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New Dietary Ingredient Notification for β-Nicotinamide Mononucleotide (NMN)

Submitted by the Notifier:
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Prepared by the Agent of the Notifier:
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July 25, 2022

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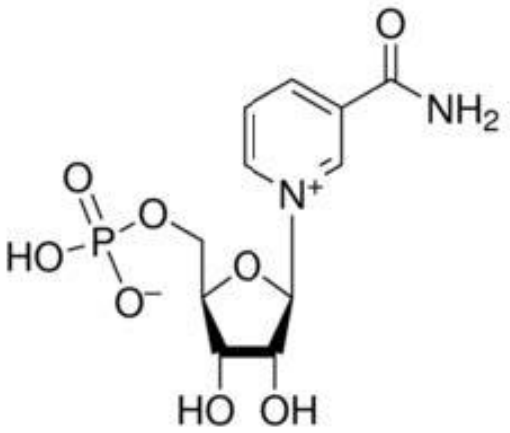
Section I: Cover Letter

Consumer Safety Officer
Office of Nutrition, Labeling and Dietary Supplements (HFS-810)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5100 Paint Branch Parkway
College Park, MD 20740

DEAR SIR OR MADAM:

The undersigned, (b) (4), submits this new dietary ingredient notification under section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act with respect to the new dietary ingredient, β-Nicotinamide Mononucleotide (NMN), that the notifier intends to market as a bulk dietary ingredient.

NMN is a member of the vitamin B_3 family, as defined by the National Academy of Medicine,[1] and is commercially produced by the notifier using a non-pathogenic and non-toxigenic production organism as a ≥98% pure white to off white crystalline powder. NMN has a molecular formula of $C_{11}H_{15}N_2O_8P$, a molecular weight of 334.22 g/mol, and the structural formula show in the figure below:



Relevant identifiers and/or synonyms of NMN include:

- InChIKey: DAYLJWODMCOQEW-TURQNECASA-N
- CAS Registry Number: 1094-61-7
- IUPAC name: 3-Carbamoyl-1-[5-*O*-(hydroxyphosphinato)-β-D-ribofuranosyl]pyridinium

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NMN has been legally present in conventional foods as an article used for food in a form in which the food is not chemically altered. It is, therefore, exempt from the requirement to submit a 75-day premarket notification pursuant to section 413(a)(1) of the Federal Food, Drug, and Cosmetic Act (see sections III.C.5 and III.C.6.ii and Appendix A). As such, this new dietary ingredient notification is being submitted voluntarily.

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(b) (4) (agent of the notifier)

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Section II: Table of Contents

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Figures and Tables

Appendices

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Section III: Body of the Notification

III.A Administrative

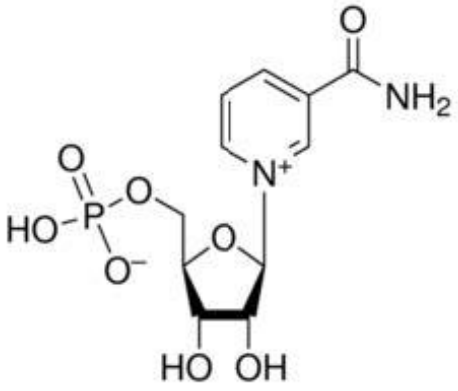
III.A.1 Description of β-Nicotinamide Mononucleotide

The subject of this new dietary ingredient (NDI) notification is β-Nicotinamide Mononucleotide (NMN), manufactured and sold as a bulk dietary ingredient for use as a dietary ingredient in dietary supplements, by Inner Mongolia Kingdomway Pharmaceutical Limited with its principal offices at:

Tuoketuo Industrial Park
Hohhot
Inner Mongolia
China
010206

The dietary ingredient, NMN, is a "vitamin" (in accordance with the definition of niacin (aka, vitamin B_3) given by the National Academy of Medicine[1]; see also sections II.B.1 and III.C.4), a dietary substance for use by man to supplement the diet by increasing the total dietary intake (see section III.C.5), and a "metabolite" of a vitamin (with respect to the common vitamin B_3 dietary ingredient, nicotinamide; see section III.C.4) pursuant to section 201(ff)(1)(A, E, and F) of the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The notifier's NMN ingredient is a white to off white crystalline powder of not less than 98.0% purity that is produced using a non-pathogenic and non-toxigenic production organism**.** NMN has a molecular formula of $C_{11}H_{15}N_2O_8P$ and a molecular weight of 334.22 g/mol. Its structural formula is shown in the figure below:



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Relevant identifiers and/or synonyms of NMN include:

- InChIKey: DAYLJWODMCOQEW-TURQNECASA-N
- CAS Registry Number: 1094-61-7
- IUPAC name: 3-Carbamoyl-1-[5-*O*-(hydroxyphosphinato)-β-D-ribofuranosyl]pyridinium

Inner Mongolia Kingdomway Pharmaceutical Limited intends that dietary supplements containing its NMN bulk dietary ingredient will be marketed to adults in forms appropriate to dietary supplements (e.g., tablet, capsule, powder) at levels that do not exceed 800 mg NMN per serving or 800 mg NMN per day by adults (e.g., take one 800 mg serving daily, take one 400 mg serving twice daily).

It is expected that the use of bulk NMN supplied by Inner Mongolia Kingdomway Pharmaceutical Limited as a dietary ingredient in dietary supplements by its customers will be in full compliance with all applicable laws and regulations regarding such use and will be within the use recommendations specified above by Inner Mongolia Kingdomway Pharmaceutical Limited in this NDI notification.

III.A.2 Trade Secrets and Confidential Commercial Information

Pursuant to section 413(a)(2) of the FD&C Act, information contained in this NDI notification that is considered trade secret or otherwise confidential commercial information shall be kept confidential. Below we have identified information in this notification that we believe is trade secret or confidential commercial information including explanations for the basis of this belief. Additionally, we have marked this information where it appears in the notification by preceding all such information with, "**[BEGINNING OF CONFIDENTIAL INFORMATION]**" and following it with, "**[END OF CONFIDENTIAL INFORMATION]**."

Information we believed to be trade secret or confidential commercial information:

- Subsections III.B.2 and III.B.3 in their entireties and including the content of their referenced appendices (i.e., Appendices B–E).
    - The information contained in these sections is trade secret because it contains commercially valuable details regarding the manufacturing methods and practices, specifications (including internal methods of analysis), and stability test parameters used to determine the shelf-life of NMN.
- Subsection III.C.2.i (in its entirety) providing detailed summaries of toxicological studies on the notifier's NMN article of commerce as well as the parts of Appendix G containing the full laboratory reports of these studies.

- The information contained in these subparts and the associated appendix documents are confidential commercial information in order to preserve the ability of the notifier to publish the methods and results of these studies in a peer review scientific journal. Part of the publishing requirement is the attestation to the journal that studies reported in a submitted manuscript have not been published elsewhere. The summaries contained in subsection III.C.2.i provide a level of detail that would preclude the ability to make such attestation.

III.A.3 Safety Narrative for Dietary Supplements Containing the bulk NDI

Inner Mongolia Kingdomway Pharmaceutical Limited expects that all other ingredients (i.e., non-dietary ingredient ingredients) contained in any dietary supplement containing the company's bulk NMN dietary ingredient marketed in the United States by the company's customers (internal or external) will be approved food or color additives or will be generally recognized as safe (GRAS) for their intended uses (e.g., bulking agents, binders, capsules and capsule components, color additives, or other processing aids) and, therefore, would not be expected to present a safety concern in a finished dietary supplement under its stated conditions of use.

Inner Mongolia Kingdomway Pharmaceutical Limited also expects that any other dietary ingredients contained in any dietary supplement containing the company's bulk NMN dietary ingredient marketed in the United States by the company's customers (internal or external) will be used in full compliance with the requirements of all applicable laws and regulations pertaining to use of pre-DSHEA dietary ingredients or NDIs, as the case may be.

NMN has been the subject of a thorough safety assessment as described in subsections III.C.1–6 below and summarized here. The safety of this dietary ingredient is supported by toxicological studies (both in vitro genetic toxicity and studies in animals), clinical studies in humans without occurrence of serious adverse events, and its history of use. The totality of evidence for the safety of NMN is comprised of this aforementioned data and also relates to the robust quality control standards for NMN as documented in section III.B below.

Data from a ninety-day repeated-dose oral toxicity studies in rats (summarized in subsection III.C.2.i.e) was used to determine a no-observed-adverse-effect level (NOAEL) for NMN of 1150 mg/kg bw/day in male and female Han:WIST rats. A safety factor was applied to the NOAEL to establish an acceptable daily intake (ADI) in humans of 11.5 mg/kg bw/day. The ratio of highest daily intake level (800 mg; equivalent to 11.4 mg/kg bw in a 70 kg human) of NMN recommended under the recommended conditions of use of any dietary supplements containing Inner

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Mongolia Kingdomway Pharmaceutical Limited's NMN (EDI) to the ADI was calculated to be 0.99.

As noted in subsection III.C.5, exposure to niacins occurring naturally in foods were not considered relevant to the safety assessment of NMN due to the absence of associated adverse events. Exposure to NMN (or related niacins) from the different dietary supplements as well as a single functional food (i.e., a single, limited GRAS use (see Appendix A)) is consider substitutive as these are consumed intentionally for the purpose of supplementation with NMN. Considering the EDI/ADI ratio in the context of data establishing a lack of genotoxicity, the history of use of NMN, and other data and information presented in section III.C that is corroborative of the safety of NMN supports a reasonable expectation of safety of any dietary supplements containing NMN under the stated conditions of use.

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III.B Establishment of Identity

III.B.1 Detailed Description of the Identity of β-Nicotinamide Mononucleotide

Inner Mongolia Kingdomway Pharmaceutical Limited's NMN is a white to off white crystalline powder comprised of not less than 98.0% purity on a dry basis and meeting food grade specifications that is produced using a non-pathogenic and non-toxigenic production organism**.** NMN has a molecular formula of $C_{11}H_{15}N_2O_8P$ and a molecular weight of 334.22 g/mol. Its structural formula is shown in Figure 1.

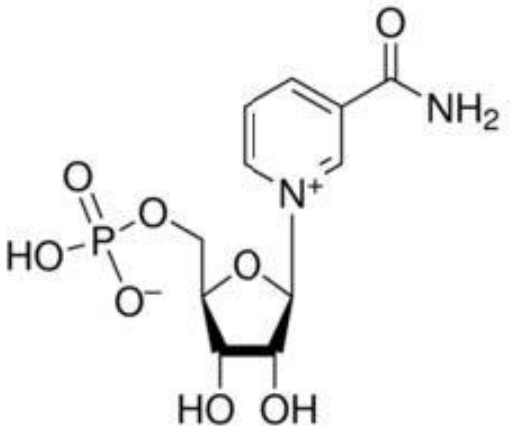

Figure 1. Structural Formula of NMN

Relevant identifiers and/or synonyms of NMN include:

- InChIKey: DAYLJWODMCOQEW-TURQNECASA-N
- CAS Registry Number: 1094-61-7
- IUPAC name: 3-Carbamoyl-1-[5-*O*-(hydroxyphosphinato)-β-D-ribofuranosyl]pyridinium

Biologically, NMN is an intermediate of the salvage pathway of nicotinamide adenine dinucleotide (NAD^+) biosynthesis. NMN is the direct precursor to NAD^+, and, therefore, together with classical dietary vitamin B_3 precursors, meets the definition of "niacin" (aka, vitamin B_3) as "nicotinamide (nicotinic acid amide), nicotinic acid (pyridine-3-carboxylic acid), and derivatives that exhibit the biological activity of nicotinamide" as given by the National Academy of Medicine (formerly Institute of Medicine).[1]

A note regarding terminology: Regardless of formal scientific definitions as given above, the term niacin has been commonly used to mean nicotinic acid specifically and the term niacinamide has been commonly used to mean nicotinamide

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specifically. To avoid confusion, in this NDI notification, the specific terms, nicotinic acid (NA) and nicotinamide (Nam), will be used wherever practical, and when used, the generic term "niacin(s)" will mean vitamin B_3 (i.e., Nam, NA, and derivatives that exhibit the biological activity of Nam) unless specifically stated otherwise.

[BEGINNING OF CONFIDENTIAL INFORMATION]

III.B.2 Manufacturing Methods and Practices

III.B.2.i Good Manufacturing Practice

NMN from Inner Mongolia Kingdomway Pharmaceutical Limited is produced (b) (4) (see Appendix B).

III.B.2.ii Raw Materials

Raw materials used in the production of NMN are (b) (4) (see Appendix B), are prepared and handled (b) (4) .

III.B.2.iiii Manufacturing Narrative and Flowchart

NMN is manufactured (b) (4) (as depicted schematically in Figure 2):

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III.B.3 Specifications

The specifications for the food-grade product NMN, along with the specification methods (see Appendix C), which have been validated for their stated purposes, are listed below in Table 1.

Table 1. NMN Specifications

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III.B.3.i Batch Analysis

Production conformity and consistency of NMN are (b) (4)

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III.B.3.ii Shelf-Life Stability

A two-year shelf–life from the time of manufacture has been recommended as an appropriate expiration period for NMN. This recommendation is based upon (b) (4) (see Appendix E).

[END OF CONFIDENTIAL INFORMATION]

III.B.4 Identity References

Reprints or photocopies of the full text of all published and unpublished identity references cited in section III.B that have not already been included in appendices to other subsections of the identity section are contained in Appendix F (Identity References) of this notification.

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III.C Safety Assessment

III.C.1 Comprehensive Safety Profile of β-Nicotinamide Mononucleotide

NMN has been the subject of a thorough safety assessment as briefly summarized in this subsection and summarized in greater detail in subsections III.C.2–6 below. The totality of evidence for the safety of NMN is comprised of pivotal and corroborative data, as described in subsections III.C.2–6, and also relates to the robust quality control standards for this ingredient as documented in section III.B above.

NMN meets the definition of a vitamin as defined by the National Academy of Medicine[1] and is one of several vitamin B_3 compounds that can support the biosynthesis of NAD^+ in humans. Metabolism of niacins is well understood and NMN is the direct precursor to NAD^+ in the salvage pathway (the primary pathway of NAD^+ biosynthesis) and the only compound capable of directly supporting mitochondrial NAD^+ biosynthesis.[2-7] More recent work has identified a specific NMN transporter that is expressed in mammals in the gastrointestinal mucosa, pancreas, liver, and some other tissues, and the small intestine has been identified as the primary absorption site.[8] Additionally, recent work has demonstrated the ability of orally ingested NMN to increase concentrations of Nam and NAD^+ or their metabolites in a variety of mammalian tissues.[8-13]

The notifier (Inner Mongolia Kingdomway Pharmaceutical Limited) sponsored a Good Laboratory Practice (GLP) compliant 90-day repeated-dose oral toxicity study in rats that was performed in accordance with OECD test guideline 408.[14] The test item was the notifier's NMN product of commerce and was administered at constant volume in a distilled water vehicle at doses of 0, 300, 725, and 1150 mg/kg bw/day. The unscheduled death of a single high-dose male animal during the study was ruled due to gavage error. No other unscheduled deaths occurred, and no adverse effects or signs of toxicity that could be attributed to the test item were observed. While some statistically significant findings were observed during the study, due to their low magnitude and the absence of correlated clinical observations or histopathological findings, they were considered either adaptive or, in the majority of instances in which a dose response and associated changes in organ weights were absent, indicative of normal variation. The NOAEL was determined to be 1150 mg/kg bw/day, the highest dose tested.

Two additional subchronic repeated-dose oral toxicity studies using other NMN substances of similar purity to the notifier's article of commerce were located during our literature searches. One of these studies was also reported as GLP and OECD 408 compliant and administered NMN to rats at doses of 0, 375, 750, or 1500 mg/kg bw/day for 90-consecutive days followed by a 28-day recovery period.[15] No unscheduled deaths occurred during the study, and no adverse effects or signs of

toxicity that could be attributed to the test item were observed. While some statistically significant findings were observed they were of low magnitude, mostly without associated findings in other parameters, and resolved following the recovery period. A few changes in clinical chemistry parameters, organ weights, and histopathology that were considered treatment related were not considered adverse, either due to their likely adaptive nature and absence following the recovery period or their low grade and absence of human counterparts. The NOAEL was determined to be 1500 mg/kg bw/day, the highest dose tested.

In the other subchronic study, rats were administered NMN at doses of 0, 500, 1000, or 2000 mg/kg bw/day for 91-consecutive days followed by a 14-day recovery period.[16] The GLP status of this study was not reported nor was any test guideline stated. Based on approximately 15% decreased body weight compared to controls in both male and female rats at the high dose at the end of the treatment phase (there was a trend towards reversal over the recovery phase), the NOAEL was determined as 1000 mg/kg bw/day, the mid dose. Other various statistically significant changes that occurred in study parameters were slight in magnitude and absent at the end of the treatment period or were species-specific lesions without human counterparts.

The three subchronic studies briefly summarized above are corroborated by an acute oral toxicity study in rats (no mortality, signs of toxicity, or gross pathological lesions following a single dose of 2666.6 mg/kg bw/day NMN),[15] three subacute oral toxicity studies in rats, a subacute oral toxicity study in mice, and a subacute oral toxicity study in beagle dogs. In 7- and 14-day repeated dose studies in rats, maximum tolerated doses (MTD) were determined as 5000 (the single dose tested) and 1000 (the low-mid dose) mg/kg bw/day, respectively.[16] In a GLP, OECD 407 14-day study in rats (using the notifier's article of commerce), the NOAEL in females was determined as 2400 mg/kg bw/day (the highest dose tested) while in males, the LOAEL was 1200 mg/kg bw/day (the high-mid dose).[17] In mice, the MDT following 7 days of administration was determined to be 2680 mg/kg bw/day while in beagle dogs the MDT was determined to be 1340 mg/kg bw/day following 14 days of administration (the highest doses tested in each species).[18]

The genetic toxicity of NMN has also been investigated. The notifier conducted a battery of GLP studies according to OECD test guidelines. NMN did not exhibit genotoxic potential in a bacterial reverse mutation test (OECD 471) at concentrations up to 5000 µg/plate,[19] an in vitro mammalian chromosomal aberration test (OECD 473) at concentrations up to 2000 µg/mL,[20] or an in vivo mammalian micronucleus test (OECD 474) doses up to 2000 mg/kg bw.[21] Another NMN substance of similar purity did not exhibit genotoxic potential in a bacterial reverse mutation test (OECD 471) at concentrations up to 5000 µg/plate or an in vitro mammalian micronucleus test (OECD 487) at concentrations up to 2000 µg/mL (GLP status of these tests was not reported).[15]

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Two clinical trials were located in which evaluations of safety were primary outcomes. In these studies, NMN did not cause any adverse effects on evaluated parameters and no serious adverse events occurred.[11, 12] Serious adverse events or adverse clinical effects were also not observed in an additional four clinical trials located in which safety parameters were not primary outcomes.[13, 22-24] In general, NMN was well tolerated in humans at daily dosages up to 2000 mg and durations up to 12 weeks. While it is uncertain exactly how closely some of the NMN test items used in the located clinical trials relate to the notifier's NMN, these studies are, nonetheless, corroborative as relates to the safety of the ingredient in general.

Finally, the results of the above studies are corroborated by the history of use of NMN as natural component of most food as well as a GRAS substance (see Appendix A). While, due to the much lower levels of exposure and lack of robust exposure data, this line of evidence is not adequate in and of itself to establish an adequate basis of safety for the recommended use of NMN as a dietary ingredient in dietary supplements, it is, nonetheless, supportive of the preclinical and clinical data summarized above.

The safety of notifier's recommended use of NMN is also supported by the ULs for Nam established by authoritative scientific bodies (i.e., Expert Group on Vitamins and Minerals and European Food Safety Authority) based on studies in humans.[25, 26] These UL provide margins of exposure (MoE) over the notifier's recommended conditions of use at molar equivalents of approximately 2 and 3-fold, respectively. While the UL for Nam established by the National Academy of Medicine does not provide an MoE, this UL was established for niacins in general and was based on the non-pathological flushing effect caused by NA only.[1] Neither Nam nor NMN have been found to cause flushing and mechanistic studies have elucidated associated structural components that are unique to NA.[27, 28] Furthermore, the United States Food and Drug Administration has affirmed that both Nam and NA are GRAS as nutrient supplements without limitation. Published safety studies and safety assessments on another related substance, nicotinamide riboside chloride salt (NRc), have not supported the use of this substance at level as high as recommended by the notifier for NMN.[29, 30] However, the substances are not chemically identical and the notifier's NMN is not a salt form, the safety of NMN is based on similarly conducted toxicological studies on the notifier's article of commerce as well as toxicological studies on other NMN substances, and clinical studies using NRc provide support for a greater MoE than would be derived from the preclinical studies only.[31-40] There is, additionally, some evidence in the preclinical work to suggest NOAELs and LOAELs in NRc subchronic rat studies may have been conservatively determined based on adaptive rather than adverse changes.

In considering risk characterization of the recommended use of NMN as a dietary ingredient in dietary supplements, it is reasonable to employ the principle of

selecting the highest NOAEL under the lowest LOAEL in selection of a critical NOAEL for this purpose. While use of the NOAEL of 1500 mg/kg bw/day from the 90-day study by Cros et al. could be justified under this principle, we also considered the added relevance (including access to its the full data set), of the 90-day study conducted in rats on the actual article of commerce of the notifier. Therefore, 1150 mg/kg bw/day was selected as the pivotal NOAEL for human risk characterization.

The subacute MTD of 1340 mg/kg bw/day NMN in a second (and non-rodent) species—the beagle dog—while insufficient to establish safety on its own (due to limited study parameters, short duration, and lack of characterization of the test item) is corroborative of the results of the 90-day study in rats as are the clinical trials without serious adverse events or adverse effects on study parameters (such as vital signs and clinical chemistries) in which NMN has been ingested by humans up to 2000 mg/day for 14-days, 1200 mg/day for 6 weeks, and 250 mg/day for 12 weeks.

To establish an ADI in humans, a safety factor of 100 (based on multiplicatively combined uncertainty factors of 10 to account of intraspecies variation and 10 to account for interspecies variation) was considered appropriate, based on the totality of evidence, to apply to the critical NOAEL (1150 mg/kg bw/day derived from the 90-day study on the notifier's article of commerce). This extrapolation (NOAEL/safety factor; 1150 mg/kg bw/day /100) results in an ADI in humans of 11.5 mg/kg bw/day (equivalent to 805 mg daily using a reference adult human weight of 70 kg).

As noted in subsection III.C.5, exposure to NMN occurring naturally in foods was not considered relevant the establishment of an ADI based on this safety assessment of NMN due to their unavoidable presence in the background diet and the absence of associated adverse events. Exposure to NMN from the different dietary supplements as well as a single functional food (i.e., a single, limited (GRAS) use (see Appendix A)) is considered substitutive as these are consumed intentionally for the purpose of supplementation with NMN.

Based on the notifier's recommended intake of the bulk dietary ingredient in dietary supplements at levels not to exceed 800 mg NMN per day (approximately 11.4 mg/kg bw for a 70 kg human), the NOAEL allows for an adequate MoE (NOAEL/EDI; 1150 mg/kg / 11.4 mg/kg) of 101-fold), and the EDI/ADI ratio is 0.99. An EDI/ADI ratio ≤ 1 supports a conclusion that the recommended use of NMN as a dietary ingredient provides reasonable assurance that the ingredient does not present a significant or unreasonable risk of illness or injury.

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III.C.2 Toxicology Studies

III.C.2.i Toxicological Studies on the Notifier's β-Nicotinamide Mononucleotide Article of Commerce

Inner Mongolia Kingdomway Pharmaceutical Limited sponsored the independent investigation of the potential mutagenic activity and repeated-dose oral toxicity of its NMN article of commerce in the battery of studies that is summarized below in sections III.C.2.i.a–III.C.2.i.e (see Appendix G).[14, 17, 19-21] The studies were conducted in compliance with GLP in GLP certified facilities (b) (4)).

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III.C.2.i.a Bacterial Reverse Mutation Assay

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III.C.2.i.b In vitro Mammalian Chromosomal Aberration Study

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III.C.2.i.c In vivo Mammalian Micronucleus Study

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III.C.2.i.d Fourteen-day Repeated-Dose Oral Toxicity Study

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III.C.2.i.e Ninety-day Repeated-Dose Oral Toxicity Study

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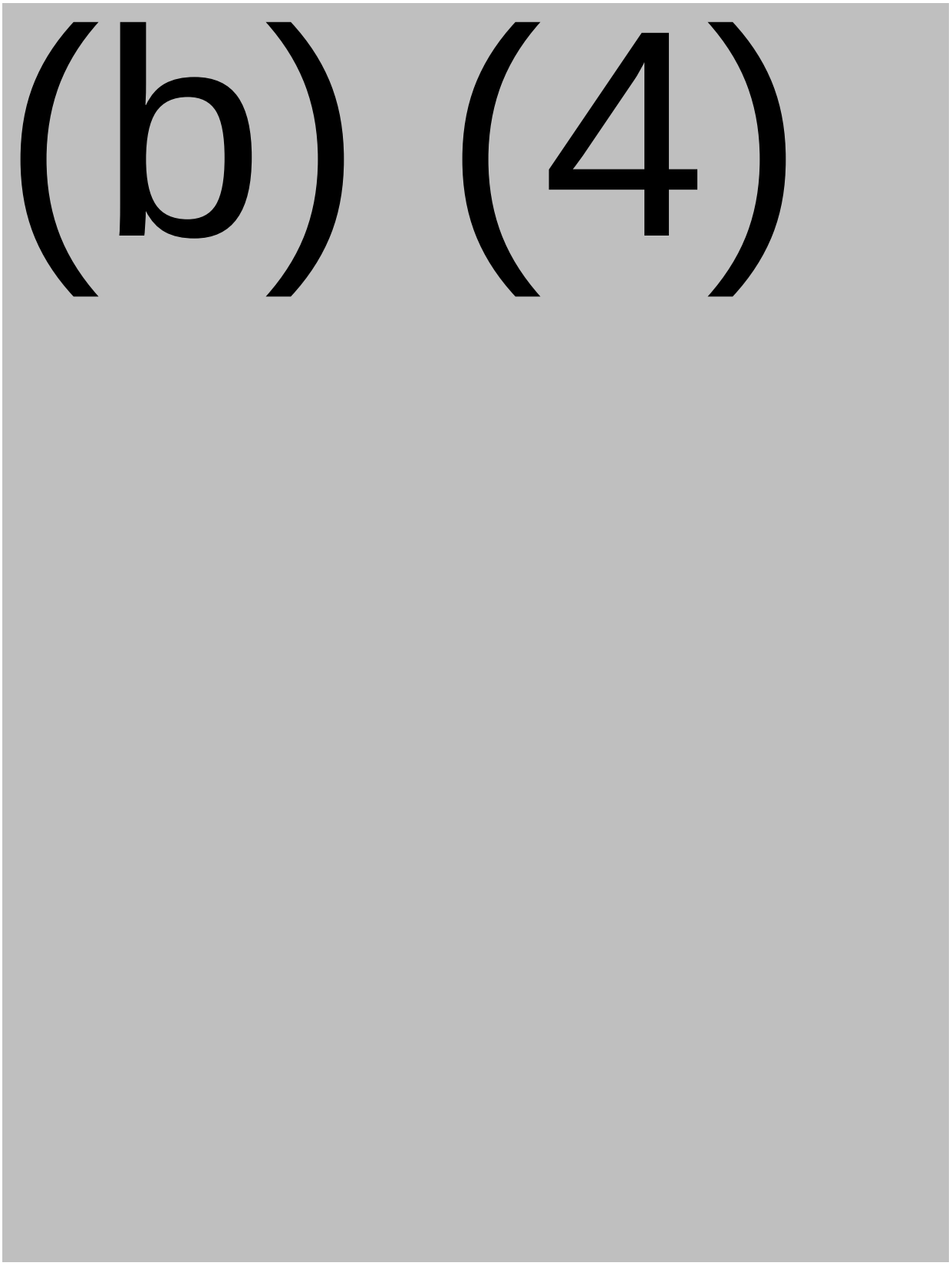

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[END OF CONFIDENTIAL INFORMATION]

III.C.2.ii Toxicological Studies on Other NMN Substances

Three preclinical safety evaluations of other NMN substances were located during our literature searches and are summarized below.

III.C.2.ii.a Genetic Toxicity

The genotoxic potential of a 99.03% pure synthetic NMN compound (branded as NMN-C® and supplied by Seneque SA, Switzerland) was evaluated in a bacterial reverse mutation test and an in vitro mammalian micronucleus test[15]:

- The bacterial reverse mutation test was conducted using *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* (WP2 pKM101) tester strains according to OECD TG 471 (GLP status of the study was not reported). Results for strain TA1537 were unanalyzable due to excessive cytotoxicity at the top three concentrations with metabolic activation using the pre-incubation procedure. Under all other experimental conditions, NMN-C® failed to induce gene mutations by base pair changes or frameshifts up to the maximum recommended test concentration of 5000 µg/plate, in the presence and absence of metabolic activation, under the applied plate incorporation and pre-incubation experimental conditions.
- The in vitro mammalian micronucleus test was conducted in CHO-K1 Chinese Hamster Ovary cells according to OECD TG 487 (GLP status of the study was not reported). NMN-C®, up to the maximum recommended test concentration of 2000 µg/mL, did not induce statistically significant or concentration related increases in micronuclei in the cytoplasm of CHO-K1 cells in short-term experiments with and without metabolic activation or long-term experiments without metabolic activation.

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III.C.2.ii.b Acute Toxicity

The acute oral toxicity of NMN-C® was evaluated in female Sprague-Dawley (SD) rats according OECD TG 423 (GLP status of the study was not reported).[15] Three rats were administered a single dose of 2666.6 mg/kg bw NMN-C® by gavage and observed for 14-days. No mortality, clinical signs of toxicity, or abnormal effects on body weight development were observed during the observation period, and no gross pathological lesions were observed at necropsy. The authors reported the study was conducted as a limit test; however, it is unclear why 2666.6 mg/kg bw was chosen as the dose or why, given the dose was <5000 mg/kg bw, a second group was not tested at the same dose.

III.C.2.ii.c Subacute Toxicity

You et al. 2020

The subacute toxicity of an undescribed NMN compound (supplied by Shokou Life Tech, Japan) was evaluated in mice and dogs.[18] Four groups of three to five healthy male C57BL6J mice were administered either a saturated solution (67 mg/mL) of NMN dissolved in water (vehicle-control) or the control alone either once or twice daily for seven consecutive days. Mice received 0 (vehicle control once daily), 0 (vehicle control twice daily), 1340 mg/kg bw/day (NMN solution once daily), or 2680 mg/kg bw/day (NMN solution twice daily) NMN by gavage at a dose volume of 20 mL/kg bw. Two groups of 10 beagle dogs (sex not reported) were administered the same saturated solution at 10 mg/mL by gavage twice daily for 14 consecutive days, resulting in received daily dosages of 0 or 1340 mg/kg bw/day. The experiments were conducted sequentially: (1) two groups of mice gavaged once daily, (2) two groups of mice gavaged twice daily, and (3) two groups of dogs gavaged twice daily.

All animals were observed once daily at the cage side for clinical signs of adversity (e.g., "behavior, mental status, gland secretion, respiration status, feces characters, genitals, and death") and body weight measurements were made. Blood was collected for serum analyses of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), blood urea nitrogen (BUN), uric acid UA), total cholesterol (TC), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides (TG), and insulin (mice only). In mice only, following sacrifice, liver weights were obtained, and histopathological examinations of the liver and kidney were conducted.

No mortality occurred in either mice or dogs and clinical signs were comparable between treated and control animals. A statistically significant decrease in body weight gain was observed compared to controls in the 1340 mg/kg bw/day group mice (the treated animals appear to have not gained any weight); however, the

magnitude in terms of percent difference was not reported. While in the 2680 mg/kg bw/day group mice, body weight gain was decreased in both the control and treated groups without statistical significance between groups (it appears that both control and treated groups lost weight). In dogs, body weight gain was statistically significantly increased in the treated group compared to the control.

There were no statistically significant differences in clinical chemistry parameters in the 1340 mg/kg bw/day group mice although ALT and AST activities were slightly higher relative to control. In the 2680 mg/kg bw/day group mice, ALT was statistically significantly increased, AST was non-significantly increased more than double, and BUN, TC, and LDL were statistically significantly decreased compared to the controls. AST/ALT ratios did not differ statistically significantly from controls in either group. In the dogs, CREA and UA were statistically significantly increased compared to controls. No other alterations in clinical chemistry parameters were observed among the groups; however, serum insulin was non-significantly lower in the treated mice compared to controls.

At necropsy in mice, liver weight to body weight ratio was statistically significantly decreased in 1340 mg/kg bw/day while in the 2680 mg/kg bw/day group mice the ratio was slightly, but non-significantly decreased. No histopathological alterations were observed in the in the livers (and presumably kidneys although histopathology examination results were not specifically reported) of either group.

The MTD of repeated administration of NMN by gavage for seven consecutive days in male C57BL6J mice is considered to be 2680 mg/kg bw/day while the MTD of NMN administered by gavage to beagle dogs for 14 consecutive days was 1340 mg/kg bw/day.

Turner et al. 2021

Two subacute studies (7- and 14-days) of a 99.9% pure NMN compound (branded as Restorin® NMN and supplied by Seragon, USA) were conducted in order to gather data for a subchronic study.[16] Note, no test guidelines or GLP compliance were reported for these studies.

Restorin® NMN was administered by gavage to two groups of five SD rats/sex at doses of 0 (vehicle-control) or 5000 mg/kg bw/day for seven consecutive days. Animals were observed daily, body weights were obtained on Days 1 and 7, and the animals were sacrificed on Day 8 and subjected to gross necropsy. Treatment was well tolerated, although body weight development and food consumption were statistically significantly reduced in the treated animals, and no treatments-related finding were observed at necropsy (no details were reported). Based on these findings, 5000 mg/kg bw/day was selected as the high dose for the 14-day study.

In the 14-day study, doses of 0, 500, 1000, 3000, or 5000 mg/kg bw/day of Restorin® NMN were administered by gavage to five groups of seven SD rats/sex. The animals were observed cage-side daily, and detailed clinical examinations and measurement of body weight and food consumption were conducted weekly. Blood (Day 15 just prior to sacrifice following overnight food deprivation) and urine samples (over 4–6h in metabolic cages during the last 3 days of treatments) were collected for clinical pathology investigations. Details regarding necropsy and histopathology examinations were not provided.

One high-dose male was found in the moribund condition on Day 14 and sacrificed early. The animal exhibited ptosis, mild piloerection, mild passivity prostration, dyspnea, and weight loss were observed; however, no specific necropsy or histopathology results or cause of death were reported. Nonetheless, the authors appear to have considered the animal's condition to be treatment related as they reported, "Restorin® NMN dosed at 5,000 mg/kg/day for 14 days was associated with mortality". Clinical observations reported were mild piloerection in one other male and all females at the high dose and dehydration and weight loss in the majority of females at the high dose.

Overall mean body weight gain and mean food consumption were statistically significantly decreased in the 3000 and 5000 mg/kg bw/day group males and 5000 mg/kg bw/day group females, and in the males the decreased mean body weights were exceed the threshold of 10%. Several clinical pathology parameters were affected in 3000 and 5000 mg/kg bw/day males and/or females and correlated with histopathological finds in the livers and kidneys at the two highest doses. Additional histopathological alterations were observed in the glandular stomach, spleen, and thymus. No adverse effects were observed in the 500 or 1000 mg/kg bw/day dose groups. Based on these findings, 2000 mg/kg bw/day was selected as the high dose for the 91-day subchronic study.

III.C.2.ii.d Subchronic Toxicity

Cros et al. 2021

In a GLP study conducted according to OECD TG 408, NMN-C® was evaluated in male and female SD rats administered 0 (vehicle-control), 375, 750, or 1500 mg/kg bw/day by gavage for 90 consecutive days followed by a 28-day recovery period.[15] Ten animals of each sex/group were used for the main study and the recovery groups were comprised of 5 control and high-dose animals/sex.

No mortality, adverse clinical signs, abnormal behavior or functional neurological deficits, toxicologically relevant alterations in body weight development, or ophthalmological alterations were observed during the treatment or recovery phases. While a statistically significant reduction in mean body weight was

observed in the female high-dose group the magnitude (6%) was below the threshold considered toxicologically relevant and was compensated during the recovery period.

Statistically significant, dose-related increases in ASL, ALT, and AST (dose related in females only) were observed in males and females. However, the magnitudes of change were small (ALT increase remained well under 2-fold in females (110.87%) and all other increases were <1-fold) and were fully recovered following the 28-day no treatment phase. The authors considered all other statistically significant changes in clinical pathology parameters to be sporadic occurrences, although this conclusion is difficult to interpret as several were dose related and/or remained statistically significant following the recovery period and historical control data were not provided to place statistically significant findings in context. Nonetheless, the general low magnitudes of change absence of correlating findings in other study parameters appear to support the authors' conclusion.

Absolute liver weight and liver weight relative to body weight were statistically significantly increased in the high-dose males; however, the magnitudes of change were slight and were fully recovered after 28-days without treatment. Liver weights were not affected in the females. Dose-related statistically significant increases were also observed in kidney weights (absolute and relative) of the male high-dose group and a dose-related decrease in absolute spleen weight was observed in the female high-dose group. Additionally, absolute thyroid/parathyroid was statistically significantly increased in the male high-dose group. Again, it was difficult to view the observed changes in the context of normal biological variation as historical control data were not provided; however, all of these organ weight changes, as well as a few sporadic statistically significant changes in the lower dose groups only were fully recovered following the 28-days without treatment.

No gross pathological lesions were observed at necropsy. During the histopathological examination, treatment related effects were observed in the liver, kidneys, and harderian glands. Low grade, centrilobular hepatocellular hypertrophy, without evidence of degeneration or necrosis, was observed in the livers of 5 of 10 high-dose. The finding was not present in male rats of the satellite group following the 28-day recovery period and was not observed in female rats; thus, the nature of the lesions and correlated minor increases in liver enzymes and liver weights likely reflect adaptive changes due to increased metabolic demand.

Low grade chronic progressive nephropathy (CPN) was observed in the kidneys of mid- (5 of 10) and high-dose (8 of 10) males and correlated with the observed increases in kidney weights but lacked associated finding in clinical chemistry or urinary parameters. CPN is known to occur spontaneously in rats, but in this study, it was dose related and was only partially recovered (4 of 10) after discontinuation of dosing for 28 days. Nonetheless, due to the low severity and absence of associated

clinical pathology, the finding, while treatment related, was considered non-adverse. Additionally, CPN is a rodent-specific finding (with male rats distinctly predisposed compared to female rats) that is not relevant in humans.[42]

Lymphocytic infiltration of the harderian glands was observed in both sexes of all main groups (including control) with a dose-related increase in frequency and severity. Frequency of occurrence was slightly higher in males compared to females and severity increased from minimal in controls to slight-to moderate (the authors reported grades as means) at the mid and high doses. The lesion was not present in satellite control group at the end of the recovery period and was partially recovered in both incidence and severity in the satellite high-dose group. While lymphocytic infiltration of the harderian glands is known to occur spontaneously in rats, in this study it also appeared to be treatment related. However, it was considered a non-adverse effect due to the lack of other inflammatory cells, edema, degenerative or necrotic changes, squamous metaplasia of acini or ducts, or alterations in glandular tissue or ocular structure.

Based on these results the NOAEL was determined to be 1500 mg/kg bw/day, the highest dose tested.

Turner et al. 2021

In another subchronic study, Restorin® NMN was administered to groups of male and female Crl:CD® SD rats by gavage at doses of 0, 500, 1000, or 2000 mg/kg bw/day for 91-consecutive days.[16] The high dose was selected based on the 14-day study summarized in section III.C.2.ii.c above, and the study was conducted in compliance with GLP. Ten rats/sex were included in each dose group and two satellite groups of 5 rats/sex were administered the control and high-dose and followed for an additional 14-day recovery period without treatment. An additional group of 15 rats (10 main and 5 satellite)/sex were administered nicotinamide riboside (NR) chloride (a vitamin B_3 that has received FDA's no questions letter with respect to GRAS uses (GRN 635) and that can be metabolically converted to NMN in the salvage pathway (see section III.C.4 and Figure 3)) at the molar equivalent of the high dose (1740 mg/kg bw/day) as a comparator (note, findings in the NR group were generally similar to those in the 2000 mg/kg bw/day NMN group and are not further discussed in this summary).

Cage-side observation of clinical signs were made at least twice daily, detailed clinical examinations and body weight and food consumptions measurements were made weekly. Ophthalmological examines were conducted during the last week of treatment. Blood samples for clinical pathology were collected on the day of sacrifice (Day 92 for the main groups and Day 106 for the satellite groups). The authors also noted that urine sediment was examined microscopically but did not report collection procedures. "Full gross pathology" was performed on all animals

and histopathology was performed on a "comprehensive range of tissues" in control and high-dose group animals as well as any gross lesions observed at necropsy.

No mortality or treatment-related clinical signs were observed throughout the treatment and recovery phases. Male and female body weight development was adversely affected during the treatment phase in the 2000 mg/kg bw/day group and was associated with statistically significantly decreased food consumption. Body weight development was also statistically significantly reduced compared to control in the 1000 mg/kg bw/day males; however, the reduction remained under 10% relative to control in this group. There was a trend towards reversal during the recovery period as indicated by statistically significant increases in body weight gain in the male animals compared to controls and body weight gain in the females becoming non-significant (as compared to the statistically significantly decreased body weight gain compared to control observed during the treatment phase).

Hematological findings were somewhat difficult to interpret as in text reporting differed from the referenced supplementary table, which was incomplete. According to the text, hemoglobin was slightly but statistically significantly increased in males at 2000 mg/kg bw/day compared to controls and neutrophil count and percent were slightly increased while lymphocyte percent was slightly decreased in the 2000 mg/kg bw/day males (statistical significance not reported). These findings were not present at the end of the recovery period, and no significant differences in hematological parameters were observed in the female treatment groups compared to the controls.

Statistically significant dose related increases were observed in ALP and ALT at 2000 mg/kg bw/day in males and females and ALT was also statistically significantly increased at 1000 mg/kg bw/day in males. While slight (<1-fold), these changes were associated with statistically significant, dose-related increases in absolute liver weight (high-dose males) and liver weight relative to body weight (high-dose males, mid- and high-dose females) and "small areas of zonal hypertrophy" in the livers of some high dose animals. These findings were not observed in satellite animals following the recovery period.

Sodium was slightly but statistically significantly increased with a dose response in high-dose females, and dose-dependent, statistically significant decreases were observed in creatinine (high-dose females), LDH (high-dose males), and phosphorus (mid- and high-dose males). Statistically significant decreases in total cholesterol were observed in males of all dose groups with a reverse dose response as well as low-dose females. Several others sporadic changes were observed. None of these changes were observed in the satellite group animals.

Administration of the test item was associated with decreased urinary pH (all groups) and urine volume (mid dose, high dose, and positive control) and increased

specific gravity (mid dose, high dose, and positive control); no significant microscopic changes were observed. The noted changes were not present following the recovery period. The lower pH was considered due to urinary excretion of the acidic test item, and the increases in specific gravity were slight and considered incidental (and together with several of the observed hematological and clinical chemistry changes are best attributed to mild dehydration).

No treatment related macroscopic lesions were observed at necropsy while several statistically significant alterations in organ weights were observed. Statistically significant increases in absolute kidney weight (high-dose males) and kidney to body weigh ratios (mid-dose males and high-dose males and females) were dose-related and correlated with the histological finding of hyaline droplets in the proximal renal tubules of some male rats at 2000 mg/kg bw/day. While kidney weight changes did not persist at the end of the recovery period, the authors did not comment on renal histology following recovery. The authors correctly note that alpha $_{2\mu}$-globulin nephropathy is a male rat-specific condition,[43] and while their reporting of the lesion was incomplete, its occurrence in only treated male rats (at the high dose and positive control) and concomitant occurrence with early stage CPN in several high-dose animals (although the authors did not specifically note which rats) is, nonetheless, suggestive of the diagnosis. As discussed above, CPN is also a rodent-specific finding that is not relevant in humans.[42]

Absolute brain and spleen weights were decreased in high-dose females and males, respectively. Brain, heart, and testes weights relative to body weight were increased in high-dose males while ovary weight relative to body weight was increased in the high-dose and positive control females. These changes were not associated with correlating histopathology and were considered either due to the decreased body weights or as incidental findings unrelated to administration of the test item. Other histological lesions observed were considered background or individual findings and were not otherwise described. Based on reduced body weights and elevated ALT at 2000 mg/kg bw/day, the NOAEL was determined as 1000 mg/kg bw/day. While we agree with the NOAEL on the basis of suppressed body weight development, we opine that changes in liver enzymes and weights at the high dose were likely adaptive. Unfortunately, the authors' reporting of histological findings in the liver were incomplete, and while likely consistent with adaptive induction, we were unable to form an independent conclusion with respect to this finding.

III.C.3 Human Studies on NMN Substances

Two human studies in which safety assessment was reported as a specific aim were located. The first was a non-randomized open-label clinical trial in which the safety of single doses of 100, 250, and 500 mg of NMN (96–97% purity and supplied by Oriental Yeast Co. Ltd., Japan) were evaluated in 10 healthy male volunteers.[11]

(b) (4)

NMN did not cause adverse clinical signs or symptoms or adverse effects on heart rate, blood pressure, body temperature, ophthalmic parameters, sleep quality or evaluated laboratory parameters 5 hours after administration.

The second was a randomized double-blinded placebo-controlled clinical trial in which 30 healthy adults were given 125 mg NMN (Mitsubishi Corporation Life Sciences Limited, Japan) twice daily (250 mg/day) or matching placebo for 12 weeks and followed for an additional 4 weeks.[12] Subjects' symptom diaries and vital signs and clinical chemistry and hematology parameters were evaluated at baseline and every 4 weeks through the end of the recovery period. Body composition measurements were made at baseline and the end of the treatment period. Treatment compliance was 96.4 and 96.7% in the NMN and placebo groups, respectively. No serious adverse events occurred, and the rate of adverse events was comparable among the NMN and placebo groups with only one adverse event in each group (gastrointestinal symptoms) being considered due to treatment (the placebo subject withdrew from the study after 56 days of continuous symptoms while the transient event in the NMN subject did not cause withdrawal). No abnormal vital signs or body composition measurements were observed in any subjects of either group during the study, and no abnormal or clinically relevant differences in clinical chemistry parameters occurred.

An additional four clinical trials investigating various uses of NMN were located.[13, 22-24] These are summarized with respect to safety in Table 2 below.

Table 2. Safety Summary of NMN Clinical Trials

Author, Date	Test Item	Daily Dosage	Duration	Subjects	Design	Key Findings
Yoshino et al. (2021)[13]	NMN-NOS	250 mg	10 weeks	25 PM, PD♀adults	RCT	No AEs reported. No effects on body composition, VS, or CP.*
Liao et al. (2021)[22]	NMN-NOS	300, 600, or 1200 mg	6 weeks	48 athletic ♂& ♀adults	RCT	No AEs reported. No effects on body composition or VS. No ECG or CPET abnormalities.
Kim et al. (2022)[23]	NMN-NOS	250 mg	12 weeks	108 elderly ♂& ♀adults	RCT	No AEs reported.

(b) (4)

Author, Date	Test Item	Daily Dosage	Duration	Subjects	Design	Key Findings
Pencina et al. (2022)[24]	MIB-626	1000 or 2000 mg	14 days + 28 day follow up without Tx	32 OWOOH middle-aged to older ♂ & PM ♀	RCT	Well tolerated. No serious AEs AE frequency similar among MIB-626 & P groups. 1 high-dose subject withdrawn due to AE (diarrhea). No SS effects on CP or CS effects on VS or QTc interval. Mild AST & ALT elevation in 1 P and 1 low dose subject; return to baseline on discontinuation.

Abbreviations: ♀, female; ♂, male; AE, adverse event; ALT, alanine aminotransferase activity; AST, aspartate aminotransferase; CP, clinical pathology (e.g., hematology, clinical chemistry); CPET, cardiopulmonary exercise test; CS, clinically significant; ECG, electrocardiogram; MIB-626, microcrystalline unique polymorph NMN formulation to optimize absorption; NOS, not otherwise specified as to form; OWOOH, overweight or obese but otherwise healthy; P, placebo; PD, prediabetic; PM, postmenopausal; RCT, randomized double-blinded placebo-controlled clinical trial; SS, statistically significant differences; Tx, treatment; VS, vital signs.
*Published comment argued study results are questionable due to ineffective randomization.[44] The authors responded with their disagreement.[45]

III.C.4 Absorption, Distribution, Metabolism, and Excretion (ADME)

NMN is an intermediate metabolite of the endogenous salvage pathway of NAD^+ biosynthesis (Figure 3) formed when Nam and 5-phosphoribosyl-1-pyrophosphate are reacted on the enzyme Nam phosphoribosyltransferase (NamPT).[2, 3] NMN is then converted to NAD^+ by NMN adenylyltransferase (NMNAT) via the addition of ATP. NamPT is rate-limiting in the salvage pathway and is subject to negative feedback inhibition by both the oxidized and reduced forms of NAD (i.e., NAD^+ and NADH), which can be bypassed by supplying NMN or NR directly.[2, 4] In addition to metabolic reduction-oxidation reaction functions (i.e., redox interconversions of NAD^+ and NADH and NAD phosphate ($NADP^+$) and NADPH), NAD^+ can be catabolized to form regulatory proteins (sirtuins, adenosine diphosphate (ADP)-ribosyltransferases (ARTs), and poly(ADP-ribose) polymerases (PARPs)) and calcium mobilizing messengers (ADP-ribose and cyclic ADP-ribose), resulting in the salvage of Nam to replete the NAD^+ pool ($NADP^+$ can also be metabolized to form calcium mobilizing messenger NA ADP phosphate).[3-6]

Additionally, the NAD^+ catabolizing enzymes are inhibited by Nam, suggesting that in addition to the salvage pathway, a Nam degradation pathway may be important as part of NAD^+ regulation. Indeed a human gene encoding a Nam-*N*-methyltranferase has been identified,[5] and following acute administration of 100, 250, or 500 mg NMN to 10 healthy human males in a non-randomized, non-blinded setting, plasma levels of Nam metabolite 1-*N*-methylnicotinamide and its metabolites *N*-methyl-2-pyridone-5-carboxamide and *N*-methyl-4-pyridone-5-carboxamide were elevated over baseline (with the latter two metabolites being

statistically significantly increased with a dose response).[11] These metabolites were also elevated compared to placebo in plasma and/or skeletal muscle of prediabetic overweight or obese women after 10 weeks of supplementation with 250 mg NMN in a randomized, double-blinded, placebo-controlled trial.[13] Additionally, basal NAD^+ was increased compared to placebo in peripheral blood monocytes, and while it was not increased in the skeletal muscle of the subjects, the presence of increased Nam metabolites was indicative of increased NAD^+ turnover.

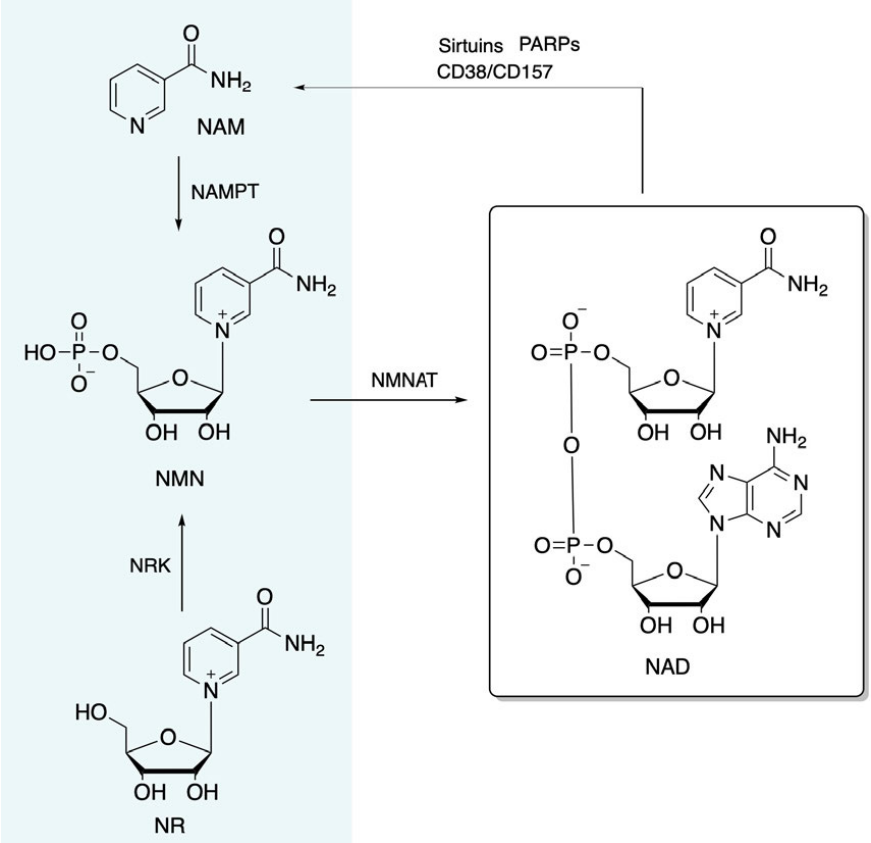

Figure 3. NAD salvage pathway. Adapted from Hong et al., 2020[3]

In addition to the salvage pathway from Nam, NMN can be formed by phosphorylation of NR by NR kinase (NRK), which then enters the NAD^+ biosynthetic pathway at NMNAT as described above and shown in the bottom part of Figure 3.[2-5] This alternate may be considered part of the salvage pathway, as extracellular Nam can be converted to NMN by NamPT secreted by various cell types, which in turn has been reported to be converted to NR by CD73 (an extracellular surface 5'-nucleotidase) and transported back into cells via an equilibrative nucleoside transporter.[5, 7, 46] A *de novo* pathway to NAD^+ biosynthesis,

(b) (4)

as well as a pathway from dietary NA, also exist but are not described here as they do not proceed through NMN as an intermediate.

It has been reported that orally administered NMN must first be converted to NR in order for uptake by mammalian cells to occur after which intracellular NR is converted back to NMN and that this subsequent conversion by NRK is rate limiting when NMN or NR are provided as NAD precursors.[7, 47] However, this mechanism does not explain the rapid kinetics reported in mice following intraperitoneal (IP) or oral administration of NMN (see below).[9, 48] More recently, extracellular conversion of NMN to NR by CD73 has been demonstrated to be weak in a recombinant human CD73 model, and in vitro experiments in human cancer cell lines suggest CD73 is unnecessary for utilization of extracellular NMN (although they also suggest conversion to NR or Nam is necessary, at least in the MCF-7 human breast cancer cell line).[49]

In contrast, a specific NMN transporter has been identified.[8] Preliminary experiments, based on the hypothesis that upregulation of transport of NMN into cells might occur when intracellular NAD^+ concentrations decline, identified a gene (*S1c12a8*) of the SLC12 family of electroneutral cation-chloride-couple transporters that was upregulated in mouse primary hepatocytes, pancreatic islet cells, and hippocampal neurospheres when conversion of Nam to NMN was blocked by inhibiting NamPT. A close homolog to *S1c12a8*, in which the membrane-spanning amino acid sequences are conserved, occurs in humans (and other species). *S1c12a8* was found to be highly expressed in the small intestine and pancreas and moderately expressed in the liver and white adipose tissue and expression was also induced in mouse NIH3T3 fibroblasts. To determine whether *S1c12a8* encodes an NMN transporter, mouse primary hepatocytes were incubated with NMN in the presence of a CD73 inhibitor (adenosine-5'-[α,β-methylene] diphosphate), nucleoside transporter inhibitor (dipyridamole), and NamPT inhibitor (FK866) to ensure no potential conversion of NMN to NR, uptake of NR into cells, or conversion of Nam to NMN, respectively. In comparison to control cultures, intracellular NMN was significantly increased following 1 minute of incubation with NMN. The same experimental conditions were repeated in cells knocked down for *S1c12a8* or *Nrk1* (the gene that encodes the intracellular NRK enzyme in these cells), and intracellular increases in NMN following 1 minute of incubation were completely absent and unaffected in the former and latter knockdown (KD) conditions, respectively.

K_m, V_{max}, and specificity of the S1c12a8 protein were then evaluated using *S1c12a8*-overexpressing NIH3T3 (S1c12a8-OE) cells, which lack detectible extracellular CD73 and CD38 (cyclic ADP ribose hydrolase) activities, and ^{3}H-labeled NMN. For the specificity experiments, membrane fraction of the S1c12a8-OE and control cells were combined with phospholipid bilayers of deproteinized erythrocyte plasma membrane to create proteoliposomes. The calculated K_m (34.1 ± 8.3 μM)

and V_{max} (11.5 ± 1.2 pmol/min/mg) were consistent with NMN concentration ranges detected in mouse plasma and erythrocytes. Excess amounts of NAD^+, AMP, NA mononucleotide, or Nam did not exhibit significant competition with the same excess concentration of ^{3}H-NMN while unlabeled NMN and NR did show competition in the proteoliposome experiments. The IC_{50}'s for NMN and NR were determined as 22.8 ± 3.6 µM and 77.4 ± 10.8 µM, respectively. As NR has not been demonstrated to reach this concentration, even under pathophysiological conditions, the protein was considered specific for NMN under physiological conditions. Nonetheless, NMN specificity was confirmed definitively using doubly labeled NMN (O18-D-NMN) or NR (O18-D-NR) with S1c12a8-OE or control NIH3T3 cells incubated for 5 minutes (note, it is presumed the test items were doubly labeled with the stable isotopes oxygen-18 (^{18}O) and deuterium (^{2}H); however, the authors did not report further details). Under treatment with 25 µM of O18-D-NMN, O18-D-NMN concentrations were increased 4-fold in S1c12a8-OE cells compared to controls and there was no conversion of O18-D-NMN to O18-D-NR. Conversely, treatment with 25 µM of O18-D-NR resulted in equivalent low levels of O18-D-NMN synthesis and transport of O18-D-NR in both S1c12a8-OE cells and controls without statistically significant differences.

Next the ability of S1c12a8 to transport NMN in vivo was evaluated in mice knocked down for *S1c12a8* expression in the small intestine, resulting in approximate reductions of 60 and 50% in the jejunum and ileum, respectively (note, the S1c12a8 protein was not expressed in the duodenum). Following oral administration of 500 mg/kg bw NMN to *S1c12a8*-KD or controls, no increase in plasma NMN levels were observed in the *S1c12a8*-KD mice while statistically significant increases were observed in the controls after 5 minutes. In the *S1c12a8*-KD mice, plasma Nam levels tended to be higher compared to controls, which may be attributed to conversion of NMN to Nam although the difference was not statistically significant. NAD^+ levels were also decreased in jejunal (statistically significant) and ileal (nonsignificant) cells in the *S1c12a8*-KD mice compared to controls. Whole body *S1c12a8* knockout (KO) mice or controls were administered 500 mg/kg bw O18-D-NMN by gavage for measurement of direct uptake of NMN in the jejunum and ileum and was clearly detectable in the control mice, but statistically significantly reduced by 46 and 36%, respectively, in the jejunum and ileum of the *S1c12a8*-KO mice. Experiments incubating primary hepatocytes from the control and *S1c12a8*-KO mice with O18-D-NMN and O18-D-NR confirmed the experiments in S1c12a8-OE NIH3T3 cells, showing similar results.

Consistent with the decrease in intracellular NAD^+, *S1c12a8* expression was decreased in the ileum of aged mice. Therefore, young and aged mice were administered 500 mg/kg bw NMN by gavage. While NMN plasma levels were lower in aged compared to young mice at all timepoints, the fold increases in NMN were greater in the aged compared to the young mice, and the ileal NAD^+

concentrations approached those of the young mice with fold increases being equivalent. Finally, NAD^+ concentrations were statistically significantly reduced in the ilea of aged, but not young, *S1c12a8*-KD mice while young *S1c12a8* overexpressing mice exhibited statistically significant increases in ilea NAD^+ concentrations.

Overall, the results of this study demonstrate the presence of a mammalian NMN-specific transporter that is highly expressed in the gut and pancreas and at least moderately expressed in some other tissues, notably the liver. Furthermore, upregulation of the transporter occurs when tissue NAD^+ concentrations decline (e.g., age, increased demand) indicating a mechanism for responding to a demand for NAD^+ and maintaining homeostasis. Finally, the small intestine was identified as the major site of dietary absorption of NMN. While these results stand in contrast to other reports that suggest conversion of NMN to NR is necessary before cellular uptake can occur, it is possible that conversion may be necessary in some tissues but not others or that different conditions alter the route by which NMN is taken up in some tissues. Nonetheless, the study by Wilk et al. calls into question the ability of CD73 to convert NMN to NR.[49] In fact, it appears that serum may contain an enzyme capable of converting NMN to NR and it is noted that the studies by Nikiforov et al. and Ratajczak et al. both used serum to supplement their culture media suggesting a possibility that the supposed conversion by CD73 was artifactual.[7, 47] Regardless, it is clear that NMN can be utilized by cells to support the biosynthesis of NAD^+ whether by direct uptake by a transporter or conversion to NR. More work is needed in order fully elucidate the mechanism of cellular NMN uptake.

Thus, in summary, there are three potential general points of entry to the biosynthesis of NAD^+ through NMN in humans as follows: Nam via salvage or dietary supply, NR via salvage or dietary supply, and NMN via dietary supply. As noted above, additional entry points supplied by tryptophan (i.e., *de novo* synthesis), NA (Preiss-Handler pathway), or NA riboside (NAR) are not described in this safety assessment as they do not proceed through NMN. It is also noted that biosynthesis of NAD^+ via the *de novo* pathway is insufficient to provide for physiological needs,[7] and the *de novo* pathway also does not participate in feedback regulation of NAD^+ biosynthesis (in which NamPT is central),[2] suggesting a central role of the salvage pathway in regulating intracellular concentrations of NAD and Nam. However, it is also noteworthy that in the particular case of mitochondrial NAD^+ biosynthesis, an NMN utilizing pathway from these other entry points is suggested by the ability of NA and NAR to raise mitochondrial NAD^+ concentrations. NMN is the only cytosolic NAD^+ precursor that contributes directly to mitochondrial concentrations of NAD^+ as NMNAT is the only enzyme of the biosynthetic pathway present within the mitochondrial matrix, while all necessary pathway enzymes are present in both the cytosol and the nucleus.[7] Thus, cytosolic NAD^+ derived from the *de novo*

pathway, NA, or NAR must either back-convert to NMN via reverse flux through cytosolic NMNAT, hydrolysis by Nudix pyrophosphatases within peroxisomes followed by export to the cytosol, or via extracellular conversion of NAD^+ to Nam via cADP-ribose synthases and then to NMN and/or NR followed by cellular uptake as described above.[6, 7] For reference, the full extent of known and hypothesized NAD^+ metabolism is shown in Figure 4.

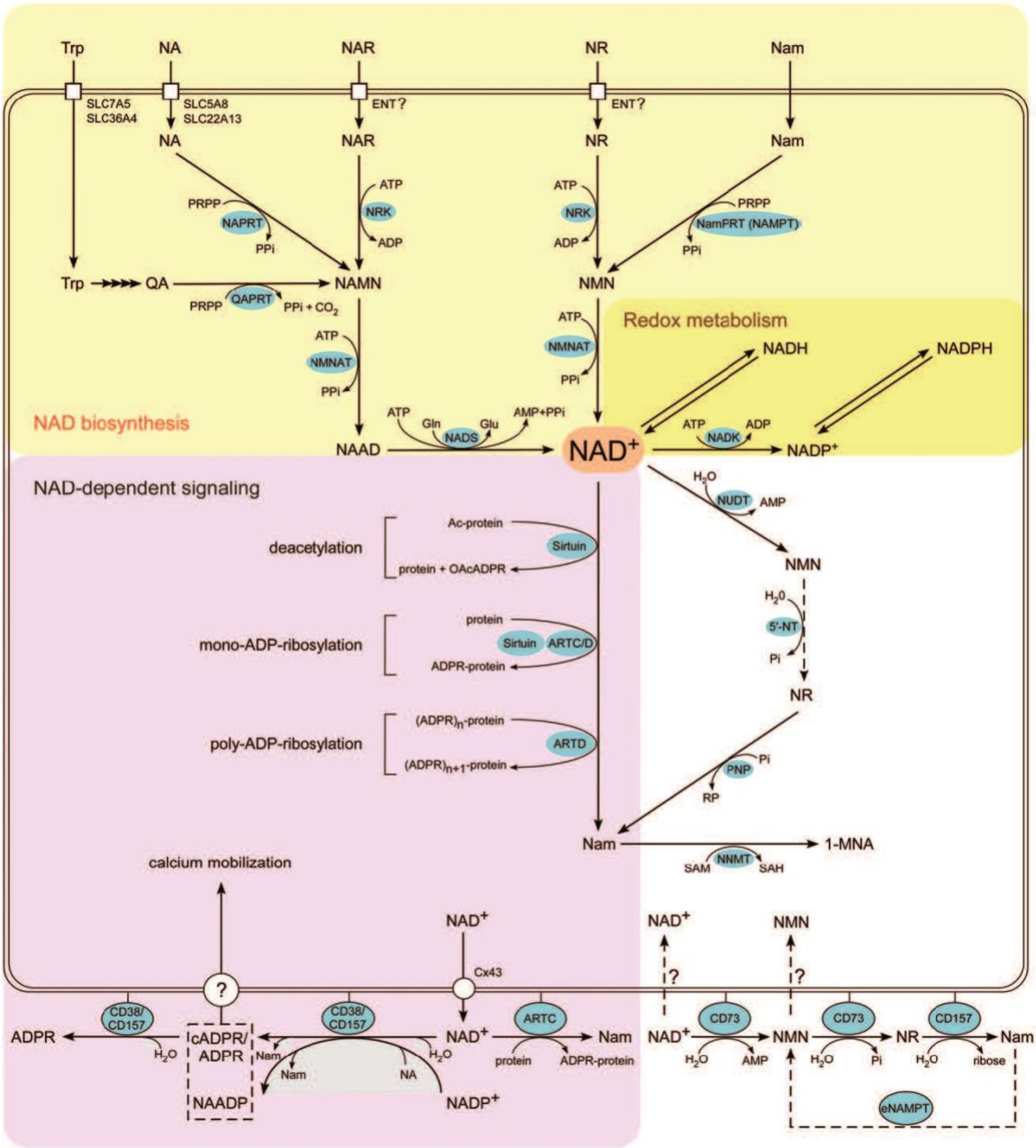

Figure 4. NAD metabolism. Adapted from Nikiforov et al., 2015[6]

(b) (4)

The absorption and tissue distribution of NMN has been studied in laboratory animals. Following administration of a single dose of 500 mg/kg bw NMN IP to male and female wild-type C57BL/6J mice, peak NMN concentrations approximately 15-fold greater than baseline were observed in the liver, pancreas, and white adipose tissue (WAT) at 15 minutes, retuning to baseline by 30 minutes.[48] Concomitant rises in NAD^+ concentrations were observed and sustained at 60 minutes in all three tissues. In another experiment of the same study, following treatment of diabetic C57BL/6J mice receiving a high-fat diet (HFD; approximately 42, 42, and 15% of calories from fat, carbohydrate, and protein, respectively) with 500 mg/kg bw/day NMN by IP injection for 7 (females) or 10 (males) consecutive days, NAD^+ concentrations (which are depleted in the diabetic mice) were restored in the liver and WAT and were moderately, but statistically significantly, increased in skeletal muscle compared to control mice receiving the HFD alone.

NMN kinetics were further investigated at lower doses by the oral route in male C57BL/6N mice in both acute and long-term (6 months) experiments.[9] Fasted mice received single doses of 0 (vehicle-control) or 300 mg/kg bw NMN by gavage after which plasma NMN exhibited a step increase within 2.5 minutes, peaked at 10 minutes (statistically significant), and returned to baseline at 15 minutes in the treated mice. Hepatic NAD^+ concentrations non-statistically significantly increased concomitantly beginning 15 minutes following administration, with continued rising through 30 minutes, and remained elevated at 60 minutes. Additionally, non-statistically significant increases in tissue NAD^+ concentrations were observed in skeletal muscle and the cerebral cortex, but not in WAT or brown adipose tissue (BAT), 60 minutes following administration. Because the observed tissue NAD^+ concentrations were not statistically significantly elevated compared to controls, a radio-label experiment was conducted to confirm whether conversion of the orally administered NMN to NAD^+ occurred. Double-labeled [^{13}C7, D2']-NMN (C13-D-NMN) was administered by gavage at 0 and 300 mg/kg bw and traced in hepatic and soleus muscle tissue at 10- and 30-minutes post administration. C13-D-NAD^+ was clearly detectable in the liver, but not the soleus muscle, after 10 minutes and approximately doubled by 30 minutes, and C13-D-NAD^+ was clearly detectable in the soleus at 30 minutes; thus, confirming the rapid absorption and tissue conversion of orally administered NMN to NAD^+. Next, NMN was administered to mice in drinking water at 0, 100, and 300 mg/kg bw/day for 12 months, and at 6 months an interim sacrifice was conducted to obtain tissue samples of liver, skeletal muscle, WAT and BAT for determination of NAD^+ concentrations. Non-statistically significant trends of dose-related increases were observed in hepatic tissue and BAT NAD^+ concentration while no differences compared to controls were observed in skeletal muscle and WAT. Thus, orally administered NMN was rapidly absorbed and converted to NAD^+ in at least some tissues acutely and may result in slight dose-

(b) (4)

dependent increases in the steady state of tissue NAD^+ concentrations in some metabolically active tissues following chronic administration in mice.

Another study investigated the ability of orally administered NMN to affect brain NAD^+ concentrations in C57BL/6J mice.[10] Six male mice/group were administered, by gavage, a single dose of either 400 mg/kg bw NMN dissolved in phosphate buffered saline or the vehicle alone and sacrificed, by carbon dioxide asphyxiation 45 minutes later, and brain tissues were prepared for analysis of NAD^+ concentrations. Compared to controls, the NAD^+ concentration in brain tissue of the treated mice was statistically significantly increased (+40%) suggesting that orally administered NMN is able to cross the blood brain barrier and rapidly convert to NAD^+, although further confirmation is required for a definitive conclusion.

In a rat study, male Wistar rats were administered 0 or 45 µmol/kg NMN (equivalent to 15 mg/kg bw) by IP injection and urine (collected over 24h prior to injection and at various intervals over 48h following injection), blood (collected every 30 min over 6h), and liver (collected after 3h) samples were collected for measurement of urinary metabolites and whole blood and liver total NAD.[50] No direct measurements of NMN were made in any of the collected samples. A molar equivalent of Nam was also measured for comparison to NMN, and rats on the study were fed with a niacin-free diet in order that total NAD levels would be derived only from dietary tryptophan and the injected vitamins. Following administration of NMN, the sums of urinary metabolites per time interval tended to peak during the interval from 0–3h (the exception was Nam *N*-oxide, which peaked from 3–6h) after which they gradually returned to baseline over 24–48 hours while excretion of Nam metabolites peaked at the 3–6h interval (urinary excretion of unmetabolized Nam peaked at the 0–3h interval). Excretion of metabolites following administration of Nam were statistically significantly greater during the 3-6h interval compared to excretion of metabolites following administration of NMN, while total NMN metabolites tended to domination (although not statistically significantly) during the intervals from 6–12 hours. Interestingly, control data was not shown but is presumed to match baseline measurements. While the authors failed to measure unmetabolized NMN or report the sum and statistical significance of total excretion of Nam and NMN metabolites from time zero to various intervals, they suggested that the statistically significantly higher excretion following Nam administration during the 3–6h interval is representative of higher salvage pathway NAD^+ biosynthesis and longer retention time of NMN relative to Nam due to the ability of NMN to bypass feedback regulation of NAD^+ conversion. No significant differences were observed in whole blood compared to control from 0–6h or liver total NAD concentrations 3h following administration (note, this was also true with administration of Nam).

In contrast to the blood results in rats administered NMN IP, which measured only total NAD (i.e., NAD^+ + NADH), in a randomized, double-blinded, placebo-

controlled clinical trial, NAD^+ and NA mononucleotide levels were statistically significantly increased compared to placebo in whole blood of healthy humans given 250 mg/day NMN for 12 weeks.[12] These levels were statistically significant compared to placebo at 4, 8, and 12 weeks and returned to baseline at Week 16 following 4 weeks without treatment. These results in humans as well as the results of urinary metabolite analyses in rats are in general agreement with plasma results reported by Irie et al. and Yoshino et al. following oral administration of NMN to humans as descried above.[11, 13]

The lack of statistically significant differences in rat liver total NAD concentrations 3h following IP administration of 45 µmol/kg bw lacks comparators while several studies have reported effects on liver NAD+ concentrations. For example, in a study by Yoshino et al., mice received 500 mg/kg bw NMN IP and while liver NAD^+ concentrations were elevated over baseline, the authors did not report whether the elevation was statistically significant nor did they measure levels at timepoints beyond 60 minutes.[48] And in a mouse study, compared to controls, hepatic NAD^+ concentrations were statistically significantly dose-dependently increased (approximately ≥ 13-fold at the low dose and ≥ 17-fold at the high dose) in C57BL/6J mice administered 0, 1340 or 2680 mg/kg bw/day NMN by gavage for seven consecutive days.[18] In this study, serum Nam concentrations were also statistically significantly increased, approximately 40-fold, in beagle dogs administered 1340 mg/kg bw/day NMN by gavage for 14 consecutive days.

As illustrated in the extensive discussion above, while the anabolic pathways leading to the biosynthesis of NAD^+ are known and the catabolism of NAD^+ proceeds through the same known pathways regardless of the precursor, details of NAD^+ regulation and compartmentalization; the forms in which precursors are absorbed, enter cells, and are eliminated; and whether and how the specific pharmacodynamics of NAD^+ vary according to the kinetics of the precursor ingested remain to be fully elucidated.

III.C.5 History of Use

Following a June 9, 2021 independent conclusion of GRAS status, the notifier's NMN has been introduced to the market, as of September 2021, in the United States as a GRAS ingredient in a protein powder at a level of 30 mg/serving. The mean and 90th percentile maximum estimated daily intakes in U.S. population aged 2 years and older are 40.1 mg/person (p)/day (0.51 mg/kg body weight (bw)/day) and 75.1 mg/p/day (0.95 mg/kg bw/day), respectively; however, no qualitative or quantitative post market exposure data has been generated. NMN is, also, a natural component of conventional foods. Its levels have been assayed in a limited number of fruits and vegetables as well as beef and shrimp and were found to range from 0.0 to 1.88 mg/100 g (0.00188%) in food.[9] If present at the maximum assayed concentrations

of 1.88 mg/100 g in edamame, 1.60 mg/100 g in avocado, or 0.22 mg/100 g shrimp, this would result in consumption of 1.6 mg NMN in a typical 85 g serving of edamame, 0.8 mg NMN in a typical 50 g serving of avocado, or 0.24 mg NMN in a typical 110 g serving of shrimp (based on the US FDA reference amounts customarily consumed per eating occasion for fresh vegetables, avocado, and shellfish, respectively).

While no qualitative or quantitative data regarding ingestion of NMN occurring naturally in conventional foods was located, as NAD^+ is necessary to the survival of all cells, NMN is presumed to be present at some level in most food stuffs consumed by humans. For this reason, exposure to naturally occurring NMN, or other niacins, in conventional foods was considered as normal background to any effects noted in preclinical or clinical studies or case reports of patients that received supplemental niacins. Additionally, as levels of niacins occurring naturally in foods have never been associated with adverse effects,[1] the history of consumption of any NMN occurring naturally in food was not considered relevant to the safety assessment. Nonetheless, while not relevant to this safety assessment, this history of use clearly establishes the legal presence of NMN in conventional foods as an article used for food in a form in which the food is not chemically altered.

In addition to the history of use in food, a general Internet search revealed that a number of NMN products are currently marketed as dietary supplements with typical serving sizes ranging from 50 to 500 mg and sometimes with recommendations to take multiple servings daily, which in some cases could result in ingestion of up to 1000 mg daily.

III.C.6 Other Evidence of Safety

III.C.6.i Other Niacins

Because NMN is a member of the B_3 family of vitamins (aka niacins) its safety assessment should be evaluated and put into perspective with information establishing the safety of uses of other members of this family. In this respect, established Dietary Reference Intakes (DRI) for classical forms of niacin (i.e., NA and Nam) are relevant to discuss as are safety data and evaluations related to another novel niacin, NR. No formal preclinical toxicological investigations meeting current internationally accepted test protocols have been conducted on Nam or NA. Because of this, authoritative scientific bodies have relied on human data in establishing Tolerable Upper Intake Levels (UL) for Nam and NA. For purposes of comparison, the molar equivalents to NMN are given below:

- 1 mg NA = 2.71 mg NMN
- 1 mg Nam = 2.74 mg NMN

- 1 mg NR chloride (NRc) = 1.15 mg NMN

III.C.6.i.a National Academy of Medicine (United States)

The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients of the Food and Nutrition Board (FNB) of the National Academy of Medicine (formerly Institute of Medicine) has established DRIs (i.e., Adequate Intakes (AI), Recommended Dietary Allowances (RDA), and ULs) for niacin.[1] Niacin was defined as "nicotinamide (nicotinic acid amide), nicotinic acid (pyridine-3-carboxylic acid), and derivatives that exhibit the biological activity of nicotinamide".

AIs are:

- 2 mg niacin[1] per day for infants 0–6 months
- 4 mg niacin per day for infants 7–12 months

RDAs are:

- 6 mg niacin equivalents (NE)[2] per day for children 1–3 years
- 8 mg NE per day for children 4–8 years
- 12 mg NE per day for children 9–13 years
- 16 mg NE per day for adolescent (14–18 years) and adult males
- 14 mg NE per day for adolescent and adult females
- 18 mg NE per day for adolescent and adult females during pregnancy
- 17 mg NE per day for adolescent and adult females during lactation

ULs are:

- Not Determinable for infants 0–12 months
- 10 mg niacin per day for children 1–3 years
- 15 mg niacin per day for children 4–8 years
- 20 mg niacin per day for children 9–13 years
- 30 mg niacin per day for adolescents 14–18 years (including females during pregnancy and lactation)

[1]Preformed niacin defined as nicotinamide, nicotinic acid, and derivatives that exhibit the biological activity of nicotinamide.
[2]NEs are used because the amino acid L-tryptophan can be used for in vivo biosynthesis of niacin. 1 mg niacin = 1 mg NE; 60 mg L-tryptophan = 1 mg NE.

(b) (4)

- 35 mg niacin per day for adults (including females during pregnancy and lactation)

As there was no evidence of adverse effects related to niacins that occur naturally in foods, such exposures were not considered by the FNB in determining ULs. ULs for niacin were based on flushing of the skin (enough such that it affects an individual's decision to continue taking the vitamin) that can occur following ingestion of NA from fortification of foods, dietary supplements, or pharmaceutical drugs. While flushing does not appear to be causally associated with forms of niacin other than NA, nonetheless, the ULs were applied to all forms of niacin as they were considered protective against potential adverse effects of Nam at higher doses.

Gastrointestinal effects, increased blood glucose, and liver injury have been associated with ingestion of NA from dietary supplements or pharmaceuticals at high doses (≥1000 mg/daily).[1, 51, 52] Liver toxicity appears to be more common in individuals taking a sustained release preparation of NA but has been reported with immediate release NA as well. Gastrointestinal effects and liver injury have also been associated with ingestion of Nam from dietary supplements at dosages ≥3,000 mg/day, and a negative effect on insulin sensitivity has been reported at 2,000 mg/day.[1, 51, 53] Other adverse effects, such as ocular and skin effects, have also be documented at high doses of NA.[1, 52] For purposes of comparison the molar equivalents of NNM to 35 mg NA, 1000 mg NA, 2000 mg Nam, and 3000 mg Nam are 95, 2715, 5474 mg, and 8210 mg, respectively. These molar equivalents (with the exception of the dose based on flushing, which is not applicable to NMN as discussed further below) offer a wide MoE compared to the notifier's proposed use of NMN as a dietary ingredient in dietary supplements and are strongly corroborative of the results observed in toxicological studies of NMN.

III.C.6.i.b Flushing

Transient visible flushing of the skin accompanied by sensations of tingling, burning, itching, and/or headache is a well-known vasodilatory adverse effect associated with ingestion of 30 mg or more of NA and thought to be mediated by prostaglandins (PGs). Flushing has not been associated with ingestion of naturally occurring NA, likely due to low concentrations in food as compared to the high bolus doses encountered by fortification of foods, supplementation, and pharmaceutical uses. While flushing is considered a largely harmless, non-pathological effect, it is often perceived as uncomfortable, resulting in discontinuation of supplemental or therapeutic use of NA and, theoretically, may result in exacerbation of mild postural hypotension in the elderly. Therefore, flushing has been the basis of the establishment of ULs for NA.[1, 25, 26]

(b) (4)

Because of the flushing observed with use of NA, Bean and Spies investigated the structural features associated with the effect in human male (n=47) and female (n=7) adults and children (n=4) using a number of pyridine (including NA and Nam) and pyrazine compounds and the objective measure of change in skin temperature at 15 points of the face and neck.[27] Flushing was specific to compounds containing a carboxyl radical at the 3-position of the pyridine ring. Nam, which contains an amide group at this position, or other compounds without this structural feature, did not cause flushing. NMN, as a metabolite of Nam, also contains an amide group at this position, and we have found no reliable documented evidence of flushing occurring with the Nam or NMN forms of vitamin B_3, regardless of the source.

In a more recent study investigating the mechanism of NA-induced flushing, GPR109A, a G-protein-coupled receptor, previously shown to mediate antilipolytic effects of NA in adipocytes, was shown to mediate NA-induced flushing involving release of PGD_2 and PGE_2 in the skin of mice.[28] GPR109A, an NA receptor expressed in adipocytes and immune cells, is referred to as PUMA-G in mice and HM74A in humans. When NA binds the receptor in adipocytes, a signal cascade results in reduced activity of hormone-sensitive lipase, which in turn results in the downstream effects on plasma lipid concentrations associated with NA, but not Nam. First the investigators demonstrated that administration of NA to mice resulted in a transient dose-dependent vasodilation of the skin. They then demonstrated that NA-induced flushing was absent in cyclooxygenase-1 knockout mice, but not in endothelial nitric oxide synthase knockout mice, demonstrating the role of PGs, but not nitric acid, in NA-induced flushing, and then further demonstrated, in mice deficient in various downstream PG receptors that PGD_2 and PGE_2 are the specific PGs involved, which is consistent with evidence in humans. Next it was demonstrated that PUMA-G deficient mice did not respond with a vasodilatory effect when administered NA while mice heterozygous for a null allele of PUMA-G and wild-type mice responded with similar vasodilatory effects. However, when administered with PGD_2 or serotonin, PUMA-G knockout mice exhibited a full flushing response. Northern blot and polymerase chain reaction analyses demonstrated the expression of PUMA-G in the skin of mice, and further analyses demonstrated this expression within immune cells of the skin. The ability of wild-type macrophages to respond to NA via PUMA-G was then demonstrated in vitro. Finally, bone marrow chimeras were created by transplanting PUMA-G knockout mice with either wild-type or PUMA-G knockout bone marrow. Before transplantation and after transplantation with PUMA-G knockout bone marrow, the PUMA-G deficient mice did not exhibit cutaneous vasodilation when administered NA; however, 12-weeks following transplantation with wild-type bone marrow the wild-type chimeras began to exhibit cutaneous vasodilation following administration of the NA, and expression of PUMA-G was demonstrated in both their bone marrow and bone marrow-derived dermal cells.

In summary, the flushing mechanism of NA has been demonstrated as due to the release of prostaglandins in the same pathway responsible for its lipid-lowering effects. The flushing effect is considered non-pathological but may result in discontinuation of treatment and postural hypotension in susceptible individuals. Further the carboxyl radical at the 3-position of the pyridine ring is implicated in this activity. Other niacins, including NMN, which have an amide at the 3-position, have not been reliably associated with flushing and do not possess lipid-lowering activity. Based on the evidence presented, there is no reason to consider the ULs established on NA-induced flushing as relevant to the use of NMN (or Nam).

III.C.6.i.c United States Food and Drug Administration

The US Food and Drug Administration (FDA) affirmed, on November 16, 1983, that NA and Nam are GRAS for use as nutrient supplements with no limitations other than current good manufacturing practice pursuant to 21 CFR §184.1530 and 21 CFR §184.1535.

At the recommendation of the President of the United States, in 1969, that FDA critically review the safety of GRAS food substances (such as those on FDA's "GRAS list"), the Select Committee on GRAS Substances, in 1979, reviewed the safety of NA and Nam and, based on an extensive review of the scientific literature,[54] opined, "There is no evidence in the available information on niacin (nicotinic acid) or niacinamide (nicotinamide) that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future."[55]

An NDI notification (report number 882) was submitted to FDA on behalf of ChromaDex, Inc. for use of an NRc compound (NRc-C) as the sole dietary ingredient in a dietary supplement at levels up to 180 mg/day for up to 90 days, was filed by FDA on August 24, 2015 (with additional information submitted on October 13 and 30, 2015), and received the agency's letter of acknowledgement without objection on November 3, 2015, indicating no current challenge to the safety of the recommended use of the dietary ingredient or its status as a dietary ingredient. The basis for determining a reasonable expectation of safety was the derivation of a UL of 3 mg/kg bw/day, or 180 mg/day assuming a body weight of 60 kg, by application of a 100-fold safety factor to the NOAEL determined from a 90-day repeated-dose oral toxicity study in rats (section III.C.6.i.f contains a summary of published safety data on NRc-C). For purposes of comparison, the molar equivalents of NMN to 180 mg NRc-C is 207 mg.

An FDA GRAS notice (GRN 635) was found in the FDA GRAS Notices Inventory database for NRc-C. GRAS notice number GRN 635 received FDA's no questions response letter on August 3, 2016, indicating no current challenge to the safety of

the ingredient for its intended use a source of vitamin B_3 in vitamin waters, protein shakes, nutrition bars, gum, chews, and powdered beverages at a maximum level of 0.0057% by weight as consumed. The estimated users-only dietary exposure to NRc-C from its intended uses in foods by consumers aged 2 and older was 51 mg/person/day (0.8 mg/kg body weight (bw)/day) at the mean and 145 mg/person/day (2.2 mg/kg bw/day) at the 90th percentile. Based on the pivotal toxicological data described in GRN 635 (see section III.C.6.i.f below), a UL of 3 mg/kg bw/day, or 180 mg/day assuming a body weight of 60 kg, was estimated by applying a 100-fold safety factor to the NOAEL determined from a 90-day repeated-dose oral toxicity study in rats. For purposes of comparison, the molar equivalents of NMN to 180 mg NRc-C is 207 mg.

A second NDI notification (report number 1062) was submitted on to FDA on behalf of ChromaDex, Inc. in order to present new information for the purpose of amending the recommended conditions of use of NRc-C. The notification was filed by FDA on December 27, 2017 (with additional information submitted on February 5 and March 6, 2018) and received the agency's letter of acknowledgement without objection on March 7, 2018, indicating no current challenge to the safety of the amended recommended use of the dietary ingredient in a dietary supplement at levels up to 300 mg/day without any restrictions on duration of use. While details of the new information presented is not publicly available in the FDA docket it included additional genetic toxicity, reproductive and developmental toxicity studies in rodents, safety pharmacology and subacute toxicity studies in juvenile dogs, and long-term studies in humans. For purposes of comparison, the molar equivalents of NMN to 300 mg NRc-C is 345 mg. While these levels are lower than the notifier's proposed use levels of NMN, the safety of NMN was based on similarly conducted toxicological studies on the notifier's article of commerce as well as toxicological studies on other NMN substances, and there is, additionally, some evidence to suggest higher NOAELs and use levels of NR may also be appropriate (see section III.C.6.i.f below).

III.C.6.i.d Expert Group on Vitamins and Minerals (United Kingdom)

The Expert Group on Vitamins and Minerals (EVM) evaluated the long-term safety of niacin supplements and established Guidance Levels after determining that data were insufficient to establish Safe Upper Limits (SULs).[25] Guidance Levels were defined as "determination[s] of doses of vitamins and minerals that potentially susceptible individuals could take daily on a life-long basis, without medical supervision in reasonable safety." It was noted that Guidance Levels "must not be confused with, or used as, SULs." Niacin (vitamin B_3) was defined as "the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (nicotinic acid amide), and the coenzyme forms of the vitamin."

The Guidance Level for NA was established as 17 mg/day (equivalent to 0.28 mg/kg bw/day in a 60 kg adult) for supplements only. This guidance level was based on consistent reports of flushing at intakes of 50 mg/day or greater, which was considered a LOAEL. EVM noted that long-term NA doses of 3000 mg/day or more were associated with hepatotoxicity, hyperglycemia, gastrointestinal complaints, ocular side effects, and gout, and that lower levels of sustained release NA have been associated with higher rates of gastrointestinal effects and hepatotoxicity and that its Guidance Level is not applicable to sustained release formulations.

The Guidance Level for Nam was established as 560 mg/day (equivalent to 9.3 mg/kg bw/day in a 60 kg adult) for supplements only. This guidance level does not apply to pregnant women. The guidance level was based on two studies in type 1 diabetics using 25 and 42 mg/kg bw/day Nam, respectively, in which adverse effects were not observed. EVM noted sparse evidence from case reports that long-term Nam doses of 3000–9000 mg/day have been associated with liver dysfunction. For purposes of comparison, the NMN molar equivalent of 560 mg Nam is 1533 mg and is corroborative of our safety assessment of the notifier's NMN and recommended use levels based on preclinical toxicological studies. Furthermore, this Guidance Level is based on a NOAEL in humans and the corroborative evidence from case reports suggests a human LOAEL in the NMN equivalent range of 8210–24631 mg/day, suggesting a wide MoE compared to the notifier's proposed use of NMN as a dietary ingredient in dietary supplements.

III.C.6.i.e European Food Safety Authority

At the request of the European Commission (EC), the Scientific Committee on Food (SCF) of the of the European Food Safety Authority (EFSA) provided EC with an opinion on ULs for NA and Nam.[26]

ULs for NA were determined as follows, based on flushing using a NOAEL of 30 mg/day from a clinical trial:

- 2 mg per day for children 1–3 years
- 3 mg per day for children 4–6 years
- 4 mg per day for children 7–10 years
- 6 mg per day for children 11–14 years
- 8 mg per day for adolescents 15–17 years
- 10 mg per day for adults (not applicable to females during pregnancy or lactation due to inadequate data)

SCF noted that forms of toxicity more severe than flushing (such as hepatotoxicity, including potentially life-threatening liver injury, glucose intolerance, and ocular effects) principally occur with doses of NA >500 mg/day.

ULs for Nam were determined as follows, based on NOAELs of approximately 25 mg/kg bw/day in clinical studies in subjects with, or at risk for, diabetes:

- 150 mg per day for children 1–3 years
- 220 mg per day for children 4–6 years
- 350 mg per day for children 7–10 years
- 500 mg per day for children 11–14 years
- 700 mg per day for adolescents 15–17 years
- 900 mg per day for adults (not applicable to females during pregnancy or lactation due to inadequate data)

SCF noted that only a single case of hepatotoxicity, at a high dose of 9000 mg/day, has been reported in association Nam (with the exceptions of patients who had also been give NA during the course of treatment). However, they also noted that safety data on Nam is limited as, in contrast to NA, it has not been extensively studied in humans at doses ≥3000 mg/day. SCF also noted that flushing does not occur with Nam, that gastrointestinal effects are rare, and that no glucose intolerance or other significant adverse effects have been reported in clinical trials in subjects with, or at risk for, diabetes. For purposes of comparison, the molar equivalent of NMN to 900 mg Nam is 2463 mg while the molar equivalents to 3000 and 9000 mg/day are 8210 and 24631 mg/day, respectively. These data, too, corroborate our safety assessment of the notifier's NMN and recommended use levels based on preclinical toxicological studies.

In response to a Novel Food Application by ChromaDex, Inc., at the request of the EC, EFSA's Panel on Nutrition, Novel Foods and Allergens (NDA) provided an opinion on the safety of the intended use of ChromaDex's NRc (NRc-C) in food supplements, as a source of niacin for the healthy adult population, at levels up to 300 mg/day.[56] In addition to the published toxicological studies and clinical trials, NDA also reviewed unpublished repeated-dose toxicology studies in dogs and developmental and reproductive toxicity studies in rats.

The panel determined repeated-dose toxicity NOAELs of 300 mg/kg bw/day in rats and dogs, NOAELs for fertility and reproductive performance of 675 and 1088 mg/kg bw/day in male and female rats, respectively (the highest doses tested), and NOAELs for maternal and embryo-fetal toxicity of 325 mg/kg bw/day each; however, the observed embryo-fetotoxicity was determined to be secondary to maternal toxicity. The panel also considered kinetic data and human data (in which

NRc-C did not cause adverse effects at doses up to 2000 mg/day for up to 12 weeks). The panel concluded that NRc-C is safe for its intended use in healthy adults, excluding pregnant or lactating females, at doses up to 300 mg/day and in pregnant or lactating females at doses up to 230 mg/day. While 300 mg/day provides a MoE of only 70-fold relative to the general toxicity NOAEL of 300 mg/kg bw/day in rats, the NDA concluded this margin was acceptable based on available human data for both NRc and Nam and the approximately 6-fold MoE of the NR molar equivalent to Nam relative to EFSA's UL for Nam. The safe level of exposure in pregnant or lactating females was based on the observed maternal toxicity in the embryo-fetal developmental toxicity study and an approximately 100-fold MoE. The panel also concluded that Nam is a bioavailable metabolite of supplemental NRc-C. For purposes of comparison, the molar equivalents of NMN to 230 and 300 mg NRc-C are 264 and 345 mg, respectively. While these levels are lower than the notifier's proposed use levels of NMN, the safety of NMN was based on similarly conducted toxicological studies on the notifier's article of commerce as well as toxicological studies on other NMN substances, and there is, additionally, some evidence to suggest higher NOAELs and use levels of NR may also be appropriate (see section III.C.6.i.f below).

III.C.6.i.f Published Studies on NRc

A battery of GLP (with the exception of the 14-day study) toxicological studies were conducted an NRc of >99% purity manufactured by ChromaDex, Inc. (NRc-C). in accordance with OECD (or in the case of the acute oral toxicity, US FDA) test guidelines (TGs) as reported below.[29] An additional GLP 90-day oral toxicity study in rats was conducted in accordance with OECD TGs on an NRc of >97% purity produced by a Elysium Health (NRc-E) and is also reported.[30]

Bacterial Reverse Mutation Test of NRc-C

In order to evaluate the mutagenic potential of NRc-C, a bacterial reverse mutation test was conducted using *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and *E. coli* (WP2 *uvrA*) tester strains in accordance with OECD TG 471.[29] Under the experimental conditions applied, NRc-C failed to induce gene mutations by base pair changes or frameshifts in the genome of the strains used at concentrations up to the maximum recommended test concentration of 5000 µg/plate in the presence and absence of metabolic activation.

In vitro Mammalian Chromosomal Aberration Test of NRc-C

In order to evaluate the clastogenic potential of NRc-C, an in vitro mammalian chromosomal aberration assay was conducted using human peripheral blood lymphocytes in accordance with OECD TG 473.[29] NRc-C was not clastogenic up to the maximum recommended concentration for non-cytotoxic compounds (5

mg/mL) with and without metabolic activation in this test system under the applied conditions.

In vivo Mammalian Micronucleus Test of NRc-C

In order to evaluate the in vivo genotoxic potential of NRc-C, a mammalian micronucleus assay was conducted in groups of male and female SD rats in accordance with OECD TG 474.[29] NRc-C was negative for producing micronuclei in this in vivo rat micronucleus test at concentrations up to the limit dose of 2000 mg/kg bw.

Acute Oral Toxicity Study of NRc-C

Oral administration of a single dose of 5000 mg NRc-C/kg bw did not cause mortality or acute toxicity in male or female SD rats in a study conducted in accordance with the Guidance for Industry, Single Dose Acute Toxicity Testing for Pharmaceuticals from the United States Food and Drug Administration.[29] The LD_{50} was concluded as greater than 5000 mg/kg bw.

Fourteen-day Repeated-Dose Oral Toxicity Study of NRc-C

The conduction of the 14-day study was loosely structured according to OECD TG 407 to obtain further information related to the potential oral toxicity of NRc-C in order to provide data for dose selection for a 90-day oral toxicity study.[29] Five SD rats/sex/group were administered NRc-C (formulated in a water vehicle) by gavage at doses of 0 (vehicle-control), 750, 1500, 2500, or 5000 mg/kg bw/day for 14 days. The rats were observed cage side for morbidity, mortality, and clinical signs during the treatment phase. Body weight measurements and detailed clinical examinations were conducted on Days 1, 8, and 15 (body weight was also measured prior to treatment). Food consumption was measured on Days 8 and 15. All animals were sacrificed on Day 15 and subjected to gross pathological examination. No functional observation battery (FOB), clinical pathology, or histopathology examinations were conducted.

Statistically significant decreases in body weight were observed in the 2500 (-7–8%) and 5000 (-8–9%) mg/kg bw/day group males compared to the control group males, and an 8% decrease in food consumption was also observed in the 5000 mg/kg bw/day group. No differences in body weights or food consumption were observed in the female groups. No gross lesions were observed in any groups at necropsy. Due to the approximately 10% reduction in body weight in male rats following oral administration of NRc-C, the MTD was considered <5000 mg/kg bw/day for purposes of dose selection for the 90-day study.

Ninety-day Repeated-Dose Oral Toxicity Study of NRc-C

In order to evaluate the potential health hazards, including identification of toxic effects and target organs, of repeated oral exposure to NRc-C, and to determine a

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NOAEL, a 90-day study was conducted in accordance with OECD TG 408.[29] The study was conducted according to the recommendations of the AAALAC and CPCESA.

Methods: Four groups of 10 SD rats/sex/group were administered NRc-C dissolved in water (vehicle) by gavage at doses of 0, 300, 1000, and 3000 mg/kg bw/day for 90-days. An additional group was administered an equimolar dose (1260 mg/kg bw/day) of Nam by gavage as a positive control at the high dose.

All tests and examinations were conducted according to above stated guideline, except it was not specifically reported whether detailed clinical examinations were conducted, no FOB was conducted, and the hematological evaluation did not include platelets. Urinalysis was added as an optional examination.

Results: No mortality or clinical signs were observed during the study. Statistically significant reductions in body weight and food consumption were observed throughout the study in the high-dose males compared to controls, reaching 17%, and similar decreases were observed in the positive control (Nam) group males. Statistically significant decreases in body weight (<10%) and food consumption were also observed transiently in the low- and mid-dose group males beginning Weeks 3 and 6, respectively. Body weight development was not affected in the females; however, transient reductions in food consumption were observed in all female groups.

Statistically significant dose-related increases in total white blood cell (WBC) and neutrophil counts were observed in both sexes of the high-dose and positive control groups, and monocyte count was also statistically significantly increased in the females of both groups. Additionally, WBCs were statistically significantly increased in the mid-dose males and neutrophils were statistically significantly increased in the mid-dose females. The observed changes were not correlated with any inflammatory changes during the histopathological examinations.

ALT, alkaline phosphatase, and TGs were statistically significantly increased, and chloride was statistically significantly decreased, in both sexes of the high-dose and positive control groups compared to controls while in the females of both groups, gamma-glutamyl transferase was also statistically significantly increased and sodium was statistically significantly decreased. Additionally, AST was statistically significantly increased in the Nam group females. In the mid-dose group females only, ALT and TGs were statistically significantly increased, and sodium was statistically significantly decreased. These changes are shown as percent differences from the negative controls in the table below. Bile acids were also reported to be statistically significantly increased in the high-dose group, but no details (e.g., sex differences, positive control) were reported. No alterations in hematological or

clinical chemistry parameters compared to controls were observed in the low-dose group.

Table 3. Statistically Significant Clinical Chemistry (% change vs control)

	NRc-C		Nam
	1000 mg/kg bw/day	**3000 mg/kg bw/day**	**1260 mg/kg bw/day**
Males			
ALT	NS	87.0%	78.5%
ALP	NS	31.8%	39.7%
TG	NS	157.7%	97.9%
Cl	NS	-4.1%	-4.0%
Females			
ALT	36.4%	117.2%	123.2%
AST	NS	NS	25.0%
ALP	NS	72.9%	88.2%
GGT	NS	59.0%	73.3%
TG	64.5%	117.9%	206.6%
Sodium	-0.7%	-2.4%	-1.4%
Chloride	NS	-3.9%	-3.1%

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; NS, Not Statistically Significant; TG, tryglycerides.

Urine volume was increased in the high-dose and positive control males and was considered treatment-related, possibly in correlation with histopathological lesions of the adrenal glands. Urine pH was decreased in the high-dose males and females, but not in the positive control group.

Small testes were observed bilaterally in the high-dose and positive control males at necropsy and was correlated to degeneration/atrophy observed in these groups during the histopathological examination. A subcutaneous nodule correlated with an adenocarcinoma of the mammary gland was observed in a single high-dose male and was considered an individual finding known to occur spontaneously in SD rats. Multiple statistically significant alterations in absolute and relative to body weight organ weights were observed in both sexes of the high-dose and positive control groups and males of the mid-dose group while in the mid-dose females, only liver weight relative to body weight was statistically significantly increased compared to controls. Among the organ weight alterations, the increases in liver and kidney weights relative to body weight appeared to be dose-related in both sexes while increases in heart and ovary weights relative to body weight appeared dose-related

in females (absolute organ weight data was not presented). In the low dose group, sporadic decreases were observed in the absolute brain and heart weights in males while no absolute alterations were observed in females and no relative alterations were observed in either sex. The organ weight alterations observed in the liver, kidneys, testes, epididymides and ovaries were considered to be treatment-related in the high-dose and positive control groups. The remaining alterations were considered due to random biological variation or secondary to the observed effects on body weight in males.

Centrilobular hepatocellular hypertrophy described as "enlarged hepatocytes containing granular eosinophilic cytoplasm, follicular cell hypertrophy, characterized by enlarged follicular epithelium, which contained pale eosinophilic cytoplasm and small clear vacuoles, and hepatocyte single cell necrosis" was observed in the livers of the high-dose and positive control group animals of both sexes. Chronic progressive neuropathy was also observed in these animals. As mentioned above, in the male rats only of these groups, degeneration/atrophy of the seminiferous tubules was observed. Some tubules also contained degenerating germ cells. In the females of these groups, hypertrophy of corpora lutea was observed in the ovaries. These effects were all considered to be treatment-related adverse effects. Additionally, hypertrophy of the zona glomerulosa of the adrenal cortex was observed in both sexes of the high-dose and positive control group animals but was considered non-adverse, and in the discussion of results, the authors also specifically note that thyroid follicular hypertrophy occurred (no further details provided). All other microscopic findings observed in the various groups were considered incidental due to their random occurrences (data not provided).

Conclusions: Repeated administration by gavage of 0, 300, 1000, and 3000 mg/kg bw/day of NRc-C for 90 days resulted in adverse effects in SD rats in the liver, kidneys, testes, epididymides, and ovaries at 3000 mg/kg bw/day. At 1000 mg/kg bw/day statistically significant increases in liver (both sexes) and kidney (males only) weights and the clinical pathology parameters (females only) of neutrophils, ALT, and TG were also considered adverse. While, due to the presence of dose responses, the authors determined these results adverse in the 1000 mg/kg bw/day group, they also considered these effects mild and potentially adaptive, noting that the increases in ALT and TG were below the typical twofold threshold for biological relevance and that correlating histopathological findings were not observed in kidneys (note, read-down histopathological examinations of the liver, kidneys, thyroids, testes, epididymides (male), ovaries (female), and adrenals were performed due to microscopic findings in these organs at 3000 mg/kg bw/day, and while the authors only specifically stated the absence of findings in the kidneys, due to an absence of reporting of positive findings in the other organs examined at 1000 mg/kg bw/day, their failure to specifically report an absence of microscopic findings in those organs is not presumed to imply that findings were present). While we agree

that the findings at 1000 mg/kg bw/day may be adaptive rather than adverse, a more robust review of the data (not provided in the published article) would be required to make such a determination with confidence. As such we accept the determinations of the authors, that the LOAEL in male and female SD rats was 1000 mg/kg bw/day based on the dose-related increases in liver and kidney weights, neutrophil count, ALT, and TG and the NOAEL was 300 mg/kg bw/day. Additionally, adverse effects similar to those observed in the 3000 mg/kg bw/day NRc-C group were observed in the positive control group that received 1260 mg/kg bw/day of Nam (a dose equimolar to 3000 mg/kg bw/day NRc-C).

Ninety-day Repeated-Dose Oral Toxicity Study of NRc-E

In order to evaluate the potential health hazards, including identification of toxic effects and target organs, of repeated oral exposure to NRc-E, and to determine a NOAEL, a 90-day study with a 28-day recovery period was conducted in accordance with OECD TG 408.[30]

Methods: Ten CD IGS SD (CrL: SD) rats/sex/group were administered NRc-E dissolved in distilled water (vehicle) at concentrations to provide for uniform administration by gavage of a dose volume of 5 mL/kg bw. Four groups were administered doses of 0 (vehicle-control), 300, 500, and 1200 mg/kg bw/day for 90-days. Four satellite groups of five CrL: SD rats/sex/group were administered the same doses for 90-days and then followed for a 28-day untreated recovery period following the final dose administration.

All tests and examinations were conducted according to study protocols and in compliance with above stated guideline, except as noted below; urinalysis was added as an optional examination. As the study was published in 2020, it is unclear from the reporting whether parameters added at the 2018 update of the OECD 408 TG, such as low- and high-density lipoprotein-cholesterol and thyroid hormones and weights, were omitted as a deviation from protocol or because the study was conducted at an early date according to the previous OECD TG 408 update. Additionally, in a deviation from protocol, prostate and seminal vesicles with coagulating glands were not weighted.

Results: No mortality occurred during the study and no test item-related clinical signs (no further details reported) or ophthalmological alterations were observed. A treatment-related decrease in body weight (-13%) was observed in the high-dose male group compared to controls, becoming statistically significant on the final treatment day. The decrease was somewhat recovered in the high-dose male satellite group at the end of the recovery period in that it was no longer statistically significant. The decreased body weights were accompanied by non-significant decreases in body weight gain, food consumption, and feed efficiency. No

differences in body weights, body weight gain, food consumption or feed efficiency were observed in the female animals.

All statistically significant differences in hematological, clinical chemistry, and urinalysis parameters observed in the treatment groups compared to controls remained within their respective historical control ranges and were not correlated to any histopathological findings or clinical signs. Additionally, these changes did not occur with a dose-response, were not observed in the satellite groups, and/or occurred only in the satellite groups. Therefore, the observed changes were not considered to be toxicological relevant.

No statistically significant differences in absolute organ weights were observed in either sex of any group. In the male animals, statistically significant differences in adrenal, brain, and liver weights relative to body weight observed in the high-dose main group were not observed in the recovery group and, likewise, statistically significant differences in heart and testes weights relative to body weight observed in the high-dose recovery group were not observed in the main group, while kidney weights relative to body weight were statistically significantly increased in both the main and recovery high-dose male groups. No correlating histopathology, clinical pathology, or absolute organ weight changes were observed; therefore, these were attributed to the decreased body weight in the high-dose males. A statistically significant decrease observed in the heart-to-brain weight ratio in the low-dose male main group was not considered toxicologically relevant due to the lack of dose response and correlating histopathology. In the females, a statistically significant dose-related increase in the liver-to-body weight ratio observed in the high-dose main group was not considered to be toxicologically relevant due to the lack of correlating clinical pathology and histopathology.

No test item-related macroscopic or microscopic findings were observed during necropsy or the histopathological examination, respectively (no further details reported).

Conclusions: Following repeated administration by gavage of 0, 300, 500, and 1200 mg/kg bw/day of NRc-E for 90 days the NOAEL in male CrL: SD rats was determined to be 500 mg/kg bw/day on the basis of reduced body weight development in the 1200 mg/kg bw/day group. In females, the NOAEL was determined to be 1200 mg/kg bw/day; the highest dose tested.

In addition to the preclinical studies reported above, a dozen human studies investigating effects of NR were located and are summarized in the table below with respect to safety (studies in which NR was administered in combination with other substances were not evaluated). Overall, NR appears to be well tolerated in humans when administered at daily dosages up to 2000 mg for up to 12 weeks.

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Table 4. Safety Summary of NR Clinical Trials

Author, Date	Test Item	Daily Dosage	Duration	Subjects	Design	Key Findings
Trammell et al. (2016)[31]	NRc-C	100, 300, or 1000 mg	Single dose x 3 occasions	12 healthy♂ & ♀adults	RCT	No serious or dose-related AEs reported. 2 subjects reported flushing at mid dose. 2 subjects reported feeling hot at high dose.
Airhart et al. (2017)[32]	NRc-C	250, 500, 1000, or 2000 mg Escalating dose design	8-days	80 healthy♂ & ♀adults	OL	No AEs attributable to test item. No CS effects on VS or CP.
Martens et al. (2018)[33]	NRc-C	1000 mg	6 weeks	30 older ♂& ♀adults with IVEF	RCT-X	No serious AEs. 14 total AEs in 7 total subjects: Tx phase (nausea, flushing, leg cramps, increase bruising); P phase (headache, skin rash, flushing, fainting, drowsiness). 2 dropouts due to AE during P condition. No SS effects on VS or glucose regulation. No CS effects on CP.
Dollerup et al. (2018*, 2019, & 2022))[34, 57, 58]	NRc-C	1000 or 2000 mg	12 weeks	40 OSOH middle-aged to older ♂	RCT	No serious AEs. 6 minor AEs—4 in Tx (pruritus, sweating, bloating, transient stool change), 2 in P (reflux, periodic loose stool). No abnormal CP in Tx group.
Conze et al. (2019)[35]	NRc-C	100, 300, or 1000 mg	8 weeks	140 OWOH ♂& ♀adults	RCT	No serious or dose-related AEs reported. No flushing reactions. No CS effects on VS or CP.
Elhassan et al. (2019)[36]	NRc-C	1000 mg	21 days	12 elderly ♂	RCT-X	Well tolerated with no AEs reported. No adverse effects on CP.
Dolopikou et al. (2020)[37]	NR-NOS	500 mg	Single dose	12 young & 12 old healthy ♂	RCT-X	No mention of AEs Subjects were instructed to "immediately report" any serious AEs.
Zhou et al. (2020)[38]	NRc-C	500–2000 mg Escalating dose design	5–9 days	5 HHF	Not reported	No Tx-related AEs observed. 1 subject withdrawn for change unrelated to Tx

(b) (4)

Author, Date	Test Item	Daily Dosage	Duration	Subjects	Design	Key Findings
Remie et al. (2020)[39]	NRc-C	1000 mg	6 weeks	13 OWOH ♂& ♀adults	RCT-X	Well tolerated with no AEs reported. No SS or CS effects on CP.
Brakedal et al. (2022)[40]	NRc-C	1000 mg	30 days	30 PD-N	RCT	Well tolerated; no AEs attributable to Tx. 98% compliance. Minor AEs recorded: 7 Tx phase subjects experienced 12 AEs; 3 P subjects experienced 3 AEs No adverse effects on VS, CP, or CSx.

Abbreviations: ♀, female; ♂, male; AE, adverse event; CP, clinical pathology (e.g., hematology, clinical chemistry); CS, clinically significant; CSx, clinical symptoms; HHF, hospitalized with stage D heart failure; IVEF, impaired vascular endothelial function; NOS, not otherwise specified as to form; OL, open-label non-randomized clinical trial; OSOH, obese, sedentary but otherwise healthy; OWOH, overweight but otherwise healthy; P, placebo; PD-N, newly diagnosed with Parkinson Disease, treatment naïve subjects; RCT, randomized double-blinded placebo-controlled clinical trial (-X, crossover design); SS, statistically significant differences; Tx, treatment; VS, vital signs.
*Safety outcomes from the Dollerup et al. study were reported in the 2018 publication.

While NOAELs in the preclinical toxicological studies on NRc were lower than NOEALs observed in the preclinical toxicological studies on NMN, the two substances are slightly different (and the NR studies were performed on a salt form of the substance), and in considering the safety of NMN, a higher weight of relevance should be considered with respect to studies performed using NMN itself, especially with respect to studies performed on the notifier's article of commerce. Additionally, the clinical trials utilizing NR, as well as ULs for Nam established by authoritative scientific bodies (if dismissing the flushing effect attributed to NA only) for Nam are corroborative of the safety of NR at higher doses than extrapolation from preclinical NOAELs would suggest and of the results of clinical trials and preclinical studies using NMN.

III.C.6.ii Current Regulatory Status

A thorough search for the current regulatory status of NMN, relevant to its use in food and/or dietary supplements in the United States, was conducted. A summary of pertinent search results is shown below:

- Inner Mongolia Kingdomway Pharmaceutical Limited's NMN is GRAS for use in a protein powder at addition levels up to 32.5 mg per 26 g serving. The mean and 90th percentile estimated dietary exposures to NMN from this use for the U.S. population aged 2 years and older are 40.1 mg/person (p)/day

(0.51 mg/kg body weight (bw)/day) and 75.1 mg/p/day (0.95 mg/kg bw/day), respectively.

- An NDI notification (report number 1240) for use of an NMN compound as bulk dietary ingredient at levels up to 300 mg/day by adults was filed by FDA on December 27, 2021 (with additional information submitted on February 1 and 14, 2022) and received an objection letter from the agency on the basis that the notification failed to adequately establish that a dietary supplement containing the dietary ingredient would be reasonably expected to be safe under its recommended conditions of use.
- An NDI notification (report number 1234) for use of an NMN compound as bulk dietary ingredient at levels up to 300 mg/day by adults was filed by FDA on November 15, 2021 and received an objection letter from the agency on the basis that the notification failed to adequately establish that a dietary supplement containing the dietary ingredient would be reasonably expected to be safe under its recommended conditions of use.
- Two NDI notifications (report numbers 1174 and 1189) for use of an NMN compound as dietary ingredient in the dietary supplement "Reju-Me" at levels up to 500 mg/day by adults were filed by FDA on August 25, 2020 and December 3, 2020. NDI notification report number 1174 was not evaluated for an adequate basis of safety due to a regulatory issue (due to the recommended use by the sublingual route, the product containing the dietary ingredient did not meet the definition of a dietary supplement) with the notification. NDI notification report number 1189 received an objection letter from the agency on the basis that the notification failed to adequately establish a reasonable expectation of safety of the dietary supplement containing the dietary ingredient under its recommended conditions of use.

III.C.6.iii Non-pathogenicity and Non-toxigenicity

The production organism is the non-pathogenic and non-toxicogenic yeast, *S. cerevisiae* (commonly known as baker's yeast or brewer's yeast), which is widely used in food production, including baking, brewing beer, and winemaking. *S. cerevisiae* is a multipurpose approved food additive for direct addition to human foods pursuant to 21 CFR 172.896.

III.C.6.iv Allergenicity

NMN does not contain or have added, and is manufactured in a facility free of, all nine major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, soybeans, and sesame) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally,

NMN does not contain gluten, oats, celery, mustard, sesame seeds, sulfur dioxide and sulfites or any derivatives or products of the aforementioned.

No reports of allergic reactions to NMN were found in our investigations.

III.C.6.v Reported Adverse Events

Since the 2020 market introduction of NMN by Inner Mongolia Kingdomway Pharmaceutical Limited, the company has not received any reports of adverse events or customer complaints associated with the ingredient.

FDA databases that may contain information pertaining to safety concerns or adverse events (AE) were accessed and searched on July 14, 2022. No FDA letters regarding a safety concern to companies that market products containing NMN were located. Searches of FDA's Recalls, Market Withdrawals, & Safety Alerts search engine and FDA archives did not find any mention of safety concerns for NMN products. Two AE reports (AERs) related to NMN products were located in FDA's Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS). Both AEs were reported for the same "Ultra NMN" product and were logged in the CAERS on consecutive days (although the actual date of the AE was reported for only one of the two). The first logged AER reported NMN as suspect in a 67-year-old individual, and a serious event outcome (i.e., disability) was reported related to the following signs and symptoms:

- "Abdominal pain, Abdominal tenderness, Diarrhoea, Headache, Nausea, Vomiting projectile",

The second logged AER reported NMN as suspect in a female of unreported age, and a non-serious outcome was reported related to the following signs and symptoms:

- "Abdominal pain lower, Abdominal pain upper, Abdominal tenderness".

It is possible that the two AERs represent duplicate reporting of the same AE. Additionally, based on the absence of FDA issued warning letters, product recalls, or other information, it does not appear that FDA considered these events as a sufficient post-market signal to cause concern about the product.

CAERS currently contains records of 176,563 AER records submitted to FDA from January 2004 through December 31, 2021 (the date of the last data set release). Thus, the frequency of occurrence of AERs related to NMN within the CAERS data set is <0.002%. Additionally, CFSAN notes the following:

> "*The adverse event reports about a product and the total number of adverse event reports for that product in CAERS only reflect information AS REPORTED and do not represent any conclusion by FDA about whether the*

product actually caused the adverse events. For any given report, there is no certainty that a suspected product caused a reaction. Healthcare practitioners, firms, agencies, consumers, and others are encouraged to report suspected reactions; however, the event may have been related to a concurrent underlying condition or activity or to co-consumption of another product, or it may have simply occurred by chance at that time."

It is also noted that AERs vary in quality and reliability and CAERS may contain duplicate reports.

III.C.7 Safety and Toxicology References

Reprints or photocopies of the full text of all published and unpublished references cited in section III.C are contained in Appendix G (Safety and Toxicology References) of this notification.

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