

## Safety Assessment of Nicotinamide Riboside, a Form of Vitamin B3

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11 **Safety Assessment of Nicotinamide Riboside, a Form of Vitamin B3**

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## Safety Assessment of Nicotinamide Riboside

### Abstract

Nicotinamide riboside (NR) is a naturally occurring form of vitamin B3 present in trace amounts in some foods. Like niacin, it has been shown to be a NAD<sup>+</sup> precursor in the biosynthesis of NAD<sup>+</sup>. The safety of Niagen, a synthetic form of NR was determined using a bacterial reverse mutagenesis assay (Ames), an in vitro chromosome aberration assay, an in vivo micronucleus assay, and acute, 14-day and 90-day rat toxicology studies. Niagen was not genotoxic. The oral LD50 was > 5000 mg/kg. Based on the results of a 14-day study, a 90-day study was performed comparing Niagen at 300, 1000 and 3000 mg/kg/day to an equimolar dose of nicotinamide at 1,260 mg/kg/day as a positive control. Results from the study show that Niagen had a similar toxicity profile to nicotinamide at the highest dose tested. Target organs of toxicity were liver, kidney, ovaries, and testes. The lowest observed adverse effect level (LOAEL) for Niagen was 1000 mg/kg/day, and the no observed adverse effect level (NOAEL) was 300 mg/kg/day.

**Keywords:** Niagen; nicotinamide riboside; nicotinamide; 90-day subchronic oral toxicity; genotoxicity

## Safety Assessment of Nicotinamide Riboside

### Introduction

Niacin refers to nicotinamide, nicotinic acid and derivatives that exhibit the biologic activity of nicotinamide. Nicotinamide riboside (NR) is a single chemical moiety containing nicotinamide and ribose (Figure 1) and, because it is a precursor of nicotinamide adenine dinucleotide (NAD), is considered to be a form of vitamin B3 (reviewed in Chi and Sauve 2013; Penberthy and Kirkland, 2012; Bogan and Brenner, 2008). Although there is no evidence of adverse effects from naturally occurring niacin in foods, it is recognized that for nutrients, there is a daily level of intake or threshold above which the risk of adverse effects increase (defined as the tolerable upper intake level (UL)) (Institute of Medicine, 1998). A UL for NR has not been established by an authoritative or regulatory body. Therefore, to establish a UL for Niagen™, a synthetic form of NR, pivotal genotoxicity and toxicology studies were conducted.

Niacin is essential for the formation of the pyridine nucleotide coenzymes NAD and nicotinamide adenine dinucleotide phosphate (NADP). Both coenzymes function indispensably in oxidation-reduction reactions involved in glucose, fatty acid, ketone body, and amino acid catabolism. Once inside the cells, nicotinic acid is converted to NAD by the

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biosynthetic (Preiss-Handler) pathway and intracellular NAD glycosidases then release nicotinamide, which circulates to other tissues where it is absorbed and functions as a precursor for NAD and NADP formation (Preiss and Handler, 1957a; Preiss and Handler, 1957b; Preiss and Handler, 1958a; Preiss and Handler, 1958b; Collins and Chaykin, 1972). In contrast, nicotinamide and nicotinamide riboside are converted to NAD by way of nicotinamide mononucleotide in the salvage pathway (Rongvaux et al., 2002; Martin et al., 2001; Revollo et al., 2004; Bieganski et al., 2004). When nicotinic acid and nicotinamide are in excess, both are methylated in the liver and excreted in the urine (Mrochek et al., 1976).

Because animal toxicology studies on nicotinic acid and nicotinamide are limited and do not comply with current standardized testing protocols, such as OECD (Unna, 1939; Handler and Dann, 1942; Chen et al., 1938; OECD SIDS, 2002), authoritative bodies have used the results clinical studies in which high doses of nicotinic acid and nicotinamide have been administered to derive the ULs. The most severe forms of toxicity after nicotinic acid ingestion are hepatotoxicity and glucose intolerance, and occur at doses greater than 500 mg/day. Flushing has also been reported, and generally occurs at doses greater than 50

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11 mg/day. As a result, it is considered to be the most sensitive endpoint of nicotinic acid  
12 effects. In contrast, after ingestion of supplemental nicotinamide, no cases of flushing or  
13 glucose intolerance have been reported and only one case of hepatitis was reported  
14 following the ingestion of greater than 3 g/day for several days (Winter and Boyer, 1973).  
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22 Although nicotinamide does not appear to be associated with flushing, the IOM  
23 established a UL of 35 mg/day for adults 19 years and older for both nicotinic acid and  
24 nicotinamide based on flushing because it is considered to be protective against potential  
25 adverse effects. The European Commission Scientific Committee on Food (SCF)  
26 established a UL of 10 mg acid/day for nicotinic acid based on flushing (European  
27 Commission Scientific Committee on Food, 2002). In contrast, the SCF established a  
28 separate UL of 900 mg/day for nicotinamide based on hepatic function in diabetic subjects  
29 ((European Commission Scientific Committee on Food, 2002).  
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11 **Materials and Methods**

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15 Bacterial Reverse Mutagenicity (Ames Assay)

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18 Bacterial reverse mutation assays were performed in compliance with the  
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20 Organization for Economic Co-operation and Development (OECD) Principles of Good  
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22 Laboratory Practices (GLP) and Guideline No. 471. Niagen (>99% nicotinamide riboside  
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24 chloride; CAS No. 23111-00-4) was supplied by ChromaDex, Inc. 2-aminoanthracene, 2-  
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26 nitrofluorene, sodium azide, 9-aminoacridine, and 4-nitroquinoline-*N*-oxide were obtained  
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28 from Sigma Aldrich Chemical Co., Inc. Aroclor 1254-induced rat liver S9 homogenate was  
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30 obtained from Xenometrix AG. *Salmonella typhimurium* TA98, TA100, TA1535, and  
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32 TA1537 were obtained from the National Collection of Type Cultures. *Escherichia coli*  
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34 WP2 *uvrA* (pKM101) was obtained from Xenometrix.

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41 The mutagenicity of Niagen was determined using the plate incorporation and pre-  
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43 incubation methods. In the plate incorporation method, 50, 159, 501, 1582, and 5000 µg  
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45 Niagen was mixed with selective top agar containing 0.6-0.8% agar, 0.5% NaCl, the tester  
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47 strains *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *Escherichia coli* WP2 *uvrA*  
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pKM101, histidine and biotin or tryptophan (depending on the type stain used), with and without of a metabolic activation system (S9 mix; 5-30% of the Aroclor 1254-induced rat liver S9 homogenate, NADP, glucose-6-phosphate, magnesium chloride, and potassium chloride) at 45-50°C. The mixture was overlaid onto solidified Vogel-Bonner minimal E basal agar (Vogel and Bonner, 1956), and after the selective top agar solidified, the plates were incubated at 37°C for 67 hr. The plates were examined for the presence of a background lawn and precipitate, and the number of revertant colonies were counted manually. In a confirmatory assay, the tester strains *S. typhimurium* TA98, TA100, TA1535 and TA1537 or *Escherichia coli* WP2 *uvrA* pKM101, and 99, 265, 699, 1869, or 5000 µg Niagen were preincubated at 37°C for 30 min in the presence or absence of the S9 mix. Molten selective top agar containing 0.6-0.8% agar, 0.5% NaCl, and histidine and biotin or tryptophan (depending on the type stain used) was then added and the resulting mixture was plated and incubated at 37°C for 67 hr. The plates were examined for the presence of a background lawn and precipitate, and the number of revertant colonies were counted manually.



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12 Importantly, all experiments were performed in triplicate with vehicle and strain-  
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14 specific positive controls. In the presence of the S9 mix, the positive controls for all strains  
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16 was 2-aminoanthracene. In the absence of the S9 mix, the positive controls for strains  
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18 TA98, TA100 and TA1535, TA1537, and WP2 *uvrA* pKM101 were 2-nitrofluorene,  
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20 sodium azide, 9-aminoacridine, and 4-nitroquinoline-*N*-oxide, respectively. Niagen was  
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22 considered cytotoxic if there was a 50% reduction in the mean number of revertants per  
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24 plate compared to the mean vehicle control and/or at least a moderate reduction in the  
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26 background lawn. Niagen was considered mutagenic if there was a concentration-related  
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28 increase in the number of revertants per plate in at least one tester strain over a minimum of  
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30 two increasing concentrations of Niagen. In the case of the strains TA98, TA100, and WP2  
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32 *uvrA* pKM101 the result was considered positive if the mean number of revertants was  
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34 equal to or greater than two times the number of revertants obtained with the negative  
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36 control. In the case of the strains TA1535 and TA1537 the result was considered positive if  
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38 the mean number of revertants was equal to or greater than three times the number of  
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40 revertants obtained with the negative control.  
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12 In vitro Chromosomal Aberration Assay  
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15 In vitro chromosomal aberration assays were performed in compliance with the  
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17 OECD Principles of GLP and Guideline No. 473. Niagen (>99% nicotinamide riboside  
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19 chloride) was supplied by ChromaDex, Inc. Cyclophosphamide monohydrate and ethyl  
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21 methanesulphonate were obtained from Sigma Aldrich Chemical Co., Inc. Aroclor 1254-  
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23 induced rat liver S9 homogenate was obtained from Xenometrix AG. Human peripheral  
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25 blood lymphocytes (PBLs) were obtained from whole blood harvested from a healthy donor  
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27 who was approximately 35 years-old, had no history of smoking or alcoholism, and had not  
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29 received medication one month prior to the blood draw. The whole blood was cultured in  
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31 RPMI 1640 medium containing 10% fetal bovine serum, heparin, EDTA, amphotericin,  
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33 penicillin, streptomycin, and phytohaemagglutinin (PHA) at 37°C and 5% CO<sub>2</sub> for 3 days  
34  
35 per OCED Guideline 473 and International Conference on Harmonisation of Technical  
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37 Requirements for Pharmaceuticals for Human Use (ICH)-harmonized guidances on  
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39 genotoxicity testing of pharmaceuticals.  
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47 To determine the clastogenic activity of Niagen, the PHA-stimulated whole blood  
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49 cultures were centrifuged and the resulting PBLs were resuspended in RPMI 1640  
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11 containing 10% fetal bovine serum, amphotericin, penicillin, streptomycin, either vehicle  
12 (water), 1.25, 2.5, or 5 mg/ml of Niagen, or the appropriate positive control  
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14 (cyclophosphamide monohydrate and ethyl methanesulphonate), supplemented with either  
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16 phosphate buffered saline or the metabolic activation system (S9 mix; 10% of the Aroclor  
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18 1254-induced rat liver S9 homogenate, 4mM NADP, 5 mM glucose-6-phosphate, 8 mM  
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20 magnesium chloride, and 33 mM potassium chloride). The mixtures were then incubated at  
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22 37°C and 5% CO<sub>2</sub> for 3 and 19 hours, at which point colchicine was added to the cultures  
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24 to a final concentration of 2 µg/ml. Three hours later, the cells were harvested by  
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26 centrifugation at 800 to 1000 rpm for approximately 10 minutes, resuspended in 0.56 %  
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28 pre-warmed potassium chloride, and incubated at room temperature for 25 to 30 minutes.  
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30 The cell suspension was then centrifuged 800 to 1000 rpm for approximately 10 minutes,  
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32 the resulting supernatant was discarded, and the cellular pellet was resuspended and  
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34 incubated in cold fixative (acetic acid: methanol (1:3)) at room temperature for 10 to 15  
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36 minutes. This process was repeated 3 additional times with one incubation at 4 °C for a  
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38 minimum of 1 hour followed by two incubations at room temperature for 10 to 15 minutes.  
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40 After the final incubation, the cells suspension was dropped onto clean, cold slides, which  
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42 were then gently dried over a flame, stained with 5% Giemsa, and scored for the presence  
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of metaphase cells and the presence of aberrations. To determine the mitotic index, which was used as an indicator of cytotoxicity, a minimum of 1000 cells were scored for each group and the total number of metaphases was divided by the number of cells counted. The quotient was then multiplied by 100. To determine the types of aberrations (chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks, deletions, and fragments) a minimum of 300 metaphases containing 46 +/- 2 centromere regions were counted and the number of cells containing one or more different types of aberrations were recorded. The data was the subjected to a one-tailed Fisher Exact test. Niagen was considered cytotoxic if there was a 45 +/- 5% reduction in the mitotic index compared to the vehicle control. Niagen was considered mutagenic if there was a concentration-related and statistically significant increase ( $p < 0.05$ ) in the number of chromosome aberrations.

### In vivo Micronucleus Assay

The in vivo micronucleus assay performed in compliance with the OECD Principles of GLP, OECD Guideline No. 474, and the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals

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11 (CPCESA), Government of India. The rats were obtained from Harlan Laboratories.

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14 Nutrilab Rodent Pellet feed was obtained from Provimi Animal Nutrition. Niagen (>99%  
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16 nicotinamide riboside chloride; CAS No. 23111-00-4) was supplied by ChromaDex, Inc.

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19 Niagen (>99% nicotinamide riboside chloride) was supplied by ChromaDex, Inc.

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22 Cyclophosphamide was obtained from Sigma Aldrich Chemical Co., Inc.

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25 All rats were housed at three rats per sex per cage, acclimatized for at least 5 days  
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27 prior to treatment, and, except for the overnight fast prior to euthanasia on day 91, provided  
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29 feed and water *ad libitum* throughout the study. Prior to dosing the rats were randomized by  
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31 body weight to two groups (n=6/sex/group). At dosing, a single dose of vehicle (water),  
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33 500, 1000, and 2000 mg/kg of Niagen or 40 mg/kg cyclophosphamide was administered by  
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35 gavage at a rate of 10 ml/kg body weight. Twenty-four hours after dosing the vehicle, 500  
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37 mg/kg Niagen-, 1000 mg/kg Niagen-, 2000 mg/kg Niagen-, and 40 mg/kg  
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39 cyclophosphamide-treated groups were euthanized by carbon dioxide asphyxiation. Forty-  
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41 eight hours after dosing, an additional vehicle and 2000 mg/kg Niagen-treated group was  
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44 also euthanized by carbon dioxide asphyxiation. The animals were observed for mortality at  
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1 and 2 hours and then twice daily after dosing for 2 days. Clinical signs were monitored 1 and 2 hours after dosing and then once daily for 2 days.

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

### Acute Toxicity Study

The acute toxicity study was performed in female Sprague Dawley SD rats in compliance with the OECD Principles of GLP, the Guidance for Industry, Single Dose Acute Toxicity Testing for Pharmaceuticals from the United States Food and Drug Administration, and the recommendations of the AAALAC and CPCESA, Government of

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11 India. The rats were obtained from Harlan Laboratories. Nutrilab Rodent Pellet feed was  
12 obtained from Provimi Animal Nutrition. Niagen (>99% nicotinamide riboside chloride)  
13 was supplied by ChromaDex, Inc.  
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20 All rats were housed two to three rats per sex per cage, acclimatized for at least 5  
21 days prior to treatment, and, except for the overnight fast prior to dosing, provided feed and  
22 water *ad libitum* throughout the study. Prior to dosing the rats were randomized by body  
23 weight to two groups (n=5/sex/group). At dosing, a single dose of vehicle (water) or 5000  
24 mg/kg of Niagen was administered by gavage at a rate of 10 ml/kg body weight. Over the  
25 course of the following 14 days all rats were monitored for morbidity, mortality, and visible  
26 clinical signs. Detailed clinical examinations were conducted on day 1, 8 and 15 and  
27 included evaluations of the skin, fur, eyes, mucous membranes, occurrence of secretions  
28 and excretions and autonomic activity, changes in gait, posture, response to handling, and  
29 presence of clonic or tonic movements, stereotypes, or bizarre behaviors. Body weights  
30 were recorded prior to treatment on day 1 and then on day 8 and 15. Food consumption for  
31 each cage was measured on day 8 and 15 and food consumption per rat was calculated by  
32 dividing the total food consumption during the interval per cage by the number of rats  
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multiplied by the number of days. On day 15, all animals were euthanized and examined for gross pathological changes. Analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing the Niagen and vehicle-treated group for each sex. All quantitative variables, like body weight, body weight gain, and food consumption were subjected to Student's t-test. Males and females were considered separately for each analyses and a p value of <0.05 was considered statistically significant.

### 14-Day Repeat Dose Study

A 14 day repeat-dose study was conducted in Sprague Dawley rats in compliance with the OECD Guideline 407, but for 14 days instead of 28 days, and the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCESA), Government of India. The rats were obtained from Harlan Laboratories. Nutrilab Rodent Pellet feed was obtained from Provimi Animal Nutrition. Niagen (>99% nicotinamide riboside chloride) was supplied by ChromaDex, Inc.



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All rats were housed two to three rats per sex per cage, acclimatized for at least 5 days prior to treatment, and provided feed and water *ad libitum* throughout the study. Prior to dosing the rats were randomized to five groups (n=5/sex/group) according to body weight. During the 14-day treatment period, each group was gavaged daily with either vehicle (water) or 750, 1500, 2500, or 5000 mg/kg/day of Niagen at a rate of 10 mL/kg body weight. Dose formulation analyses showed that Niagen was completely soluble in water and the dose formulations were homogeneous and contained the targeted concentrations of nicotinamide riboside. Stability analyses showed that when Niagen was dissolved in water, nicotinamide riboside was stable up to 6 hours at room temperature and 7 days at 2-8 °C. During the 14 day-treatment period all rats were monitored for morbidity, mortality, and visible clinical signs. Detailed clinical examinations were conducted on day 1, 8 and 15 and included evaluations of the skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity, changes in gait, posture, response to handling, and presence of clonic or tonic movements, stereotypes, or bizarre behaviors. Body weights were recorded prior to treatment on day 1 and then on day 8 and 15. Food consumption for each cage was measured on day 8 and 15 and food consumption per rat was calculated by dividing the total food consumption during the interval per cage by the

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number of rats multiplied by the number of days. On day 15, all animals were euthanized and examined for gross pathological changes. All analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing the Niagen and vehicle-treated group for each sex. All quantitative variables, like body weight, body weight gain, and food consumption were tested for normality and homogeneity of variances within the group before performing a one-factor ANOVA by treatment. When the data were found to be non-optimal, the data were log transformed prior to performing the ANOVA. Comparisons of the means differences between the Niagen- and vehicle-treated groups was performed using Dunnett's post-hoc test. When normality/homogeneity was significant, even after transformation, the data was subjected to the Kruskal-Wallis test followed by Dunn's post-hoc test. Males and females were considered separately for each analyses and a p value of <0.05 was considered statistically significant.

### Subchronic Toxicity Study

A 90-day oral subchronic toxicity study in Sprague Dawley rats was conducted in compliance with the OECD Principles of GLP, the OECD Guideline 408 for testing of chemicals, and the recommendations of the AAALAC and CPCESA, Government of India.

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The rats were obtained from Harlan Laboratories. Nutrilab Rodent Pellet feed was obtained from Provimi Animal Nutrition. Niagen (>99% nicotinamide riboside chloride) was supplied by ChromaDex, Inc. Nicotinamide ( $\geq 99.5\%$ ; CAS No. 98-92-0) was obtained from Sigma Aldrich.

All rats were housed two to three rats per sex per cage, acclimatized for at least 5 days prior to treatment, and, except for the overnight fast prior to euthanasia on day 91, provided feed and water *ad libitum* throughout the study. Prior to dosing the rats were randomized by body weight to 5 groups (n=10/sex/group). During the 90-day treatment period, each group was gavaged daily with either vehicle (water), 300, 1000, 3000 mg/kg of Niagen or 1260 mg/kg/day of nicotinamide, which is equivalent to 3000 mg/kg/day of Niagen on a molar basis. Dose formulation analyses showed that both Niagen and nicotinamide were completely soluble in water and the dose formulations contained the targeted concentrations of nicotinamide riboside or nicotinamide. Stability analyses showed that when Niagen and nicotinamide were dissolved in water, both nicotinamide riboside and nicotinamide were stable up to 6 hours at room temperature and 7 days at 2-8 °C. The parameters evaluated during the study were twice daily checks for mortality, daily

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evaluations for clinical signs, weekly detailed clinical examinations, and weekly body weight and food consumption measurements. Ophthalmological examinations were performed prior to treatment and prior to sacrifice. On day 91, after urine was collected individually from all animals after an overnight fast, the animals were anesthetized, and blood was collected from the sublingual vein for hematology, coagulation, and clinical chemistry evaluations. Then the animals were euthanized by exsanguination under deep anesthesia and subjected to necropsy and gross pathological examination. Hematological parameters included differential leukocyte count (DLC), reticulocyte, leukocyte, erythrocyte, eosinophil, neutrophil, lymphocyte platelets, basophils, monocytes, and large unstained cell (LUC) counts, hemoglobin (Hgb), hematocrit, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean hemoglobin concentration (MCHC), prothrombin time (PT), and activated partial thromboplastin time (APTT). Plasma clinical chemistry parameters included total protein, albumin, bile acids, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGTP), globulin, alkaline phosphatase (ALP), total cholesterol (TC), triglycerides, glucose, blood urea nitrogen (BUN), creatinine, inorganic phosphorous (Pi), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and chloride (Cl) levels. The organs that were collected,

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weighed, and preserved included the adrenals, aorta, bone marrow smear, brain including medulla/pons, cerebellum and cerebrum, caecum, colon, duodenum, epididymides, esophagus, eyes with optic nerve, biceps femoris muscles, femur bone with joint gross lesions, heart, ileum with Peyer's patches, jejunum, kidneys, liver, lungs with main bronchi and bronchioles, mandibular lymph nodes, mesenteric lymph nodes, mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, seminal vesicles and coagulating glands, rectum, salivary glands (mandibular, parotid and sublingual), sciatic nerve, skin (inguinal region), spinal cord at 3 levels - cervical, mid-thoracic and lumbar, spleen, sternum with marrow, stomach, testes, thymus, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus with cervix, and vagina. The preserved tissues were processed and embedded in paraffin, sectioned and stained with Haematoxylin and Eosin. Histopathology was performed on all preserved organs of vehicle control, positive control nicotinamide and Niagen high dose. Liver, kidneys, thyroids, testes, epididymides (male), ovaries (female) and adrenals were examined at lower dose Niagen groups as treatment-related microscopic lesions were found in high dose group. Urine was analyzed for volume, osmotic pressure, specific gravity, pH, and concentrations of glucose, bilirubin, ketone, blood, protein, urobilinogen, nitrate and the presence of leukocytes. All analyses were conducted using

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two-tailed tests for a minimum significance level of 5% comparing the Niagen and vehicle-treated group for each sex. All quantitative variables, like body weight, body weight gain, and food consumption were tested for normality and homogeneity of variances within the group before performing a one-factor ANOVA by treatment. When the data were found to be non-optimal, the data were log transformed prior to performing the ANOVA.

Comparisons of the means differences between the Niagen- and vehicle-treated groups was performed using Dunnett's post-hoc test. When normality/homogeneity was significant, even after transformation, the data was subjected to the Kruskal-Wallis test followed by Dunn's post-hoc test. Males and females were considered separately for each analyses and a p value of <0.05 was considered statistically significant.

## Safety Assessment of Nicotinamide Riboside

### Results

#### Bacterial Reverse Mutagenicity (Ames Assay)

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

#### In vitro Chromosomal Aberration Assay

Niagen was not cytotoxic to ex vivo human peripheral blood lymphocytes at any of the concentrations used in the study as determined by the mitotic index (data not shown), and, compared to the vehicle control, did not increase the number of aberrant metaphases when incubated with or without S9 mix for 6 hours (Table 2). Moreover, the types of

### Safety Assessment of Nicotinamide Riboside

aberrations (chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks) detected in the vehicle- and Niagen-treated cells were similar. In contrast, the positive controls, cyclophosphamide and ethyl methansulphonate, significantly increased the number of aberrant metaphases ( $p < 0.05$ ), characterized as chromatid gaps, chromosomal gaps, chromosomal breaks, chromatid breaks, deletions, and fragments, thus confirming the sensitivity and validity of the assay. Similar results were also found when the lymphocytes were incubated with increasing amounts of Niagen for 22 hours in the absence of the S9 mix (data not shown). Niagen was therefore not clastogenic under the conditions used in the study.

### In vivo Micronucleus Assay

No mortalities or clinical signs of toxicity were observed in any of the rats receiving Niagen. In addition, bone marrow analyses showed that compared to the negative control, the administration of 500, 1000, and 2000 mg/kg of did not result in cytotoxicity or increase the percentage of polychromatic erythrocytes at either 24 or 48 hours of administration (Table 3). In contrast, the positive control cyclophosphamide induced a statistically significant ( $p < 0.05$ ) increase in the percentage of polychromatic erythrocytes



### Safety Assessment of Nicotinamide Riboside

at 24 hours demonstrating both the sensitivity and validity of the assay. Therefore, Niagen was not genotoxic under the conditions used in this study.

### Acute Toxicity Study

No mortalities were observed in males or females at the single tested dose of 5000 mg/kg of Niagen. No clinical signs were observed in male and female rats. No statistically significant changes were observed in body weight in both sexes. Cumulative body weight gain (days 1-15) was significantly lower in female rats as compared to vehicle-treated group. Because change in day 15 body weight was minimal (-3%), this was considered as treatment related but non-adverse. There were no statistically significant changes in food consumption in either sex. There were no gross pathological lesions observed in male and female rats. The LD50 for Niagen was determined to be greater than 5000 mg/kg.

### 14-day Toxicology Study

Minimal reduction in mean body weight compared to the vehicle-treated group was observed in male rats at 2500 mg/kg/day (7-8% reduction) and 5000 mg/kg/day (8-9% reduction) on Days 8, 11, 14 and 15. A test article related decrease in overall (Day 1-14)

## Safety Assessment of Nicotinamide Riboside

feed consumption was observed at 5000 mg/kg/day (8%) in male rats. No test item-related changes were observed in body weight and feed consumption in female rats. No gross pathological lesions were observed in male and female rats. Based on these results, the doses of 300, 1000 and 3000 mg/kg/day were chosen for the 90-day subchronic toxicity study in rats.

### 90-day Subchronic Toxicity Study

#### *Mortality, body weight and feed consumption*

No treatment related mortality or clinical signs were observed at any dose level in this study. Compared to vehicle-treated controls, a significant ( $p < 0.05$ ) treatment-related decrease in body weight (17% reduction) was noted in male rats at the high dose (3000 mg/kg/day) of Niagen; a similar decrease in body weight was observed at an equimolar dose (1260 mg/kg/day) of nicotinamide (Figure 2). A significant ( $p < 0.05$ ) 8-9% reduction body weight ( $< 10\%$ ) was observed at the 300 and 1000 mg/kg/day dose of Niagen in male rats. This decrease was  $< 10\%$  and therefore not considered to be adverse. No statistically significant differences in body weights were seen in Niagen- or nicotinamide-treated

### Safety Assessment of Nicotinamide Riboside

females. In male rats, decreases in feed consumption were noted at 3000 mg/kg/day of Niagen (9-14%) and 1260 mg/kg/day of nicotinamide (9-17%) throughout the treatment period. Decreases in feed consumption also occurred at 300 mg/kg/day on days 57-64 and at 1000 mg/kg/day on days 50-57. In female rats, decreases in feed consumption occurred at days 1-8 in the nicotinamide treated group and at days 15-22 in the 3000 mg/kg/day Niagen group.

### *Hematological, clinical chemistry and urinalysis tests*

Similar treatment-related changes in hematology parameters were observed at the high dose Niagen- and nicotinamide-treated groups (Table 4). Statistically significant ( $p < 0.05$ ) treatment related increases in white blood cells (WBC) and neutrophils occurred in both males and females. Statistically significant ( $p < 0.05$ ) increases in monocytes were also noted in females treated with 3000 mg/kg/day of Niagen and 1260 mg/kg/day of nicotinamide. At 1000 mg/kg/day of Niagen, significant increases in WBC and neutrophils were observed in males and females, respectively. There were no significant changes in hematological parameters at male or female rats treated with 300 mg/kg/d of Niagen. Importantly, the significant effects were not associated with any inflammatory changes in

### Safety Assessment of Nicotinamide Riboside

any of the organs examined. All other changes in hematology parameters, including those determined to be statistically significant, were considered to be due to normal biological variation, and not due to the administration of the test item.

Niagen produced statistically significant ( $p < 0.05$ ) increases at 3000 mg/kg/day in ALT, ALP, GGT, triglycerides and bile acids; the effects on ALT and triglycerides were significant at 1000 mg/kg/day of Niagen in females (Table 5). Comparable effects on clinical chemistries were seen in the nicotinamide-treated group. A minimal but statistically significant decrease in sodium in females and chloride in males and females was noted at 3000 mg/kg/day of Niagen. Similar results were seen in the nicotinamide-treated group. Significant reductions ( $p < 0.05$ ) in sodium were also observed in males and females treated with 1000 mg/kg/day. All other changes in clinical chemistry parameters were considered incidental as the magnitude of change minimal and were considered to be due to normal biological variation.

Urinalysis showed increased urine volume in both nicotinamide and high dose Niagen- treated males. This effect was considered a treatment-related effect, which may have been correlated with microscopic changes in the adrenals. Urine pH was decreased in

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10 Safety Assessment of Nicotinamide Riboside

11 males and females in the 3000 mg/kg/day Niagen high dose males (20%) and females  
12 (13%), but not the males and females treated with nicotinamide. The slightly acidic pH may  
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14 have been due to the excretion of test item and an acidic metabolite.  
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20 *Gross Pathology*  
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23 Bilateral small-size testes was observed in 3000 mg/kg/day Niagen- and  
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25 nicotinamide-treated males was considered to be treatment-related and associated with  
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27 degeneration/atrophy of the seminiferous tubules.  
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31 One single incidence of auxiliary region subcutaneous nodule microscopically  
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33 associated with adenocarcinoma of mammary gland was observed in one Niagen high dose  
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35 male. This neoplastic change was considered an incidental tumor of spontaneous origin as it  
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37 is reported to occur naturally in young Sprague Dawley male rats (Ikezaki et al., 2011).  
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41 All other single or low incidences of gross pathologic findings observed in different  
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43 groups were considered incidental and not related to test item as they were randomly  
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45 distributed in different groups and were not dose-dependent.  
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12 *Organ Weights*

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15 At 3000 mg/kg/day, there were statistically significant reductions in absolute organ  
16 weights of brain, spleen, testes, epididymides, prostate, thyroid/parathyroid, pituitary and  
17 heart in males; brain and pituitary absolute organ weights were reduced and liver and  
18 kidney absolute organ weights were increased in females. In the nicotinamide-treated  
19 group, there were statistically significant reductions in absolute organ weights of brain,  
20 spleen, testes, epididymis, prostate, thyroid/parathyroid, pituitary, thymus and heart in  
21 males; brain and pituitary absolute organ weights were reduced and liver and ovary weights  
22 were increased in females. At 1000 mg/kg/day, there were statistically significant  
23 reductions in absolute organ weights of thyroid/parathyroid, pituitary and heart in males; no  
24 effects on absolute organ weights were seen in females. At 300 mg/kg/day, there were  
25 statistically significant reductions in absolute organ weights of brain and heart in males; no  
26 effects on absolute organ weights were seen in females. Relative organ weight changes in  
27 the 3000 mg/kg/day Niagen-treated rats were similar to those of animals ingesting an  
28 equimolar dose of nicotinamide (Table 6). Treatment-related organ weight changes were  
29 observed in liver, kidneys, testes, epididymides and ovaries in 3000 mg/kg/day Niagen- and  
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## Safety Assessment of Nicotinamide Riboside

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

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

## *Histopathological Findings*

Treatment-related histopathological changes were observed in liver, thyroid, kidneys, testes, epididymides, ovaries and adrenals in both the 3000 mg/kg/day Niagen-

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11 treated and nicotinamide-treated groups. All other single or low incidences of microscopic  
12 findings observed were considered incidental and not related to test item as they were  
13 randomly distributed among groups. Importantly, the treatment-related histopathological  
14 changes noted in Niagen at the high dose were similar to the findings observed in the  
15 equimolar nicotinamide group.  
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24 In the livers of 3000 mg/kg/day Niagen-treated and nicotinamide-treated and treated  
25 males and females centrilobular hepatocellular hypertrophy, characterized by enlarged  
26 hepatocytes containing granular eosinophilic cytoplasm, follicular cell hypertrophy,  
27 characterized by enlarged follicular epithelium which contained pale eosinophilic  
28 cytoplasm and small clear vacuoles, and hepatocyte single cell necrosis were reported and  
29 considered a treatment-related adverse change. In the kidneys, chronic progressive  
30 nephropathy characterized by presence of foci or areas of basophilic tubules, with or  
31 without simple tubular hyperplasia, hyaline casts, atrophic tubules, dilated tubule, focal  
32 glomerular sclerosis/atrophy and mononuclear cell infiltration was seen.  
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46 In male rats, both 3000 mg/kg/day Niagen-treated and nicotinamide-treated rats  
47 exhibited degeneration/atrophy of seminiferous tubules characterized by the presence of  
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### Safety Assessment of Nicotinamide Riboside

some tubules containing degenerating germ cells. Some tubules were also depleted of all germ cells and lined only by Sertoli cells and while others were partially depleted of germ cells. Degenerating tubules contained multinucleated germ cells, spermatid head retention, Sertoli cell cytoplasmic vacuolation and disorganization of germ cells. Reduced sperm and cell debris in epididymal lumen in nicotinamide and Niagen high dose males were considered treatment-related effects.

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

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

## Safety Assessment of Nicotinamide Riboside

### Discussion

The safety of a synthetic form of NR was determined in an Ames assay, in vitro chromosome aberration assay, in vivo micronucleus assay and acute, 14-day and 90-day rat toxicology studies to derive a UL.

The determination of a UL for any vitamin is based on well-established principles of risk assessment and relies on data concerning adverse health effects from excessive nutrient intakes in epidemiologic studies, clinical trials, and experimental studies. Several factors associated with these various data sources influence the derivation of a UL. Among the most important of these factors are the intake at which adverse effects occur (the LOAEL) and the maximum level of intake, which is always less than the LOAEL, at which no adverse health effects are observed (the NOAEL) (IOM, 1998).

There are no animal toxicology studies on nicotinic acid and nicotinamide that comply with current standardized testing protocols from which to derive a UL. Therefore, authoritative bodies have used the results clinical studies in which high doses of nicotinic acid and nicotinamide have been administered. A UL of 35 mg/day for adults was

### Safety Assessment of Nicotinamide Riboside

established for both nicotinic acid and nicotinamide by the IOM (1998) based on the flushing in humans as the critical adverse effect. However, nicotinamide does not induce flushing when either given as an intravenous injection or orally at high-doses to patients with diabetes (SCF, 2002), and therefore, it is currently thought that the flushing effects are related to the presence of the carboxyl group on the pyridine nucleus of nicotinic acid (Figure 1)(European Commission Scientific Committee on Food, 2002). NR also lacks this carboxyl group and thus may not produce the flushing response that is associated with high nicotinic acid intakes (Figure 1). Furthermore, in vitro studies have shown that NR does not induce the GPR109A-mediated calcium flux, which is believed to be required for nicotinic acid-induced flushing (Benyo et al., 2005; Canto et al., 2012). It is therefore appropriate to derive the ULs for nicotinamide and NR based on endpoints other than flushing.

Consistent with this, the European Commission and UK Expert Group on Vitamins and Minerals derived independent ULs for nicotinamide and nicotinic acid. The SCF (2002) set the UL for nicotinamide of 900 mg/day based on long-term studies in patients with Type 1 diabetes mellitus, at dosages of 2-3 g of nicotinamide per day whereas the UK

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11 Expert Group on Vitamins and Minerals set that UL at 500 mg/day (UK Expert Group on  
12 Vitamins and Minerals, 2003) based on human studies where large doses (up to 3000  
13 mg/day for periods of up to 3 years) appeared to be well tolerated.  
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20 NR is not genotoxic. In addition, NR is thought to exhibit the same toxicity profile  
21 as nicotinamide because evidence from a single dose pharmacokinetic study in humans  
22 suggests that it is metabolized in a manner similar to nicotinamide (unpublished results).  
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24 Because there are no publicly available 90-day studies on either nicotinamide or NR, a  
25 toxicology study was completed where 300, 1000, or 3000 mg/kg body weight/day of NR,  
26 or 1260 mg/kg body weight/day of nicotinamide, which is equivalent to 3000 mg/kg/day  
27 dose of NR on a molar basis, was administered to rats over the course of 90 days. Adverse  
28 effects at 3000 mg/kg body weight/day of NR included treatment-related adverse effects in  
29 liver, kidneys, testes, epididymides and ovaries. These effects included increases in clinical  
30 chemistry parameters related to hepatocyte damage (ALT, ALP, and GGT) and a  
31 corresponding increase in liver weight, centrilobular hepatocellular hypertrophy and single  
32 cell necrosis. In addition, thyroid follicular cell hypertrophy and increased kidney weight  
33 with exacerbation of chronic progressive nephropathy were observed. Statistically  
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## Safety Assessment of Nicotinamide Riboside

significant but minor reductions in sodium and chloride were seen and microscopically associated with hypertrophy of zona glomerulosa in adrenals at 3000 mg/kg. Importantly, these effects also occurred in the nicotinamide groups with a similar magnitude.

Niagen administration at 1000 mg/kg/day dose level resulted in treatment-related organ weight changes in liver and kidney and increases in neutrophils, ALT and triglycerides, which were statistically significant in female rats only. Although these changes were considered adverse, based their dose-dependent responsiveness, the increases in ALT and triglycerides occurred only in one gender and were below the 2-fold increase that is typically used as the cut-off for a biologically significant effect in the absence of histological results (Hall et al., 2012). The kidney weight increases at this dose also occurred in the absence of corresponding histopathology. Therefore, the liver and kidney effects at 1000 mg/kg/day were considered to be treatment-related, but mild and potentially adaptive in nature due to prolonged exposure to this form of niacin. There were no treatment related adverse effects noted at 300 mg/kg/day, although there was a slight decrease (8%) in overall body weight (Day 90) at 300 mg/kg/day, which was considered

## Safety Assessment of Nicotinamide Riboside

adaptive. The NOAEL and LOAEL for NR were determined to 300 and 1000 mg/kg body weight/day, respectively.

A UL for human exposure to NR is derived by application of a 100-fold safety factor to the NOAEL determined from this 90-day study; the UL for nicotinamide riboside is 3 mg/kg/day or 180 mg/day, assuming a body weight of 60 kg. Importantly, because the UL for NR falls below the ULs for nicotinamide and the adverse effects associated with ingestion of NR were similar those found with equimolar amounts of nicotinamide, this level of intake provides an adequate margin of safety to protect consumers from the adverse effects associated with NR ingestion.

## Acknowledgements

We would like to thank Syngene International Limited for performing all studies and WR Grace for manufacturing Niagen.

## Declaration of Conflicting Interests

Dietrich Conze, Juan Crespo-Barreto, and Claire Kruger are employed by ChromaDex, Inc.

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10 Safety Assessment of Nicotinamide Riboside

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15 This research received no specific grant from any funding agency in the public,  
16 commercial, or not-for-profit sectors.  
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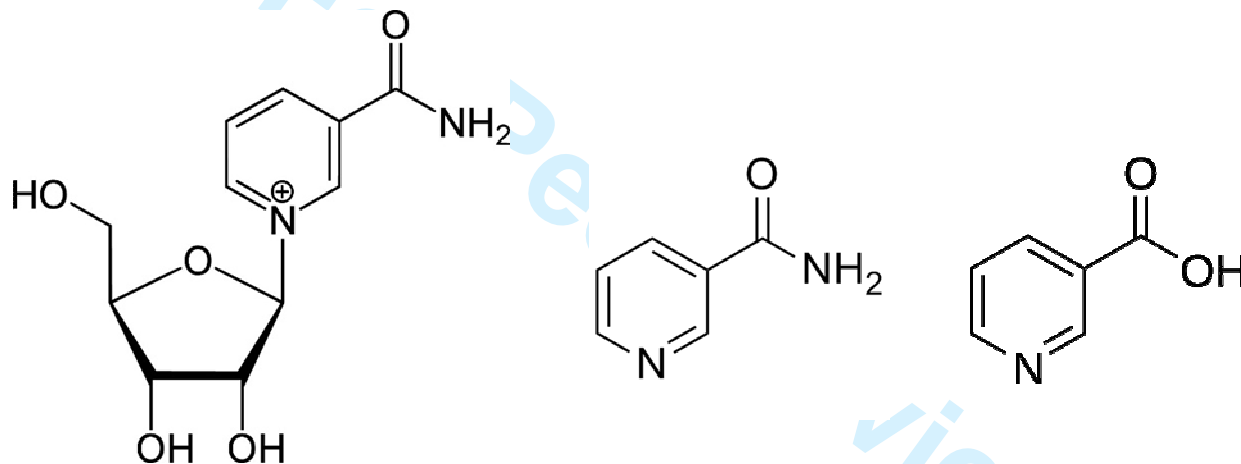
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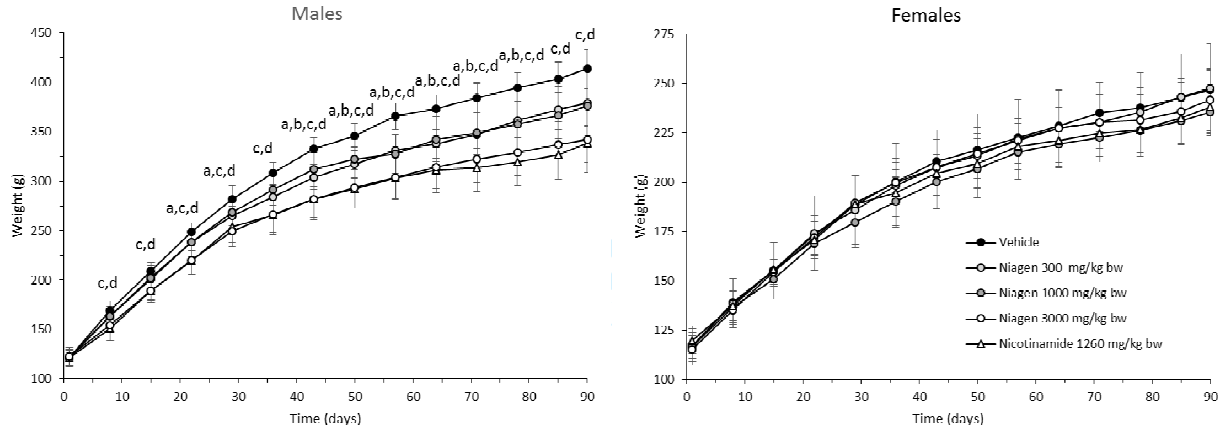
## Figures and Tables



**Figure 1.** Structure of nicotinamide riboside (left), nicotinamide (middle), and nicotinic acid (right).

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Safety Assessment of Nicotinamide Riboside



**Figure 2.** Body weights of male and female rats treated with vehicle control, nicotinamide riboside or nicotinamide for 90-days. “a”, “b”, “c”, and “d” denote significant (p<0.05) differences between rats treated with 300, 1000, and 3000 mg/kg/day of Niagen, or 1260 mg/kg/day of nicotinamide, respectively, and rats treated with the vehicle control.

## Safety Assessment of Nicotinamide Riboside

Table 1. Ames Assay Results

Test Item		Test Concentrations (µg/plate)	Mean colonies/plate				
			Frameshift types		Base-pair types		Frameshift and base-pair types
			TA98	TA1537	TA1535	WP2uvrA pKM101	
<i>Plate Incorporation method</i>							
With S9 Mix	Water	-	22 ± 3	8 ± 2	10 ± 3	149 ± 3	101 ± 1
	Niagen	50	27 ± 6	8 ± 2	12 ± 3	153 ± 3	108 ± 6
		159	23 ± 4	11 ± 3	8 ± 2	152 ± 3	103 ± 3
		501	22 ± 5	8 ± 3	10 ± 2	142 ± 4	104 ± 3
		1582	26 ± 1	9 ± 3	12 ± 2	144 ± 8	103 ± 1
		5000	24 ± 4	10 ± 8	11 ± 6	144 ± 6	104 ± 2
	2-Aminoanthracene	4	597 ± 14	83 ± 3	108 ± 4	-	883 ± 12
	2-Aminoanthracene	30	-	-	-	736 ± 6	-
Without S9 Mix	Water	-	21 ± 4	8 ± 4	12 ± 4	119 ± 6	104 ± 2
	Niagen	50	27 ± 4	9 ± 2	14 ± 5	121 ± 16	106 ± 3
		159	23 ± 6	9 ± 5	14 ± 3	116 ± 6	108 ± 5
		501	27 ± 4	7 ± 2	17 ± 5	111 ± 10	111 ± 6
		1582	26 ± 3	7 ± 4	18 ± 7	122 ± 14	121 ± 15
		5000	27 ± 4	8 ± 3	18 ± 4	122 ± 17	121 ± 3

## Safety Assessment of Nicotinamide Riboside

Table 1. Ames Assay Results

Test Item	Test Concentrations (µg/plate)	Mean colonies/plate					
		Frameshift types		Base-pair types		Frameshift and base-pair types	
		TA98	TA1537	TA1535	WP2uvrA pKM101	TA100	
2-Nitrofluorene	2	228 ± 10	-	-	-	-	
9-Aminoacridine	4	-	82 ± 3	-	-	-	
Sodium Azide	1	-	-	124 ± 3	-	526 ± 7	
4-Nitroquinoline- <i>N</i> -oxide	4	-	-	-	621 ± 3	-	
<i>Preincubation method</i>							
With S9 Mix	Water	-	22 ± 6	8 ± 1	10 ± 4	105 ± 6	108 ± 5
	Niagen	99	21 ± 5	6 ± 1	9 ± 1	96 ± 11	104 ± 6
		265	22 ± 4	6 ± 1	8 ± 1	107 ± 16	103 ± 6
		699	26 ± 5	9 ± 2	14 ± 1	86 ± 17	117 ± 4
		1869	22 ± 3	8 ± 2	15 ± 4	110 ± 10	105 ± 13
		5000	26 ± 6	5 ± 1	9 ± 1	99 ± 2	112 ± 4
2-Aminoanthracene	4	571 ± 6	90 ± 7	101 ± 5	-	919 ± 8	
2-Aminoanthracene	30	-	-	-	562 ± 18	-	
Without S9 Mix	Water	-	20 ± 8	9 ± 1	14 ± 2	96 ± 4	104 ± 6



## Safety Assessment of Nicotinamide Riboside

Table 1. Ames Assay Results

Test Item	Test Concentrations (µg/plate)	Mean colonies/plate				
		Frameshift types		Base-pair types		Frameshift and base-pair types
		TA98	TA1537	TA1535	WP2uvrA pKM101	TA100
Niagen	99	16 ± 1	9 ± 4	12 ± 2	98 ± 7	103 ± 9
	265	18 ± 5	8 ± 1	11 ± 2	95 ± 4	109 ± 3
	699	19 ± 4	8 ± 4	11 ± 3	91 ± 4	103 ± 8
	1869	17 ± 3	6 ± 4	9 ± 1	92 ± 7	113 ± 7
	5000	22 ± 2	6 ± 3	9 ± 1	94 ± 7	120 ± 4
2-Nitrofluorene	2	216 ± 4	-	-	-	-
9-Aminoacridine	4	-	92 ± 4	-	-	-
Sodium Azide	1	-	-	126 ± 7	-	419 ± 9
4-Nitroquinoline- <i>N</i> -oxide	4	-	-	-	505 ± 16	-

“-“ denotes not tested

Values are mean +/- standard deviation

## Safety Assessment of Nicotinamide Riboside

**Table 2: Summary of Results- In vivo Micronucleus test**

Parameters	Dose (mg/kg bw)					Positive Control 40
	Vehicle Control 0	Niagen 500	Niagen 1000	Niagen 2000 (a)	Niagen 2000 (b)	
<i>Males</i>						
Sampling time (hours)	24	24	24	24	48	24
PCE's with micronuclei (%)	0.00	0.00	0.00	0.00	0.00	<b>0.28*</b>
Range	0-1	0	0-1	0	0	<b>8-15</b>
Mean P/E ratio	0.34	0.37	0.39	0.38	0.39	0.40
<i>Females</i>						
Sampling time (hours)	24	24	24	24	48	24
PCE's with micronuclei (%)	0.00	0.00	0.00	0.00	0.00	<b>0.27*</b>
Range	0	0	0	0	0	<b>7-13</b>
Mean P/E ratio	0.40	0.43	0.44	0.44	0.41	0.40

## Safety Assessment of Nicotinamide Riboside

**Table 3: Summary of Significant Changes-Hematological Parameters Day 91**

Parameters	Doses (mg/kg/day)				
	Vehicle 0	Niagen 300	Niagen 1000	Niagen 3000	Nicotinamide 1260 <sup>^</sup>
<b>Males</b>					
White Blood Cells	8.47±1.97	9.16±1.54	<b>11.11 ±1.77*</b>	<b>13.51± 2.64*</b>	<b>14.11±1.66*</b>
Total Neutrophils	2.12±0.7	2.30±1.67	2.86±1.20	<b>5.76± 1.47*</b>	<b>6.02±0.7*</b>
Total Monocytes	0.28 ±0.09	0.33±0.30	0.40±0.19	0.42±0.09	0.45±0.12
<b>Females</b>					
White Blood Cells	6.25±1.20	6.23 ±1.84	7.40±1.01	<b>10.38± 1.51*</b>	<b>9.99±2.64*</b>
Total Neutrophils	0.93±0.31	1.11±0.57	<b>1.63±0.29*</b>	<b>3.36± 1.19*</b>	<b>4.13±1.56*</b>
Total Monocytes	0.16 ±0.07	0.15±0.05	0.15±0.04	<b>0.32±0.08*</b>	<b>0.33±0.21*</b>

Abbreviations: WBC etc

Values represented as Mean ± SD

\*Significantly higher than the vehicle control group at  $p < 0.05$ .<sup>^</sup>: equimolar ratio to Niagen 3000 mg/kg/d

## Safety Assessment of Nicotinamide Riboside

**Table 4: Summary of Significant Changes-Clinical Chemistries Day 91**

Parameters	Doses (mg/kg/day)				
	Vehicle 0	Niagen 300	Niagen 1000	Niagen 3000	Nicotinamide 1260 <sup>^</sup>
<i>Males</i>					
ALT (U/L)	85.23±26.11	75.94±12.20	106.17±45.34	<b>159.35 ±27.56*</b>	<b>152.11± 25.50*</b>
AST (U/L)	122.18±27.34	116.10±13.94	138.23±49.03	132.93±23.63	139.47±26.99
ALP (U/L)	99.85±22.69	120.94±16.68	110.96±18.67	<b>131.63 ±19.73*</b>	<b>139.48± 24.88*</b>
GGT (U/L)	3.09±0.90	4.13±1.22	3.47±1.20	3.82±1.22	3.81±1.16
Trig (mg/dL)	49.84±18.96	59.30±29.48	69.65±29.91	<b>128.34± 47.88*</b>	<b>98.57 ±34.72*</b>
Sodium (mmol/L)	139.15 ±3.37	141.03 ±6.05	137.99 ±3.52	137.1 ±4.7	135.88 ±1.9
Chloride (mmol/L)	101.55 ±2.66	102.13 ±4.41	99.21 ±1.3	<b>97.34 ±3.24*</b>	<b>97.52 ±1.25*</b>
<i>Females</i>					
ALT (U/L)	56.09 ±15.54	56.34 ±10.47	<b>76.50 ±20.42*</b>	<b>121.81 ±22.35*</b>	<b>125.22 ±30.29*</b>
AST (U/L)	101.02 ±18.67	106.1 ±18.52	126.69 ±35.51	126.27 ±33.09	<b>126.31 ±16.24*</b>
ALP (U/L)	60.79 ±5.16	69.66 ±11.23	85.96 ±22.06	<b>105.10 ±26.99*</b>	<b>114.41 ±30.91*</b>
GGT (U/L)	3.15 ±1.17	3.9 ±0.92	3.83 ±1.42	<b>5.01 ±1.36*</b>	<b>5.46 ±1.75*</b>

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## Safety Assessment of Nicotinamide Riboside

**Table 4: Summary of Significant Changes-Clinical Chemistries Day 91**

Parameters	Doses (mg/kg/day)				
	Vehicle 0	Niagen 300	Niagen 1000	Niagen 3000	Nicotinamide 1260 <sup>^</sup>
Trig (mg/dL)	28.38 ±6.9	28.24 ±7.92	<b>46.69 ±12.97*</b>	<b>61.85 ±23.31*</b>	<b>87.00 ±38.48*</b>
Sodium (mmol/L)	137.71 ±1.28	137.86 ±1.25	<b>136.68 ±1.46*</b>	<b>134.42 ±1.22*</b>	<b>135.83 ±1.17*</b>
Chloride (mmol/L)	102.12 ±0.96	101.43 ±1.13	100.69 ±1.52	<b>98.12 ±1.63*</b>	<b>98.93 ±1.33*</b>

Abbreviations: ALT AST ALP GGT etc

Values represented as Mean ± SD

\*Significantly higher than the vehicle control group at  $p < 0.05$ .

<sup>^</sup>: equimolar ratio to Niagen 3000 mg/kg/day

## Safety Assessment of Nicotinamide Riboside

**Table 5: Summary of Significant Changes-Organ Weight Ratio Relative to Body Weight At Day 91**

Parameters	Dose (mg/kg/day)				
	Vehicle 0	Niagen 300	Niagen 1000	Niagen 3000	Nicotinamide 1260 <sup>^</sup>
<b>Males</b>					
Terminal Body Wt.	395.7 ±18.36	<b>363.56 ±23.22 *</b>	<b>354.23 ±21.85 *</b>	<b>317.21±25.8 *</b>	<b>312.87±26.74 *</b>
<b>Organ/body weight</b>					
Liver	2.958 ±0.143	3.013 ±0.163	<b>3.200 ±0.18 *</b>	<b>3.600±0.272 *</b>	<b>3.703 ±0.116 *</b>
Kidneys	0.715 ±0.047	0.701 ±0.033	<b>0.777 ±0.02 *</b>	<b>0.876±0.063 *</b>	<b>0.833 ±0.086 *</b>
Brain	0.507 ±0.027	0.526 ±0.038	<b>0.550 ±0.024 *</b>	0.572 ±0.042	<b>0.583 ±0.043 *</b>
Heart	0.368 ±0.019	0.356 ±0.018	0.373 ±0.015	0.384 ±0.02	0.387 ±0.015
Thymus	0.068 ±0.011	0.076 ±0.015	<b>0.086 ±0.014 *</b>	0.07 ±0.009	0.068 ±0.008
Adrenals	0.012 ±0.001	0.013 ±0.001	<b>0.014 ±0.001 *</b>	<b>0.014 ±0.001 *</b>	<b>0.015 ±0.001 *</b>
<b>Females</b>					
Terminal Body Wt.	232.29 ±8.1	234.43 ±23.28	219.51 ±9.92	216.19 ±14.75	<b>215.61 ±11.16 *</b>
<b>Organ/body weight</b>					
Liver	2.902 ±0.191	3.003 ±0.327	<b>3.295 ±0.181*</b>	<b>4.046 ±0.174 *</b>	<b>4.465 ±0.239 *</b>

## Safety Assessment of Nicotinamide Riboside

**Table 5: Summary of Significant Changes-Organ Weight Ratio Relative to Body Weight At Day 91**

Parameters	Dose (mg/kg/day)				
	Vehicle 0	Niagen 300	Niagen 1000	Niagen 3000	Nicotinamide 1260 <sup>^</sup>
Kidneys	0.676 ±0.053	0.645 ±0.06	0.678 ±0.058	<b>0.822 ±0.044 *</b>	<b>0.766 * ±0.019</b>
Brain	0.78 ±0.034	0.788 ±0.055	0.801 ±0.047	0.798 ±0.041	0.781 ±0.031
Heart	0.392 ±0.016	0.393 ±0.015	0.398 ±0.022	<b>0.433 ±0.032 *</b>	<b>0.424 * ±0.029</b>
Thymus	0.09 ±0.011	0.097 ±0.024	0.093 ±0.013	0.087 ±0.014	0.084 ±0.017
Adrenals	0.027 ±0.003	0.028 ±0.003	0.027 ±0.002	0.029 ±0.004	0.027 ±0.003
Ovaries	0.036 ±0.005	0.037 ±0.005	0.036 ±0.006	<b>0.045 ±0.008 *</b>	<b>0.049 ±0.007 *</b>

Values represented as Mean ± SD

\*: Significantly higher / lower than the vehicle control group at  $p < 0.05$ .

<sup>^</sup>: equimolar ratio to Niagen 3000 mg/kg/day