both published and unpublished papers for the same study were available (Wilson 2015 Study No. 14NBHC and Trammell et al. 2016; Airhart et al. 2017; Avery 2017 Study No. AV151003; Guo 2018 Study No. 160312; Dollerup et al. 2018; Conze et al. 2019 and Schacter 2018 Study No. 15NRHC). In addition, FSANZ identified three additional relevant publications (Ratajczak et al. 2016; Liu et al. 2018; Martens et al. 2018).

2.4.1 Human Studies

Open-label, non-randomised study of pharmacokinetics of NRC in healthy adults. Airhart et al. 2017. Regulatory status: Not GCP¹

A non-randomised, open-label pharmacokinetics study of eight healthy volunteers (6 female and 2 male aged 33 ± 8 years) was undertaken in which NRC, manufactured by the applicant, was self-administered orally as: a single dose of 250 mg on Day 1 and 2, 250 mg twice daily on Day 3 and 4, 500 mg twice daily on Day 5 and 6, and 1000 mg twice daily on Day 7 and 8. On the morning of Day 9, subjects completed a 24 hour pharmacokinetic study after receiving 1000 mg NRC. NR and NAD⁺ concentrations were determined in blood from samples drawn on Day 1 and on Day 9 at 0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24 h. During the 24 hour pharmacokinetic standardised study meals without dairy products (a source of tryptophan) were provided. Paired Student's t tests were used to compare baseline and Day 9 average concentration at steady state values, baseline and pre-final dose concentrations and baseline and peak concentrations for NR and NAD⁺.

NR was detected in whole blood samples collected prior to treatment on Day 1, varying by 2.1 fold between subjects from 0.016 to 0.034 µmol/L. Seven subjects had an increase in whole blood NR concentration from baseline to Day 9, with levels ranging from -10% to +127%. Following the Day 9 dose, maximum whole blood NR concentrations (C_{max}) were observed at 3 hours post-dose in four participants, with negligible changes observed in NR levels in the other four participants. For the four subjects who exhibited a distinct C_{max}, a first-order elimination rate constant of 0.26 ± 0.04 h⁻¹ and a half-life of 2.7 h were calculated.

Mean fasting NAD⁺ concentrations measured at baseline were 27 ± 6 µmol/L, NADH was not detected and NMN was detected at or just above the lower limit of quantification. NAD⁺ concentrations were higher compared to baseline at Day 9 in all subjects, with the average steady state concentration on average 2-fold higher than baseline (1.34 to 2.66 fold higher), with a mean increase of 26.7 µmol/L (p = 0.001). Changes in whole blood NR and NAD⁺ concentrations were correlated highly across the 8 subjects, with the Pearson correlation coefficient of 0.85 (R² = 0.72, p = 0.008).

The authors noted the variation in the bioavailability of a 1000 mg dose of NR among individuals, with only half of participants observing an increase in NR levels of 100% or more. The author suggests several possible reasons for this including the instability of NR in the blood, degradation of NR in the gastrointestinal tract to nicotinamide and following

¹ GCP: Good Clinical Practice

absorption, metabolism to NMN, and conversion to NAD⁺ or NR. The authors suggested that further study of metabolism of NR would be required.

6-week NRC supplementation study, with randomised double-blind crossover design, in middle-aged and older adults (Martens et al. 2018). Regulatory status: Not GLP² or GCP.

A randomised, placebo-controlled, crossover trial of NRC supplementation (500 mg NRC twice daily or placebo) was undertaken for six weeks to evaluate the tolerability and efficacy for elevating NAD⁺ metabolite levels in healthy women and men. Thirty participants aged 55-79 with a mean BMI of 24 ± 4 kg/m² were randomised and 24 individuals completed the study. The concentrations of NAD⁺ and metabolites were measured in peripheral blood mononuclear cells. NAD⁺ and NAAD concentrations increased significantly in the NRC group compared to the control group (NAD⁺: 16.3 ± 13.9 vs 10.1 ± 6.81 pmol/mg protein; mean ± SD, p < 0.05; NAAD: 1.4 ± 2.14 vs 0.26 ± 0.54 pmol/mg protein, p < 0.05) however increases in NADP, Nam and NMN did not reach statistical significance. NR was not detected in either group.

Pharmacokinetics of NRC in an 8-week repeat-dose study in healthy adults. Schacter 2018; Conze et al. 2019.

A randomised double-blind, placebo-controlled four-arm parallel study was undertaken investigating the effects of NRC supplementation on urinary MeNam and other NR metabolites in the urine and blood of healthy adults.

Participants with a BMI of 25-30 kg/m² received 100 mg, 300 mg or 1000 mg NRC or placebo (microcrystalline cellulose) in four capsules each day after breakfast for 8 weeks. Subjects were instructed to avoid foods that contain high amounts of vitamin B3 or tryptophan during the two week run-in and eight week test periods, and to maintain current levels of physical activity. No significant differences in demographics, anthropometric measurements or vital signs were observed between treatment groups. Fasting blood and urine samples were taken on Day 0, 7, 14, 28 and 56 for NR metabolite analysis. One hundred and forty adults were analysed in the intention to treat (ITT) population and 128 in the per protocol (PP) population.

In the ITT population, the urinary MeNam concentration increased in a dose dependent manner at eight weeks in the 100 mg, 300 mg and 1000 mg dose groups compared to placebo, reaching significance in the 300 mg and 1000 mg dose groups (placebo: 4.1 ± 1.9; 100 mg: 6.6 ± 4.9; 300 mg: 10.6 ± 16.1; 1000 mg: 17.8 ± 8.8 ng/μg creatinine).

Whole blood NAD⁺ concentration increased in the ITT population for the three NR groups in a dose-dependent manner compared to placebo at week 1, 2 and 8, reaching significance in the 300 mg and 1000 mg groups.

Dose-dependent increases in plasma Nam concentration were observed with increasing doses of NRC in the ITT populations, reaching significance in the 1000 mg group compared to placebo (approximately 44 vs 22 ng/mL). Urinary 2-PY concentration increased in a dose-dependent manner at eight weeks in the ITT population, reaching significance in the 300 mg and 1000 mg dose groups compared to placebo (placebo: 15 ± 7 ng/μg creatinine; 100 mg: 30 ± 24; 300 mg: 51 ± 55; 1000 mg: 113 ± 51).

Similar results to those for the ITT population were observed in the PP population.

² GLP: Good Laboratory Practice

12-week NRC supplementation study, with randomised double-blind design, in obese men (Dollerup et al. 2018). Regulatory status: Not GCP

An investigator-initiated block randomised, double-blinded, placebo controlled parallel clinical trial was undertaken of 40 healthy, sedentary Caucasian men with a BMI > 30 kg/m², aged between 40 and 70 years. Participants were randomly assigned to receive an oral supplement of NRC (1000 mg twice daily or placebo) for 12 weeks. First morning urine was collected 12 hours after the final NR dose for quantification of NR and NAD-derived metabolites and normalised to creatinine concentrations. Urinary NR metabolites were compared using the unpaired 2-sample t test. NR supplementation significantly increased all NAD⁺ and NR-derived metabolites ($p < 0.01$) as shown in **Table 1** for β -NR, Nam, MeNAM, nicotinamide oxide (Nam oxide), 2-PY, 4-PY and nicotinic acid riboside (NAR).

Table 1. Urinary NAD⁺ metabolite concentration (mean \pm SEM normalised to creatinine concentration) after 12 weeks with placebo or 1000 mg NRC twice daily (Dollerup et al. 2018)

| Analyte | Urine concentration ($\mu\text{mol/g creatinine} \pm \text{SEM}$) | |
|-------------|---|-----------------|
| | Test group | Placebo group |
| β -NR | 0.41 \pm 0.02 | 0.32 \pm 0.01 |
| Nam | 4.51 \pm 0.64 | 1.41 \pm 0.08 |
| MeNam | 483 \pm 52.3 | 40.3 \pm 2.01 |
| Nam oxide | 12.3 \pm 1.61 | 1.50 \pm 0.22 |
| 2-PY | 738 \pm 80.5 | 73.8 \pm 10.1 |
| 4-PY | 191 \pm 20.1 | 16.8 \pm 0.84 |
| NAR | 0.73 \pm 0.15 | 0.01 (No SEM) |

Randomised double-blind crossover study of pharmacokinetics of single doses of NRC in healthy human subjects (Unpublished study, Wilson (study director) 2015, published as part of Trammell et al. 2016).

A single-centre randomised, double-blind crossover study was undertaken using GCP to determine the pharmacokinetics of NRC and its metabolites after a single dose of either 100 mg, 300 mg or 1000 mg NRC (Wilson 2015 unpublished; Trammell et al. 2016). No placebo group was used in the study. Twelve healthy subjects (6 male and 6 female) received each dose with a seven day washout between doses. NR and metabolites including Nam, MeNAM, NAD, NADP, NAR, 2-PY and 4-PY were measured in urine before the first dose and at 0-6, 6-12, and 12-24 h following dosing. Measurements in plasma and white blood cells (WBCs) were taken pre-dose and 1, 2, 4, 8, 12 and 24 h post-dose. Pharmacokinetic parameters including pre-dose corrected area under the curve $\text{AUC}_{0-24 \text{ hr}}$ ($\mu\text{mol.hr/L}$), maximum observed concentration C_{max} ($\mu\text{mol/L}$) and time of maximum concentration T_{max} (h) were calculated for each subject-dosage combination.

Plasma NR concentration did not significantly change in any group compared to pre-dose levels at any measured time point ($p > 0.05$). Urinary NR concentrations significantly decreased compared to pre-dose levels in all dose groups at most time points ($p < 0.05$).

Relative urinary Nam concentration increased significantly in the 1000 mg group compared to pre-dose levels ($p < 0.05$) but decreased in the other dose groups compared to pre-dose levels.

Dose-dependent increases in MeNam concentration were observed in plasma (C_{max} 0.22 \pm 0.19, 0.43 \pm 0.33, 0.89 \pm 0.62 $\mu\text{mol.h/L}$; $p < 0.001$) and in white blood cells. Urinary MeNam concentrations (standardised by creatinine) increased significantly in the 1000 mg group compared to pre-dose, but not in the 100 mg or 300 mg groups.

2-PY C_{max} increased in a dose-dependent manner in plasma (3.5 ± 1.3 , 6.6 ± 2.0 , 17.0 ± 5.0 $\mu\text{mol/L}$ respectively, $p < 0.001$) and white blood cells (2.7 ± 1.5 , 6.4 ± 5.8 , 12.9 ± 7.2 $\mu\text{mol/L}$; $p < 0.001$).

A dose-response relationship was observed between NRC dose and 4-PY concentrations in plasma and WBC. Plasma 4-PY C_{max} increased with increasing doses of NR : 0.44 ± 0.35 , 0.90 ± 0.37 , 2.52 ± 0.64 $\mu\text{mol/L}$ respectively for the 100 mg, 300 mg and 1000 mg doses ($p < 0.001$) and WBC 4-PY C_{max} : 0.42 ± 0.22 , 0.94 ± 0.59 , 1.91 ± 1.22 $\mu\text{mol/L}$ respectively ($p < 0.001$).

Urinary NAD concentrations decreased in all groups at all measured time points compared to pre-dose levels. Plasma and WBC NAD concentrations did not show a consistent pattern compared to pre-dose levels across groups, and were not significantly different at most time points ($p > 0.05$).

White blood cell and urine NADP concentrations standardised by creatinine were not significantly different to pre-dose levels in all NRC groups at any time point ($p > 0.05$). White blood cell NAAD concentrations increased in all groups 4 and 8 hours after dosing compared to pre-dose levels, reaching significance at 4 and 8 hours in the 300 mg group, and at 24 hours in the 1000 mg group.

Urinary NMN concentration standardised by creatinine decreased compared to pre-dose levels in all groups, reaching significance at most time points up to 12 hours ($p \leq 0.003$). White blood cell NMN concentrations did not show a consistent pattern compared to pre-dose levels across groups, and were not significantly different at any time points ($p > 0.05$).

Urinary NAR concentration standardised by creatinine increased in all groups compared to pre-dose levels 6-24 hours after dosing ($p < 0.05$). Plasma NAR concentration was higher in the 300 mg and 1000 mg groups 8 hours after dosing ($p < 0.05$), with AUC_{0-24h} 0.48 ± 0.01 , 0.62 ± 0.19 and 1.22 ± 1.00 ; $p = 0.002$.

Seven day repeat-dose study in a human volunteer (Trammell et al. 2016) Regulatory status: Not GCP

A healthy 52-year-old man, weighing 65 kg, took nicotinamide riboside chloride each morning prior to breakfast, for seven consecutive days. The dose level was reported to be 1000 mg, however it is not clear whether the dose was 1000 mg NRC, or adjusted so that he consumed 1000 mg nicotinamide riboside. Pre-supplementation blood and urine samples were collected. On the first day of study, blood was collected prior to dose ingestion and at 0.6, 1, 1.14, 2.7, 4.1, 7.7 and 8.1 h post dose. Blood was also collected 24 h after the first and the last dose. No changes in NAD^+ or metabolites were observed in the first 2.7 h, but from 4.1 h after the first dose, NAD^+ was increased in peripheral blood mononuclear cells by a factor of 2.3, and a number of metabolites of NAD^+ (including NMN, Nam, 4-PY, MeNam, NAAD, NAD^+ , NADP^+ , 2-PY and ADPR) also increased, peaking between 5 and 10 h after dose ingestion.

2.4.2 Animal Studies

Toxicokinetics assessment of NRC in rats. Avery (2017).

A toxicokinetic analysis of plasma Nam and MeNam concentrations was undertaken in a 90-day study in rats gavaged with NRC (1000 mg/kg bw/day) or a molar equivalent of nicotinamide (420 mg/kg bw/day). Blood was collected before dosing and 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dosing.

On Day 1, Nam and MeNam concentrations increased in both treatment groups 30 min after dosing. Nam concentration peaked later in the NRC group compared to the Nam treatment group in both males and females (T_{max} males: 6.0 ± 0.0 hr vs 0.67 ± 0.17 ; T_{max} females: 6.7 ± 0.7 hr vs 1.0 ± 0.0 hr), with the C_{max} lower in the NRC group compared to the Nam treatment group (C_{max} males: 154.8 ± 13.9 vs 249.8 ± 8.6 $\mu\text{g/mL}$; C_{max} females 118.7 ± 1.6 vs 385.4 ± 15.6 $\mu\text{g/mL}$). Systemic exposure to Nam on Day 1 was lower in the NRC-treated rats compared to the Nam group (AUC_{last} males: 1271.3 ± 121.7 vs 1713.0 ± 26.1 $\text{h}^*\mu\text{g/mL}$; AUC_{last} females: 882.4 ± 109.3 vs 1872.7 ± 56.8 $\text{h}^*\mu\text{g/mL}$). Similar plasma MeNam maximum concentrations were observed in the NRC and Nam groups (C_{max} males: 4.4 ± 1.3 vs 3.5 ± 0.2 (mean \pm SEM) $\mu\text{g/mL}$; C_{max} females: 2.6 ± 0.2 vs 2.5 ± 0.1 $\mu\text{g/mL}$). Systemic exposures to MeNam were also similar in both groups (AUC_{last} males: 62.5 ± 3.0 $\text{h}^*\mu\text{g/mL}$ vs 70.2 ± 1.2 $\text{h}^*\mu\text{g/mL}$; AUC_{last} females: 39.4 ± 0.9 vs 41.2 ± 0.9 $\text{h}^*\mu\text{g/mL}$ in the NRC and Nam groups respectively). Plasma MeNam concentrations peaked earlier in the NRC group compared to the Nam group (T_{max} males: 7.0 ± 3.2 vs 10.7 ± 1.3 hr; T_{max} females: 1.5 ± 0.5 hr vs 6.2 ± 3.3 hr).

Nam systemic exposure was lower in the NRC-treated rats compared to the Nam group (AUC_{last} males: 2222.5 ± 113.8 vs 4146.5 ± 132.6 $\text{h}^*\mu\text{g/mL}$; AUC_{last} females: 1931.5 ± 80.8 vs 3118.5 ± 69.8 $\text{h}^*\mu\text{g/mL}$). However, the maximum concentration of MeNam was similar for NRC and Nam groups on Day 90.

Additional animal studies were provided for assessment but cannot be disclosed because it is confidential commercial information (CCI). The toxicokinetics of NRC was adequately demonstrated.

In vitro and in vivo studies of nicotinamide riboside kinetics (Ratajczak et al. 2016)
Regulatory status: Not GLP

The test article for the studies reported in this paper was NRC, but it was manufactured by Biosynth (now Biosynth Carbosynth, Itasca, IL, USA) rather than by the applicant.

Mice with a constitutive ablation of the *Nrk1* gene were generated, and primary hepatocytes from these mice were compared *in vitro* to those from wild-type (WT) mice of the same ancestry. Levels of NAD^+ in hepatocytes of the knockout mice increased when the cells were treated with nicotinamide, but not when the cells were treated with nicotinamide riboside or NMN. In contrast, levels of NAD^+ in hepatocytes of the WT mice increased with all treatments. Thus, *Nrk1* is required for utilization of exogenous nicotinamide riboside and NMN. Decreased, but not ablated, ability to utilize exogenous nicotinamide riboside and NMN, relative to that of WT mice, was also demonstrated in the knockout mice *in vivo*, following intraperitoneal injection of the test articles. Tissues in which the effect was demonstrated were liver, kidney and brown adipose tissue. It was demonstrated that some of the nicotinamide riboside and NMN was converted to Nam in the peritoneal cavity.

Single-dose toxicokinetics findings in C57BL/6 mice (Liu et al. 2018) Regulatory status: Not GLP.

Dosing of mice occurred as part of a series of experiments to clarify the metabolism of NAD^+ . NR was synthesised in the laboratory in which the experiments were carried out, and was radiolabelled on both the nicotinamide and riboside moieties. The test system comprised female C57BL/6 mice, 12 to 14 weeks old at the time of dosing. They were maintained under standard laboratory conditions of environment and husbandry. The mice were pre-catheterised at the time of purchase. Initially, NR was administered at a dose of 50 mg/kg bw, either by intravenous (IV) bolus or by oral gavage. Intact nicotinamide riboside was detected in the blood following IV administration but not following oral administration.

Postmortem examination of the tissue labelling showed that orally administered NR was incorporated into liver NAD⁺, whereas other tissues contained minimal radiolabelled NAD⁺. In contrast, when radiolabelled NR was administered IV, label was detected in liver and kidney. The experiment was repeated at doses of 200 and 500 mg/kg bw NR, but the results were the same. In the brain, only NAD⁺ with a single label was detected, whereas nicotinamide with both labels was assimilated into NAD⁺ in skeletal muscle. The authors concluded that when NR is administered orally, it is largely converted to NMN on first pass in the liver. They further concluded that NR does not cross the brain-blood barrier, and the brain is reliant on circulating NMN.

Bioavailability studies in mice (Trammell et al. 2016) Regulatory status: Not GLP

The nicotinamide riboside used in these assays was the chloride salt and was produced under GMP conditions. Mice were male C57Bl/6J mice, kept under standard laboratory conditions. In the first single-dose study, mice were gavaged with NRC at 185 mg/kg bw (n=3/timepoint), or an equimolar dose of either NA (n=4/timepoint) or Nam (n=4/timepoint). The vehicle was saline, and a control group (n=4/timepoint) were gavaged with the vehicle. Mice were dosed 0.25, 1, 2, 4, 6, 8 and 12 h prior to being killed at approximately the same time of day, to prevent circadian rhythm effects. The medullary lobe of the liver was freeze-clamped at liquid nitrogen temperature and stored at -80°C until analysed for hepatic NAD⁺. NA produced a rapid increase in hepatic NAD⁺, with a doubling of hepatic NAD⁺ in approximately 15 min. Oral Nam led to an increase in hepatic NAD⁺ that commenced after 2 h and peaked at 8 h. The AUC of hepatic NAD⁺ generated in response to oral gavage with Nam was approximately 50% greater than that generated in response to NA. Oral gavage with NRC led to the greatest increase in hepatic NAD⁺; a fourfold increase peaking at 6 h after gavage. Hepatic concentrations of 13 NAD⁺ metabolites were also quantified, with each precursor producing a temporally distinct pattern of hepatic NAD⁺ metabolites.

In the second study, mice (15/group) were gavaged with 185 mg NRC/kg bw. The NRC was radiolabelled with ¹³C on the nicotinamide moiety and deuterium on the riboside moiety. Mice (n=3) were killed at 0, 2, 4, 6 and 8 h after dosing. At 2 h, 54% of the NAD⁺ and 32% of the NADP⁺ contained at least one radiolabel, and 5% and 6% respectively had both radiolabels. Radiolabel was also found in hepatic NAAD with similar percentage and time-course as for NAD⁺.

A repeat-dose study in mice (n=8) was also performed in which mice were intraperitoneally injected with NCR (500 mg/kg bw body weight) or saline for 6 days. Livers and hearts were freeze-clamped and prepared for metabolomics analysis. Concentrations of NAD⁺ metabolites (NMN, NAD⁺, NADP⁺, Nam, MeNam, 4-PY, NAMN, NAAD and ADPR) were significantly higher in liver, and significantly higher in heart samples, with the exception of NAD⁺, NADP⁺ and ADPR (p < 0.05).

2.4.3 In vitro studies

An unpublished in-vitro study of nicotinamide riboside metabolism in human blood measured the effect of the enzyme NR phosphorylase on nicotinamide riboside concentration in human blood (Guo et al. 2018). NR phosphorylase that is present in red blood cells (Grossman and Kaplan 1958) hydrolyses NR to nicotinamide and ribose-1-phosphate (Rowen and Kornberg 1951). Water, human whole blood, heparinised plasma and serum were spiked with ¹³CD-NR, in which the nicotinamide portion was labelled with ¹³C and the riboside portion labelled with deuterium. Samples were incubated at 4 °C or 37 °C for 0, 5 and 10 min, after which time the ¹³CD-NR and ¹³C-nicotinamide were quantified by LC-MS/MS.

Following incubation the concentration of ¹³CD-NR was similar in the heparinised plasma, serum and water samples compared to the pre-incubation levels at both time points, but the

¹³CD-NR peak area ratio of the heparinised whole blood samples decreased over time. The ¹³C-nicotinamide concentration was also similar in the heparinised plasma, serum and water samples compared to pre-incubation levels, but increased in the whole blood samples over time. Faster reaction kinetics of the decrease in ¹³CD-NR and increase in ¹³C-nicotinamide were observed at 37°C compared to 4°C in the whole blood sample.

2.5 Discussion and Conclusion

Niacin functions as a source of NAD⁺ in the body that is required for a range of cellular functions. Currently, nicotinic acid and nicotinamide are forms of niacin that are added to certain foods. Several studies have shown that NRC can be metabolised to NAD⁺ via two separate pathways (Rowen and Kornberg 1951, Bieganowski and Brenner 2004, Belenky et al. 2007, Bogan and Brenner 2008, Chi and Sauve 2013).

In order to determine the bioavailability of NRC compared to other forms of niacin FSANZ considered the body of evidence on the effect of NRC supplementation on the concentration of NAD⁺ and metabolites in blood and urine. Six human studies measured the pharmacokinetics of NRC oral supplementation in healthy individuals and its effect on blood and urine NRC metabolites including NAD⁺ compared to either pre-dose levels, placebo or varying concentrations of NRC. Duration of supplementation varied from 7 days to 12 weeks, with one crossover study measuring blood and urine metabolite concentrations for 24 hours following supplementation (Wilson 2015).

Five studies measured the effect of NRC supplementation on several NAD⁺ metabolite concentrations in human blood fractions, with concentrations increasing compared to baseline or placebo in all studies and reaching statistical significance in four studies (Wilson 2015, Airhart et al. 2017, Martens et al. 2018, Schacter 2018). Two of the three studies that compared different doses of NRC observed a dose-response relationship between NRC dose and NAD⁺ metabolite concentration in blood (Wilson 2015, Schacter 2018). Urine concentrations of NAD⁺ metabolites were in general consistent with results in human blood.

NRC has been shown to contribute to hepatic NAD⁺ levels in mice (Ratajczak et al. 2016; Liu et al. 2018), and increase in plasma MeNam concentrations in other animal studies which cannot be disclosed because it is CCI. NR is metabolised to Nam and riboside in human whole blood (Guo 2018).

No human studies were identified that compared the effects of NRC supplementation to NA or Nam supplementation. A 90-day rat study showed that similar doses of NRC or Nam caused an elevation of plasma Nam and MeNam. Similar plasma concentrations of MeNam were observed in the two treatment groups, however the maximum plasma concentration (C_{max}) and total systemic exposure (AUC) to Nam was higher (approximately 1.6- to 1.8-fold) in the Nam group compared to the NRC group. Plasma MeNam levels peaked earlier in the NRC group than in the Nam group.

The Applicant requests the use of NRC as a source of niacin in foods for special medical purposes (FSMP). As previously discussed, FSMPs are used to manage the diets of people with certain diseases, disorders or medical conditions and are used under the supervision of a medical practitioner ([FSANZ 2016](#)). The body of evidence for the effect of NRC on NAD⁺ metabolite concentration in humans related to healthy individuals, however the FSMP formulation can be modified by the supervising medical practitioner in cases where vitamin malabsorption is suspected. FSANZ notes that foods for special medical purposes do not extend to therapeutic goods which are used to treat or cure a medical condition.

No studies were identified to indicate that NRC would inhibit the absorption of other nutrients.

The doses of NRC used in the human trials ranged from 100 to 2000 mg/d (42 to 840 mg/d of niacinamide assuming equimolarity); the lowest dose being several fold higher than the Recommended Dietary Intake of niacin (14 mg/d for women and 16 mg/d for men). At these high intakes of NRC, tissue concentrations of the bioavailable forms of niacin increase markedly and would be expected to support normal physiological function. FSANZ could not find any published or registered trials in humans which included a comparator group taking Nam or NA. In the absence of human studies to establish the bioavailability of NRC compared to already permitted forms of niacin, FSANZ cannot judge with certainty the extent to which lower NRC intakes which match adult Recommended Dietary Intakes for niacin (14 mg/day in women, 16 mg/day in men) would support essential requirements, when it is the only form of vitamin B3 in the diet.

3 Safety Assessment

3.1 Objectives for the safety assessment

Objectives for the safety assessment include the following:

- To determine whether the information provided is of sufficient scope and quality to assess the safety of NRC
- To determine the maximum safe level of intake of NRC for age groups over 1 year of age
- To determine whether extrapolation of the safety data from adults to children and adolescents is appropriate
- To determine what side effects may be expected from supplementation of FSMP with NRC.

3.2 Background

3.2.1 Evaluation of the submitted data

FSANZ has assessed the submitted evidence on the safety of NRC, and information from other sources. The assessed data include information on toxicity in laboratory animals, genotoxicity, and human tolerance studies. The data are considered suitable to assess the hazard of NRC.

3.2.2 Characteristics of nicotinamide riboside chloride

The chemical characteristics of NRC are described in Section 4.

3.3 Toxicological data

Except where otherwise stated, studies reviewed as part of this safety assessment were conducted using the NRC, manufactured by the applicant for commercial production, that is the subject of this application.

Kinetics and metabolism of NRC are reviewed in Section 2.3.

3.3.1 Acute toxicity studies in animals

Acute oral toxicity study of NRC in Sprague Dawley rats. Unpublished study report, Bhoite (study director) 2014; published as part of Conze et al 2016. Regulatory status: GLP, compliant with USFDA-CDER Guidance

Sprague Dawley rats, aged 6-8 weeks at the start of treatment, were housed 2-3/cage under standard laboratory environmental and husbandry conditions, to which they were acclimatized for 5 days prior to the start of the study. Rats were assigned, 5/sex/group, to either the vehicle control group and a test group. The vehicle/control article was purified water, and stability of the test article in water over the dosing period was established prior to Day 1. Rats were fasted overnight prior to dose administration on Day 1, at a dose volume of 10 mL/kg bw, and a dose of 0 or 5000 mg NRC/kg bw.

Rats were subject to cageside examinations at 30 min, 1,2,3 and 4 h after dose administration, and twice daily for 14 days. Detailed clinical examinations were conducted prior to dose administration on Day 1, and on Days 8 and 15, and body weights were recorded according to the same schedule. Food consumption was recorded weekly from Day 1 to the end of the in-life phase. Rats were killed on Day 15 and gross necropsies were performed.

All rats survived to the end of the study, no abnormal clinical signs were observed, there were no treatment-related effects on group mean values for food consumption, and no gross pathological lesions were found in any rats. The group mean cumulative body weight gain of treated females was significantly lower than that of control females, although the group mean value for Day 15 bodyweight of treated females was only 3% lower than that of control females. The lower cumulative body weight gain was considered to be treatment-related but not adverse. No corresponding change was observed in group mean values for cumulative body weight gain or Day 15 bodyweight in males.

It was concluded that the acute lethal oral dose of NRC in the Sprague Dawley rat is >5000 mg/kg bw.

3.3.2 Short-term toxicity studies in animals

14-day repeat-dose oral toxicity study of NRC in Sprague Dawley rats. Unpublished study report, Bhoite (study director) 2014; published as part of Conze et al 2016. Regulatory status: Not GLP

Rats used in this study were 6 to 7 weeks of age at the start of treatment. They were housed 2-3/cage under standard laboratory environmental and husbandry conditions, to which they were acclimatized for 5 days prior to the start of the study. They were assigned to five groups comprising 5/sex/group. Stability of the test article in solution in purified water, the vehicle and control article, was established, and concentration and homogeneity of the Day 1 dose formulations was confirmed by analysis. Rats were gavaged daily for 14 days with NRC at 0, 750, 1500, 2500 or 5000 mg/kg bw, at a dose volume of 10 mL/kg bw.

Rats were subject to twice-daily mortality/moribundity checks, and daily clinical observations. Detailed clinical examinations were conducted on Day 1 prior to dose administration, and on Days 8 and 14. Body weights were recorded prior to treatment on several days, and also recorded prior to killing on Day 15. Food consumption was recorded on several days and finally on Day 15. Rats were killed on Day 15 and were subject to gross necropsy.

All rats survived to the end of the in-life phase and there were no abnormal clinical signs observed. There were no gross lesions on necropsy of any rat.

Statistically significant decreases in group mean bodyweight values, as compared to the control group of the same sex, were observed in male rats dosed with ≥ 2500 mg/kg bw/day, on Days 8, 11, 14 and 15. Decreases relative to the male control values on the same days were 7-8% for 2500 mg/kg bw/day males, and 8-9% for 5000 mg/kg bw/day males. In 5000 mg/kg bw/day males, the group mean value for overall food consumption was decreased by 8% relative to that of male controls, but the difference was not statistically significant.

There were no treatment-related effects of group mean values for bodyweight gain or food consumption in female rats.

The study director concluded that the decreases in group mean bodyweights were treatment-related but not adverse.

90-day repeat-dose oral toxicity study of NRC and Nam in Sprague Dawley rats. Unpublished study, Bhoite (study director) 2015; published as part of Conze et al 2016. Regulatory status: GLP; OECD guidelines.

The positive control article for this study, Nam, was purchased from Sigma-Aldrich.

Rats ranged in age from 41-55 days at the start of treatment. Rats were housed 1-2/cage under standard laboratory conditions of environment and husbandry. After 5-6 days of acclimatisation, they were assigned to five core cohort groups of 10/sex/group and two toxicokinetics (TK) cohort groups of 9/sex/group. The main cohort groups were vehicle control, positive control, and 300, 1000 or 3000 mg/kg bw/day NRC. The vehicle was purified water, in which both NRC and Nam were completely soluble. Nam was administered to the positive control group at a dose level of 1260 mg/kg bw/day, equimolar to the high dose group. The TK groups were administered either 420 mg/kg bw/day Nam, or 1000 mg/kg bw/day NRC, on an equimolar basis. Formulations were used within the pre-established stability period, and formulation concentrations were verified by analysis in Weeks 1, 7 and 13. All rats were gavaged at a constant dose volume of 10 ml/kg bw.

Observations included twice-daily mortality/moribundity checks, daily cageside observations, and weekly detailed clinical observations. Ophthalmoscopic examinations were also conducted. Body weights were recorded on Day 1 prior to dosing, and weekly thereafter, and feed consumption was recorded weekly according to the same schedule. Urine for analysis was collected overnight from core cohort rats prior to necropsy. A fasted terminal bodyweight was recorded prior to killing each rat on the last day of the in-life phase, and blood was collected for assessment of haematology, coagulation parameters and clinical chemistry. Core cohort rats were killed by exsanguination while under anaesthesia, and subject to gross necropsy. Fresh weights were recorded for adrenals, brain, heart, kidneys, liver, pituitary, spleen, thymus, thyroid/parathyroid. Fresh weights were also recorded for epididymides, testes and accessory sex glands of males, and ovaries and uteri of females. Paired organs were weighed together. A comprehensive list of organs and tissues from core cohort rats were fixed and processed for histopathology. Initial microscopic examination of the negative control, positive control and 3000 mg/kg bw/day groups was followed by examination of liver, kidneys, thyroids, testes, epididymides, ovaries and adrenals from all other core cohort rats.

There were no treatment-related effects on survival, clinical signs, ophthalmology findings, coagulation parameters, or urinalysis findings. There were no adverse findings of any kind in the 300 mg/kg bw/day group. The mid dose, 1000 mg/kg bw/day, was identified as the lowest observed adverse effect level (LOAEL) on the basis of statistically significant increases in group mean values for leucocytes (21% increase) in males and in ALT (36% increase), and triglycerides (65% increase) in females. When compared to group mean values for negative control rats of the same sex, treatment with 3000 mg/kg bw/day NRC and with 1260 mg/kg bw/day Nam was associated with decreased food consumption and significant (>10%) reduction in group mean bodyweights of males; significant increases in group mean values for neutrophils in both sexes; significant increases in group mean values for ALT, ALP and triglyceride in both sexes; significant increases in group mean values for AST and gamma glutamyl transferase (GGT) in females; significant increases in group mean values for relative weight of liver and kidneys in both sexes; significant increases in absolute weights of testes and epididymides in males; and significant increases in relative weights in ovaries in females. Both 3000 mg/kg bw/day NRC and 1260 mg/kg bw/day Nam were also associated with increased incidences in both sexes of hepatocellular hypertrophy, thyroid follicular cell hypertrophy, chronic progressive nephropathy, and hypertrophy of the zona glomerulosa of the adrenals; increased incidences in females of single-cell hepatocyte necrosis and hypertrophy of corpora lutea; and in males, increased incidences of degeneration/atrophy of tubules in the testes, increased luminal debris in the epididymides, and reduced numbers of luminal sperm. Results from the TK cohorts are presented in Section 2.3.2.

It was concluded that 300 mg/kg bw/day was the no observed adverse effect level (NOAEL), and 1000 mg/kg bw/day the LOAEL. It was noted that NRC and Nam had the same adverse effects at equimolar doses of 3000 mg/kg bw/day NRC and 1260 mg/kg bw/day Nam.

Additional animal studies were provided for assessment but cannot be disclosed because it is CCI. The toxicological assessment supports the NOAEL stated above of 300 mg/kg bw/day.

3.3.3 Long-term toxicity/carcinogenicity studies in animals

No long-term studies of NRC in animals were submitted by the applicant or located by literature search.

3.3.4 Genotoxicity assays

Genotoxicity assays of NRC include a bacterial reverse mutation assay (Ames test), a chromosomal aberration assay in human peripheral blood lymphocytes, and a micronucleus assay in rats.

Bacterial reverse mutation assay of NRC. Unpublished study report, Kamath (study director) 2015; published as part of Conze et al 2016. Regulatory status: GLP; compliant with Guideline OECD 471.

Test strains used for this assay were *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA 1537, and *Escherichia coli* strain WP2 uvrA. The vehicle and control article was water.

In the first mutagenicity assay, performed using the plate incorporation method, no positive mutagenic responses were observed with any of the test strains, in either the presence or absence of S9 activation. Dose levels tested were 50, 159, 501, 1582 and 5000 µg/plate. Similarly, in a confirmatory mutagenicity assay performed using the pre-incubation method, no positive mutagenic responses were observed with any of the test strains in either the presence or absence of S9 activation. Dose levels tested were 99, 265, 699, 1869 and 5000 µg/plate.

Standard positive control articles were used as appropriate for the strains and presence or absence of S9 mix, and the expected increases in mutations were observed, confirming the validity of the assays.

It was concluded that NRC is not mutagenic in the bacterial reverse mutation assay.

Chromosomal aberration assay of NRC using human peripheral blood lymphocytes. Unpublished study, Kamath (study director), 2016; published as part of Conze et al, 2016. Regulatory status: GLP; compliant with OECD Guideline 473

Human peripheral blood lymphocytes (HPBLs) were obtained from a healthy non-smoking donor, approximately 35 years old. The vehicle and control article was water, in which NRC was fully soluble at 500 mg/mL, the highest concentration used. NRC was tested in the presence and absence of rat liver S9.

During both the preliminary toxicity assay and mutagenicity study, the HPBLs were treated in the presence (3-6 hours) and absence of S9 activation (3-6 hours and 20-22 hours). The concentration levels tested in the preliminary study were 0.15625, 0.3125, 0.625, 1.25, 2.5 and 5 mg/mL. Neither precipitate nor toxicity were observed at any concentrations tested.

Therefore, in the mutagenicity assay NRC was tested at 1.25, 2.5 and 5 mg/mL. No precipitate, toxicity or positive mutagenic responses were observed in either the presence or absence of S9. Appropriate positive control articles induced the expected increases in chromosomal aberrations, confirming the validity of the assay.

It was concluded that NRC did not cause chromosomal aberrations under the conditions of the assay.

In vivo mammalian erythrocyte micronucleus test of NRC in Sprague Dawley rats. Unpublished study report, Pandey (study director) 2016; published as part of Conze et al 2016. Regulatory status: GLP, compliant with OECD Guideline 474

In the mutagenicity study, rats, approximately 6 weeks old at time of receipt, were acclimatized to standard laboratory environmental and husbandry conditions for 6 days before assignment to groups of 6/sex/group. In addition to a negative control group (water) and a positive control (cyclophosphamide) group, groups included three groups dosed at levels of 500, 1000 and 2000 mg/kg bw and killed after 24 h, and one group dosed with 2000 mg/kg bw and killed after 48 h. Bone marrow analyses of rats treated with NRC showed no signs of significant increases in the number of micronuclei compared to the negative control group. Bone marrow analysis of rats treated with cyclophosphamide showed the expected increase in number of micronuclei, confirming the validity of the assay.

It was concluded that NRC did not induce micronuclei under the conditions of the assay.

3.3.5 Developmental and reproductive studies in animals

Developmental toxicity study of NRC in Sprague Dawley rats. Unpublished study, Rao (study director), 2016. Regulatory status: GLP; compliant with OECD guideline 414.

Groups of pregnant rats (24/group) were gavaged daily with doses of 325, 750 or 1500 mg/kg bw/day from Gestation Days 5 to 19. Toxicological endpoints in dams included clinical signs, mortality, morbidity, body weight, food consumption and gross pathological findings on necropsy after caesarean section. Fetuses were examined for sex, weight and external, visceral and skeletal malformations.

The maternal NOAEL was identified as 325 mg/kg bw/day on the basis of significant decreases in bodyweight and food consumption at doses ≥ 750 mg/kg bw/day. The fetal NOAEL was identified as 750 mg/kg/day on the basis of decreased fetal bodyweights at 1500 mg/kg bw/day, together with increases in incidence of abnormalities commonly observed in association with maternal toxicity.

One-generation reproductive dietary toxicity in Sprague Dawley rats. Unpublished study, Ganiger (study director) 2016. Regulatory status: GLP; Compliant with OECD guideline 415.

Rats were assigned, 25/sex/group, to four groups and provided with diets containing 0, 3000, 6000 or 12000 ppm NRC in the diet, which was supplied *ad libitum*. Clinical examinations were performed and body weights and food consumption of P generation females was measured.

At birth, all pups, both dead and alive, were examined, sexed and weighed, and the P generation rats were subject to detailed necropsies. Histopathological examination was carried out on reproductive organs of P generation rats.

All rats survived to the end of the in-life phase and no treatment-related clinical signs were observed. There were no treatment related effects on body weights or food consumption of P

generation females, or on gestation length, pre-coital time, fertility index, survival indices of pups, appearance of pups on PND 1, gross findings on necropsy of P generation rats or pups, or histopathological findings of P generation rats.

A statistically significant decrease in group mean bodyweight, as compared to that of male controls, was observed during part of the study in P generation males in the 12000 ppm group. The lower group mean bodyweights were considered non-adverse.

It was concluded that the NOAEL for fertility and reproductive performance was 12000 ppm in the parental diet. Based on food consumption and bodyweight data, this was equivalent to 675.2 mg/kg bw/day in P generation males and 1088.4 mg/kg bw/day in P generation females. The NOAEL for prenatal and postnatal survival and development of pups was the same as the maternal NOAEL.

3.3.6 Human tolerance studies

A randomised double-blind crossover study of pharmacokinetics of single doses of NRC in healthy human subjects, conducted under GCP conditions, is summarised in Section 2.3.1 (unpublished study report of Wilson 2015; published as Trammell et al 2016). There were 12 (6/sex) participants in the study. Doses of NRC were 100, 300 and 1000 mg NRC. Doses were taken in capsule form. Doses of ≤ 1000 mg NRC had no effects on heart rate, systolic blood pressure, diastolic blood pressure, liver function tests (AST, ALT, GGT and total bilirubin), kidney function tests (creatinine and estimated glomerular filtration rate) and serum electrolytes. Most haematology parameters showed no significant differences between groups. A significant difference between group mean neutrophil count after the 300 mg dose and the 1000 mg dose was attributed to a single individual in the 1000 mg group having a low neutrophil count, and was therefore regarded as unrelated to NRC.

A total of five Adverse Events (AEs) were reported by five participants associated with the 100 mg dose of NRC. AEs considered to be unlikely to be related to NRC were feeling of warmth, tiredness, pruritis and headache. An AE considered to be possibly related to NRC was a decrease in haemoglobin in one participant. Seven participants reported 9 AEs in association with the 300 mg dose of NRC. AEs considered unlikely to be related to NRC included headaches (four reports), soft or loose stool (two reports), and one report of flushing and feeling warm. One AE of decreased total white blood cell count was considered possibly related to NRC. Four AEs were reported following the 1000 mg NRC. Two AEs of feeling hot, one headache and one finding of low haemoglobin were classified as unlikely to be related to NRC. All AEs were resolved without the need of any intervention. There was no association between AE severity or category with dose. There were no serious AEs reported during the study.

The short-term repeat-dose study of Airhart et al (2017) has been described in Section 2.3.1. Eight volunteers took oral doses of NRC, at dose levels ranging from 250 mg once daily to 1000 mg twice daily, for nine days. No AEs or side effects were reported by the participants. No clinically significant changes were seen in serum levels of the five pre-selected safety endpoints, which were potassium, creatine kinase, glucose, uric acid and ALT. A slight decrease in group mean serum potassium was statistically significant but all values remained within normal range. Other changes which were statistically significant but clinically within normal range were decreases in haematocrit, haemoglobin, and platelet count. White blood cell differentials were unaffected. NRC supplementation had no effect on blood pressure, body temperature, body weight, white blood cell count, or serum levels of lactate dehydrogenase (LDH), AST, sodium, chloride, blood urea nitrogen (BUN), or creatinine.

21-day NRC supplementation study, with randomised double-blind crossover design, in aged men (Elhassan et al 2019). Regulatory status: Not GLP or GCP.

This was an efficacy study rather than a tolerance study, but includes some information on human tolerance of NRC. Subjects were 12 healthy men, with a mean age of 75 years (range 72-78) and a mean BMI of 26.6 kg/m² (range 21-30). Participants were assigned to either NRC at 500 mg twice daily (1000 mg/day), or placebo. After taking the supplement or placebo for 21 days, they were given a 21-day washout period before 21 days on the alternative treatment (placebo or NRC). Subjects attended the clinical research facility for assessments at 8:00 am in a fasted state at the start of each treatment (NRC or placebo) (Days 1 and 43 of study) and at the end of each treatment (Days 22 and 64 of study). Participants were subject to medical examination, recording of AEs and compliance, collection of blood samples, measurement of blood pressure and collection of urine over 24 h. On Days 1, 22 and 64, participants were also subject to measurement of hand grip strength, muscle biopsy, glucose tolerance test, indirect calorimetry, assessment of muscle arteriovenous difference, and venous occlusive plethysmography.

All subjects completed the study. NRC was well tolerated with no adverse effects observed on clinical pathological assessments for a variety of parameters including markers of liver, kidney and thyroid function. No participants reported AEs. Four participants reported increased libido while on NRC, but no participants reported this effect while on the placebo.

Other reported results were that NRC increased NAD⁺ and Nam clearance products in muscle, downregulated energy metabolism and mitochondrial pathways, and depressed levels of circulating inflammatory cytokines. The authors concluded that oral nicotinamide riboside is available to muscles of aged people, and has anti-inflammatory effects.

6-week NRC supplementation study, with randomised double-blind crossover design, in middle-aged and older adults (Martens et al 2018). Regulatory status: Not GLP or GCP.

Subjects for this study were men and postmenopausal women in good health, with a mean age of 65 ± 7 y and a mean BMI of 24 ± 4. Exclusion criteria included peripheral artery disease, overt cardiovascular disease, abnormal blood chemistries for liver or kidney function, uncontrolled thyroid disease, alcohol dependence, severe obesity and unstable weight. A total of 24 subjects, 12/group, completed the trial. Supplements were NRC (500 mg x 2/d) or placebo, with morning and evening meals for 6 w. A washout period between interventions was not included. Adherence was measured by pill count every two weeks, and bodyweight was recorded at the same time. At prestudy screening and at the end of each intervention phase, blood samples were collected. Assessments at the end of each phase included a variety of measurements of cardiovascular function (blood pressure, aortic stiffness, carotid artery compliance, endothelium dependent dilation), metabolic function (resting metabolic rate, insulin sensitivity), exercise capacity and physical function (cardiorespiratory fitness, walking endurance, muscle strength, rate of torque development, handgrip strength, leg fatigability, dynamic balance, mobility, manual dexterity). Peripheral blood mononuclear cells were isolated from blood samples for extraction and measurement of NAD⁺ metabolites and nucleosides/nucleotides. Results of analysis of NAD⁺ metabolites and nucleosides/nucleotides are described in Section 2.3.1.

Adherence to the study treatments was excellent (>95%). NRC was well tolerated with no serious AEs. No subjects dropped out of the study during the NRC phase. Reported mild AEs during the NRC supplementation included one episode of nausea, one skin rash, one of flushing, one of leg cramps and one of increased bruising. Blood analysis showed no evidence of changes in haematology or blood chemistry, including markers of hepatic and renal function. There were no significant changes in blood lipid profiles. Oral NRC supplementation led to significantly increased NAD⁺ and NAAD in peripheral blood mononuclear cells. No significant effects of NRC supplementation were observed in the other parameters measured.

6-week NRC supplementation study, with randomised double-blind crossover design, in obese volunteers (Remie et al 2020). Regulatory status: Not GLP or GCP.

This study was not intended to be a tolerance study but was conducted to investigate the effects of NRC supplementation on insulin sensitivity, mitochondrial function and other metabolic health parameters in obese volunteers. Thirteen volunteers, including six men and seven postmenopausal women, completed the study. Inclusion criteria included age range 45 to 65 y, BMI 27 to 35, non-smoking, with sedentary lifestyle, stable bodyweight, no active diseases, and alcohol consumption of ≤ 2 servings/d. Mean age was 59 ± 5 y, and mean BMI was 30.2 ± 2.6 . Participants received 1000 mg/d NRC or placebo for 6 weeks, followed by a 4 to 7 week washout before commencing the placebo or NRC, as applicable. Compliance was determined by weekly pill count, and body weight and blood sampling were conducted according to the same schedule. Assessments and samples during each intervention included resting acetylcarnitine concentrations in skeletal muscle on Day 32, intrahepatic and intramyocellular lipid measurements on Day 36, sleeping metabolic rate on Day 36 (measured overnight in a metabolic chamber), insulin sensitivity measured by hyperinsulinaemic:euglycaemic clamp on Day 37, cardiac phosphocreatine:ATP ratio and left ventricular ejection fraction on Day 37, skeletal muscle biopsy taken on Day 37, 24-h ambulatory blood pressure on Days 38 and 39 (two days and one night), and body composition on Day 40. The skeletal muscle biopsies were used to measure NAD⁺ metabolites, mitochondrial respiration, protein content, and acylcarnitine concentrations. Glucose, free fatty acids, triglycerides, cholesterol, HDL cholesterol and inflammatory cytokine concentrations were measured in blood.

NRC at 1000 mg/d was well tolerated, with no AEs or side effects reported. Compliance was high (99.3%). Supplementation with NRC was associated with elevation in NAAD, acetylcarnitine and MeNam in skeletal muscle; slight increase in body fat-free mass; increased sleeping metabolic rate; and increased capacity to form acetylcarnitine in muscle during exercise. No effects in insulin sensitivity, mitochondrial function, hepatic or intramyocellular lipid accumulation, cardiac energy status, cardiac ejection fraction, ambulatory blood pressure, plasma markers of inflammation or energy metabolism were detected.

8-week NRC supplementation study, with randomised double-blind design, in healthy overweight volunteers (published by Conze et al 2019; unpublished study report is by Schacter (study director) 2018. Regulatory status: GCP, ICH guidelines.

Healthy men and non-pregnant, non-breastfeeding women between the ages of 40 and 60 y, with a BMI between 25 and 30, were recruited for this study provided they were willing to refrain from vitamin B3 supplements and foods high in tryptophan or vitamin B3. Exclusion criteria included diabetes, active peptic ulcer, alcohol consumption above 2 servings/d, recent history of drug or alcohol abuse, use of medical marijuana, antihypertensives or lipid-lowering medications, history of renal or hepatic disease, or history of niacin deficiency. Eligible recruits (n=140) were randomised to four groups (35/group). The drop-out rate did not exceed 2/group. Groups were supplemented with 0 (placebo), 100, 300 or 1000 mg NRC/day for eight weeks. Participants kept records of supplementation diet, and any AEs, and the records were reviewed at least twice weekly. Anthropometric parameters and vital signs were recorded at screening and on Days 0, 7, 14, 28 and 56. Blood and urine samples were collected according to the same schedule. Parameters measured in blood included haematology, electrolytes, markers of renal and hepatic function, blood lipid profiles and NAD⁺ metabolites. Urine was analysed for NAD⁺ metabolites. Results from analysis of NAD⁺ metabolites are discussed in Section 2.3.1.

Compliance with supplementation was >97% in all groups. No dose-dependent AEs were

identified. There were no serious AEs, and the type, incidence and severity of the AEs were similar across all the groups. Of 26 AEs reported in the 100 mg/day group, 24 were considered unlikely to be related to NRC. The AEs considered to be possibly related to NRC were leg pain and high blood pressure, and both were mild in intensity. Of 27 AEs reported in the 300 mg/day group, 25 were considered unlikely to be related to NRC, or not related to NRC. The two exceptions were nausea and muscle pain, and both were mild in intensity. A total of 22 AEs were reported in the 1000 mg/day group, of which 19 were considered unlikely to be related to NRC, or not related. AEs considered possibly related were sore back, muscle soreness and nausea. All were mild in intensity. All AEs were transient and resolved by the end of the study. Treatment with NRC had no effect on mean systolic blood pressure, mean diastolic blood pressure, mean heart rate, or body weight. No dose-related alterations in group mean haematology or clinical chemistry values were observed, and all group mean values remained within normal ranges. NRC had no effect on plasma homocysteine. Dose-dependent increases in metabolites of nicotinamide riboside were detected in blood and urine. Overall, there were no adverse effects of NRC at doses \leq 1000 mg/day.

12-week NRC supplementation study, with randomised double-blind design, in obese men (Dollerup et al 2018). Regulatory status: Not GCP

Forty sedentary men with a BMI >30 , otherwise healthy nonsmokers in the age range 40-70 y, were the participants in this study. Prestudy assessment included a clinical examination and electrocardiography. From two weeks prior to the start of the intervention, participants were asked to refrain from taking vitamins or other dietary supplements. Participants received oral supplementation with either 1000 mg NRC twice daily (2000 mg/day, 1000 mg morning and evening), or a placebo, for 12 weeks. Examinations prior to supplementation and at the end of the study included dual-energy X-ray absorptiometry scan, MR imaging and spectroscopy, hyperinsulinaemic:euglycaemic clamp with infusion of radioactive tracers of glucose and palmitate, indirect calorimetry, repetitive blood sampling, and biopsies of muscle and adipose tissue. Dual-energy X-ray absorptiometry scanning was used to assess body composition and bone mineral density. MR imaging and spectroscopy were used to measure hepatic lipid content, abdominal subcutaneous adipose tissue, and visceral adipose tissue. Participants kept a diet journal for three days prior to the clamp study and were asked to repeat the dietary pattern prior to the second clamp study. Urine was collected before and after each clamp period. Prior to the clamp studies, participants were also asked to refrain from alcohol and physical exercise. At six weeks, the midpoint of the intervention, a blood sample was drawn for clinical pathology and reports of AEs were recorded. Reports of AEs were also recorded at the end of the trial.

Compliance with the intervention was $>95\%$. NRC at 2000 mg/day was well tolerated. There were four reports of minor AEs in the NRC group; pruritis, excessive sweating, bloating, and transient changes in stool. All AEs were mild. No abnormalities were found in haematology or clinical chemistry at the 6-week midpoint. Supplementation with NRC had no effect on group mean results for insulin sensitivity, insulin stimulation, palmitate flux, resting energy expenditure, body composition, distributions of abdominal subcutaneous and visceral adipose tissue, respiratory exchange ratio, or oxidation rates of glucose, lipid or protein. Overall, NRC supplementation did not result in a significant difference between the treated and placebo group in hepatic lipid content, but interindividual variability led the authors to suggest that there may be responders and non-responders to NRC in this parameter. NRC supplementation was associated with a slight increase in group mean plasma triglyceride although all values remained within the normal reference range for the laboratory. NRC supplementation did not have any effect on group mean values for total cholesterol, LDL cholesterol or HDL cholesterol or any other clinical chemistry findings. It was concluded that NRC supplementation is safe at 2000 mg/day.

3.3.7 Flushing

Dermal flushing is a side effect of high doses of nicotinic acid supplementation (Sebrell and Butler 1938, Spies et al. 1938), caused by the activation of the nicotinic acid G-protein coupled receptor GPR109A in epidermal Langerhans cells (Benyo et al. 2005, Benyo et al. 2006). This activation results in the production of prostaglandins D₂ and E₂ (Kamanna et al. 2009) leading to vasodilation of capillaries.

Nicotinamide is not converted to nicotinic acid *in vivo* and the GPR109A receptor is specific for nicotinic acid (Wise et al. 2003), therefore a flushing response is not expected for either nicotinamide or nicotinamide riboside (Bogan and Brenner 2008). Two of twelve participants self-reported flushing at the 300 mg dose, but not at the 100 mg or 1000 mg dose, in the study of Trammell et al. (2016). One patient receiving NRC reported flushing in the study by Martens et al. (2018), but so did two patients receiving placebo. None of the patients in the three other human studies reviewed (Airhart et al. 2017, Dollerup et al. 2018, Conze et al. 2019) reported flushing as an AE of NRC supplementation. FSANZ concludes that in general NRC supplementation is not associated with flushing, and would not be expected to induce flushing.

3.3.8 Human allergenic potential

NRC is synthetic, with no animal or botanical origins. Purity exceeds 90%, and neither NRC or the <10% that is residual, contain any protein or any substance associated with human hypersensitivity reactions.

No case reports of allergic reactions to NRC were located by literature search.

3.3.9 Other studies

A small number of reviews and studies of nicotinamide riboside were located by literature search, that were reviewed but were not considered to be relevant to this hazard assessment. They are briefly summarized in this section.

Reviews

A review of NAD⁺ precursors by Bogan and Brenner (2008) and a review of nicotinamide riboside by Chi and Sauve (2013) summarized the metabolism of nicotinamide riboside. Bogan and Brenner (2008) noted that Nr1 is ubiquitous in mammalian tissues whereas Nr2 is found in heart, brain and skeletal muscle, but not in kidney, liver, lung, pancreas or placenta. Therapeutic potential of nicotinamide riboside in neurological disorders were mentioned in both reviews, but no adverse effects of nicotinamide riboside were mentioned.

A review by Yoshino et al (2018) described beneficial effects of nicotinamide riboside on some muscle disorders, on liver function, and on the nervous system, including some neurodegenerative disorders. They speculated on some hypothetical detrimental effects such as promotion of tumour growth, inhibition of sirtuins, or depletion of methyl donors, but did not present any evidence that these outcomes would occur. In the absence of data to show that these adverse effects occur *in vivo*, these comments are not considered to be relevant to this hazard assessment.

Mehmel et al (2020) summarized the potential therapeutic effects of nicotinamide riboside on neurodegenerative and cardiovascular disorders, and as supportive therapy in severe infections. This review of possible therapeutic effects is not relevant to the current application.

Studies

Kourtzidis et al (2016) reported that nicotinamide riboside decreases exercise performance in rats. The test article in their study was not NRC, but nicotinamide riboside made by another manufacturer. The test system comprised male Wistar rats, kept under standard conditions of environment and husbandry. Prior to study start, rats were acclimatized to swimming. Rats were assigned, 9/group, to a control group gavaged daily with saline vehicle, and a group gavaged with 300 mg/kg bw/day nicotinamide riboside. The dosing period was 21 days. At the end of the dosing period, exercise performance was tested by swimming performance in 34°C water with weights attached to the base of each rat's tail. The weight was progressively increased up to 10% of the rat's bodyweight. The group mean value for time to exhaustion of the treated rats was 35% worse than that of the control group (94 ± 53 s cf. 145 ± 59 s; $p = 0.071$). The authors noted that similar negative effects on stamina have been reported for NA, and attributed to decreased oxidation of fatty acids. This study is not considered relevant to the safety of NRC as a supplement in FSMP.

An 18-week dietary intervention in male C57BL/6JRccHsd mice (Shi et al 2019) investigated responses to high dose supplementation with nicotinamide riboside in the context of a mildly obesogenic diet. All mice were maintained under standard laboratory environmental and husbandry conditions, and fed a diet containing 40% energy from fat, intended to resemble average human consumption of fat in the Netherlands. After two weeks of acclimatisation to the diet, mice were assigned to groups, 12/group. The diet of the control group was supplemented with 30 mg nicotinamide riboside/kg diet, the recommended level, whereas the diet of the treatment group was supplemented with 9000 mg nicotinamide riboside/kg diet. The source of the nicotinamide riboside was not stated. A variety of assessments were conducted during the in-life phase and after mice were killed at the end of the dietary intervention. Compared to the control group, mice fed the high dose of nicotinamide riboside showed reduced metabolic flexibility (AUC of respiratory exchange ratio), lower glucose clearance rate, increased insulin resistance, and molecular and morphological changes in epididymal white adipose tissue. This study is not considered to be relevant to the current hazard assessment because the exposure to nicotinamide riboside was 300x the recommended level. The current hazard assessment concerns NRC added to FSMP, which are prescribed by medical practitioners and may be expected to be at the correct level of supplementation rather than an excessive level.

Effects of nicotinamide riboside supplementation on rodent adipose tissue were also investigated by Serrano et al (2020). Suckling male NMRI mouse pups (12/group) were administered the vehicle (water), resveratrol in solution (2 mg/kg bw) or nicotinamide riboside (approximately 15x the total niacin ingested daily from maternal milk) from PND 2 to 20. Pups were weaned onto a normal diet on PND 21, and habituated to a normal-fat (10% energy as fat) diet from PND 75 to 90. One PND 90, half the rats from each treatment group were switched to a purified high-fat (40% energy as fat) diet. All mice were killed on PND 164 for tissue collection. Supplementation with resveratrol or with nicotinamide riboside was associated with DNA methylation modifications in expression of *Slc27a1*, a thermogenesis-related fatty acid transporter, and *Prdm16*, a molecular determinant of brown beige adipocyte fate, in inguinal white adipose tissue. The authors concluded that resveratrol and nicotinamide riboside may have therapeutic potential in obesity prevention in the long term. This study is not considered relevant to the current assessment because the NHMRC and NZ MOH policy does not recommend supplementation with nicotinamide for children under 12 months of age (see Section 3.4).

Hamity et al (2020) induced mammary gland tumours in female Sprague-Dawley rats by IP injection of N-methyl-nitrosourea (NMU) at 21 days of age, in order to study the interaction of nicotinamide riboside and paclitaxel, and the effects of nicotinamide riboside on paclitaxel-induced peripheral neuropathy and on tumour growth. Group sizes varied from 6 to 12

depending on the parameter measured. Paclitaxel treatment was initiated within 3 d of tumour appearance. Baseline paw withdrawal thresholds to tactile and cooling stimuli were determined before paclitaxel treatment was commenced. Nicotinamide riboside (200 mg/kg bw/day) or vehicle was administered by gavage for 28 days or until tumour burden necessitated euthanasia of the rat. Paw withdrawal to tactile or cooling stimuli was assessed on days 15 and 22 after paclitaxel treatment was begun. Other assessments were place-escape avoidance behaviour on Day 24, and locomotor activity on Day 28. Tumours were subject to histopathology after rats were killed on Day 28. Supplementation with nicotinamide riboside significantly reduced paclitaxel-induced hypersensitivity and suppressed aversive behaviour in rats with that neuropathy. Loss of intraepidermal nerve fibres due to paclitaxel was reduced by nicotinamide riboside supplementation. There was some evidence that nicotinamide supplementation enhanced the tumour-suppressing action of paclitaxel. This study is not considered to be relevant to the current assessment because it concerns possible therapeutic actions of riboside chloride in subjects with cancer treated with paclitaxel. However it is noted that no adverse effects of nicotinamide riboside supplementation were reported.

Marinescu et al (2020) reported the results of a 90 day repeat-dose oral toxicity study of a high-purity synthetic NRC in Sprague-Dawley rats. The test article was manufactured under GMP 21 CFR 211/210 by Elysium Health, and had a purity of >97%. The vehicle and control article was distilled water. The study was conducted under GLP, according to the OECD Guideline 408 and the US-FDA Redbook (2000). Dosage levels were 0, 300, 500 and 1200 mg/kg bw/day. Test subjects were Sprague-Dawley rats, 7 weeks old at acquisition, and acclimatised to standard laboratory environmental and husbandry conditions for one week before study start. Rats were individually housed. Rats were assigned to main study cohorts of 10/sex/group, and recovery cohorts of 5/sex/group. The dosing formulations were administered at a volume of 5 mL/kg. In-life parameters included twice-daily mortality/moribundity checks, daily cageside observations, weekly detailed (in-hand) clinical observations, weekly body weights and weekly food consumption. Bodyweight gain and feed efficiency were calculated. Prior to study start and prior to scheduled kill, rats were subject to ophthalmologic examination. Urine was collected overnight in metabolism cages prior to scheduled kill, and blood was collected under anaesthesia immediately before rats were killed, for routine clinical pathology assessments. All rats were subject to complete necropsy and collection of a standard list of organs and tissues, with fresh weights recorded for the standard list of organs specified in the OECD guideline. All organs and tissues were fixed, and tissues from control and 1200 mg/kg bw/day rats were processed to slides and stained with haematoxylin and eosin for microscopic examination from control and 1200 mg/kg bw/day rats. All rats survived to scheduled kill, and no abnormal clinical or ophthalmological findings were observed. Group mean values for weekly body weights and body weight gains were lower than those of control males in the 1200 mg/kg bw/day males, but the difference reached statistical significance only on Day 92, when the value was 13% lower than that of control males. Group mean values for food consumption and feed efficiency of 1200 mg/kg bw/day males were not significantly different to those of control males at any time during the study. There were no biologically relevant treatment-related changes in group mean values for any haematology, coagulation, serological or urinalysis parameters. There were no treatment-related findings on necropsy or on microscopic examination of tissues. Statistically significant increases in group mean values for weights of adrenals, brain, kidneys and liver, relative to bodyweight, in 1200 mg/kg bw/day males compared to control males were attributed to the slightly lower group mean terminal bodyweight of this group. Group mean values for heart, kidneys and testes of the recovery cohort of this group were also slightly increased relative to group mean terminal bodyweight, when compared to control males. The group mean value for liver to bodyweight ratio was also increased in 1200 mg/kg bw/day females, when compared to control females, in the main study cohort. The decrease in body weight and body weight gain in the 1200 mg/kg bw/day males was conservatively interpreted as adverse, and the NOAEL in this study was identified as 500 mg/kg bw/day.

This study is considered to be less relevant to the current Hazard Assessment than the 90-day rat study reported by Conze et al (2016), because the test article, although also a synthetic NRC, was not the same NRC that is the subject of the current hazard assessment. FSANZ notes that the NOAEL identified by Conze et al (2016) is lower, at 300 mg/kg bw/day.

3.4 Assessments by other regulatory agencies

National Health and Medical Research Council (NHMRC) and New Zealand Ministry of Health (NZ MOH)

NRC is proposed by the applicant as an form of Vitamin B3 (niacin), and is rapidly converted to nicotinamide in human blood. It is therefore relevant to review the existing Upper Level of Intake (UL) for Nam established by the NHMRC and NZ MOH (2006). These are presented in **Table 2** . The values represent total intake from all sources.

Table 2: Upper Levels of Intake for Niacin as Nicotinamide

| Life stage | Age | Upper Level of Intake |
|--------------------------|------------|---|
| Infants | 0-12 mo | Intake should be breastmilk, formula or food only |
| Children and adolescents | 1-3 y | 150 mg/day |
| | 4-8 y | 250 mg/day |
| | 9-13 y | 500 mg/day |
| | 14-18 y | 750 mg/day |
| Adults | Men ≥19 y | 900 mg/day |
| | Women ≥19y | 900 mg/day |
| Pregnant women | 14-18 y | Not possible to establish, should be dietary only |
| | 19-50 y | |
| Lactating women | 14-18 y | Not possible to establish, should be dietary only |
| | 19-50 y | |

The UL for Nam of 900 mg/day set for adults by the NHMRC and NZ MOH is based on recommendations made by the European Commission in 2002. The NHMRC and NZ MOH commented that Nam is generally well tolerated at up to 3000 mg/day for periods up to 3 years. The UL is based on a NOAEL of 1800 mg/day in a number of high quality of trials, with the application of an Uncertainty Factor of 2 because the trials were largely conducted in children, and adults may not metabolise Nam as rapidly as children do, and may exhibit greater inter-individual variability. The NHMRC and NZ MOH did not set ULs for pregnant or lactating women, because of a lack of data in humans or in animals. The basis for the ULs for children and adolescents are not stated, but it appears that the adult ULs were extrapolated from trials in children, on the basis of bodyweight.

EFSA

NRC has been assessed as a novel food for use in food supplements for consumption by the healthy adult population, at up to 300 mg/day, by the EFSA Panel on Nutrition, Novel foods and Food allergens (NDA) (EFSA 2019). The Panel found no safety concerns associated with the production process, composition, specifications, batch-to batch variability or stability of NRC, and also concluded that there are no concerns regarding genotoxicity. There were no safety concerns raised by the human data. The Panel noted that the proposed level of use corresponds to a daily dose of Nam that is six-fold lower than the tolerable upper intake level for adults, excluding pregnant and lactating women, of 900 mg Nam/day set by the Scientific Committee on Food (SCF) in 2002. The SCF did not establish a UL for Nam in

pregnant or lactating women, due to a lack of data.

The Panel derived a NOAEL of 300 mg/kg bw/day from animal data, specifically the 90-day repeat-dose study in rats (Bhoite *et al* 2015, Conze *et al* 2016) summarised in Sections 3.3.1 and 3.3.3. The Margin of Exposure (MoE) between the proposed maximum daily dose of 300 mg (= 4.3 mg/kg bw/day for a 70 kg adult) and the NOAEL of 300 mg/kg bw/day is 70, which the Panel considered to be sufficient for the adult population, excluding pregnant and lactating women.

For maternal and embryo/fetal toxicity, the Panel derived a NOAEL of 325 mg/kg bw/day from the developmental toxicity study of Rao (2016) summarized in Section 3.3.6, above. The MoE between the proposed maximum daily dose of 300 mg (= 4.3 mg/kg bw in a 70 kg adult) and the NOAEL from the Rao (2016) study is 76. In the absence of data that would justify accepting a MoE less than 100 for pregnant and lactating women, the Panel concluded that daily intake of NRC by pregnant and lactating women should not exceed 230 mg/day.

3.5 Discussion and conclusion

The acute oral toxicity of NRC is low, with the oral LD₅₀ greater than 5000 mg/kg bw in the Sprague Dawley rat.

Oral gavage with ≥2500 mg/kg bw/day for ≥8 days was associated with moderate decreases in group mean bodyweights of male Sprague Dawley rats, but not females, in a 14-day study. No adverse effects were observed in Sprague Dawley rats gavaged with NRC at 300 mg/kg bw/day for 90 days, but at 1000 mg/kg bw/day, statistically significant alterations were observed in some clinical pathology parameters, including an increase in neutrophils, ALT, and triglycerides. At 3000 mg/kg bw/day NRC, significant adverse effects were observed in the same parameters and in some organ weights, and a number of microscopic lesions were observed in liver, thyroid, kidneys, adrenals and gonads, but the same adverse changes occurred in a positive control group of rats treated with an equimolar dose of Nam (1260 mg/kg bw/day).

Additional animal studies were provided for assessment but cannot be disclosed because it is CCI. The toxicological assessment supports a NOAEL of 300 mg/kg bw/day.

No chronic toxicity/carcinogenicity studies of NRC were submitted or located from other sources, but NRC did not show evidence of genotoxicity in a number of genotoxicity assays, and is rapidly metabolised to Nam. In rats, treatment with 3000 mg/kg bw/day NRC was associated with hypertrophic, but not hyperplastic, changes in some organs including liver, thyroid, and adrenal cortex (specifically, zona glomerulosa). However, treatment with an equimolar dose of Nam had the same effects.

No developmental toxicity was observed in rat pups at doses of NRC below that causing maternal toxicity. The fetal NOAEL was identified as 750 mg/kg bw/day on the basis of decreased fetal bodyweights at 1500 mg/kg bw/day, together with increases in the incidence of abnormalities commonly observed in association with maternal toxicity. In a one-generation reproductive study in rats, the NOAEL for fertility and reproductive performance was 12 000 ppm in the parental diet, the highest dose tested, equivalent to 675.2 mg/kg bw/day NRC in P generation males and 1088.4 mg/kg bw/day NRC in P generation females. In human tolerance studies of up to 12 weeks in duration, NRC was well tolerated at doses up to 2000 mg/day, a level exceeding the UL for Nam set by the NHMRC and NZ MOH for adults.

No case reports of allergic reactions to NRC were located by literature search. NRC would

not be expected to be an allergen, on the basis of its synthetic origin, purity, rapid metabolism to Nam, and low molecular weight of 290.7 Da. Most identified allergens are proteins or glycoproteins with molecular weights between 5 and 50 kDa (Woodfolk *et al* 2015).

4 Food Technology Assessment

4.1 Objectives for the food technology assessment

This assessment reviewed NRC as a permitted form of niacin for addition to FSMPs, from a food technology perspective. The assessment also considered the manufacturing process and the validity of analytical methods used to quantify and characterise NRC during production.

Niacin is permitted to be added to FSMPs in the form of NA or Nam. FSMPs can be the sole source of nutrition, therefore the form of the niacin used must be suitable and predictable, for example with respect to its stability, consistent chemical form, and delivery of niacin.

The food technology assessment provides information on chemical identification, physicochemical properties, and specifications for the substance proposed to be added to food. This assessment characterises the chemical properties of NRC, the manufacturing process, and describes the basis for the specification for this chemical form of the vitamin niacin to be included in Schedule 3 of the Code.

4.2 Chemical properties

4.2.1 Chemical names, properties, and structures

NRC is chemically synthesised, and is therefore a synthetic form of nicotinamide riboside as the chloride salt.

Chemical names and properties for NRC are listed in **Table 3**.

Table 3: Chemical properties of nicotinamide riboside chloride

| Property | nicotinamide riboside chloride |
|---------------------|---|
| Chemical name | 3-(Aminocarbonyl)-1-β-D-ribofuranosyl-pyridinium chloride (1:1) |
| Common name | Nicotinamide riboside chloride |
| Alternative names | Pyridinium, 3-(aminocarbonyl)-1-β-D-ribofuranosyl-, chloride Pyridinium, 3-carbamoyl-1-β-D-ribofuranosyl-, chloride (8Cl) N-ribosylnicotinamide |
| CAS registry number | 23111-00-4 |
| Chemical formula | C ₁₁ H ₁₅ N ₂ O ₅ ·Cl |
| Molecular mass | 290.7 g/mol |
| Solubility | Readily soluble in water |

The chemical structure of NRC is shown below. The applicant's product, Niagen® is a crystalline form of NRC. It is a single chemical entity which incorporates nicotinamide and ribose. NRC is a white to light brown powder that is soluble in water.

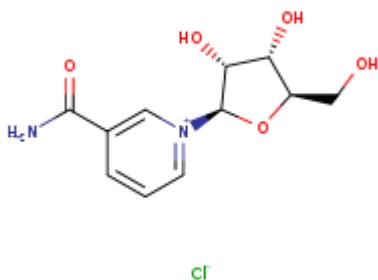


Figure 2. Chemical Structure

4.2.2 Structural identification

NRC may exist as α or β isomers (Figure 1). The applicant is seeking permission for the β form (CAS number 23111-00-4). The applicant's NRC is predominantly the β form of NRC. To confirm that NRC consists mainly of the β form NRC is analysed using nuclear magnetic resonance (NMR), which discriminates between the α and β forms. Full details of the NMR analyses were provided for assessment but cannot be disclosed because it is CCI.

4.3 Manufacturing processes

4.3.1 Production of nicotinamide riboside chloride

The applicant has provided details of the manufacturing processes for NRC including a description of the raw materials and processing aids, purification and isolation, and quality controls. Detailed information was provided as CCI information.

Manufacturing of NRC is stated to be conducted in accordance with Good Manufacturing Practices (GMP) and HACCP principles. NRC is produced via a two-step chemical synthesis process, described in section II, B.4 of the application.

According to the application, under step one, the starting material of D-ribofuranose tetraacetate is reacted with hydrochloric acid to generate the D-ribofuranose triacetate chloride. The solution is sampled for reaction completion by quantifying the amount of D-ribofuranose triacetate chloride (the reaction intermediate) via NMR. When the reaction is complete, nicotinamide is added to the mixture. The mixture is then stirred until the reaction to nicotinamide ribofuranose triacetate chloride is complete (Figure 2). 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 -ribose chloride, NIAGEN® (Figure 3).

The GRAS notification [GRN635](#) to the United States Food & Drug Administration contains a detailed flow chart for the manufacture of NRC (USFDA, 2015 – see page 23 – 24).

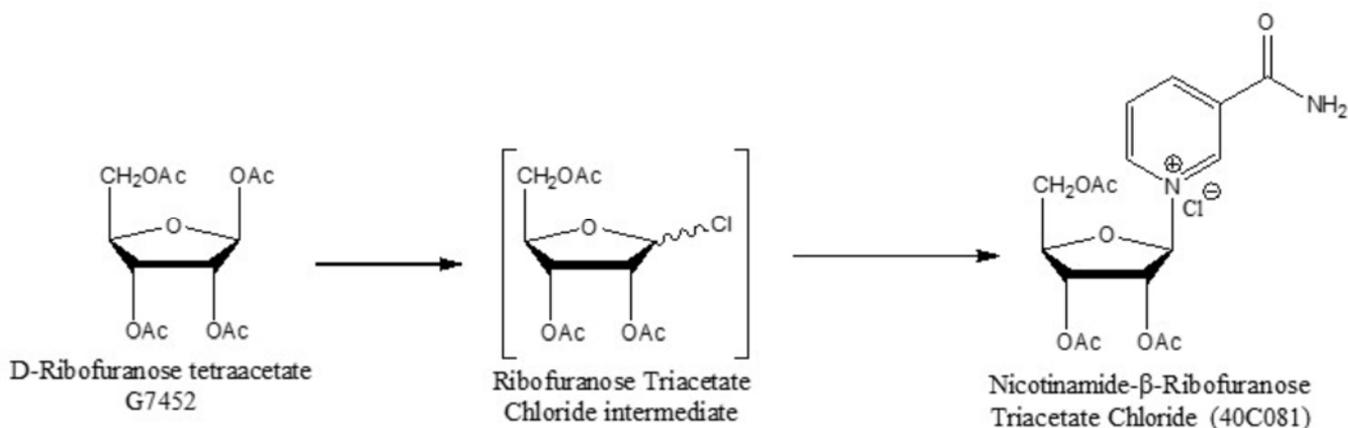


Figure 2. Step 1 Chemical Reactions

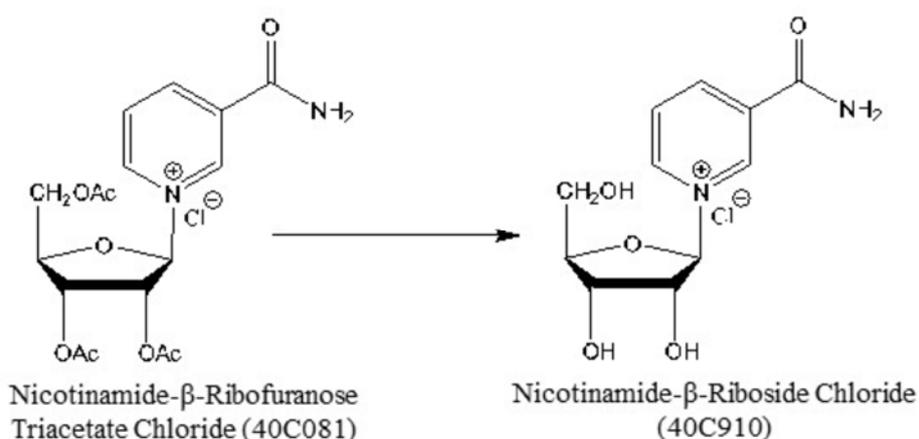


Figure 3. Step 2 Chemical Reaction

The applicant provided details on solvents and other substances used in chemical synthesis of NRC, as additional information to the application.. The solvents are as follows: acetone, methyl t-butyl ether, acetonitrile, and methanol. The substances are as follows: sodium carbonate, acetyl chloride, hydrochloric acid (gas), tributyl amine, ammonia hydroxide and sulphuric acid. Acetic acid, methyl acetate and acetamide are produced as reaction by-products and are controlled by limits in the product specification. Section 4.4 discusses the proposed specification for NRC, and includes limits for residual solvents and reaction by-products.

4.4 Product specifications

The Code (Section 1.1.1—15) requires substances to comply with relevant specifications. The specifications are listed in Schedule 3 (Identity and Purity) of the Code. NRC is not covered under existing specifications listed in Schedule 3.

As noted above, NRC has been approved for use in the EU as a novel food when in *supplement form*³. The substance has also been assessed to be added to a number of foods under GRAS notification [GRN635](#) in the USA. As such, the applicant has proposed specifications that have been defined in these jurisdictions (Table B.5-1 of the application). These specifications prescribe a minimum purity of NRC of 90%, and maximum amounts for residual solvents,

³ <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0016&from=EN>

reaction by-products, microbiological limits, and heavy metals.

The applicant provided Certificates of Analysis as CCI for five non-consecutive batches of NRC to demonstrate that the product meets the proposed specifications. Results were consistent across all batches tested for each substance. The Certificates of Analysis indicate that the substance meets S3—4 requirements for lead, arsenic, cadmium and mercury.

The specification proposed for inclusion in the Code is shown below in **Table 4**. This specification is consistent with the EU and US GRAS notification specifications, including the listing of residual solvents and reaction by-products. These parameters are proposed for inclusion in Schedule 3, as they represent and characterise the commercial proprietary product on which the safety studies were conducted.

The methods in column 3 of the table are not proposed to be included in Schedule 3, as they are either confidential to the applicant or standard methods.

Table 4. Product specifications proposed for nicotinamide riboside chloride (adapted from application)

| Parameter | Specification | Method |
|--------------------------------------|---|-------------------------------------|
| Form | Powder | visual |
| Colour | White to light brown | visual |
| Identification | Conforms by nuclear magnetic resonance (NMR) | ChromaDex method Meth-0.700.12.4 |
| Assay Nicotinamide riboside chloride | Not less than 90.0 w/w % Not more than 103 w/w % | ChromaDex method 0.700.10.2.METH3 |
| Water content | Not more than 2.0 w/w % | ChromaDex method 0.700.11.37 |
| Residual solvents | | |
| Acetone | Not more than 5000 ppm | USP** <467> |
| Methanol | Not more than 1000 ppm | USP <467> |
| Acetonitrile | Not more than 50 ppm | USP <467> |
| Methyl Tert-Butyl Ether | Not more than 500 ppm | USP <467> |
| Reaction by-products | | |
| Methyl acetate | Not more than 1000 ppm | USP <467> |
| Acetamide | Not more than 27 ppm | ChromaDex method 99-1-04-7.0-000616 |
| Acetic acid | Not more than 5000 ppm | 99.1-04-2.0-000665 |
| Arsenic and heavy metals | | |
| Arsenic | Not more than 1 ppm | USP <232>, <233>, <2232> |

| Parameter | Specification | Method |
|----------------------------|---------------------------|--------------------------|
| Mercury | Not more than 1 ppm | USP <232>, <233>, <2232> |
| Cadmium | Not more than 1 ppm | USP <232>, <233>, <2232> |
| Lead | Not more than 0.5 ppm | USP <232>, <233>, <2232> |
| Microbiological Parameters | | |
| Total plate count | Not more than 1000 CFU*/g | AOAC*** or equivalent |
| <i>Escherichia coli</i> | Absent in 10 g | AOAC or equivalent |
| Yeast and Mould | Not more than 100 CFU/g | AOAC or equivalent |

*CFU = colony forming units

**USP = United States Pharmacopeia

***AOAC = Association of Analytical Communities

4.5 Analytical methods for detection

As there are no internationally recognised methods for the analysis of NRC, methods developed by the applicant were submitted to FSANZ in confidence as CCI material. As stated in the application, the applicant has developed and validated a high performance liquid chromatography (HPLC) method for detection of NRC. The information provided included:

- Description of methods using HPLC
- Details of methods including reagents, standards, solution preparation, standard preparation, sample preparation and typical chromatograms
- Analytical procedures to assay for impurities specified in the specification
- Quantification of NRC

The information demonstrated that the NRC can be manufactured and assayed to a purity that would be consistent with the specifications proposed by the applicant.

If analytical methods are required to be developed for enforcement purposes, including how the amount of NRC relates to niacin label declarations, then full analytical methods and assistance may need to be requested from the applicant.

4.6 Stability in food

Stability studies specifically for NRC in a food matrix were provided by the applicant as CCI. Interim results for a 36 month study on the stability of NRC in a protein powder formulation show NRC is stable at 25°C/60%RH for at least 12 months, and based on accelerated testing is predicted to have a 24 month plus shelf life at real time conditions.

The applicant holds extensive stability data regarding the use of NRC in dietary/food supplements, and states that this stability data is highly relevant to its stability in food matrixes, such as powdered Foods for Special Medical Purposes products.

The GRAS notification [GRN635](#) to the United States Food & Drug Administration indicates that NRC is stable under different processing conditions and throughout the shelf life of selected food products (US FDA 2016).

From the application and additional information provided, the main degradation of NRC is

through hydrolysis leading to formation of Nam and ribose. Therefore water content is the main concern with regard to stability. The purpose of adding NRC to FSMPs is to increase Nam uptake, while ribose is a sugar synthesised by the body from food. The degradation products are therefore not a safety concern.

Data sheets and technical information held by the applicant would be available to manufacturers of FSMPs regarding the use of NRC (for example, any limitations around suitable product formulations and shelf life).

4.7 Food technology conclusion

The food technology assessment concludes that NRC is a synthetic form of nicotinamide riboside. NRC is a white to light brown powder that is soluble in water. Niagen® is a crystalline substance, that contains greater than or equal to 90% NRC, the remaining components being residual solvents, reaction by-products and degradation products.

NMR testing is undertaken by the manufacturer of NRC to ensure that the NRC is predominantly the β form of nicotinamide riboside. Stability tests indicate NRC is stable for up to two years in powdered FSMP formulations.

The Applicant has proposed specifications to be included in Schedule 3 for the use of NRC in FSMPs. FSANZ has developed a specification based on this information.

On the basis of the available data, NRC appears to be well characterised, and sufficiently stable in powdered FSMP products.

5 Dietary intake assessment

An estimate of the dietary intake of NR was not considered necessary for this assessment as niacin is already permitted to be added to FSMP, and the applicant is not requesting a change in the permitted level of addition. The Code also permits the addition of niacin to certain foods other than FSMPs in the form of both nicotinic acid and nicotinamide.

There are already published usual daily dietary intakes of niacin equivalents for the general population for both Australia (from the 2011-12 Australian National Nutrition and Physical Activity Survey (ABS 2014a and 2014b), and for New Zealand from the 2002 National Children's Nutrition Survey (Ministry of Health 2003; Ministry of Health 2005), and the 2008-09 Adult Nutrition Survey (Ministry of Health 2011a; Ministry of Health 2011b). For context these dietary intakes shown in **Appendix 1** (Table A1.1 for Australia and Table A1.2 for New Zealand children 5-14 years and Table A1.3 for New Zealanders aged 15 years and above). The contribution of the major food groups to dietary intakes of niacin equivalents is shown in Table A1.4 for Australia and New Zealand.

The consumption of FSMP food which in this case are nutrition/protein drinks and special dietary foods used under medical supervision, and therefore, fall outside the scope of food consumption data available from Australian or New Zealand National Nutrition Surveys (NNSs). Therefore nationally representative consumption data for these products are not available.

The hazard assessment did not identify any non-nutrient chemicals or compounds of a public health and safety concern associated with the addition of NRC to FSMP for which a dietary exposure assessment was warranted.

6 Risk Assessment Conclusions

FSANZ has assessed an application from ChromaDex Inc. to amend the Code to permit the use of NRC as a permitted form of Vitamin B3 in FSMPs. Vitamin B3 (niacin) functions as a source of NAD⁺ in the body that is required for a range of cellular functions. Several studies reviewed by FSANZ have shown that NRC is an alternative form of niacin that can be metabolised to NAD⁺.

To determine the bioavailability of NRC, FSANZ considered studies in humans and in laboratory animals on the effect of NRC supplementation on the concentration of nicotinamide adenine dinucleotide (NAD⁺) and metabolites in blood and/or urine. In human studies, NRC supplementation (100 to 2000 mg/day) in volunteers was associated with increases in blood concentrations of NAD⁺ and several NAD⁺ metabolites, relative to baseline values or placebo treatments showing that it is a bioavailable form of niacin. However, none of the studies included a comparator treatment group receiving nicotinic acid (NA) or nicotinamide (Nam) so it was not possible to establish bioequivalence to already permitted forms of niacin in the Code.

NRC has been shown to increase hepatic NAD⁺ levels in mice and increase plasma MeNam concentrations in other animal studies. A 90-day rat study showed that similar doses of NRC or Nam caused an elevation of plasma Nam and MeNam. Similar plasma concentrations of MeNam were observed in the two treatment groups, however the maximum plasma concentration (C_{max}) and total systemic exposure (AUC) to Nam was higher (approximately 1.6- to 1.8-fold) in the Nam group compared to the NRC group. Plasma MeNam levels peaked earlier in the NRC group than in the Nam group.

FSANZ concludes that based on the available evidence in laboratory animals and humans NRC is a bioavailable form of niacin which at intakes ranging from 100 to 2000 mg/day in humans would be expected to support normal physiological function. In the absence of human studies to establish the bioavailability of NRC compared to already permitted forms of niacin, FSANZ cannot judge with certainty the extent to which lower NRC intakes which match adult Recommended Dietary Intakes for niacin (14 mg/day in women, 16 mg/day in men) would support essential requirements, when it is the only form of vitamin B3 in the diet.

No evidence was identified to indicate that NRC would inhibit the absorption of other nutrients.

The acute oral toxicity of NRC is low. No adverse effects were observed in rats gavaged with NRC at 300 mg/kg bw/day for 90 days, but statistically significant changes in some clinical pathology parameters were observed in rats dosed with 1000 mg/kg bw/day. In the same study, a number of adverse effects were observed in rats dosed with 3000 mg/kg bw/day, but the same adverse changes occurred in a positive control group of rats treated with an equimolar dose of Nam (1260 mg/kg bw/day). Additional animal studies also supported a NOAEL of 300 mg/kg bw/day.

No chronic toxicity/carcinogenicity studies of NRC were submitted or located from other sources, but NRC was not genotoxic and no pre-neoplastic lesions were observed in the 90 day rat study. In a developmental study in rats, the fetal NOAEL was identified as 750 mg/kg bw/day on the basis of decreased fetal bodyweights at 1500 mg/kg bw/day, together with increases in the incidence of abnormalities commonly observed in association with maternal toxicity. In a one-generation reproductive study in rats, the NOAEL for fertility and reproductive performance was 12 000 ppm in the parental diet, the highest dose tested, equivalent to 675.2 mg/kg bw/day NRC in P generation males and 1088.4 mg/kg bw/day NRC in P generation females.

In human tolerance studies of up to 12 weeks in duration, NRC was well tolerated at doses

NRC would not be expected to be an allergen, on the basis of its rapid metabolism to Nam and low molecular weight. No case reports of allergic reactions to NRC were located by literature search.

Since NRC is metabolised to Nam it is important to consider NRC intakes relative to the upper level of intake (UL) for Nam. The maximum daily intake of NRC proposed in the Application was 1000 mg/day, which assuming equimolar conversion, is equivalent to 420 mg Nam. This is less than half the UL for Nam in non-pregnant, non-lactating adults (900 mg), and is also below the UL for children aged 9-13 years (500 mg/day) and adolescents aged 14-18 years (750 mg/day). It is above the UL for children aged 1-3 years (150 mg/day) and 4-8 years (250 mg/day), however it is expected that FSMPs are prescribed by, and will be used under the supervision of medical practitioners, at intakes that would not exceed the UL.

The margin of exposure (MOE) between the NOAEL of 750 mg/kg/day for fetal toxicity in a rat developmental study, and the maximum intake of 1000 mg/day NRC for a pregnant woman weighing 60 kg was 45. The corresponding MOE between the NOAEL in the one-generation reproductive toxicity in rats and proposed maximum human intake was approximately 66. On the basis of the low MOEs (<100) use at the maximum proposed NRC intake level in pregnant or lactating women is not supported. However, as for paediatric use, lower intakes approximating Recommended Daily Intakes of niacin, prescribed and supervised by a medical practitioner, do not represent a safety concern in pregnant or lactating women.

The NRC manufactured by the Applicant is a crystalline substance, that contains greater than or equal to 90% NRC, the remaining components being residual solvents, reaction by-products and degradation products. The nicotinamide riboside moiety in the NRC is predominantly the β form. Stability tests indicate NRC is stable for up to two years in powdered FSMP formulations. FSANZ has developed specifications based on information provided by the Applicant.

In conclusion, FSANZ considers that based on the best available evidence in laboratory animals and humans, NRC is a bioavailable form of niacin which at intakes ranging from 100 to 2000 mg/day would be expected to support normal physiological function. However none of the studies included a comparator treatment group receiving NA or Nam, therefore it was not possible to establish bioequivalence to forms of niacin already permitted in the Code. On that basis it is not possible for FSANZ to establish with certainty whether lower NRC intakes which match adult Recommended Dietary Intakes for niacin would support essential requirements, when it is the only form of vitamin B3 in the diet.

NRC is not expected to represent a safety concern when prescribed, and used under medical supervision at intakes below the UL for Nam, and for pregnant or lactating women, at levels of intake consistent with Recommended Dietary Intakes for niacin.

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Appendix 1: Usual daily intake of niacin equivalent from foods and proportion of niacin equivalent from food groups for Australia and New Zealand

The usual daily intake of niacin equivalent from food is provided in the **Table A1.1** for Australia and **Tables A1.2** and **A1.3** for New Zealand. The contribution of the major food groups to dietary intakes of niacin equivalents is given in **Table A1.4**.

The national nutrition survey data used for these assessments were:

- The 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS) (ABS 2014a and 2014b)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS) (Ministry of Health 2003; Ministry of Health 2005)
- The 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS) (Ministry of Health 2011a; Ministry of Health 2011b).

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information is available on the FSANZ website on [the national nutrition surveys used to conduct dietary exposure assessments](#).

The contribution of foods to niacin intake presented in this Appendix was obtained from published sources. The majority of the contribution data for Australia came from the published reports from the 2011-12 NNPAS (ABS 2014a). All results are based on day 1 food consumption data only from each of the nutrition surveys.

2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), undertaken by the Australian Bureau of Statistics (ABS) as part of the 2011-13 Australian Health Survey, is the most recent food consumption data for Australia. This survey includes food consumption data from a sample of 12,153 Australians aged from 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents (n=7,735) also completing a second 24-hour recall on a second, non-consecutive day. The data were collected from May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Consumption and respondent data from the survey were incorporated into the Harvest program from the Confidentialised Unit Record Files (CURF) dataset (ABS 2014). These data were weighted during the calculations undertaken in Harvest.

2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)

The 2002 NZ CNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5–14 years. The data were collected during the school year from February to December 2002. The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children's nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements. These data were weighted during the calculations undertaken in Harvest.

2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

The 2008 NZ ANS provides comprehensive information on food consumption for a sample of 4,721 respondents aged 15 years and above. The survey was conducted on a stratified sample over a 12-month period from October 2008 to October 2009. The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZ ANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures. These data were weighted during the calculations undertaken in Harvest.

Table A1.1. Usual daily intake of niacin equivalents from foods for Australia (mg/day)

| | Age group (years) | | | | | | | |
|--|-------------------|----------|----------|-----------|-----------|-----------|-----------|-------------|
| | 2-3 | 4-8 | 9-13 | 14-18 | 19-30 | 31-50 | 51-70 | 71 and over |
| Percentiles | Males | | | | | | | |
| 5 | 19 | 23 | 25 | 31 | 35 | 33 | 30 | 25 |
| 10 | 21 | 24 | 28 | 33 | 38 | 36 | 33 | 28 |
| 15 | 22 | 25 | 30 | 36 | 41 | 39 | 35 | 30 |
| 25 | 23 | 27 | 33 | 39 | 44 | 42 | 38 | 33 |
| 50 | 26 | 30 | 38 | 45 | 51 | 49 | 44 | 39 |
| 75 | 30 | 34 | 45 | 52 | 59 | 56 | 51 | 45 |
| 85 | 31 | 36 | 49 | 56 | 63 | 60 | 55 | 49 |
| 90 | 33 | 38 | 51 | 59 | 66 | 63 | 58 | 51 |
| 95 | 35 | 40 | 55 | 63 | 70 | 68 | 62 | 55 |
| Mean intake | 26 | 31 | 39 | 46 | 52 | 49 | 45 | 39 |
| Estimated Average Requirement (EAR) | 5 | 6 | 9 | 12 | 12 | 12 | 12 | 12 |
| Proportion with usual intake less than EAR (%) | — | — | — | — | — | — | — | — |
| Percentiles | Females | | | | | | | |
| 5 | 17 | 20 | 21 | 22 | 23 | 23 | 22 | 21 |
| 10 | 18 | 21 | 23 | 24 | 25 | 25 | 25 | 23 |
| 15 | 19 | 22 | 25 | 26 | 27 | 27 | 26 | 24 |
| 25 | 20 | 24 | 27 | 28 | 29 | 30 | 29 | 27 |
| 50 | 23 | 27 | 32 | 33 | 34 | 35 | 34 | 32 |
| 75 | 26 | 30 | 38 | 39 | 40 | 41 | 40 | 37 |
| 85 | 28 | 32 | 41 | 43 | 44 | 44 | 43 | 40 |
| 90 | 29 | 34 | 43 | 45 | 46 | 47 | 45 | 42 |
| 95 | 31 | 36 | 46 | 48 | 49 | 50 | 49 | 46 |
| Mean intake | 23 | 27 | 33 | 34 | 35 | 36 | 35 | 32 |
| Estimated Average Requirement (EAR) | 5 | 6 | 9 | 11 | 11 | 11 | 11 | 11 |
| Proportion with usual intake less than EAR (%) | — | — | — | — | — | — | — | — |

Source: ABS (2014a) and (2014b).

— nil or rounded to zero (including null cells).

Table A1.2. Usual daily intake¹ of niacin equivalents from foods New Zealanders aged 5 to 14 years (mg/day)

| | | Mean | SEM | 10th* | 50th* | 90th* |
|---------------------------|------------|------|------|-------|-------|-------|
| Sex and age group (years) | | 29.3 | 0.43 | 19.2 | 27.8 | 40.8 |
| Males | 5 - 6 | 26.1 | 0.67 | 20.0 | 25.6 | 32.8 |
| | 7 -10 | 30.0 | 0.75 | 19.8 | 28.8 | 41.6 |
| | 11-14 | 37.6 | 1.25 | 24.1 | 36.0 | 52.9 |
| | Total 5-14 | 32.3 | 0.58 | 21.0 | 30.7 | 45.4 |
| Females | 5 - 6 | 21.6 | 0.72 | 16.2 | 21.0 | 27.7 |
| | 7 -10 | 26.0 | 0.59 | 17.8 | 25.1 | 35.3 |
| | 11-14 | 28.0 | 1.28 | 23.0 | 27.7 | 33.4 |
| | Total 5-14 | 26.0 | 0.61 | 18.9 | 25.3 | 33.9 |

* Percentiles.

¹ Usual intake. These data were adjusted for intra-individual variation using PC-SIDE

Source: 2002 NZ CNS, Ministry of Health (2003)

Table A1.3. Usual daily intake¹ of niacin equivalents from foods New Zealanders aged 15 years and above (mg/day)

| | | Mean | 10th | Median (50th) ² (95% CI) | 90th ² | Inadequate intake (%) ³ |
|-------------------------------------|-----------|------|------|--|-------------------|------------------------------------|
| Total population 15 years and above | | 36.9 | 23.1 | 34.7 (33.5-35.9) | 53.3 | 0.0 |
| By sex and age group (years) | | | | | | |
| Males | 15-18 | 46.1 | 38.3 | 45.7 (41.7-49.7) | 54.6 | 0.0 |
| | 19-30 | 50.6 | 31.2 | 47.4 (41.9-52.9) | 74.0 | 0.0 |
| | 31-50 | 46.7 | 36.1 | 45.8 (42.2-49.4) | 58.4 | 0.0 |
| | 51-70 | 39.0 | 24.6 | 36.6 (34.0-39.2) | 56.1 | 0.1 |
| | 71+ | 32.0 | 24.1 | 31.4 (29.8-33.0) | 40.6 | 0.0 |
| | Total 15+ | 43.9 | 30.0 | 42.2 (40.4-44.0) | 60.0 | 0.0 |
| Females | 15-18 | 30.1 | 19.1 | 28.4 (26.3-30.5) | 43.1 | 0.2 |
| | 19-30 | 32.0 | 24.1 | 31.2 (28.1-34.3) | 40.9 | 0.0 |
| | 31-50 | 32.6 | 22.7 | 31.7 (30.1-33.3) | 43.7 | 0.0 |
| | 51-70 | 28.3 | 20.0 | 27.7 (26.4-29.0) | 37.4 | 0.0 |
| | 71+ | 24.2 | 16.8 | 23.3 (22.3-24.3) | 32.7 | 0.4 |
| | Total 15+ | 30.3 | 20.7 | 29.1 (28.1-30.1) | 41.3 | 0.1 |

¹ Usual daily intake. These data were adjusted for intra-individual variation using PC-SIDE.

² Percentiles.

³ Calculated by probability analysis.

Source: 2008 NZ CNS, Ministry of Health (2012).

Table A1.4. Major food group contributions to dietary intakes of niacin equivalents

| Major food group | Contribution (%)* | | |
|--|-----------------------------------|------------------------------|--------------------------------------|
| | Australia 2 years and above | New Zealand 5-14 years | New Zealand 15 years and above |
| Non-alcoholic beverages | 6.3 | NR | 7.6 |
| Cereals and cereal products | 18.3 | 23 | 24.5 |
| Cereal based products and dishes | 13.1 | 4 | 4.9 |
| Fats and oils | 0.1 | NR | 0 |
| Fish and seafood products and dishes | 5.0 | 3 | 5.8 |
| Fruit products and dishes | 1.7 | NR | 2.8 |
| Egg products and dishes | 1.0 | NR | 2.0 |
| Meat, poultry and game products and dishes | 30.7 | 19 | 25.3 |
| Milk products and dishes | 7.4 | 6 | 8.0 |
| Dairy & meat substitutes | 0.2 | NR | NR |
| Soup | 1.4 | NR | 0.9 |
| Seed and nut products and dishes | 1.5 | NR | 1.6 |
| Sauces, dips and condiments | 0.3 | NR | 1.8 |
| Vegetable products and dishes [#] | 6.4 | 7 | 11.4 |
| Legume and pulse products and dishes | 0.3 | NR | NR |
| Snack foods | 0.4 | NR | 0.3 |
| Sugar products and dishes | 0.0 | NR | 0.6 |
| Confectionery and cereal/nut/fruit/seed bars | 0.7 | NR | 0.6 |
| Alcoholic beverages | 1.5 | NR | 1.2 |
| Special dietary foods | 0.7 | NR | 0.4 |

NR: Not reported.

* Data are presented on an all respondents basis and data for day 1 are presented for all surveys.

[#] Two categories for New Zealand data were combined which were 'vegetables' and 'potatoes, kumara and taro'.