Nicotinamide Mononucleotide (NMN)

Prepared for

SyncoZymes (Shanghai) Co., Ltd. (SyncoZymes)
The Manufacturer of NMN
Contact person: Wei Zhu, Ph.D.
Chief Executive Officer
No. 1199 Landian Road, Pudong New District, Shanghai, 201318,
P.R. CHINA
Tel: +86-21-68187180; Fax: +86-21-68187179
Email: zhuw@syncozymes.com

and

(b) (4)

Prepared by

(b) (4)

TABLE OF CONTENTS

Figures

SECTION 4. SAFETY INFORMATION

Pursuant to 21 United States Code (U.S.C.) §350b(a)(2) [section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)], on June 29, 2020, we submit a New Dietary Ingredient (NDI) notification for beta-nicotinamide mononucleotide (β-NMN) or NMN.

Part 1. NDI Identity Information

1.1. Identity of the NDI

Common name: nicotinamide mononucleotide (NMN), beta-nicotinamide mononucleotide, or β-NMN.

Other names: beta-nicotinamide ribose monophosphate; nicotinamide ribonucleoside 5'-phosphate, nicotinamide nucleotide, and nicotinamide D-ribonucleotide.

International Union of Pure and Applied Chemistry (IUPAC) name: [(2R,3S,4R,5R)-5-(3-carbamoylpyridin-1-ium-1-yl)-3,4-dihydroxyoxolan-2-yl]methyl dihydrogen phosphate or 3-carbamoyl-1-[5-*O*-(hydroxyphosphinato)-β-D-ribofuranosyl]pyridinium.

Chemical Abstracts Service (CAS) number: 1094-61-7.

Molecular Formula: $C_{11}H_{15}N_2O_8P$.

Molecular weight: 334.22.

Chemical structure:

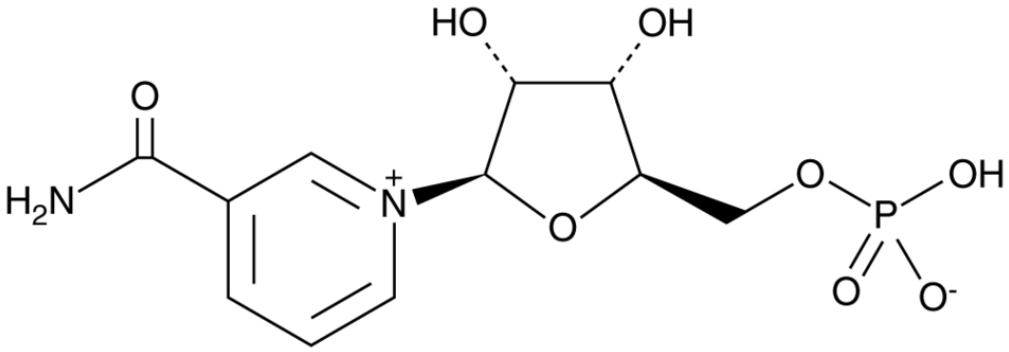


Figure 1. Chemical Structure of NMN

The terms, NMN and β-NMN, are interchangeably used in the literature

CONFIDENTIAL

NMN exists in two anomeric forms namely alpha and beta (Poddar et al., 2019). The beta anomer is the active form between these two with a molecular weight of 334.221 g/mol. Unless noted otherwise, NMN refers to β-NMN. Alpha form of NMN is made through a chemical

synthesis (Shen et al., 2021). However, NMN made via biological manufacturing process (either by fermentation or enzymatic synthesis) is stereospecific to a β form (Shen et al., 2021).

NMN and NR Share the Same Metabolic Pathway

CONFIDENTIAL

NMN is a bioactive nucleotide, which is naturally formed by the reaction between a nucleoside containing ribose and nicotinamide (NAM) and a phosphate group. It generally exists in two anomeric forms: alpha and beta. Between the two, the beta anomer is the active form (Poddar et al., 2019).

NAM and nicotinamide ribose (NR) are converted to NMN, which is the precursor of nicotinamide adenine dinucleotide (NAD^+), a co-substrate of NAD^+-dependent enzymes essential for biological processes, such as redox homeostasis, gene expression, ribonucleic acid (RNA) processing, genomic stability, immunity and inflammation, and energy metabolism (Roos et al., 2021). NMN is the main source of the salvage pathway to generate NAD^+.

NMN serves as an intermediate in the metabolism of NAM to NAD. NR is a precursor of NMN. However, NMN is metabolized extracellularly to NR that is then taken up by the cell and converted back to NMN before being metabolized into NAD^+(Poddar et al., 2019; Figure 4). Thus, it is concluded that NMN and NR have the overlapping metabolic effects.

Thus, the pharmacokinetics and safety studies of NR were briefly discussed in this NDI notice in addition to that of NMN.

Justification of NMN as an NDI

CONFIDENTIAL

The proposed NDI, like nicotinamide ribose (NR) which has established an NDI status (NDIN 1062), is a metabolite of nicotinamide which is a component of vitamin B3 (or niacin). Vitamin B3 is composed of nicotinamide and nicotinic acid. As shown in Figures 6 and 7 of the original submission, (b) (4) . Thus, it fits the definition of a dietary ingredient under section 201(ff)(1)(F) of the FD&C Act.

References for Part 1.1.

Poddar SK, Sifat AE, Haque S, Nahid NA, Chowdhury S, Mehedi I. Nicotinamide mononucleotide: Exploration of diverse therapeutic applications of a potential molecule. Biomolecules. 2019;9.

Roos J, Zinngrebe J, Fischer-Posovszky P. Nicotinamide mononucleotide: A potential effective natural compound against insulin resistance. Signal Transduct Target Ther. 2021;6:310.

Shen Q, Zhang SJ, Xue YZ, Peng F, Cheng DY, Xue YP, Zheng YG. Biological synthesis of nicotinamide mononucleotide. Biotechnol Lett. 2021;43(12):2199-2208.

1.2. Description of the Evidence Verifying the Identity of the NDI

CONFIDENTIAL

The NMN has been identified by (b) (4)). Details are presented in Appendix A.

The NMN which is manufactured (b) (4) .

(b) (4) . Details are described in Appendix A.

1.3. NDI Manufacture

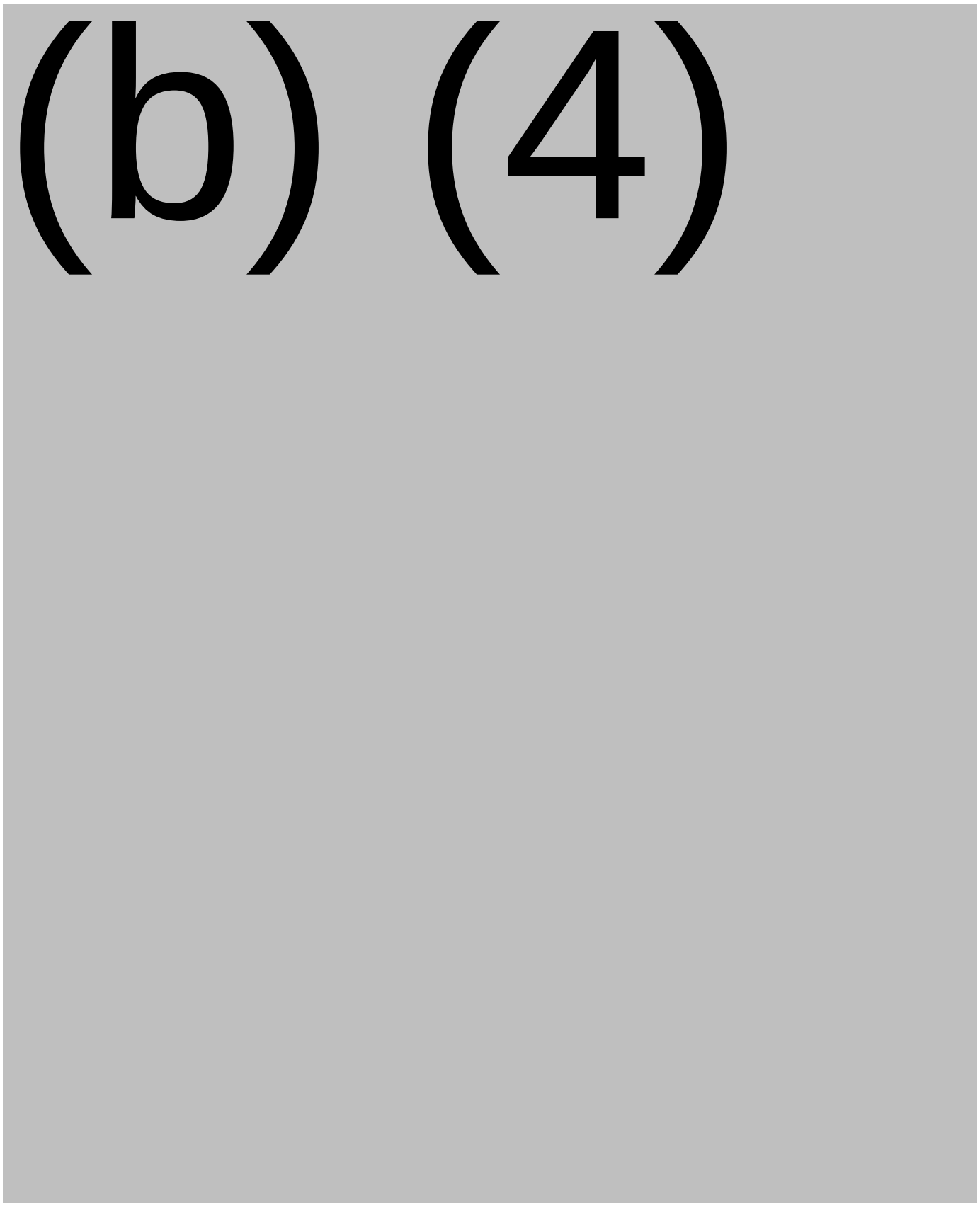
The Entire 1.3. is CONFIDENTIAL.

1.3.1. Raw Materials

CONFIDENTIAL

(b) (4)

(b) (4)


(b) (4)

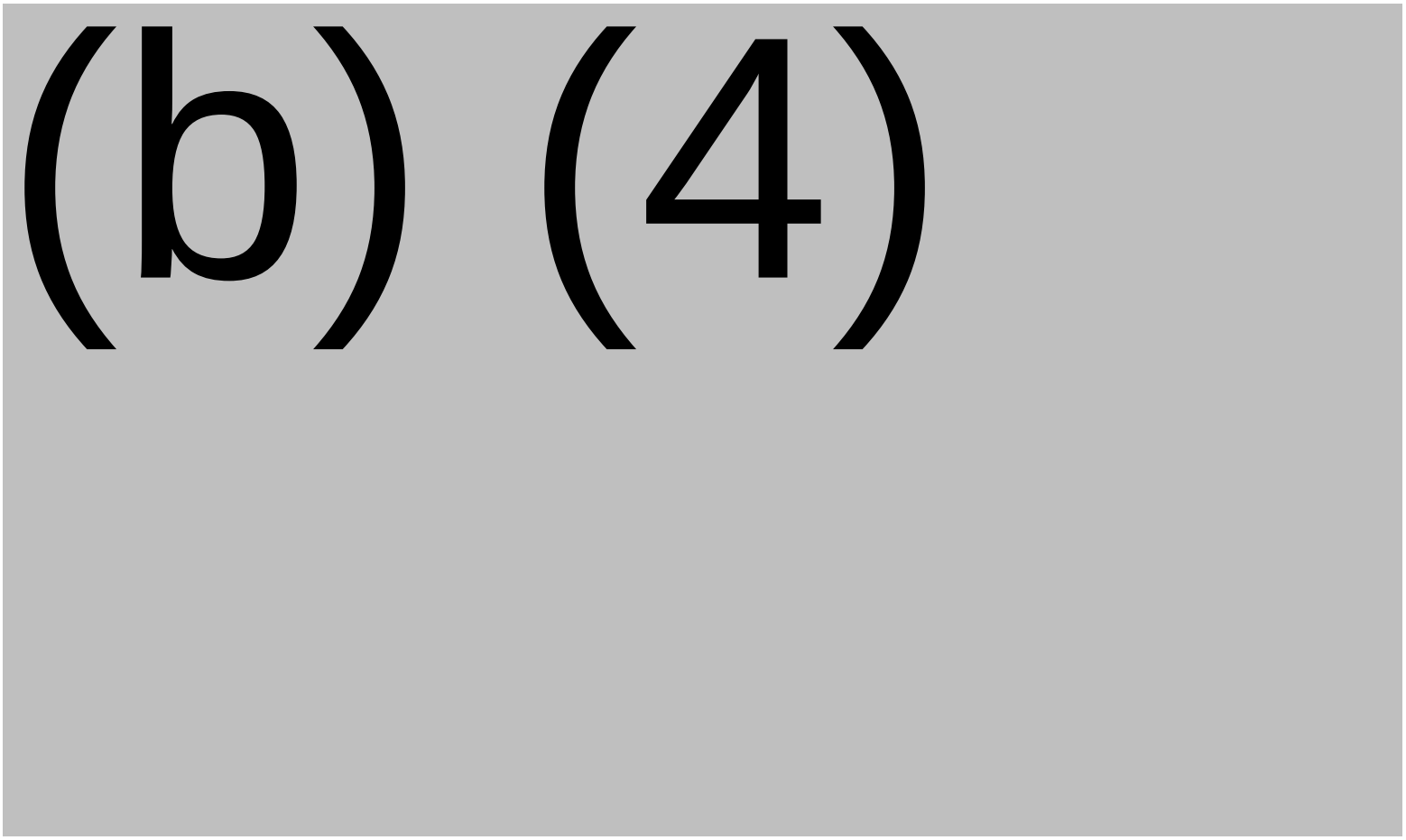
(b) (4)

Enzymatic Reactions Involved with Each Enzyme

CONFIDENTIAL

(b) (4)

is shown in Figure 2.



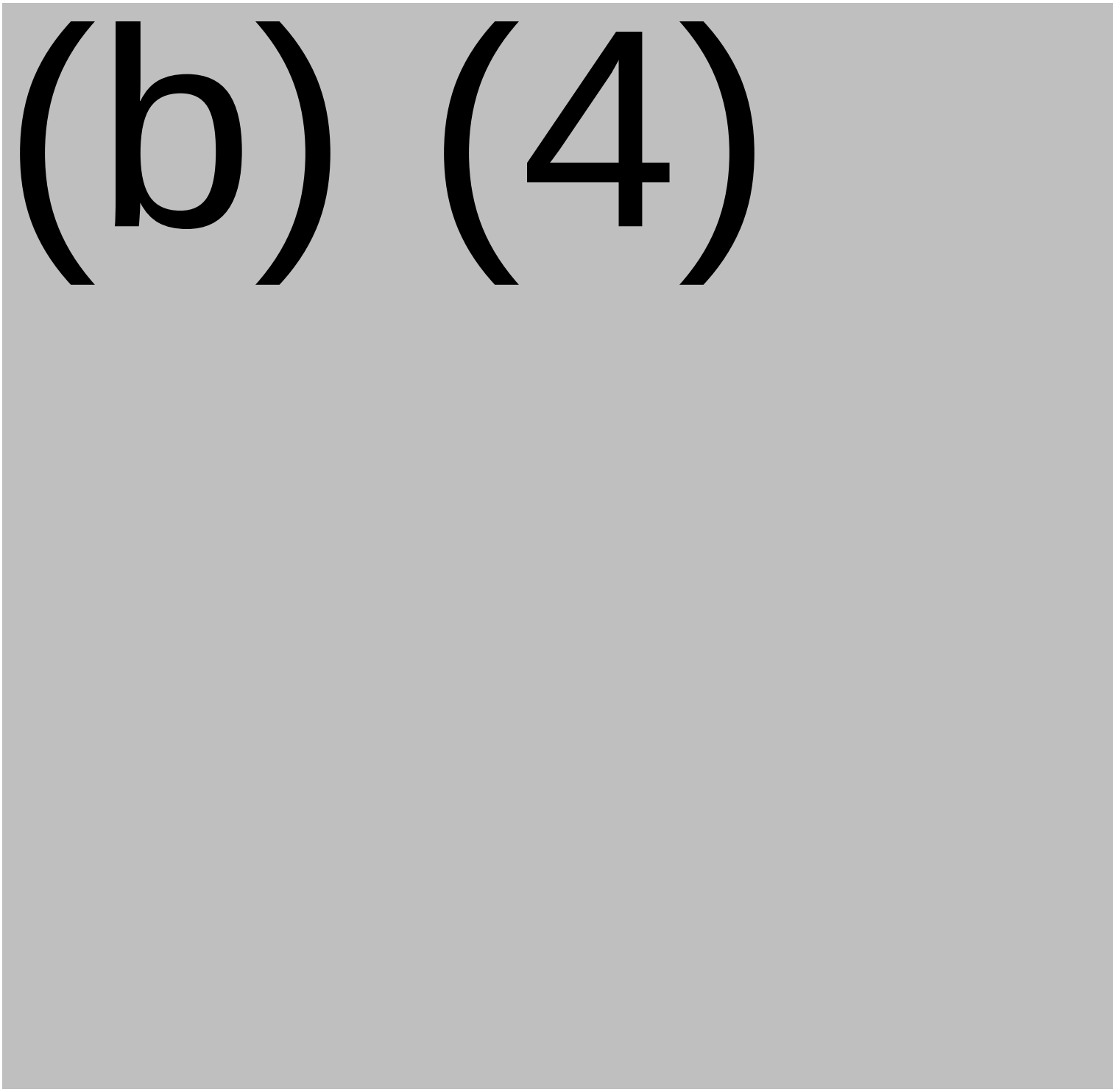
1.3.2. Manufacturing Process of NMN

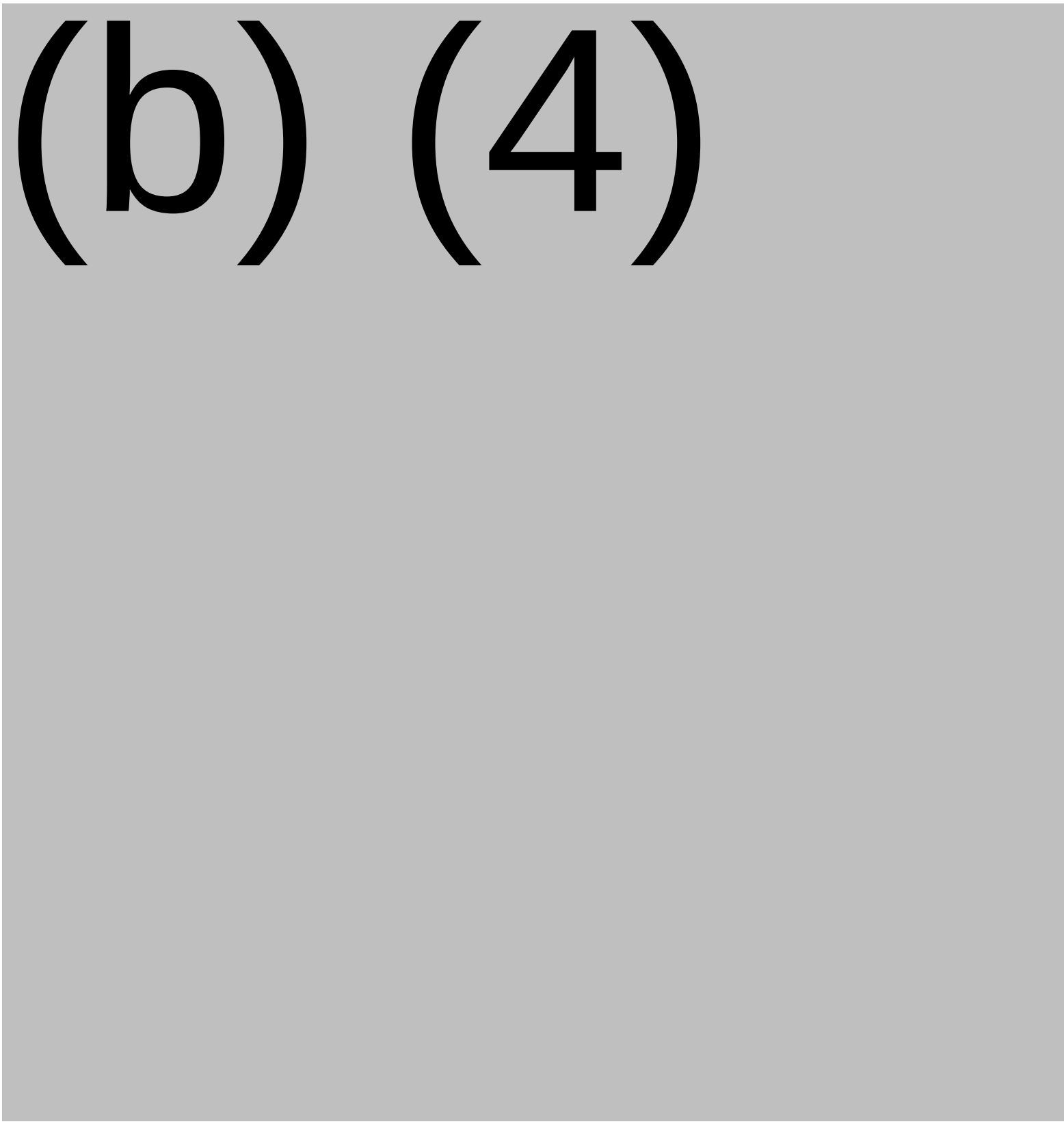
CONFIDENTIAL

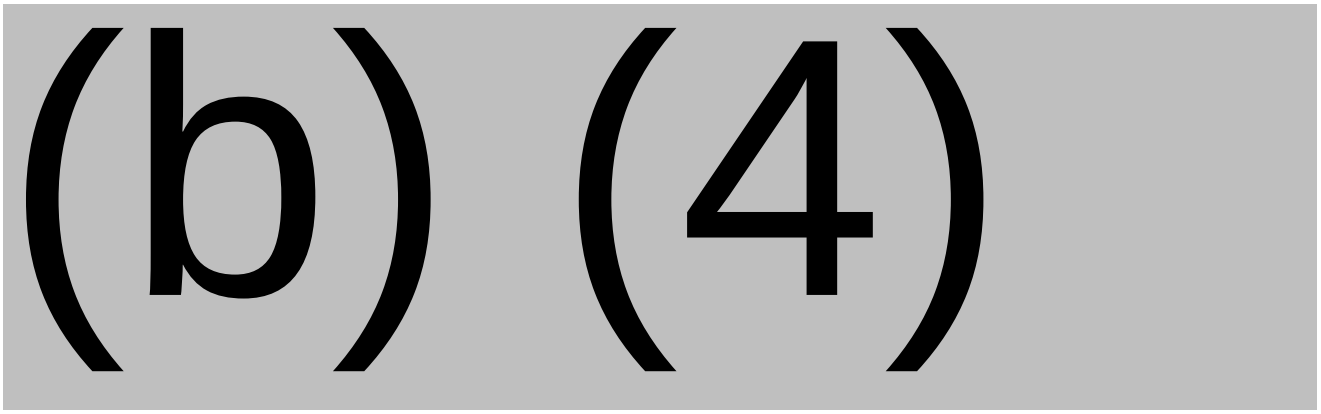
The main production process of NMN is composed of (b) (4)

(b) (4)

.

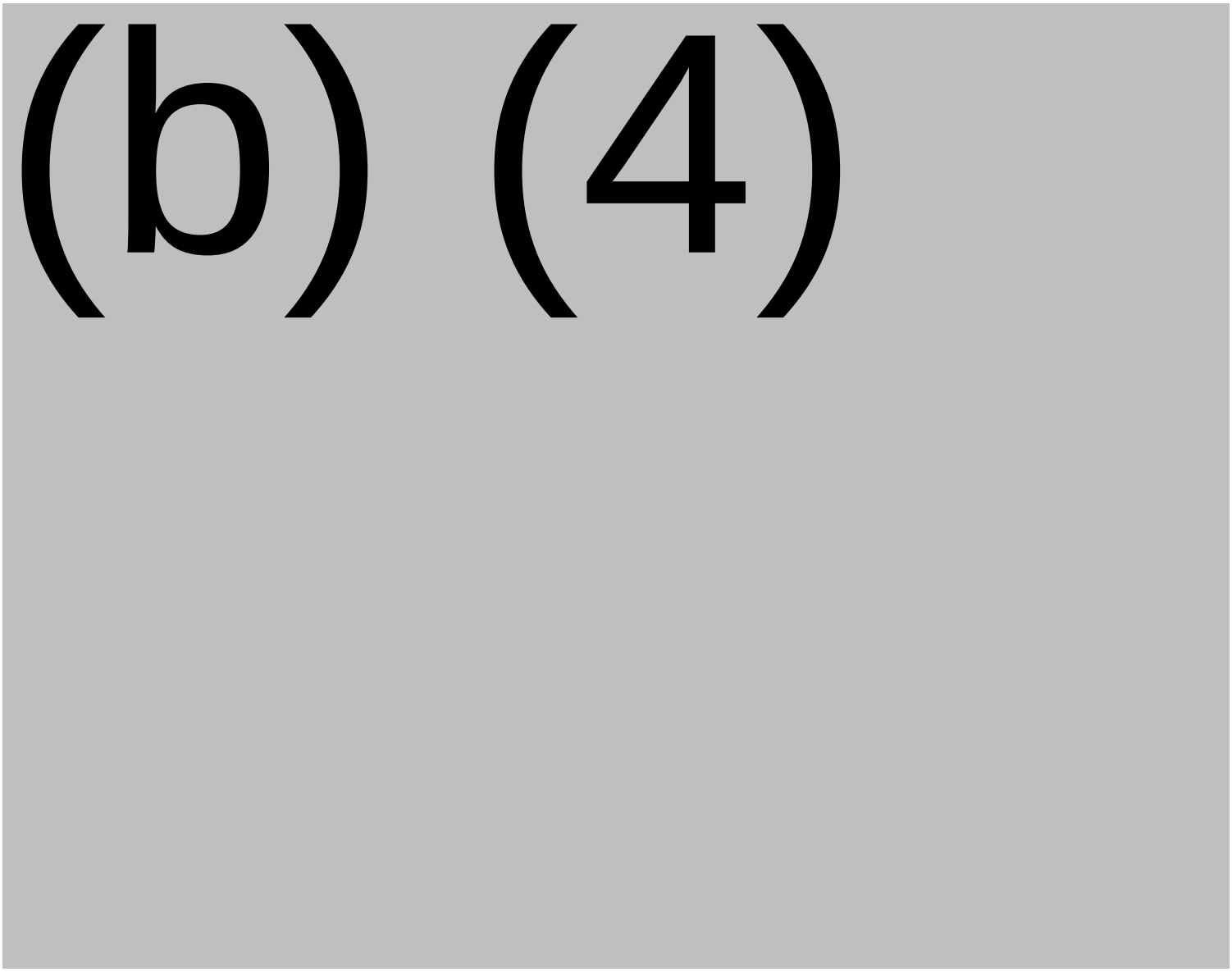

(b) (4)

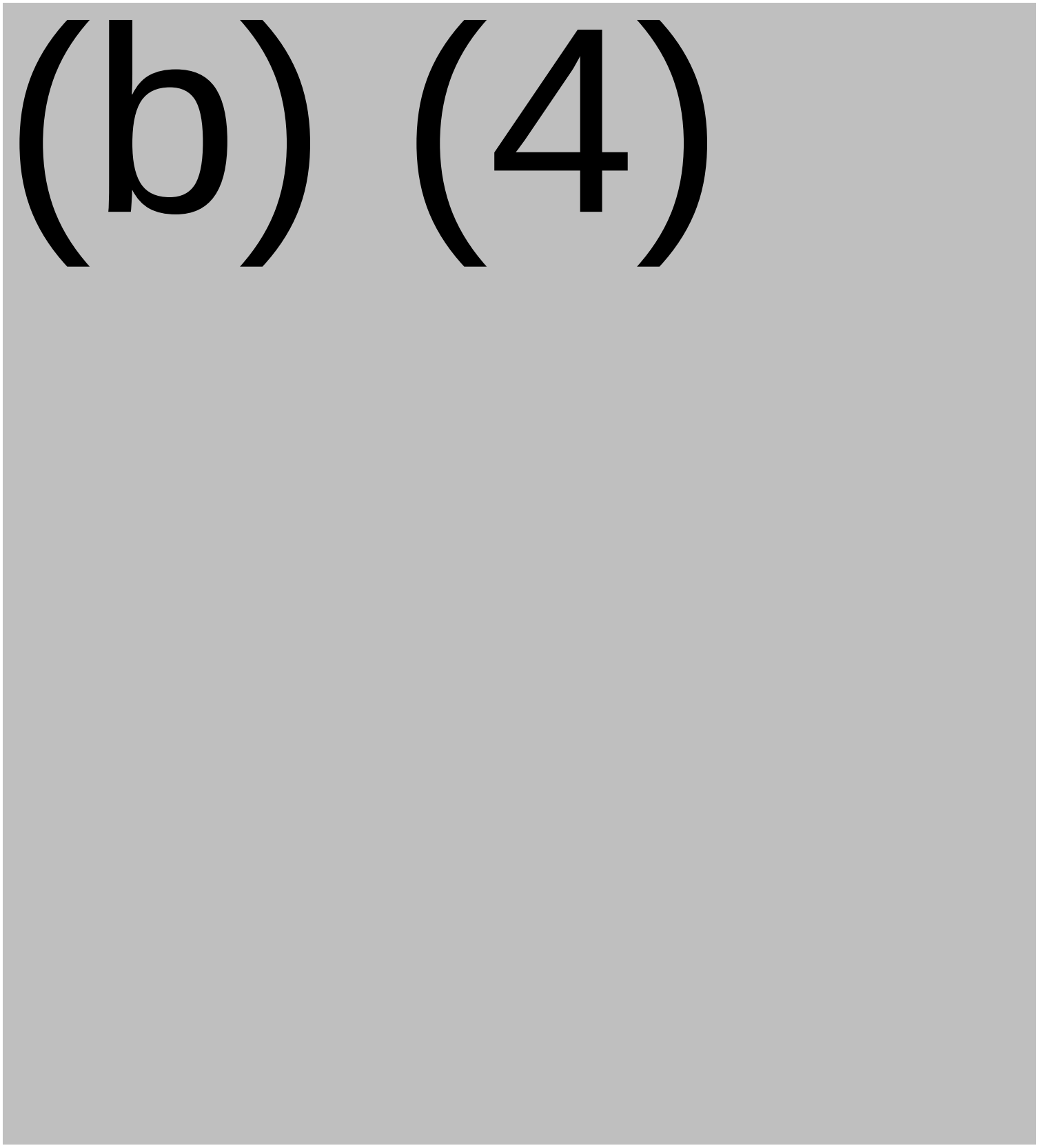

(b) (4)

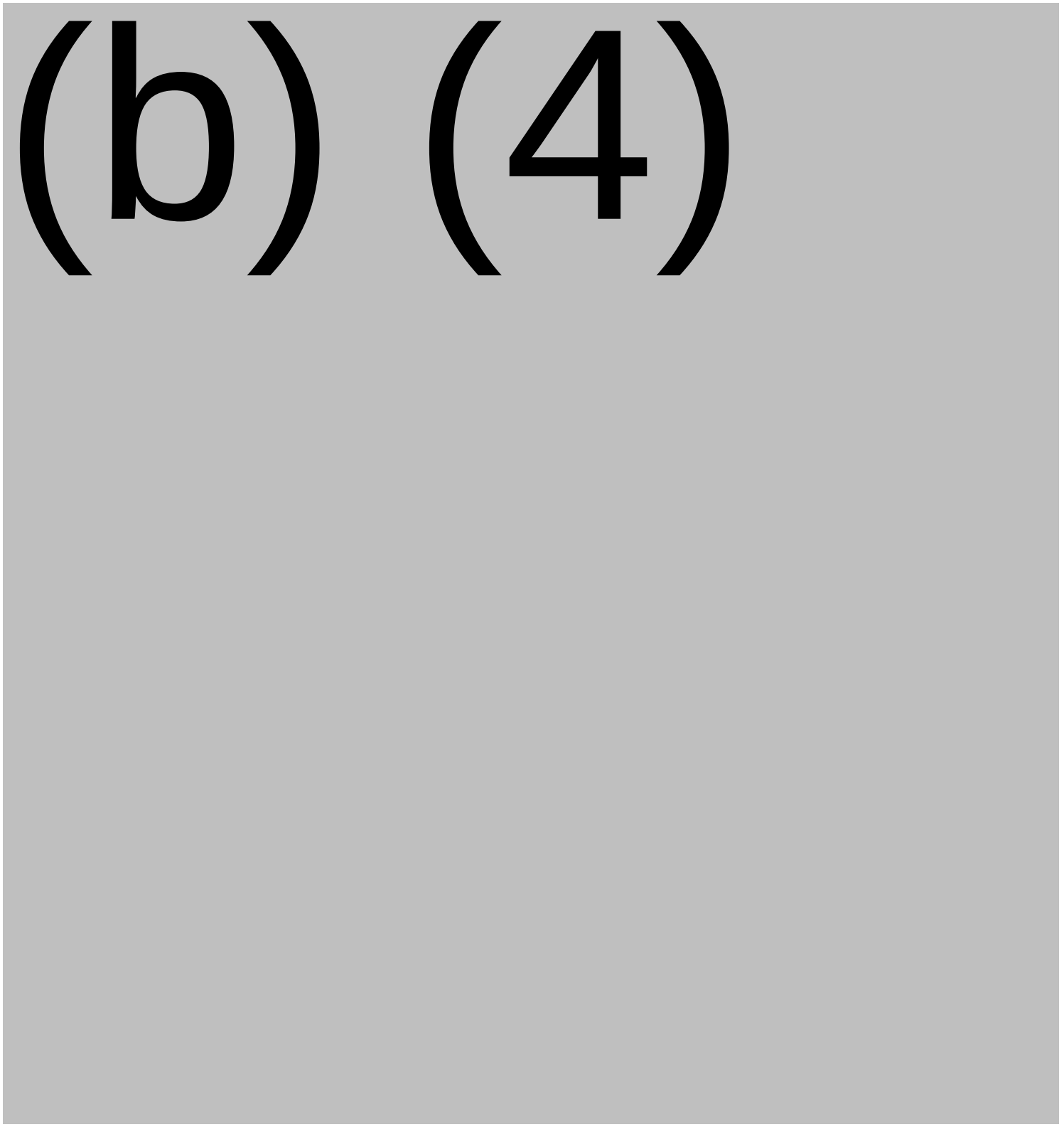


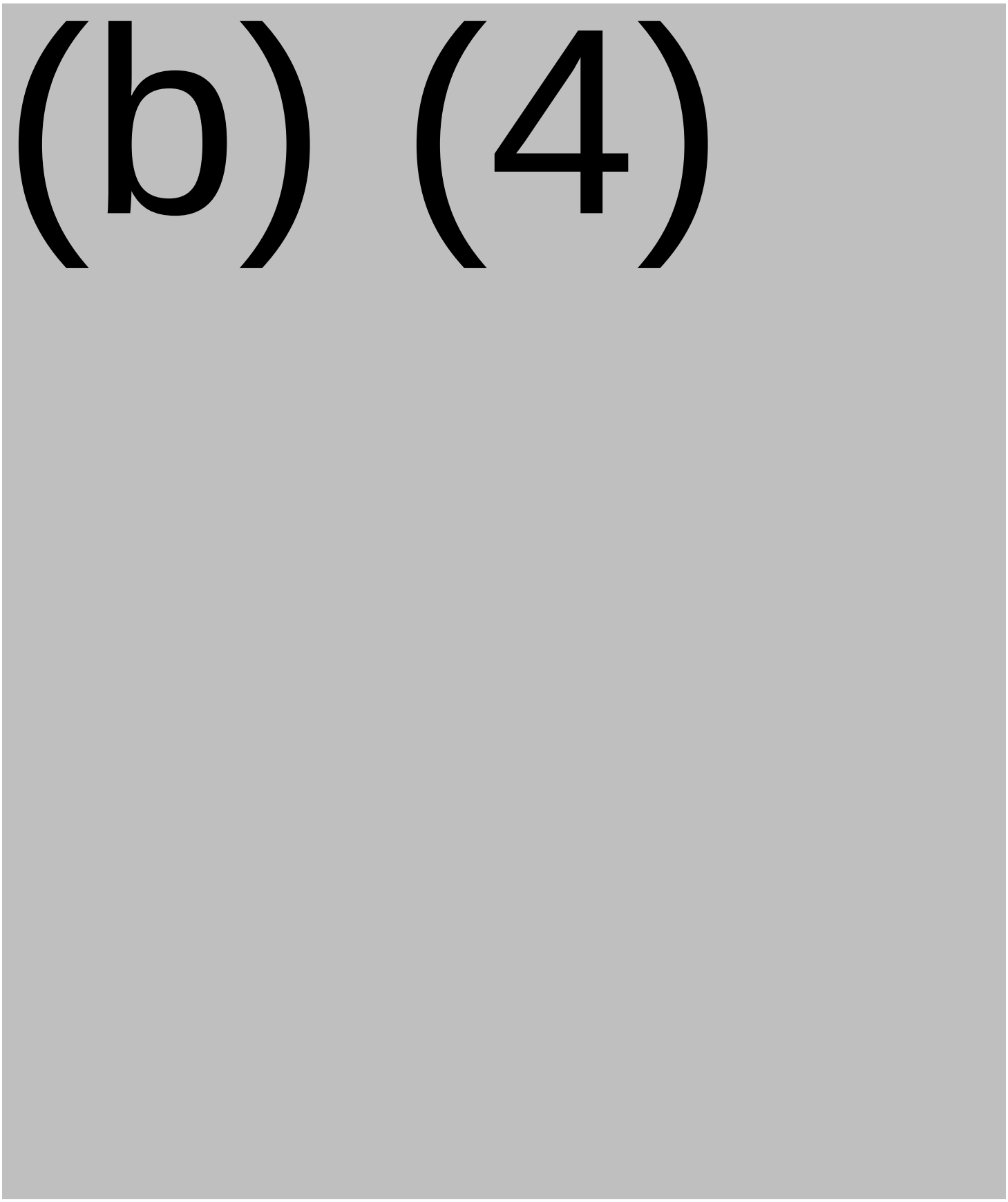
3. **Introduced genes for NMN biosynthesis**

CONFIDENTIAL



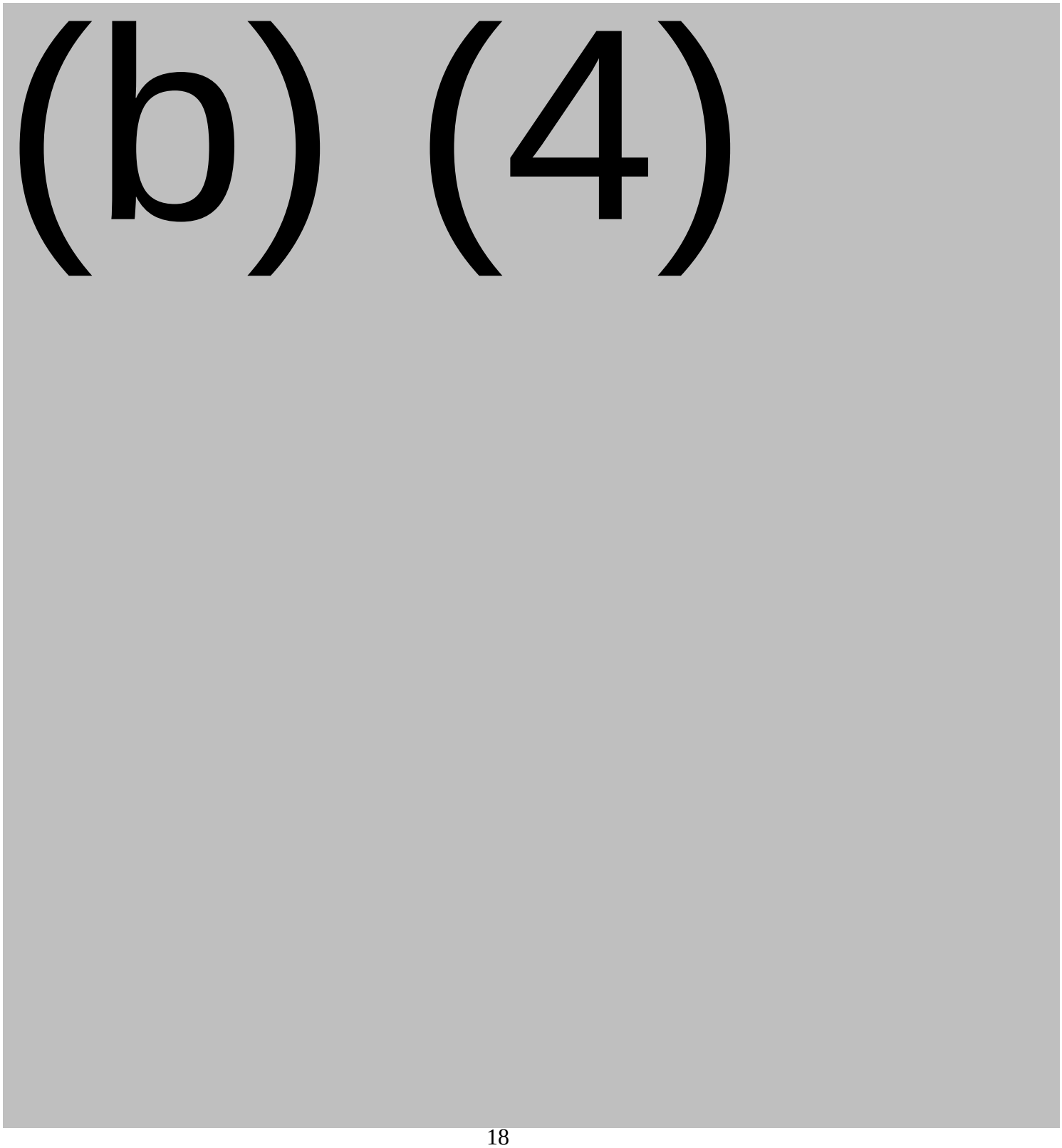

(b) (4)

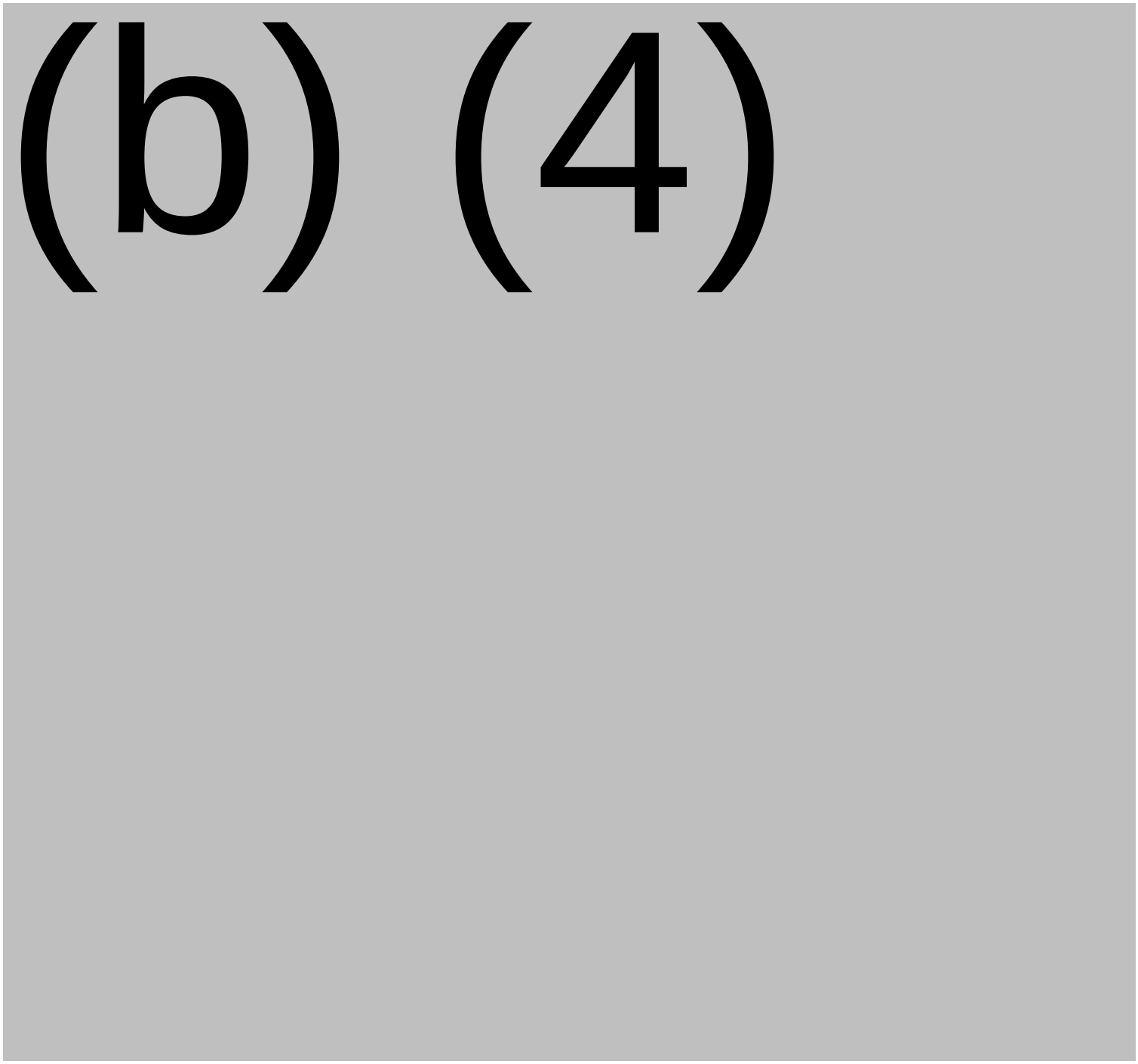

(b) (4)

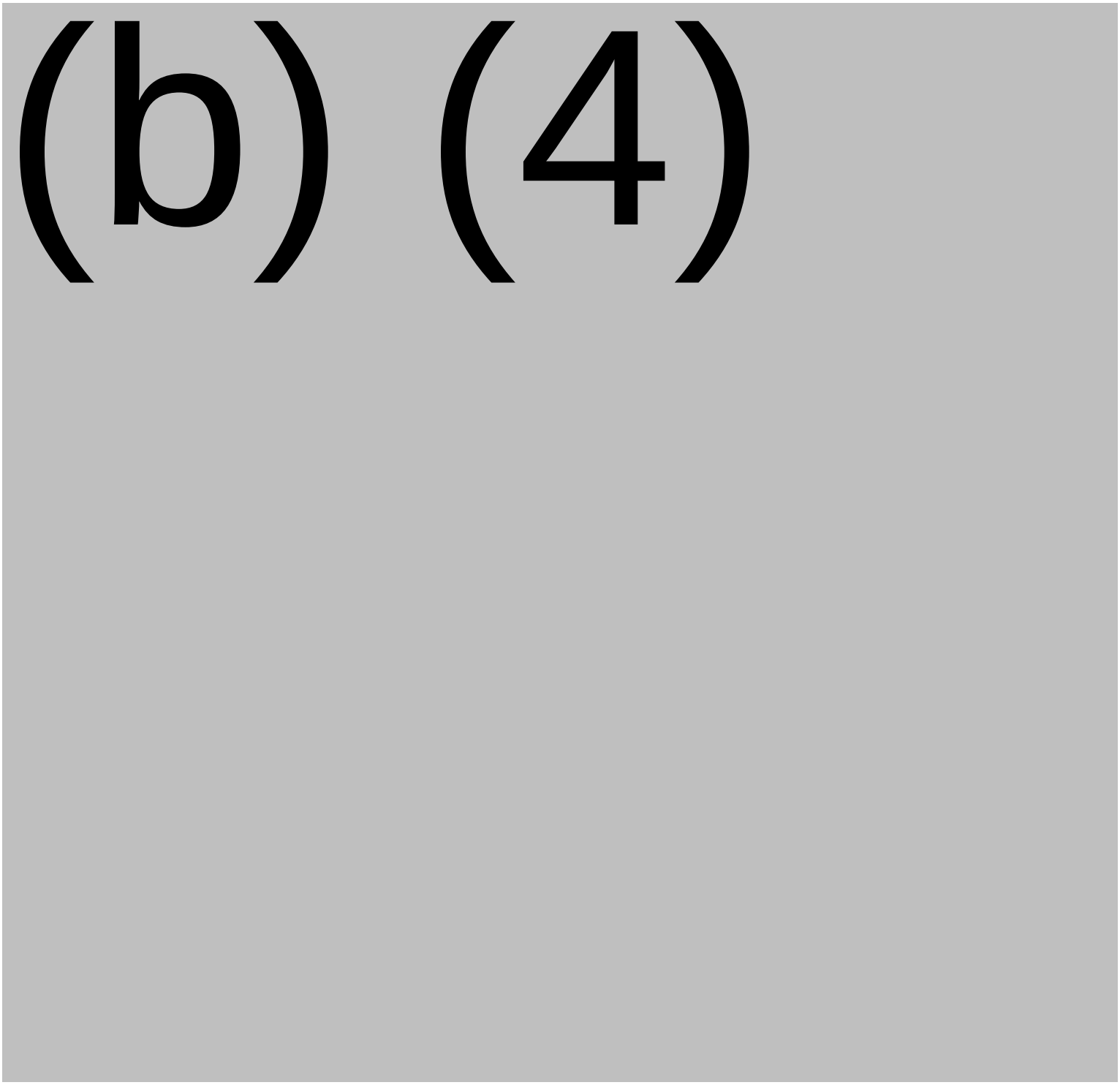

(b) (4)

1.3.3. NDI Specifications

CONFIDENTIAL

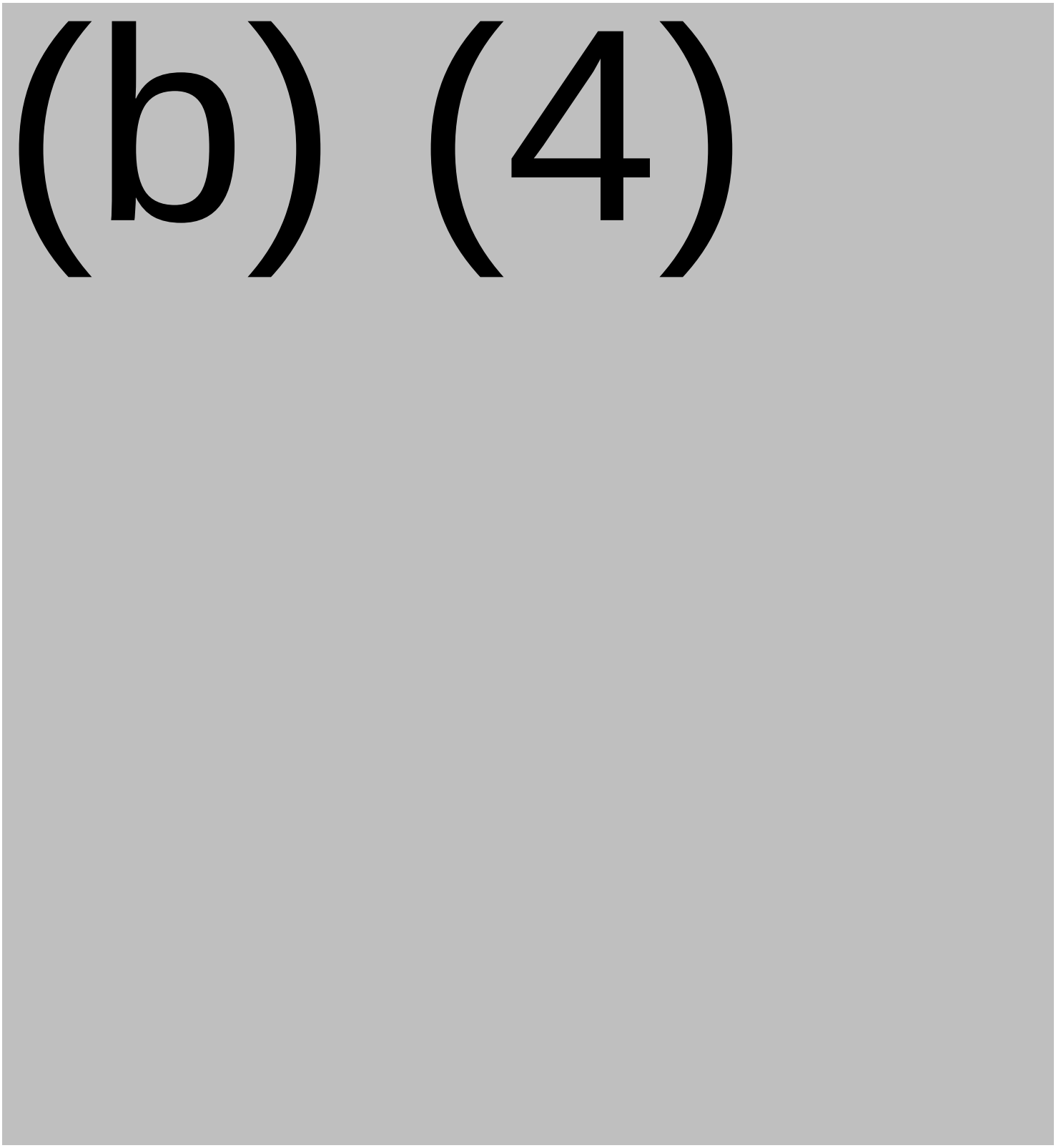



(b) (4)


(b) (4)

1.3.4. Stability of NMN

CONFIDENTIAL




(b) (4)

Part 2. Dietary Supplement Manufacture

Not applicable.

Part 3. History of Use or Other Evidence of Safety

3.1. History of Use

3.1.1. Description of the Relationship between the Historically Consumed Material and the NDI or Dietary Supplement Containing the NDI

NMN is prevalent in many foods that are safely consumed by humans as part of a normal diet. NMN comes from a variety of food sources (Table 9; Mills et al., 2016). Vegetables, such as broccoli and cabbage, contain 0.25-1.12 and 0.0-0.90 mg NMN/100 g, respectively, and fruits like avocado and tomato contain 0.36-1.60 and 0.26-0.30 mg NMN/100 g, respectively. Raw beef has 0.06-0.42 mg NMN/100 g (Mills et al., 2016). In addition, GRN 635 (FDA, 2016) describes the content of NAM-related substance in organic and commercial bovine milk (Table 10).

Table 9. Food Sources of NMN

Food	mg/100 g food
Edamame	0.47 – 1.88
Broccoli	0.25 – 1.12
Cucumber seed	0.56
Cucumber peel	0.65
Cabbage	0.0 – 0.90
Avocado	0.36 – 1.60
Tomato	0.26 – 0.30
Mushroom	0.0 – 1.01
Beef (raw)	0.06 – 0.42
Shrimp	0.22

Adopted from Mills et al. (2016).

Table 10. Levels of NR and Related Metabolites in Fat-Free Bovine Milk

Metabolite, µM	Organic milk (n=4)	Commercial milk (n = 4)
Nicotinamide	5.6 ± 1.2	5.2 ± 1.7
Nicotinamide riboside	1.9 ± 0.49	3.1 ± 0.80
Nicotinic acid riboside	<0.4	<0.4
Nicotinic acid	<1.0	< 1.0
Nicotinamide mononucleotide	<0.4	<0.4
NADH	<0.08	<0.08
NAD	<0.08	<0.08
NaMN	<0.03	<0.03
NADP	<0.02	<0.02
NAAD	<0.008	<0.008

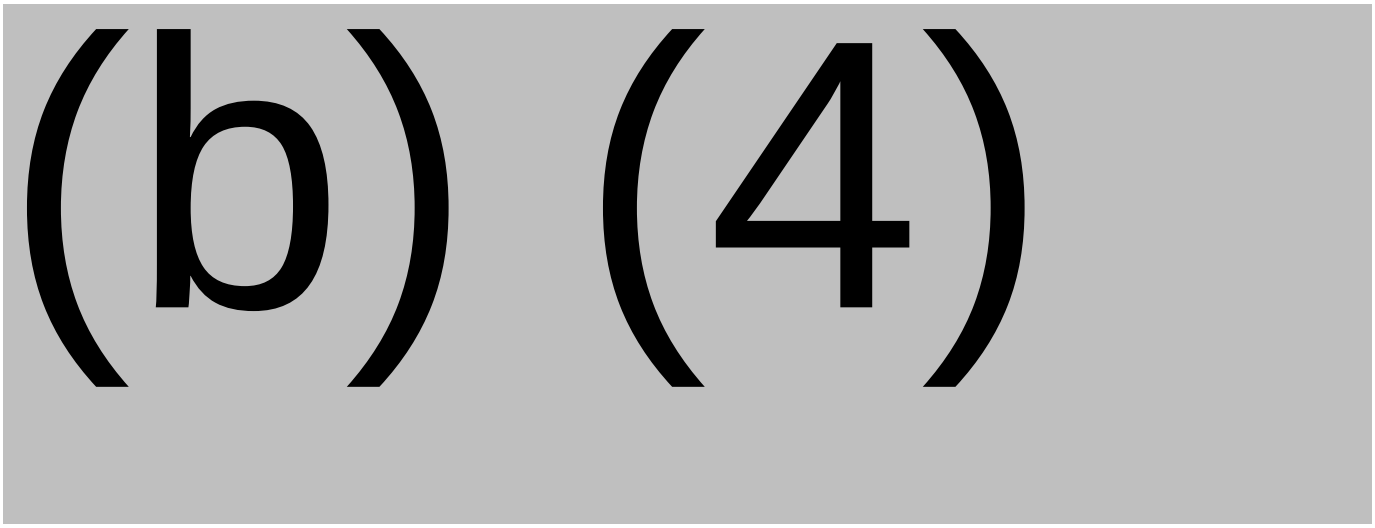
Method = LC-MS/MS (Adopted from GRN 635, FDA, 2016); N=4; NAAD = nicotinic acid adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; NaMN = nicotinic acid mononucleotide.

Niacin and NR

Vitamin B_3 (niacin) is a naturally occurring substance found in meat, poultry, fish, eggs, and green vegetables (Mackay et al., 2012). It is a combination of nicotinic acid and NAM. NR is a pyridine-nucleoside form of niacin containing an associated ribose bond, in addition to NAM.

Sales Record

CONFIDENTIAL



3.1.2. Adverse Events (AEs) Associated with Historically Consumed Material

CONFIDENTIAL

In the past 2 years, no AEs were reported by Japanese consumers.

3.1.3. Alternative Rationale for Reasonable Expectation of Safety Based on History of Use

CONFIDENTIAL

NMN shares similar properties with other NAD^+ precursors: NR, nicotinic acid, and NAM (Sauve, 2008). Nicotinic acid and NAM have several disadvantages in their therapeutic application compared to NMN. NAM may cause hepatotoxicity or flushing. Furthermore, a recent preclinical study suggested that it resides in rat bodies for less time compared to NMN (Kawamura et al., 2016; Knip et al., 2000). Niacin or nicotinic acid is associated with adverse effects, like cutaneous flushing, when administered as an immediate release formulation, while the sustained release formulations may cause hepatotoxicity (Pieper, 2003). Among the NAD^+ precursors, NR and NMN are exceptions, with fewer unfavorable side effects reported for these two metabolites (Cantó et al., 2015). No flushing has been reported by consumers of NMN.

References for Part 3.1.

Cantó C, Menzies KJ, Auwerx J. NAD(+) metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. Cell Metab. 2015;22:31-53.

FDA. 2016. GRN 635. Nicotinamide riboside chloride, filed by ChromaDex, Inc.

Kawamura T, Mori N, Shibata K. β-Nicotinamide mononucleotide, an anti-aging candidate compound, is retained in the body for longer than nicotinamide in rats. J Nutr Sci Vitaminol (Tokyo). 2016;62:272-6.

Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, Gale EA. European Nicotinamide Diabetes Intervention Trial Group. Safety of high-dose nicotinamide: A review. Diabetologia. 2000;43:1337-45.

MacKay D, Hathcock J, Guarneri E. Niacin: Chemical forms, bioavailability, and health effects. Nutr Rev. 2012;70:357-66.

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 2016;24:795-806.

Pieper JA. Overview of niacin formulations: Differences in pharmacokinetics, efficacy, and safety. Am J Health Syst Pharm. 2003;60:S9-14.

Sauve AA. NAD^+ and vitamin B3: From metabolism to therapies. J Pharmacol Exp Ther. 2008;324:883-93.

3.2. Other Evidence of Safety

CONFIDENTIAL

Regulatory Status of NMN

NMN serves as an intermediate in the metabolism of NAM to NAD. NR is a precursor of NMN. Thus, our review includes the regulatory status of NAM and NR.

Regulatory Status of NR

The Food and Drug Administration (FDA) has issued a ‘no question’ letter to one Generally Recognized as Safe (GRAS) notice related to food uses of NR (GRN 635 submitted by ChromaDex, Inc., 2016). The intended use is as a source of vitamin B_3 in food and drink products, such as vitamin water, protein shakes, nutrition bars, gum, and chews at a maximum use level of 0.027% by weight. In this GRAS notice, toxicity-related studies on NR from the literature supported the safety of use of NR. The FDA did not question the acceptability and suitability of these studies to establish the safety of NR for the proposed food uses.

On November 3, 2015 and June 29, 2018, the FDA notified ChromaDex of the acceptance of the NDI notices for NR in accordance with 21 CFR §190.6 (c) (NDIN 882 – daily doses up to 180 mg, and NDIN 1062 - daily doses up to 300 mg/day).

Regulatory Status of NAM

NAM has been safely used under 21 CFR §184.1535.

The Institute of Medicine (IOM) and the Scientific Committee on Food (SCF, 2002) established the Tolerable Upper Intake Level (UL) values of 35 and 10 mg/day for nicotinic acid and NAM, respectively. At higher doses, peripheral vasodilation (i.e., flushing) was observed. The IOM noted "The UL for niacin applies to synthetic forms obtained from supplements, fortified foods, or a combination of the two" (IOM, 1998). The flushing response was found to be mediated by the binding of nicotinic acid to the GPR109A receptor in cases of high supplemental niacin consumption (GRN 635; FDA, 2016). However, NMN may not cause flushing upon consumption of high doses in humans; Cantó et al. (2012) reported that NMN and its metabolic precursor, NR, do not bind GPR109A in mouse Hea1.6 and human HEK293T cell lines. The European Commission (SCF, 2002) and the UK Expert Group on Vitamins and Minerals (EVM, 2003) have established the UL values for NAM as 900 and 500 mg/day. These were derived without consideration of the flushing effects of nicotinic acid. Thus, these values established in Europe are likely to be more appropriate for the support of the safety of the intended uses of NR or NMN than the IOM's UL value of 35 mg/day, which is based on the flushing effects of supplementary nicotinic acid.

Accordingly, the US FDA had no questions regarding the estimated consumption of up to 145 mg of NR chloride in GRN 635. In addition, the FDA had no objection on the NDI notices of NR at daily doses of 180 and 300 mg/person (NDIN 882 and 1062).

It is reasonable to expect that a similar conclusion on flushing can be extended to NMN.

Regulations in Canada

NAM has been approved as a Natural Health Product (NHP) ingredient (Health Canada, 2021).

Regulations in Europe

NAM has been approved as a Novel Food. The UL values for NAM of 900 mg/day and 500 mg/day have been established by the European Commission (SCF, 2002) and the United Kingdom Expert Group on Vitamins and Minerals (EVM, 2003), respectively. Due to methodological limitations of available animal studies, these limits were based on studies in which human subjects consumed up to 3 g NAM/day for up to 3 years.

References for Part 3.2.

Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 2012;15:838-47.

Expert Group on Vitamins and Minerals (EVM) (2003). Niacin (Nicotinic Acid and Nicotinamide). In: *Safe Upper Levels for Vitamins and Minerals: Report of the Expert Group on Vitamins and Mineral*s. London, UK: Food Standards Agency (FSA), Expert Group on Vitamins and Minerals (EVM), pp. 52-61, 334. Available at: https://cot.food.gov.uk/committee/committee-on-toxicity/cotreports/cotjointreps/evmreport.

FDA. 2016. GRN 635. Nicotinamide riboside chloride, filed by ChromaDex, Inc.

FDA 2015. NDIN 882. Nicotinamide riboside, filed by ChromaDex.

FDA. 2018. NDIN 1062. Nicotinamide riboside, filed by ChromaDex.

Health Canada. 2021. Available at: HC Chemical Substance - Nicotinamide mononucleotide.pdf.

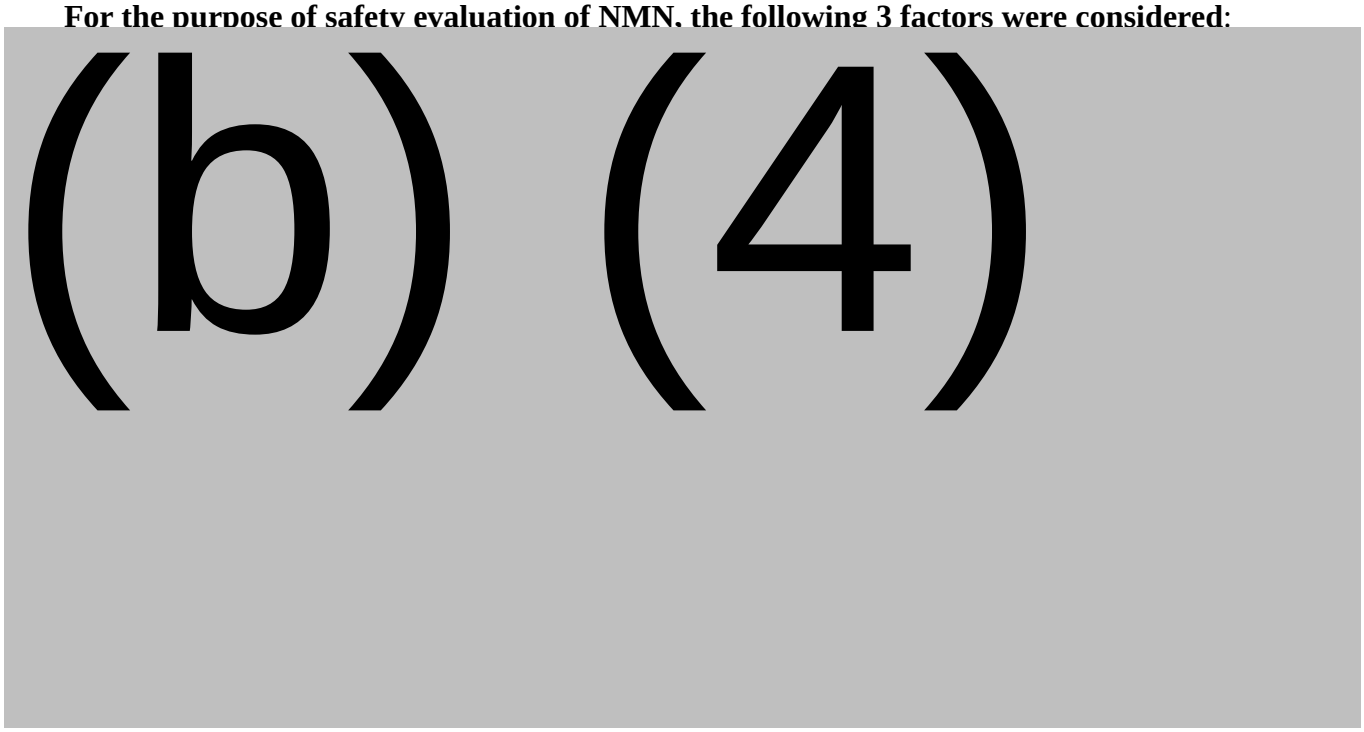
Institute of Medicine (IOM, 1998). Niacin: In: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B(6), Folate, Vitamin B(12), Pantothenic Acid, Biotin, and Choline*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline, and the Subcommittee on Upper Reference Levels of Nutrients). Washington (DC): National Academy Press (NAP), pp. 123-149. Available at: http://www.nap.edu/openbook.php?record_id=6015&page=123.

Scientific Committee on Food (SCF) (2002). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Levels of Nicotinic Acid and Nicotinamide (Niacin) (expressed on 17 April 2002). (SCF/CS/NUT/UPPLEV/39 Final). Brussels Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out80j_en.pdf.

3.3. Safety of NMN

CONFIDENTIAL

For the purpose of safety evaluation of NMN, the following 3 factors were considered:



3.3.1. Pharmacokinetics of NMN and NR

CONFIDENTIAL

Pharmacokinetics of NMN

NAM and NR are converted to NMN, which is the precursor of NAD^+, a co-substrate of NAD^+-dependent enzymes essential for biological processes as important as redox homeostasis, gene expression, RNA processing, genomic stability, immunity and inflammation, and energy metabolism (Roos et al., 2021). NMN is the main source of the salvage pathway to generate NAD^+. In human cells, NMN is available as a source of cellular energy.

Although cells are capable of synthesizing NAD^+ from tryptophan *de novo*, it is thought that a major source of NAD^+ production comes from salvage pathways (Belenky et al., 2007). In other words, most NAD^+ is recycled via salvage pathways rather than generated *de novo* to maintain NAD^+ levels. The majority of NAD^+ is salvaged from NAM, the product of CD38 and poly(ADP-ribose) polymerases, or from various forms of niacin taken up in the diet, including NAM, niacin, NR, and NMN. Rate of NAD^+ synthesis in mammals is mostly determined by the first step in the salvage pathway that converts NAM to NMN.

NMN can be synthesized from NAM and 5'-phosphoribosyl-1-pyrophosphate by Nampt (Figure 6; Yoshino et al., 2018). It can also be synthesized from NR by the NR kinase-mediated

phosphorylation reaction. NMN adenylyl-transferases convert NMN into NAD^+. Human red blood cells contain a total of approximately 50 mg of NMN (Mills et al., 2016).

It was reported that NMN rapidly appears in plasma, liver, white adipose tissue, and pancreas in wild-type mice within 15 minutes after one bolus intraperitoneal injection of NMN (500 mg/kg) (Yoshino et al., 2011). Then NMN is immediately utilized for NAD^+ biosynthesis, leading to markedly increased NAD^+ (2- to 3-fold) concentrations in the liver over 60 minutes (Yoshino et al., 2011).

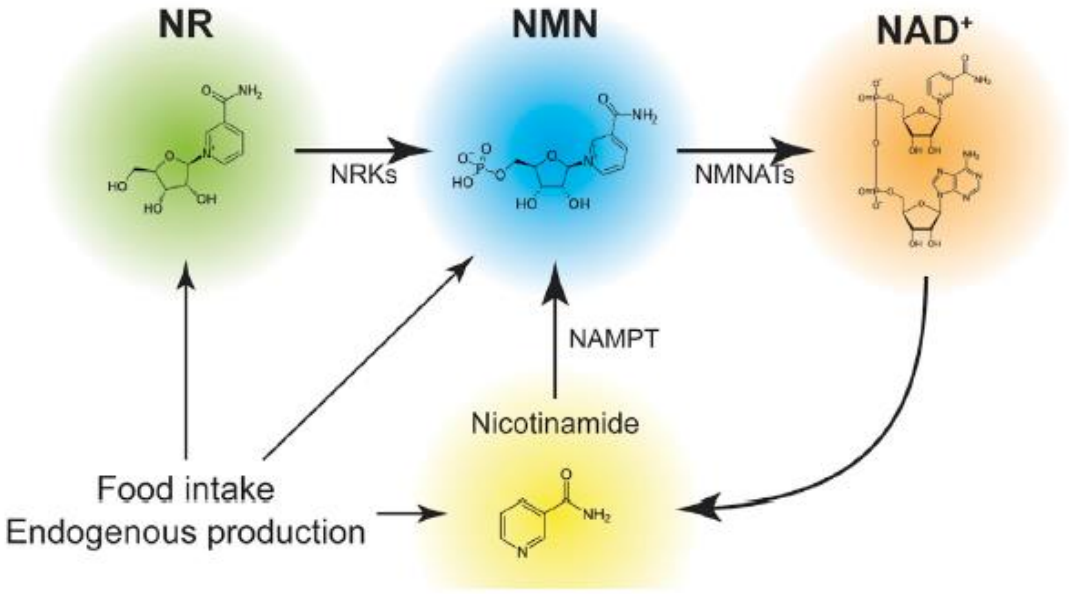


Figure 6. Metabolic Pathways for NR and NAM to NMN
Adopted from Yoshino et al. (2018)

Although the above-mentioned findings demonstrated the bioavailability of NMN, the mechanism of NMN uptake into cells or tissues is currently unclear and under debate. Two possible mechanisms have been proposed for NMN uptake (Figure 7).

1) The first hypothesis is the direct uptake of NMN, presumably through specific transporter(s). This possibility is supported by the fact that intraperitoneal or oral administration of NMN (300–500 mg/kg) immediately (within 5 minutes) increases NMN in plasma, and then, NAD^+ content in peripheral organs (Mills et al., 2016; Yoshino et al., 2011). This suggests the presence of an active NMN uptake system in the gut and other organs.
2) Another hypothesis is extracellular dephosphorylation of NMN into NR by ectonucleotidases (e.g., CD73) before uptake. It is possible that extracellular NMN is

transported into cells or tissues and utilized for NAD^+ biosynthesis in a tissue- and cell-type-specific manner. In other words, NMN is metabolized extracellularly to NR that is then taken up by the cell and converted back to NMN before being metabolized into NAD^+(Poddar et al., 2019; Figures 4 and 5). In other words, the ubiquitously expressed Nicotinamide riboside (NR) kinase 1 (NRK1) helps the subsequent conversion of NR to NMN which is further metabolized into NAD+. The results may explain the overlapping metabolic effects observed with the two compounds.

Due to overlapping metabolic effects of NR and NMN, the pharmacokinetics and safety studies of NR was briefly discussed in this NDI notoce in addition to that of NMN.

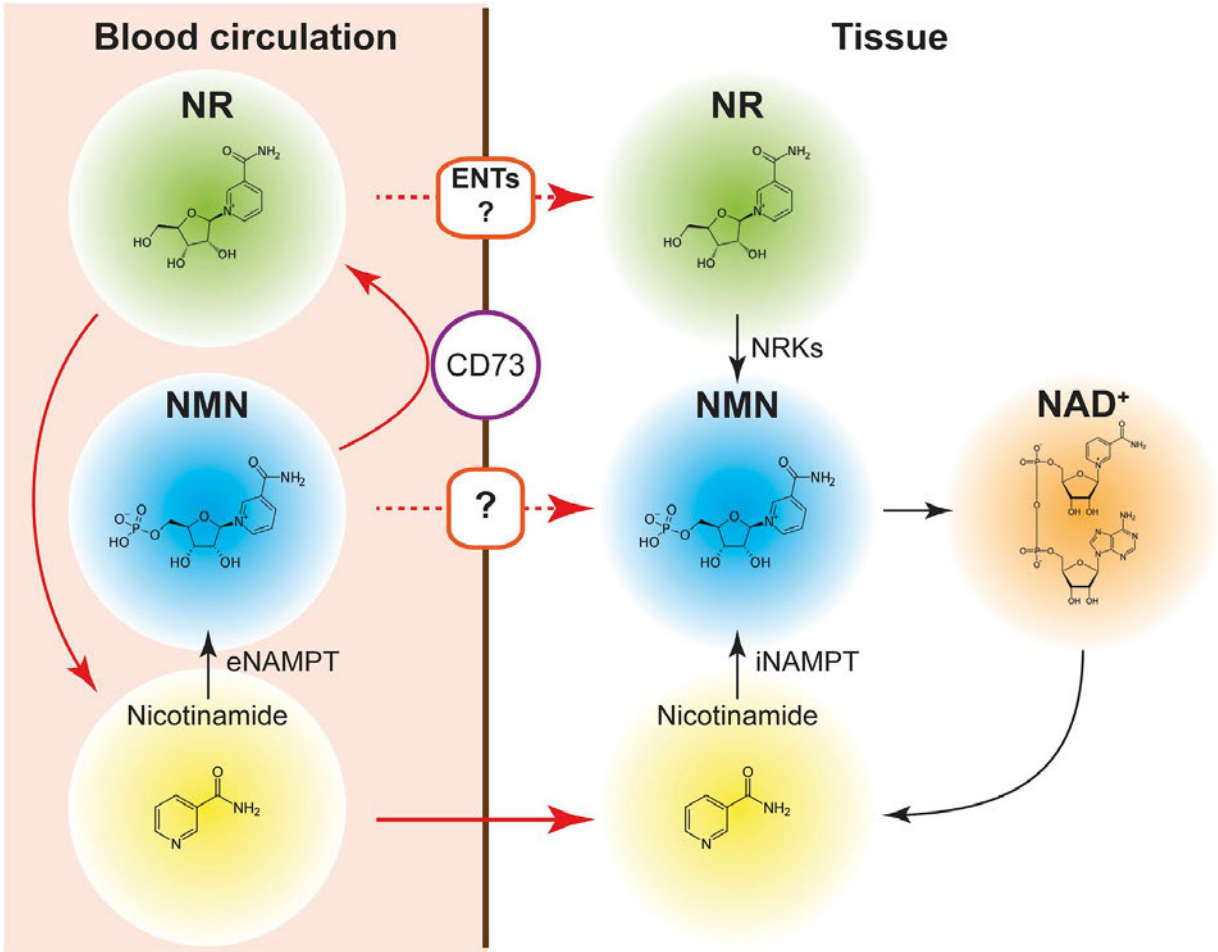


Figure 7. Uptake of NMN and NR *In Vivo*
Adopted from Yoshino et al. (2018).
Where eNAMPT= extracellular nicotinamide phosphoribosyltransferase; ENTs = equilibrative nucleoside transporters; iNAMPT = intracellular NAMPT.

Pharmacokinetic studies of NMN in Humans

The Study by Irie et al. (2020)

Irie et al. (2020) investigated the pharmacokinetics of NMN metabolites in 10 healthy male subjects. The subjects received 100, 250, or 500 mg of NMN (purity of 96-97%) in capsules orally at 9 a.m. after an overnight fasting and followed by 5 hours at rest with drinking only water freely. Metabolites of NMN, such as methylnicotinamide (MeNAM), N-methyl-2-pyridone-5-carboxamide (2-PY), and N-methyl-4-pyridone-5-carboxamide (4-PY) were identified in the plasma. The mean plasma concentrations of 2-PY and 4-PY were 1,423 and 237 nM at baseline, respectively. These values were comparable to those in a study by Rutkowski et al. (2003) that reported the mean serum concentrations of 2-PY and 4-PY in healthy subjects as 830 nM and 260 nM, respectively. NMN administration significantly increased plasma concentrations of 2-PY and 4-PY in a dose-dependent manner. The peak concentrations of 2-PY and 4-PY were observed at 5 hours after administration (100, 250, and 500 mg: 2-PY, 2,500, 2,800, and 4,400 nM, respectively; 4-PY, 400, 450, and 700 nM, respectively). The incremental areas under the curve of 2-PY were positively correlated with those of 4-PY. This study showed that the administration of NMN was degraded into 2-Py and 4-Py proportionally. The plasma concentration of MeNAM tended to moderately increase. The mean peak concentrations of MeNAM were 253, 292, and 316 nM in the 100, 250, and 500 mg groups, respectively. The incremental areas under the curve of MeNAM were correlated with those of 2-PY and 4-PY, although the incremental areas in 2-PY and 4-PY varied largely by the subjects in this study. Precise kinetics of NMN were not evaluated in this study.

The study by Pencina et al. (2022)

Pencina et al. (2022) reported the pharmacokinetics and pharmacodynamics of a microcrystalline NMN (source, NA). Overweight or obese, middle-aged or older men and postmenopausal women (16 men, 16 women; mean age of 63.9 years; mean BMI 29.1 kg/m^2) consumed placebo, 1,000 mg NMN (once a day), 2,000 mg NMN (divided into 2 doses) for 14 days. The blood and urine concentrations of NMN, NAD^+, and NAD^+ metabolome were determined.

NMN Levels: There was statistically significant difference between NMN-treated groups and placebo in blood NMN concentrations on Day 14. Low-dose of NMN (1000 mg once-daily) was associated with an average 2.7- and 1.7-times increase above baseline and twice-daily treatment resulted in 4.5- and 3.7 times increase in mean C_{max} and AUC_{last}, respectively, compared to placebo. NMN C_{max} and AUC_{last} were higher in participants treated with a high-dose (2,000 mg/day) regimen than in those treated with the low-dose (1,000 mg/day) regimen. No significant sex differences in blood NMN exposures (C_{max} and AUC_{last}) were observed across treatment groups. The NMN AUC_{last} values were not significantly associated with BMI and age.

NAD Levels: NMN treatment was associated with a dose-related increase in blood NAD levels from baseline to Day 14. On Day 1, blood NAD levels showed only a modest increase from baseline to 24 hours in NMN-treated participants. NAD levels were substantially higher on Day 14 than at baseline or day 1 in NMN groups compared with placebo group. Increases in NAD levels from baseline to day 14 were related to NMN dose and increase in NMN levels from baseline to day 14 (control vs. low-dose vs. high-dose: C_{max}, 1.36 vs. 23 vs. 40.4 ng/mL; AUC_{last} NAD values,14.1 vs. 459 vs. 867 h.ug/mL; both NMN groups vs. placebo, $p<0.001$, $R^2=0.57$). The AUC_{last} NAD values were not significantly associated with sex, BMI, and age (all $p>0.05$).

Circulating levels of NAD Metabolome: Among the circulating metabolites of NAD, 2-PY was the most abundant. Circulating concentrations all four metabolites (2-PY, NAM, Me NAM, and NR) increased from baseline to 24 hours on Day 1. Metabolite concentrations were higher on days 8 and 14 than on day 1 and baseline in both NMN-treated groups, but did not differ on days 8 and 14 (C_{max} [ng/mL] for control vs. low-dose vs. high-dose at day 14: NAM, 10.2 vs. 65.2 vs 140; MeNAM, 2.0 vs. 5.87 vs. 4.23; 2PY, 103 vs. 2150 vs. 4230; NR, 0 vs. 2.1 vs. 5.8; AUC_{last} [h.ug/mL] for control vs. low-dose vs. high-dose at day 14: NAM, 63.7 vs. 787 vs. 1,810; MeNAM, 54.8 vs. 2,300 vs. 4,300; 2PY, 1,670 vs. 42,500 vs. 82,300; NR, 3.6 vs. 10.5 vs. 18.6). C_{max} and AUC_{last} for the four metabolites were not significantly associated with sex, BMI or age.

Urinary metabolites: Urinary NMN concentrations standardized to creatinine levels were similar among treatment groups with no apparent sex differences; thus, very little oral NMN is eliminated unchanged in the urine. On days 8 and 14, mean urinary NAM and 2-PY concentrations were higher in NMN-treated groups compared to placebo group (control vs. low-dose vs. high-dose at day 14 [ng/mg creatine]: NMN, 333 vs. 384 vs. 308; NAM, 174 vs. 290 vs. 703; 2PY, 17,800 vs. 131,000 vs. 255,000).

<u>The Study by Yoshino et al. (2021)</u>

In a study by Yoshino et al. (2021), NMN at daily dose of 250 mg was administered for 10 weeks in postmenopausal women with prediabetes who were overweight or obese. Plasma peripheral blood mononuclear cell (PBMC) NAD^+ content and NMN metabolites were assessed at 4 hours after placebo or NMN ingestion Plasma or skeletal muscle concentrations of 2-PY and 4-PY, and N-MeNAM increased after 10 weeks of NMN (week 0 vs. week 12: plasma 2-PY, 5E+6 vs. 1.8E+7 AU; skeletal muscle 2-PY, 1.5E+6 vs. 3.5E+6; skeletal muscle 4-PY, 1.4E+6 vs. 3.2E+6 AU; skeletal muscle MeNAM, 2.8E+5 vs. 7E+5 AU; all $P<0.05$), but not in the placebo group. Ingestion of a single 250-mg dose of NMN at the end of the 10-week treatment did not cause a further increase in PBMC NAD^+ content above the basal value as assessed in serial blood samples obtained for 240 minutes after ingestion because of the higher basal value in the NMN group. However, the 240-minute PBMC NAD^+ content area under the curve (AUC) above zero was 43% greater in the NMN group than in the placebo group ($P < 0.01$).

Pharmacokinetic study of NMN in Animals

Consistent with the findings from human studies, a study using doubly labeled isotopic NMN (C13-D-NMN) shows that orally administered NMN is rapidly absorbed and converted to NAD^+ in peripheral organs, such as liver and skeletal muscle (Mills et al., 2016). Mills et al. (2016) administered NMN (Oriental Yeast Co., 96-97% purity) at a dose of 300 mg/kg body weight (bw) by oral gavage in mice to measure plasma NMN and hepatic NAD^+ levels over a period of 30 minutes. Plasma NMN levels exhibited a steep increase at 2.5 minutes, further increase from 5 to 10 minutes, and then went back to original levels after 15 minutes. Hepatic NAD^+ levels showed a steady increase from 15 to 30 minutes. Relatively small increases (with no statistical significance) in NAD^+ levels were observed in the liver, skeletal muscle, and cortex of the brain, but not in white or brown adipose tissue when tissue NAD^+ levels were measured 60 minutes after oral gavage of NMN. To further confirm whether orally administered NMN is utilized to synthesize NAD^+ in tissues, doubly-labeled isotopic NMN was used to trace NAD^+ in the liver and soleus muscle by mass spectrometry. Doubly-labeled NAD^+ (C13-D-NAD^+) was detected in the liver at 10 minutes post-administration, and was further increased at 30 minutes. C13-D-NAD^+ was detected in the soleus muscle at 30 minutes, but not at 10 minutes. The authors concluded that orally administered NMN is quickly absorbed, efficiently transported into the blood circulation, and immediately converted to NAD^+ in major metabolic tissues.

Pharmacokinetics of NR in Humans

A study by Trammell et al. (2016) used targeted NAD^+ metabolomics approaches and provided a comprehensive analysis of the effects of oral NR administration on PBMCs in humans and on hepatic NAD^+ metabolism in mice. In this study, NR increased the concentrations of all detected NAD^+ metabolites in PBMCs except for NAM. In mice, hepatic levels of NAM, NMN, and NAD^+ were increased approximately 4-fold 6 hours after oral administration of NR (185 mg/kg). NR was not detected in human PBMCs or mouse liver, presumably due to rapid conversion of NR to NMN by NRKs.

In the first human study by Trammell et al. (2016), a healthy adult man (52 years old) received 1,000 mg/day NR (source not specified) for 7 days. Plasma NAD^+ was increased with a single oral dose of NR (~12 to 38 μM). Plasma concentrations of Nam, 4-PY, and 2-PY were strongly elevated by oral NR (at 0 vs. 8.1 vs. 167.6 hours: 2-PY, 2 vs. 14 vs. 17 μM; 4-PY, 0.6 vs. 3.8 vs. 4.2 μM; MeNAM, 0.1 vs. 0.9 vs. 2.1 μM).

In a second human clinical trial, time-dependent PBMC NAD^+ metabolomes from 12 healthy female subjects were quantified after three different oral doses of NR (100, 300, or 1,000 mg/day NR for 3 days) in a cross-over design. NR administration in the healthy subjects significantly increased plasma NAD^+ levels commensurate with plasma NR levels. There were dose-dependent increases observed in concentrations of NAD^+ metabolite levels in PBMC (AUC

for 100 vs. 300 vs. 1,000 mg/day: nicotinic acid adenine dinucleotide [NAAD], 3 vs. 6. vs. 14 μmol h/l, P<0.001; MeNAM, 0.8 vs 1.5 vs. 7.5 μmol h/l, P<0.001; 2-PY, 20 vs. 60 vs. 205 μmol h/l, P<0.001). AUC increases of NAAD, me2PY, and MeNAM achieved statistical significance with respect to lower doses of NR. The study also reported that NAAD, which was not thought to be en route for the conversion of NR to NAD^+, is formed from NR. Thus, the authors postulated that the rise in NAAD is a highly sensitive biomarker of effective NAD^+ repletion.

The averaged peak concentration of MeNAM, 2-PY, and NAAD increased proportionally with increased NR doses. Of these metabolites, only NAAD was below the detection limit in individuals before they took NR, qualifying this metabolite as a biomarker of supplementation. NAAD was elevated from below the limit of quantification to ~1 mM to ~29 mM (29 times increase from the baseline) in 24 hours AUC. This was contrary to most NADP metabolites present before supplementation, such that the AUC attributable to supplementation was a time-zero baseline subtracted AUC. Thus, NAAD may be qualified as a biomarker for supplementation. NeNAM exhibited no tendency towards higher cellular concentrations with higher doses of NR. At the 8-hour peak, the average concentration of NAAD was elevated to 0.56±0.26, 0.74±0.27, and 1.24±0.51 mM in PBMCs of volunteers taking 100, 300, and 1,000 mg single doses of NR, respectively. NMN tended to rise and NAD^+ rose to higher concentrations of ~2 and 20 mM, respectively, in people taking 300 and 1,000 mg doses of NR versus people taking 100 mg doses. Thus, 100 mg supplementation produced an average increase of ~4±2 mM in PBMC NAD^+, whereas the higher doses produced average increases of ~6.5±3.5 mM in PBMC NAD^+.

Pharmacokinetics of NR in Mice

In a mouse study by Trammell et al. (2016), male C57Bl6/J mice were intraperitoneally (i.p.) injected with either saline (n =8) or NR chloride (500 mg/kg bw) (n = 6) for 6 days. Livers and hearts were freeze-clamped and prepared for metabolomic analysis. The steady-state levels of hepatic NAD^+ and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were much more responsive to NR than the steady state levels of cardiac NAD^+ and $NADP^+$. However, cardiac NAD^+ metabolism was clearly elevated on the basis of statistically significant elevation of NMN, NAM, MeNAM, and 4-PY. Among these metabolites, only NMN was elevated in the heart by approximately two-fold.

Summary

In summary, oral administration of NMN or NR increased the blood concentrations of NAD, MeNAM, 2-PY, and NR in a dose dependent manner in humans and animals. The data indicate that the metabolic fate of NMN and NR are similar as NMN is a metabolite of NR.

Reference for 3.3.1.

Belenky P, Racette FG, Bogan KL, McClure JM, Smith JS, Brenner C. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD^+. Cell. 2007;129:473-84.

Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, Yamaguchi S, Yamashita K, Shigaki S, Ono T, Yukioka H, Okano H, Nabeshima YI, Imai SI, Yasui M, Tsubota K, Itoh H. Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocr J. 2020;67:153-60.

Kawamura T, Mori N, Shibata K. β-Nicotinamide mononucleotide, an anti-aging candidate compound, is retained in the body for longer than nicotinamide in rats. J Nutr Sci Vitaminol (Tokyo). 2016;62:272-76.

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 2016;24:795-806.

Pencina K, Lavu S, Dos Santos M, Beleva YM, Cheng M, Livingston D, Bhasin S. MIB-626, an Oral Formulation of a Microcrystalline Unique Polymorph of β-Nicotinamide Mononucleotide, Increases Circulating Nicotinamide Adenine Dinucleotide and its Metabolome in Middle-aged and Older Adults. J Gerontol A Biol Sci Med Sci. 2022:glac049.

Roos J, Zinngrebe J, Fischer-Posovszky P. Nicotinamide mononucleotide: a potential effective natural compound against insulin resistance. Signal Transduct Target Ther. 2021;6:310.

Rutkowski B, Slominska E, Szolkiewicz M, Smolenski RT, Striley C, Rutkowski P, Swierczynski J. N-methyl-2-pyridone-5-carboxamide: a novel uremic toxin? Kidney Int Suppl. 2003:S19-21.

Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, Dellinger RW, Li Z, Abel ED, Migaud ME, Brenner C. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat Commun. 2016;7:12948.

Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 2011;14:528-36.

Yoshino J, Baur JA, Imai SI. NAD(+) intermediates: The biology and therapeutic potential of NMN and NR. Cell Metab. 2018;27:513-28.

3.3.2. Mutagenicity and Genotoxicity Studies

CONFIDENTIAL

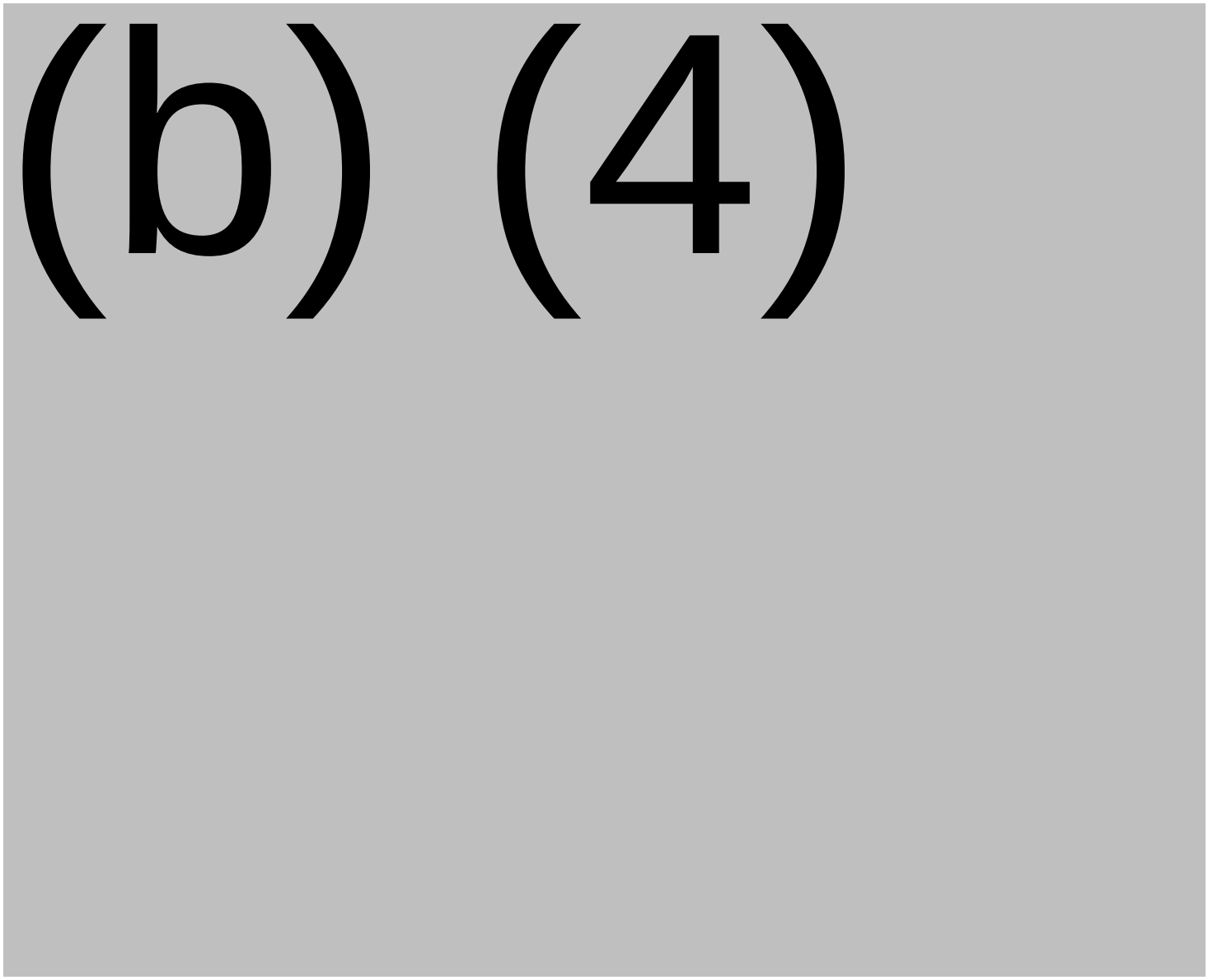
This review includes mutagenicity or genotoxicity studies of NMN and NR. Because NMN is a metabolite of NR, the data from these studies may be relevant when evaluating the safety of NMN. Thus, studies of NR were included in this review in addition to the studies of NMN.

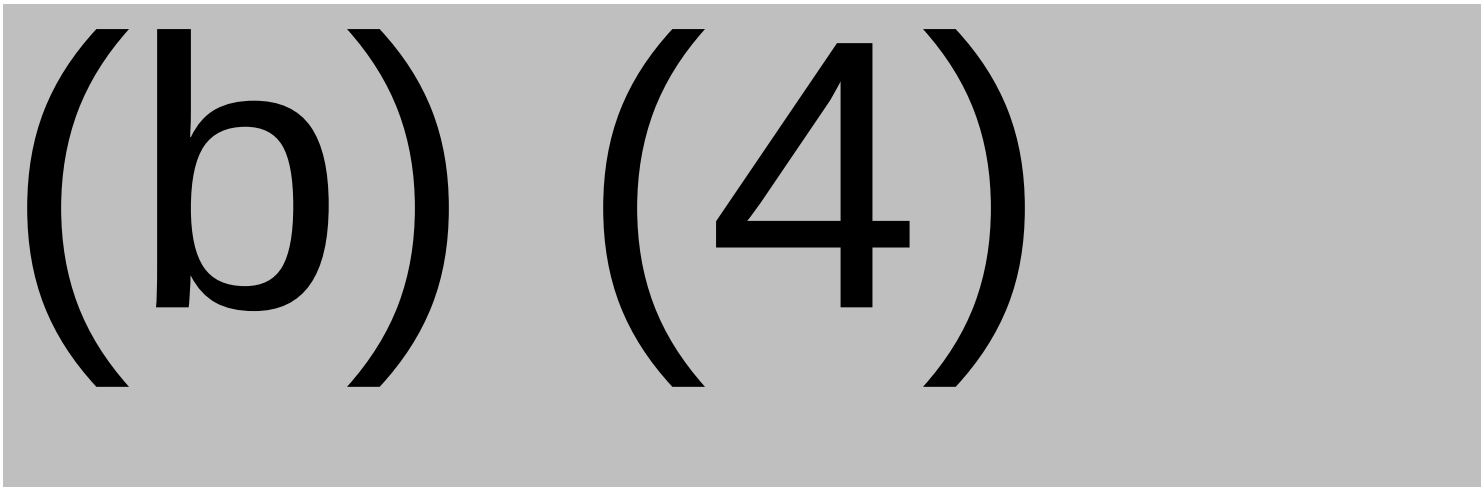
3.3.2.1. Mutagenicity and Genotoxicity Studies of SyncoZymes' NMN

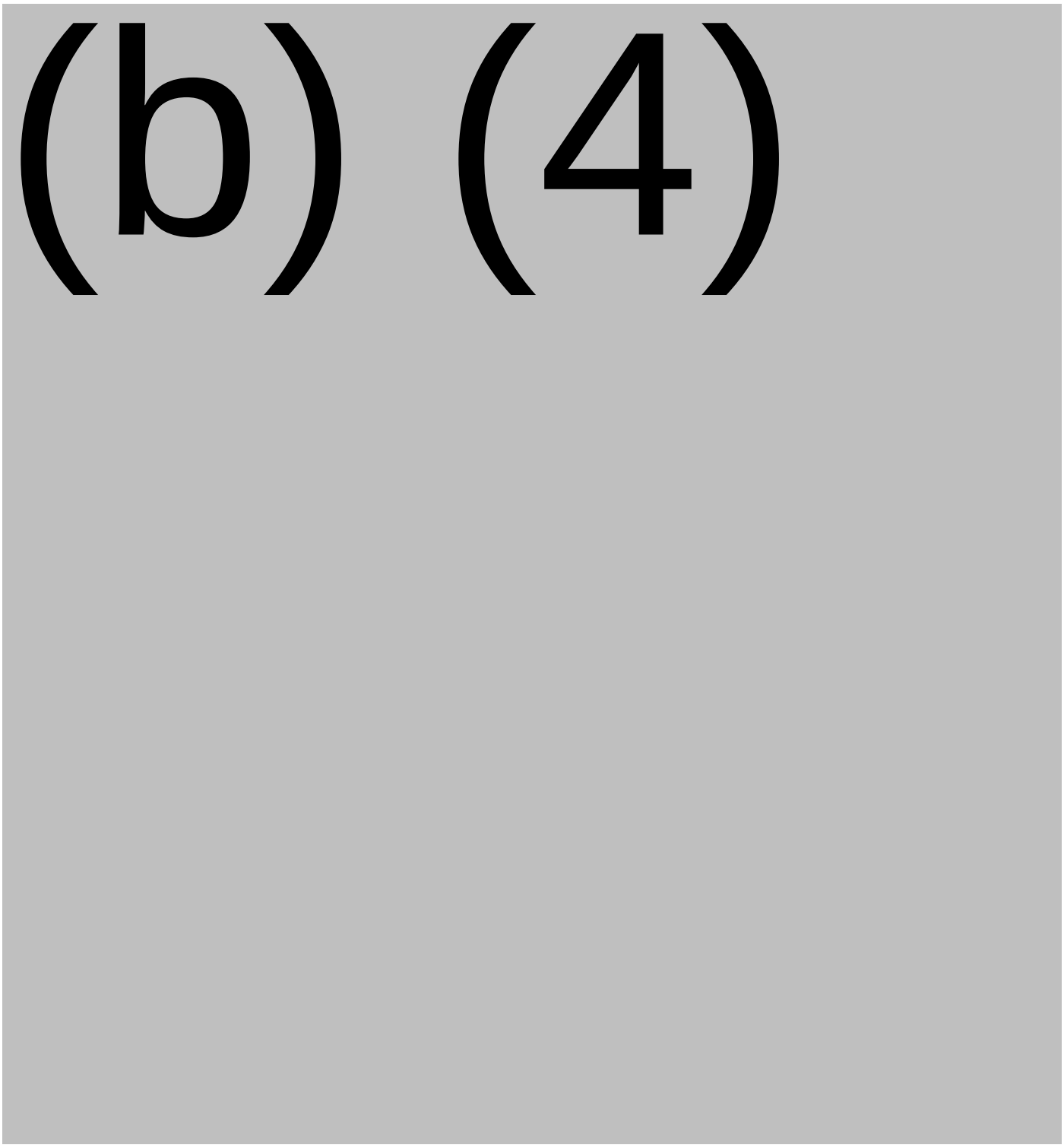
CONFIDENTIAL

(b) (4)

(b) (4)


(b) (4)


(b) (4)


(b) (4)

3.3.2.2. Mutagenicity and Genotoxicity Studies of Other Sources of NMN

CONFIDENTIAL

Cros et al. (2021) examined the safety of a highly pure, synthetic form of NMN (NMN-C®, 99.03% purity, for Seneque SA, France, by RoowinSA, France).

In a study by Cros et al. (2021) a bacterial reverse mutation test (Ames test) was conducted in *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* EP2(pKM101). The strains were exposed to concentrations of NMN, including 60, 190, 560, 1,670, or 5,000 µg/plate, with or without metabolic activation (S9). Number of revertant colonies per plate were counted and recorded by automatic colony counter. The test revealed no mutagenic effect of NMN. However, NMN induced cytotoxicity at concentrations above 0.56 mg/plate for the TA1537 strain with pre-incubation and metabolic activation. Overall, NMN was considered to be non-mutagenic and non-pro-mutagenic under the experimental conditions.

In addition, the genotoxic potential of NMN was further examined using an *in vitro* micronucleus assay with Chinese hamster ovary (CHO) cells exposed to 320, 800, or 2,000 µg/mL for 4 hours with or without metabolic activation. It was further tested in the long term for up to 1.5 to 2 times the normal CHO cell cycle. In contrast to the positive controls (mitomycin C, colchicine, and cyclophosphamide), no concentration of NMN increased the number of micronuclei compared with the negative control.

3.3.2.3. Mutagenicity Studies of NR

CONFIDENTIAL

The safety of a synthetic form of NR (Niagen™, supplied by ChromaDex, Inc., US) was determined using a bacterial reverse mutagenesis assay, an *in vitro* chromosome aberration assay, and an *in vivo* micronucleus assay (Table 12; Conze et al., 2016).

In the bacterial reverse mutagenesis assay (the Ames test), *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvr*A pKM101 were exposed to doses of NR ranging from 50 to 5,000 µg with or without the metabolic activator, the S9 mix. NR did not increase the number of revertant colonies. Human peripheral blood lymphocytes were used in the *in vitro* chromosomal aberration assay and exposed to 1.25, 2.5, or 5 mg/mL NR with or without the S9 mix. There was no increase in the number of aberrant metaphases when exposed to NR. NR was not cytotoxic or clastogenic. In the *in vivo* micronucleus assay, SD rats were exposed to single doses of 500, 1,000, or 2,000 mg/kg bw NR. No mortalities or clinical signs were observed in the SD rats. There was no increase in the number of PCE percentages. These studies demonstrated that NR was not mutagenic or genotoxic.

Table 12. Genotoxicity Studies Showing No Mutagenicity or Genotoxicity of NMN and NR

Test system	Dose	Reference
Studies of SyncoZymes NMN		
(b) (4)		
Studies of Other Sources of NMN (synthetic, 99.3% purity)		
Salmonella typhimurium TA98, TA100, TA1535, TA1537, and *E. coli* EP2(pKM101)	60, 190, 560, 1670, or 5000 µg/plate NMN	Cros et al., 2021
Chinese hamster ovary (CHO) cells	320, 800, or 2,000 µg/mL	
Studies of NR		
Salmonella typhimurium TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvr*A pKM101 ± S9 mix	50 to 5,000 µg/plate NR	Conze et al., 2016
Human peripheral blood lymphocytes ± S9 mix	1.25, 2.5, or 5 mg/mL NR	
Bone marrow from SD rats	500, 1,000, and 2,000 mg/kg bw NR	

NR = nicotinamide riboside.

References for 3.3.2.

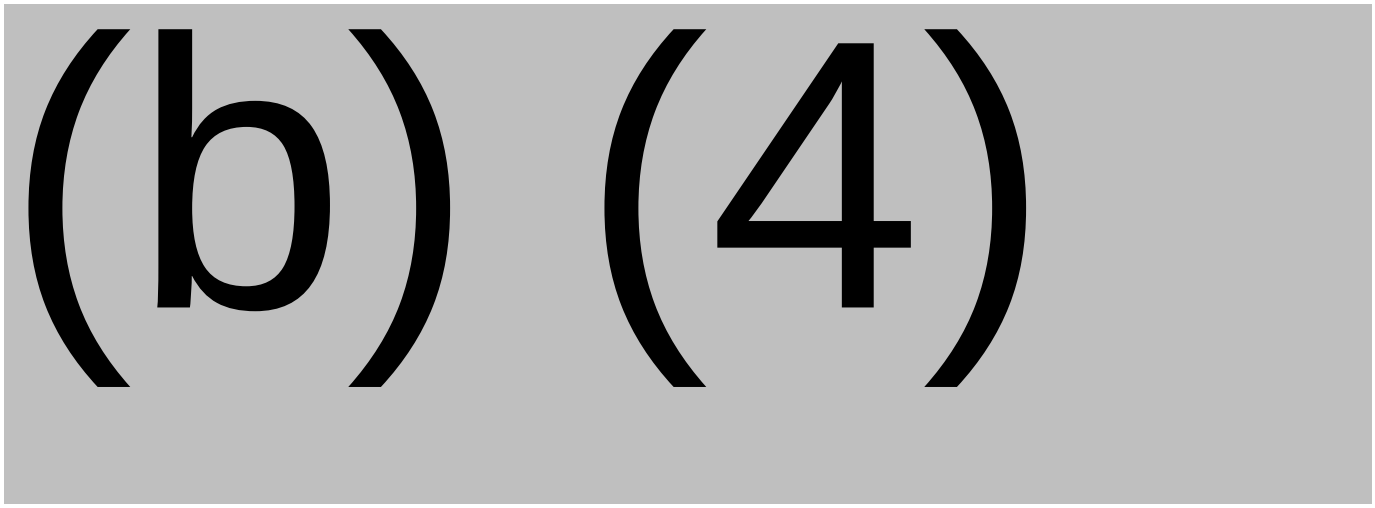
(b) (4)

Conze DB, Crespo-Barreto J, Kruger CL. Safety assessment of nicotinamide riboside, a form of vitamin B3. Hum Exp Toxicol. 2016;35:1149-60.

Cros C, Cannelle H, Laganier L, Grozio A, Canault M. Safety evaluation after acute and sub-chronic oral administration of high purity nicotinamide mononucleotide (NMN-C®) in Sprague-Dawley rats. Food Chem Toxicol. 2021;150:112060.

3.3.3. Animal Toxicity Studies of NMN and NR

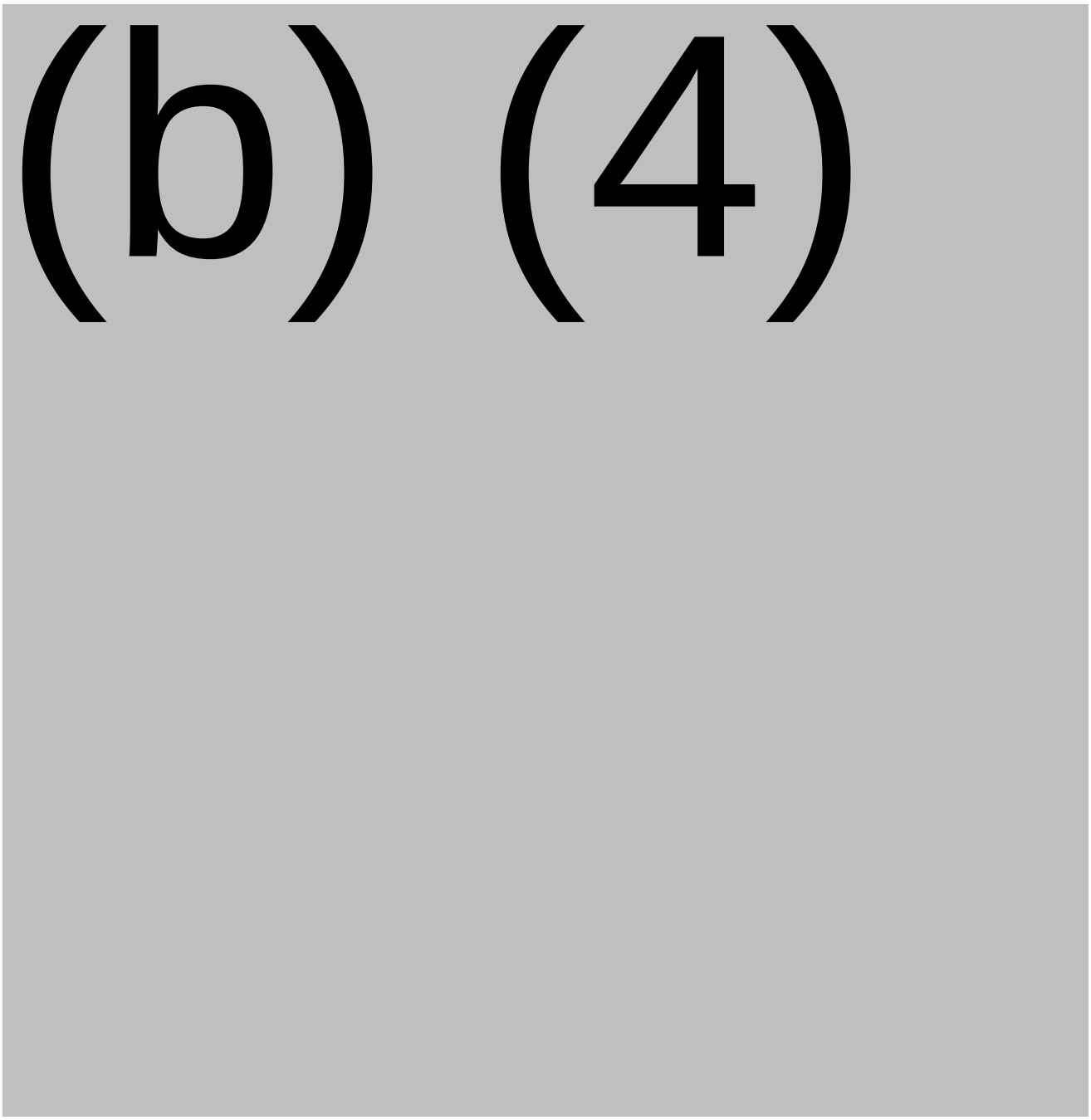
CONFIDENTIAL



Other Sources of NMN				
Sprague-Dawley rats aged 7 weeks	0 or 2,666.6 mg/kg body weight NMN	Single dose, observed for 14 d	NOAEL: 2666 mg/kg bw	Cros et al., 2021
C57BL6J mice aged 8 weeks	0 or 1,340 mg kg/bw /day NMN (single dose); 2680 mg/kg bw/day NMN (divided into two doses)	7 d	NOAEL: 2,680 mg/kg bw/d, the highest dose tested	You et al., 2020
Beagle dogs aged 4 years	0 or 1,340 mg/day NMN	14 d	NOAEL: 1,340 mg/kg bw/d, the highest dose tested	You et al., 2020
Sprague-Dawley rats aged 7 weeks	0, 375, 750, or 1,500 mg/kg bw/day NMN	90 d	NOAEL, 1,500 mg/kg bw/d, the highest dose tested	Cros et al., 2021
Mice, wild type C57BL/6N	0 or 300 mg/kg bw/d NMN (source - Oriental Yeast, Japan)	12 mo (from 5 to 17 mo of age)	NOAEL, 300 mg/kg bw/d, the highest dose tested	Mills et al., 2016

3.3.3.1. Acute Toxicity Studies of NMN in Rats

CONFIDENTIAL



Acute Toxicity Study of Other Sources of NMN

Cros et al. (2021) examined the acute oral toxicity of synthetic NMN (NMN-C®, Roowin SA, France, 99.03% purity) in female SD rats (n=3, age 7 weeks, mean body weight of 161.8 g) using the acute toxic class method (limit test, Organisation for Economic Co-operation and Development [OECD] guideline No 423). Rats received 2,666.6 mg/kg bw NMN by oral gavage, and clinical observations were performed at 30 minutes, 2 hours, and 4 hours after administration

and then once daily for 2 weeks. The test revealed that at an oral limit dose of 2,666 mg/kg body weight synthetic NMN did not lead to any mortality or treatment-related adverse signs.

Taken together, NMN has a LD_{50} value of >12-15 g/kg bw in rats. A compound that has a LD_{50} value of 5 g/kg bw or higher in rats is classified as 'practically non-toxic' and a LD_{50} value of 15 g/kg bw or higher as 'relatively harmless' (Altug, 2003). NMN belongs to the group that has the lowest toxicity rating and, thus, the use of NMN in foods and beverages is not expected to pose a safety concern.

3.3.3.2. Subacute Toxicity Study of Other Sources of NMN

Subacute Toxicity of NMN in Mice by You et al. (2020)

You et al. (2020) examined the subacute toxicity of NMN in C57BL6J mice (8 weeks old, 20-30 g body weight) and beagle dogs (4 years old, 9-11 kg body weight). The mice were randomly divided into four groups (n=3 to 5) that received one of the following by oral gavage for 7 days: water once daily, 1,340 mg kg/bw/day NMN in water (once daily), water twice daily, or 2,680 mg/kg bw/day NMN in water (divided into 2 doses). Behavior, gland secretion, respiration status, feces character, mortality, liver and kidney function indicators (serum alanine aminotransferase [ALT], aspartate aminotransferase [AST], creatine, blood urea nitrogen [BUN], and uric acid), liver NAD^+, and signs of toxicity or injury were observed once per day after NMN administration.

Mice receiving 1,640 mg/kg bw/day NMN had significantly increased hepatic NAD^+ levels compared to the control (0.7 vs. 0.01, $P<0.000182$). Body weight gain was decreased in the NMN group (0 vs. 1.5 g, $P<0.029$), as well as the liver/body weight ratio (0.045 vs. 0.05, $P<0.036$). Transcription of 74 genes was significantly altered in the liver ($P<0.05$), with lipid processes the most significantly enriched in the NMN group.

Mice receiving 2,680 mg/kg bw/day NMN had significantly increased hepatic NAD^+ compared to the control (0.9 vs. 0.01 µmol/g, $P<0.00001$). ALT (45 vs. 25 U/L, $P<0.05$) and AST levels (24 vs. 50 U/L, $P<0.05$) were increased in the NMN group compared to the control, while BUN (6.5 vs. 8.5 mmol/L, $P<0.04$), total cholesterol (TC; 1.1 vs. 1.9 mmol/L, $P<0.05$), and low-density lipoprotein cholesterol (LDL-C; 0.2 vs. 0.4 mmol/L, $P<0.01$) were decreased in the NMN group compared to the control. Histopathological examinations of the liver found no treatment-related abnormalities. The BUN concentration was slightly decreased in the NMN-treated mice, with no difference in the serum concentrations of creatinine and uric acid between the control and NMN-treated animals. The data suggested minimal adverse effects on kidney function. The changes in TC and LDL-C were considered beneficial. No other parameters were significantly different, no mortality or clinical signs were observed, and no behavioral or morphological deficits were noted.

Subacute Toxicity of NMN in Dogs

The dogs were divided into two groups (n=5) that received either 1,340 mg/day NMN or water by oral gavage. Body weight, serum biochemistry, mortality, and signs of toxicity were observed. Because NAD^+ has a very low level in plasma and urine, serum NAM, which is converted from exogenous NMN, was measured. NAM significantly increased in the NMN-treated dogs (40 vs. 1 μmol/L, $P<0.000001$). Serum creatinine (70 vs. 30 μmol/L, $P<0.02$) and uric acid (17.5 vs. 14 μmol/L, $P<0.05$) were also increased in the NMN group compared to the control. No other parameters were significantly different, with no mortality or clinical signs, and no behavioral or morphological deficits observed. The dogs were found to be in good condition throughout the study with normal autonomic activities and clean coats. The authors therefore concluded the changes were mild and minimal. This suggests a NOAEL of 1,340 mg/kg bw/day.

3.3.3.3. Subchronic Toxicity Study of Other Sources of NMN

The Study by Cros et al. (2021)

Cros et al. (2021) examined the subchronic oral toxicity of synthetic NMN (Roowin SA, France, 99.03% purity) in SD rats (aged 7 weeks, mean body weight of 161.8 g). Rats were divided into 4 groups (n=25), which received 0, 375, 750, or 1,500 mg/kg bw/day NMN by oral gavage for 90 days, followed by a 28-day treatment-free recovery period. Parameters measured included mortality, systemic clinical signs, body weight, body weight gain, food consumption, hematology, blood chemistry, and urinalysis.

Overall, rats gained 57, 55, 48, and 59% body weight for males and 40, 36, 33, and 31% for females in the 0, 375, 750, or 1500 mg/kg bw/day NMN groups, respectively. There were no significant differences in body weight noted between the control and test animals in any groups, except for the 1,500 mg/kg bw/day females, which had a 6% lower body weight compared to the controls ($P<0.05$). However, this was not considered adverse as it was minor in magnitude. There were no differences in body weight gain in any groups, except for the 1,500 mg/kg bw/day females during the 28-day recovery period. This gain compensated for reduced body weight during the test period ($P<0.05$). The MTD—the highest dose causing no more than a 10% weight gain decrement in a subchronic toxicity study—was 1,500 mg/kg bw/day. The European Medicines Agency (EMA, 2008) described the MTD as follows: “The following are considered equivalent definitions of the toxicity-based endpoint describing the maximum tolerated dose: The US Interagency Staff Group on Carcinogens has defined the MTD as follows: ‘The highest dose currently recommended is that which, when given for the duration of the chronic study, is just high enough to elicit signs of minimal toxicity without significantly altering the animal's normal lifespan due to effects other than carcinogenicity. This dose, sometimes called the maximum tolerated dose (MTD), is determined in a subchronic study (usually 90 days duration) primarily on the basis of mortality, toxicity and pathology criteria. The MTD should not produce morphologic evidence of toxicity of a severity that would interfere with the interpretation of the

study. Nor should it comprise so large a fraction of the animal's diet that the nutritional composition of the diet is altered, leading to nutritional imbalance. The MTD was initially based on a weight gain decrement observed in the subchronic study; i.e., the highest dose that caused no more than a 10% weight gain decrement."

Differences in food consumption were incidental.

Evaluations of hematological, coagulation, biochemistry, endocrine, and urinary parameters showed some isolated significant differences of small magnitudes. Among hematological parameters, white blood cell (WBC; NMN vs. control; 11.12 vs. 8.48 $10^3/\mu L$, $P<0.01$); hemoglobin (NMN vs. control; 17.85 vs. 16.75 g/dL, $P<0.05$); hematocrit (NMN vs. control; 47.01 vs. 44.66%, $P<0.05$); and mean corpuscular volume (NMN vs. control; 52.26 vs. 49.79 fL, $P<0.01$). These outcomes were significantly different from the control among males in the 1,500 mg/kg bw NMN group. Mean corpuscular volume (low-dose vs. high-dose vs. control; 7.63 vs. 7.61 vs. 7.94 fL, $P<0.05$) was significantly different from the control among females in the 375 and 1,500 mg/kg bw NMN groups (Table 15). However, these changes were mostly small in magnitude and showed no dose dependence, so they were considered incidental.

There were significant changes in liver enzymatic activity. In males, compared to the control group, there were significant increases in the activity of alkaline phosphatase (ALP; high-dose; 29%, $P<0.01$), ALT (mid-dose and high-dose; 53% and 65%, $P<0.01$), and AST (mid-dose, 29%, $P<0.05$) during the administration of NMN but not during recovery. In females, there were also significant increases in the activity of ALP (mid-dose and high-dose; 29% and 75%, $P<0.01$), ALT (low-dose, mid-dose, and high-dose; 30%, 53%, and 111%, $P<0.05$), and AST (high-dose; 17%, $P<0.001$). However, these changes were either small in magnitude or within published physiological reference ranges for SD rats (He et al., 2017). They did not persist into the recovery period.

There were no relevant macroscopic observations attributable to NMN administration. There were increases in some absolute organ weights in males: adrenal glands (low-dose and mid-dose; 23% and 32%, $P<0.05$), kidneys (mid-dose and high-dose; 10% and 17%, $P<0.05$), liver (high-dose; 28%, $P<0.05$), and thyroid/parathyroid (high-dose; 15%, $P<0.05$). There were decreases in some organ weights in females: pituitary glands (low-dose and mid-dose; -19%, $P<0.05$) and spleens (low-dose, mid-dose, and high-dose; -19%, -19%, and -20%, $P<0.05$).

There were some significant increases in the organ weight/body weight ratio: kidneys (mid-dose and high-dose; 12% and 22%, $P<0.001$), livers (high-dose; 12%, $P<0.001$), and adrenal glands (low-dose and mid-dose; 20% and 30%, $P<0.05$). There were no differences in the female groups. However, all significant differences in absolute and relative organ weights disappeared during the recovery period.

There were some treatment-related histological alterations observed in the liver, kidneys, harderian glands, and other organs. There was centrilobular hepatocellular hypertrophy observed in 5 of 10 males in the high-dose group at a low severity (mean severity of 1.4 out of a scale of 4) that correlates to the slight increase in liver weights at this dose level. This effect vanished during the recovery period. There was lymphocyte infiltration in all groups, but such infiltration disappeared during recovery in all groups except the 1,500 mg/kg males.

Low grade chronic progressive nephropathy was observed in the 750 mg/kg bw/day (mean severity of 1.2 out of 4) and 1,500 mg/kg bw/day males (mean severity of 1.4 out of 4). This effect persisted through recovery, but only in the 1,500 mg/kg group. These changes affected the kidneys, but did not show alterations in the clinical chemistry of serum and urine that correlated with the degree of severity of chronic progressive nephropathy, such as hypoalbuminemia and albuminuria. In addition, neither chronic progressive nephropathy nor higher kidney weights were found in all female groups. Therefore, the observed chronic progressive nephropathy should be considered non-adverse given the very low severity and the absence of associated clinical pathology changes.

The authors stated the liver changes at 1,500 mg/kg bw/day were considered non-adverse. They deemed them of a metabolic nature based on the absence of associated liver dysfunction as measured by liver function indicator enzymes, degeneration, and/or necrosis. Similarly, there was an absence of chronic progressive nephropathy, and no associated clinical pathology changes. The condition was also rat specific, so it was also deemed non-adverse. Similarly, lymphocyte infiltration of harderian glands was not associated with clinical manifestations or signs of inflammation, and was also considered non-adverse.

The authors stated that their experimental results allowed determination of the NOAEL for NMN at 1,500 mg/kg/day, the highest level tested.

Table 14. Clinical Biochemistry in a Subchronic Toxicity Study of NMN in Rats

Parameter	Groups (mg/kg)			
	0	375	750	1,500
Males				
ALP, U/L	65.26±11.63	66.06±4.96	74.69±10.52	84.40±16.77**
ALT, U/L	35.25±9.82	37.49±7.00	53.86±20.42**	58.14±6.99***
AST, U/L	70.54±10.01	71.15±6.72	87.09±21.96*	82.89±9.27
Glucose, mmol/L	9.59±2.84	11.29±1.93	11.12±1.40	10.43±2.87
Urea, mmol/L	5.13±0.60	5.33±0.94	5.11±0.75	5.76±0.70
Creatinine, µmol/L	31.09±2.28	31.32±2.70	29.28±2.94	28.54±3.30
Cholesterol, mmol/L	2.46±0.27	2.05±0.21*	2.07±0.39*	2.34±0.44

Triglyceride, mmol/L	0.43±0.09	0.69±0.16	0.59±0.12	0.64±0.41
Total protein, g/L	63.44±1.83	60.48±1.41*	61.96±2.38	62.89±3.21
Albumin, g/L	42.46±1.33	41.74±1.05	43.06±2.58	44.34±1.99
Globulins, g/L	20.98±1.23	18.76±1.16**	18.91±1.88**	18.52±1.45**
Total bilirubin, μmol/L	1.31±0.40	1.11±0.40	0.99±0.31	1.07±0.54
Calcium, mmol/L	2.56±0.02	2.51±0.03*	2.48±0.04***	2.49±0.05***
Sodium, mmol/L	139.96±2.24	139.51±2.34	139.3±3.44	139.56±2.63
Potassium, mmol/L	5.11±0.42	5.85±0.54**	5.66±0.48*	5.83±0.55**
Chloride, mmol/L	100.64±1.97	98.53±2.25*	98.31±1.69*	97.92±1.46**
Inorganic phophorus, mmol/L	1.84±0.11	1.94±0.22	1.79±0.20	1.62±0.31
Blood urea nitrogen, mmol/L	2.39±0.28	2.49±0.22	2.39±0.35	2.69±0.33
LDL, mmol/L	1.21±0.57	1.03±0.55	0.99±0.66	1.06±0.55
HDL, mmol/L	4.76±10.99	0.95±0.55	1.01±0.56	1.21±0.73
Females				
ALP, U/L	37.87±4.42	46.03±5.38	49.02±11.35**	66.77±8.31***
ALT, U/L	24.10±5.19	31.76±4.09**	36.98±6.22***	50.82±5.21***
AST, U/L	67.45±9.43	68.15±2.31	73.56±6.24	79.43±5.71***
Glucose, mmol/L	7.55±0.58	9.62±1.33***	9.40±1.08***	8.85±0.91*
Urea, mmol/L	6.47±0.69	4.77±0.6***	5.13±0.74***	5.63±0.81*
Creatinine, μmol/L	39.00±4.69	36.56±2.38	35.28±2.85	33.44±2.05**
Cholesterol, mmol/L	2.94±0.50	2.66±0.38	2.57±0.30	2.55±0.26
Triglyceride, mmol/L	0.44±0.13	0.67±0.09*	0.68±0.18**	0.75±0.23***
Total protein, g/L	62.77±1.11	60.48±1.04**	60.08±2.40**	61.08±1.30
Albumin, g/L	47.25±1.34	46.72±1.10	45.25±1.88*	47.02±1.37
Globulins, g/L	15.53±1.14	13.77±1.02**	14.83±1.13	14.05±1.29*
Total bilirubin, μmol/L	1.74±0.28	1.48±0.29	1.34±0.38*	2.14±0.49
Calcium, mmol/L	2.61±0.07	2.56±0.03	2.55±0.05	2.53±0.04**
Sodium, mmol/L	140.2±1.02	140.2±1.11	139.95±1.07	140.33±1.09
Potassium, mmol/L	4.56±0.44	4.64±0.22	4.73±0.29	4.75±0.25
Chloride, mmol/L	102.29±1.64	101.18±1.94	101.54±2.07	101.44±2.70
Inorganic phosphorus, mmol/L	1.7±0.15	1.57±0.21	1.52±0.19	1.37±0.20**
Blood urea nitrogen, mmol/L	3.02±0.32	2.23±0.28***	2.39±0.35***	2.63±0.38*
LDL, mmol/L	1.36±1.01	1.28±0.94	1.22±0.86	1.31±0.81
HDL, mmol/L	1.40±1.05	1.29±1.01	1.23±0.92	1.10±0.95

n = 9-10/group.

*p<0.05; **p<0.01; ***p<0.001.
ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; HDL = high-density lipoproteins; LDL = low-density lipoproteins.

Table 15. Hematology in a Subchronic Toxicity Study of NMN in Rats

Parameter	Groups (mg/kg)			
	0	375	750	1,500
Males				
RBC, 10^6/μL	8.97±0.38	8.84±0.31	9.08±0.38	9.01±0.42
WBC, 10^3/μL	8.48±1.44	9.72±1.28	10.12±2.22	11.12±1.52**
HGB, g/dL	16.75±0.80	16.44±0.78	17.34±0.74	17.85±1.15*
HCT, %,	44.66±2.17	44.04±2.21	46.05±2.49	47.01±1.35*
MCV, fL	49.79±1.55	49.8±1.51	50.71±1.31	52.26±1.53**
MCH, pg	18.69±1.00	18.62±1.15	19.14±1.16	19.86±1.50
MCHC, d/dL	37.59±2.67	37.41±2.31	37.76±2.78	37.98±2.36
PLT, 10^3/μL	914.8±104.6	839.3±96.9	832.5±29.5	867.0±43.5
MPV, fL	7.78±0.16	7.74±0.25	7.68±0.18	7.67±0.29
Reticulocyte, %	4.60±0.63	4.29±1.16	4.90±0.67	5.10±1.30
Females				
RBC, 10^6/μL	8.52±1.15	8.16±0.20	8.18±0.43	8.14±0.56
WBC, 10^3/μL	7.06±1.50	6.49±1.08	6.20±1.61	5.57±1.99
HGB, g/dL	16.73±2.61	16.13±0.57	16.21±0.60	16.33±0.45
HCT, %,	45.26±7.62	43.42±2.25	43.65±2.08	44.01±2.88
MCV, fL	52.95±2.04	53.23±1.99	53.40±1.70	54.12±2.11
MCH, pg	19.60±0.91	19.80±0.84	19.86±1.17	20.13±1.38
MCHC, d/dL	37.06±2.21	37.27±2.54	37.19±2.08	37.24±2.31
PLT, 10^3/μL	897.8±105.1	842.0±27.6	860.5±43.0	836.4±48.5
MPV, fL	7.94±0.20	7.63±0.14*	7.77±0.34	7.61±0.35*
Reticulocyte, %	5.48±1.90	5.50±0.80	5.14±0.78	5.53±1.01

Adopted from Cros et al., 2021. n = 9-10/group.
*p<0.05; **p<0.01; ***p<0.001
HCT = hematocrit; HGB = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MPV = mean platelet volume; PLT = platelet count; RBC = red blood cell count; WBC = white blood cell count.

Table 16. Absolute Organ Weights in a Subchronic Toxicity Study of NMN in Rats

Parameter, g	Groups (mg/kg)			
	0	375	750	1,500
Males				
Body weight	398.00±25.35	408.83±17.35	391.27±26.64	382.38±16.21
Liver	12.11±4.03	13.79±1.23	13.80±1.55	15.48±1.73*
Kidneys	2.85±0.16	2.94±0.15	3.14±0.16*	3.33±0.34***
Thyroid/ Parathyroid	0.020±0.006	0.030±0.011	0.030±0.016	0.023±0.004*
Adrenal glands	0.060±0.007	0.074±0.013*	0.079±0.016**	0.070±0.010
Brain	2.04±0.11	2.10±0.10	2.08±0.13	2.03±0.10
Heart	1.56±0.17	1.47±0.09	1.51±0.20	1.55±0.12
Spleen	0.75±0.10	0.81±0.04	0.80±0.11	0.73±0.27
Thymus	0.250±0.056	0.280±0.111	0.270±0.053	0.210±0.050
Epididymides	1.400±0.189	1.530±0.247	1.620±0.179	1.450±0.145
Testes	3.77±0.31	3.87±0.22	3.81±1.21	3.88±0.20
Females				
Body weight	251.72±14.04	243.83±11.05	239.74±14.52	231.90±11.0**
Spleen	0.65±0.12	0.59±0.06	0.58±0.11	0.52±0.05*
Pituitary gland	0.016±0.003	0.013±0.001*	0.013±0.003*	0.013±0.002
Brain	1.85±0.07	1.90±0.08	1.82±0.24	1.77±0.05
Heart	1.05±0.07	1.10±0.17	1.06±0.08	0.96±0.08
Liver	7.99±0.60	7.61±0.93	7.74±0.51	7.67±0.62
Kidneys	2.20±1.43	1.81±0.12	1.85±0.15	1.88±0.14
Thymus	0.252±0.066	0.252±0.019	0.244±0.049	0.227±0.051
Adrenal glands	0.068±0.011	0.080±0.015	0.083±0.015	0.077±0.038
Ovaries & oviducts	0.204±0.163	0.150±0.027	0.175±0.039	0.143±0.017
Uterus & cervix	0.67±0.29	0.97±0.41	0.73±0.36	0.64±0.25

Adopted from Cros et al., 2021. *p<0.05; **p<0.01; ***p<0.001

Table 17. Relative Organ Weight (to body weight) in a Subchronic Toxicity Study of NMN in Rats

Parameter, g/100 g	Groups (mg/kg)			
	0	375	750	1,500
Males				
Liver	3.016±0.954	3.379±0.329	3.526±0.294	4.042±0.339***
Kidneys	0.718±0.035	0.720±0.040	0.803±0.036***	0.874±0.057***
Adrenals	0.015±0.001	0.018±0.003*	0.020±0.004***	0.017±0.002
Brain	0.514±0.036	0.515±0.036	0.534±0.045	0.531±0.040
Heart	0.392±0.039	0.360±0.025	0.386±0.042	0.404±0.020
Spleen	0.188±0.019	0.199±1.008	0.205±0.022	0.189±0.070
Thymus	0.064±0.012	0.067±0.026	0.070±0.015	0.056±0.011
Thyroid/ Parathyroids	0.005±0.001	0.007±0.003	0.009±0.004*	0.006±0.001
Pituitary	0.003±0.001	0.004±0.001	0.003±0.001	0.004±0.001

Epididymides	0.354±0.054	0.374±0.055	0.416±0.053	0.379±0.047
Testes	0.951±0.108	0.947±0.057	0.973±0.308	1.017±0.068
Females				
Brain	0.738±0.054	0.781±0.054	0.761±0.11	0.766±0.031
Heart	0.416±0.039	0.452±0.062	0.441±0.03	0.412±0.03
Liver	3.177±0.206	3.115±0.277	3.239±0.272	3.302±0.16
Spleen	0.257±0.043	0.242±0.022	0.240±0.042	0.225±0.024
Kidneys	0.877±0.577	0.744±0.047	0.773±0.045	0.813±0.057
Thymus	0.100±0.025	0.103±0.008	0.102±0.021	0.098±0.023
Thyroids/ Parathyroids	0.007±0.002	0.010±0.004	0.010±0.004	0.007±0.003
Adrenals	0.027±0.005	0.033±0.006	0.034±0.006	0.034±0.019
Pituitary	0.006±0.001	0.005±0.000	0.005±0.001	0.006±0.001
Uterus & Cervix	0.269±0.127	0.401±0.170	0.304±0.140	0.278±0.109
Ovaries & Oviducts	0.081±0.063	0.061±0.011	0.073±0.014	0.062±0.006

Adopted from Cros et al., 2021. *p<0.05; ***p<0.001

Table 18. Histology in a Subchronic Toxicity Study of NMN in Rats

	Groups							
	Vehicle		375 mg/kg		750 mg/kg		1,500 mg/kg	
	M	F	M	F	M	F	M	F
Liver – Centrilobular hepatocellular hypertrophy	-	-	-	-	-	-	5/1.4*	-
Kidneys – Chronic progressive nephropathy	-	-	-	-	5/1.2	-	8/1.4	-
Harderian glands – Lymphocyte infiltration	3/1.0	1/1.0	3/1.3	3/2.0	7/2.1	6/1.8	9/2.3	7/2.6

Adopted from Cros et al., 2021. n = 10/sex/group
*total affected/mean severity grade. Severity scale: Grade 1 = minimal/very few/very small; Grade 2 = slight/few/small; Grade 3 = moderate, moderate number/size; Grade 4 = severe, important number/large size. F = females; M = males.

3.3.3.4. Chronic Toxicity Study of Other Sources of NMN

Mills et al. (2016) determined the effects of NMN on pathophysiological changes. Three-month-old C57BL/6N mice were orally administered 0 (control), 100, or 300 mg/kg bw/day NMN (96-97% pure; Oriental Yeast Co., Japan) in drinking water for 12 months. Plasma NMN and tissue NAD^+ levels, age-associated body weight gain, energy metabolism, physical activity, insulin sensitivity, plasma lipid profile, mitochondrial respiratory capacity in skeletal muscle, eye function, bone density, and myeloid-lymphoid composition were determined. No adverse effects were reported on measured outcomes.

Summary of Toxicity Studies

For the purpose of safety evaluation, the NOAEL of 1,500 mg/kg bw/day was chosen from a subchronic toxicity study of NMN. Using the safety margin of 100, the acceptable daily intake (ADI) was calculated as 1,050 mg/person/day for an adult weighing 70 kg (please note that an average American weighs 83 kg).

References for 3.3.3.

Appendix K. Acute Toxicity Study of SyncoZymes' NMN.

Altug T. 2003. Introduction to Toxicology and Food. Boca Raton, FL: CRC Press.

Conze DB, Crespo-Barreto J, Kruger CL. Safety assessment of nicotinamide riboside, a form of vitamin B3. Hum Exp Toxicol. 2016;35:1149-60.

Cros C, Cannelle H, Laganier L, Grozio A, Canault M. Safety evaluation after acute and sub-chronic oral administration of high purity nicotinamide mononucleotide (NMN-C®) in Sprague-Dawley rats. Food Chem Toxicol. 2021;150:112060.

European Medicines Agency (EMA). ICH topic S1C(R2): Step 5. Note for guidance on dose selection for carcinogenicity studies of pharmaceuticals (EMEA/CHMP/ICH/383/1995). 2008.

He Q, Su G, Liu K, Zhang F, Jiang Y, Gao J, Liu L, Jiang Z, Jin M, Xie H. Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. PLoS One. 2017;12:e0189837.

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 2016;24:795-806.

You Y, Gao Y, Wang H, Li J, Zhang X, Zhu Z, Liu N. Subacute toxicity study of nicotinamide mononucleotide via oral administration. Front Pharmacol. 2020;11:604404.

3.3.4. Animal Toxicity Studies of NR
CONFIDENTIAL

The NOAELs for NR were determined to be 500 mg/kg bw/day for male rats and 1,200 mg/kg bw/day for female rats. The differences in NOAEL values are from different doses used in the studies of NMN.

3.3.4.1. Acute Toxicity Study of NR

Conze et al. (2016) determined the safety of Niagen™ (>99% NR, ChromaDex, Inc.) in an acute toxicity study. Male and female SD rats (n=5/sex/group) received a single dose of either the vehicle (water) or 5,000 mg/kg bw NR via oral gavage and were observed for 14 days. No mortalities, clinical signs, or gross pathological changes were noted. There were also no significant differences in body weight or food consumption. Compared to the vehicle control, the cumulative body weight was significantly decreased in females receiving NR, which was treatment-related, but not considered adverse because the change was minimal (-3%).

3.3.4.2. Subacute Toxicity Studies of NR

Conze et al. (2016) also evaluated the safety of Niagen™ in a 14-day subacute toxicity study. Male and female SD rats (n=5/sex/group) received either vehicle (water), 750, 1,500, 2,500, or 5,000 mg/kg bw/day NR by oral gavage. Compared to the vehicle group, the mean body weight was decreased by 7-8% with 2,500 mg/kg bw/day NR and 8-9% with 5,000 mg/kg bw/day NR in males. Also, overall feed consumption was decreased by 8% with 5,000 mg/kg bw/day NR in males. There were no test-related changes in body weight or feed consumption in females. No gross pathological lesions were observed in any group. Based on these results, 300, 1,000, and 3,000 mg/kg bw/day NR doses were chosen for the subchronic study in rats.

In an unpublished 7-day dose range finding study by Thorsrud (2017; cited in EFSA, 2019), NR doses at 100, 300, or 1,000 mg/kg bw/day were administered to juvenile beagle dogs by oral gavage. Toxicologically relevant findings were not observed in any of the groups.

In an unpublished 28-day repeated dose toxicity study by Thorsrud (2018; cited in EFSA, 2019), juvenile beagle dogs received 0, 100, 300, or 1,000 mg/kg bw/day NR by oral gavage for 28 days. The high dose was decreased from 1,000 to 500 mg/kg bw/day on day 9 for females and on day 10 for males due to reduced body weights and adverse clinical findings, such as salivation after dosing, abdominal contractions, diarrhea, and vomiting. After lowering the high dose to 500 mg/kg bw/day, salivation and vomiting still occurred. The 500 mg/kg bw/day dose decreased blood glucose, sodium, and potassium concentrations, prothrombin time, eosinophils, and absolute and relative testes and thyroid weights in males. In females, the 500 mg/kg bw/day dose increased AST and decreased phosphate, albumin, and fibrin in blood. The changes to glucose

with 300 mg/kg bw/day and prothrombin time with 100 and 300 mg/kg bw/day were not pronounced and not dose-related. The NOAEL was 300 mg/kg bw/day for juvenile dogs.

3.3.4.3. Subchronic Toxicity Studies of NR

A Study by Conze et al. (2016)

Conze et al. (2016) performed a 90-day subchronic toxicity study in male and female SD rats (n=10/sex/group) where they were administered vehicle (water), 300, 1,000, or 3,000 mg/kg bw/day NR or 1,260 mg/kg bw/day NAM (the positive control was equivalent to 3,000 mg/kg bw/day NR on a molar basis). Treatment-related mortality and clinical signs were not reported. The toxicity profiles of the high dose NR (3,000 mg/kg bw/day) and NAM observed in body weight, feed consumption, hematology, clinical chemistry, urinalysis, gross pathology, and histopathological findings were statistically similar.

Males displayed significant treatment-related decreases in body weight (17% reduction) in the 3,000 mg/kg bw/day and NAM groups. Furthermore, decreased feed consumption was observed in the male 3,000 mg/kg bw/day NR (9-14%) and NAM groups (9-17%). No significant differences in body weight were observed in the female NR and NAM groups. Changes in clinical chemistry parameters included increased plasma ALT (87%) and ALP (32%) in the male 3,000 mg/kg bw/day NR group and increased plasma ALT (116%), ALP (75%), and gamma-glutamyl transferase (59%) in the female 3,000 mg/kg bw/day NR group. These changes were associated with increased liver weight (87% in males and 21% in females), centrilobular hepatocellular hypertrophy, and single cell necrosis. Significant but minor reductions in plasma chloride (4.1% in males and 4% in females) and sodium (2.4% in females) were associated with hypertrophy of the zona glomerulosa in adrenals with the 3,000 mg/kg bw/day NR dose. Similar changes were observed with the NAM dose.

Treatment-related adverse effects were not observed with the lower doses of NR (300 and 1,000 mg/kg bw/day NR). In males, both lower doses reduced body weight, but were not considered adverse since the changes were less than 10%. The 1,000 mg/kg bw/day dose caused treatment-related in changes in weight (8.2%) and kidney weight (8.7%). Because there was an in the absence of corresponding histopathology in males, these changes were considered mild and adaptive. In females, there were no observed significant differences in body weights in the NR and NAM groups. The 1,000 mg/kg bw/day NR dose caused a treatment-related change in the liver weight in females (13.5%) but again, these were considered mild and adaptive. Increases in neutrophils (75%), ALT (25%), and triglyceride (TG; 65%) were also observed in the female 1,000 mg/kg bw/day group. However, changes in ALT and TG only occurred in one gender and were below the two-fold increase cutoff for biologically significant effects in the absence of histological results (Hall et al., 2012). Therefore, the liver and kidney effects at 1,000

mg/kg/day were considered to be treatment-related, but mild and potentially adaptive in nature due to prolonged exposure to this form of niacin.

There were no treatment-related adverse effects noted at 300 mg/kg/day, although there was a slight decrease (8%) in overall body weight (day 90) at 300 mg/kg/day. This was considered adaptive. The authors concluded that the NOAEL for NR was 300 mg/kg bw/day, and the lowest-observed-adverse-effect-level (LOAEL) was 1,000 mg/kg bw/day.

A Study by Marinescu et al. (2020)

Marinescu et al. (2020) investigated the safety of a highly pure, synthetic NR (>97% pure) in a 90-day oral toxicity study. SD rats consumed either vehicle control or 300, 500, or 1,200 mg/kg bw/day NR for 90 days. No mortality or clinical observations occurred at any dose during the study. In males, a small but significant decrease in body weight was observed on day 92 with the 1,200 mg/kg bw/day dose. There were no test substance-related clinical observations ophthalmological parameters, hematology, coagulation, urinalysis, and macro- and microscopic histopathology findings in males or females. Test substance-related decreases with no statistical significance were observed in food consumption and food efficiency in the high-dose male group only. However, body weights on day 92 (approximately 13% reduction) were significantly lower in the high-dose males only. However, no significant differences were noted during the recovery period. No changes in these parameters and in weekly body weights were observed in the female rats. Therefore, the NOAEL was determined to be 500 mg/kg bw/day for male rats and 1,200 mg/kg bw/day for female rats.

3.3.4.4. Reproductive and Developmental Toxicity Studies of NR

In an unpublished one-generation reproductive study by Ganiger et al. (2016; cited in EFSA, 2019), SD rats consumed diets containing 0, 3,000, 6,000 or 12,000 mg/kg feed of NR (i.e., 169, 334, or 675 mg/kg bw/day in males and 273, 543, or 1,088 mg/kg bw/day in females, respectively) before mating, during pregnancy, and through weaning. The 12,000 mg/kg feed dose lowered the body weight in males on days 43-92 by 4.6-6.5% compared to the control. This change was not considered adverse since it was a weak effect. Other endpoints, such as precoital time, gestation length, fertility parameters, pathological and histopathological examinations of the reproductive organs of adult rats, survival, and abnormalities in life and death, were not affected. In rats, the NOAEL for NR was 12,000 mg/kg feed, corresponding to 675 mg/kg bw/day in males and 1,088 mg/kg bw/day in females.

In an unpublished embryo-fetal developmental toxicity study by Geetha Rao (2016; cited in EFSA, 2019), pregnant SD rats received 0, 325, 750, or 1,500 mg/kg bw/day NR by oral gavage on gestation days 5-19. Gross pathological changes were examined in dams after caesarean section. Sex, weight, and external, visceral, and skeletal malformations were examined

in fetuses. The 750 and 1,500 mg/kg bw/day doses decreased maternal feed consumption by 6 and 12%, maternal body weight by 4 and 8%, maternal body weight gain by 11 and 28%, and maternal corrected body weight gain by 55 and 107%, respectively, during gestation when compared to the control. These doses also decreased mean fetal weight by 6 and 17%, respectively. Gravid uterine weight was decreased by 13% in the 1,500 mg/kg bw/day NR group. A dose-dependent increase was observed for late resorptions with statistical significance at 1,500 mg/kg bw/day (0.05, 0.29, 0.48, and 0.52%). These changes were considered secondary to maternal toxicity. Increased incidences of fetal anasarca were observed at 1,500 mg/kg bw/day as well as incidences of 2 small fetuses, a fetus with flexed right forelimb, and a fetus with thread-like tail. Dose-dependent increases were observed for delayed, incomplete, or poor ossification in fetuses with statistical significance at 1,500 mg/kg bw/day. Minor anomalies included increases in extra, accessory, and rudimentary ribs with statistical significance at 1,500 mg/kg bw/day (10.32, 10.26, 16.47, and 30.72% of fetuses) and hypoplastic sternum with statistical significance at 750 and 1,500 mg/kg bw/day (0, 1.28, 4.12, and 24.84% of fetuses). However, the incidence at 750 mg/kg bw/day was considered not adverse since it was within the historical control range. These skeletal findings were often associated with general toxicity or reduced body weight gain of dams. The NOAEL for maternal toxicity and embryo/fetotoxicity was 325 mg/kg bw/day.

Table 19. Animal Toxicity Studies of NR

Species	Dose	Duration	LD_{50} or NOAEL	Reference
SD rats	0 or 5,000 mg/kg bw NR (ChromaDex, US)	Single dose	Morbidity, mortality, clinical signs, body weights, food consumption	Conze et al., 2016
	0, 750, 1,500, 2,500, or 5,000 mg/kg bw/d NR (ChromaDex, US)	14 d		
	0, 300, 1,000, or 3,000 mg/kg bw/d NR (ChromaDex, US)	90 d	LOAEL = 1,000 mg/kg bw/d; NOAEL = 300 mg/kg bw /d	
Beagle dogs	0, 100, 300, or 1,000 mg/kg bw/d NR	7 d	No toxicologically relevant findings	Thorsrud, 2017; cited in EFSA, 2019
Beagle dogs	0, 100, 300, or 1,000 mg/kg bw/d NR	28 d	NOAEL = 300 mg/kg bw/d	
SD rats	0, 300, 500, or 1,200 mg/kg bw/d NR (synthetic NR, Elysium Health, US)	90 d	NOAEL = 500 mg/kg bw/d (males); 1,200 mg/kg bw/d (females)	Marinescu et al., 2020
SD rats	0, 3,000, 6,000, or 12,000 mg/kg feed of NR (195, 334, or 675 mg/kg bw/d for males; 273, 543, or 1,088 mg/kg bw/d for females)	Before mating, during pregnancy, and	NOAEL = 675 mg/kg bw/d (males); 1,088 mg/kg bw/d (females)	Ganiger, 2016; cited in EFSA, 2019

		through weaning		
Pregnant SD rats	0, 325, 750, or 1,500 mg/kg bw/d NR	Gestation d 5-19	NOAEL = 325 mg/kg bw/d	Geetha Rao, 2016; cited in EFSA, 2019

bw = body weight; d = days; LOAEL = lowest observed adverse effect level; mo = months; NOAEL = no observed adverse effect level; NR = nicotinamide riboside.

References for 3.3.4.

Conze DB, Crespo-Barreto J, Kruger CL. Safety assessment of nicotinamide riboside, a form of vitamin B3. Hum Exp Toxicol. 2016;35:1149-60.

EFSA Panel on Nutrition, Novel foods and Food allergens (NDA), Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Engel KH, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Pöting A, Poulsen M, Sanz Y, Schlatter JR, van Loveren Agnès de Sesmaisons-Lecarré H, Germini A, Knutsen HK. Safety of nicotinamide riboside chloride as a novel food pursuant to Regulation (EU) 2015/2283 and bioavailability of nicotinamide from this source, in the context of Directive 2002/46/EC. EFSA J. 2019;17:e05775.

Ganiger S (Advinus Therapeutics Limited), 2016. Nicotinamide riboside chloride: One generation reproduction toxicity study through diet in Sprague-Dawley rats. Study No G10959, 313 pp. [Unpublished; cited in EFSA, 2019].

Geetha Rao G (Advinus Therapeutics Limited), 2016. Nicotinamide riboside chloride: Embryo-fetal developmental toxicity study in Sprague Dawley rats by oral route. Study No G10957. Unpublished Study Report, 319 pp. [Unpublished; cited in EFSA, 2019].

Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A, Küttler K, Malarkey DE, Maronpot RR, Nishikawa A, Nolte T, Schulte A, Strauss V, York MJ. Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol. 2012;40:971–94.

Marinescu AG, Chen J, Holmes HE, Guarente L, Mendes O, Morris M, Dellinger RW. Safety assessment of high-purity, synthetic nicotinamide riboside (NR-E) in a 90-day repeated dose oral toxicity study, with a 28-day recovery arm. Int J Toxicol. 2020;39:307-20.

Thorsrud B (Experimur), 2017. Oral 7-day dose range finding toxicity study in juvenile dogs with NIAGEN®. Experimur Study No. 17-921. Unpublished Study Report. 172 pp. [cited in EFSA, 2019].

Thorsrud B (Experimur), 2018. Oral 28-day toxicity study in juvenile dogs with NIAGEN®. Experimur Study No. 17-940. Unpublished Study Report. 388 pp. [cited in EFSA, 2019].

3.4. Animal Efficacy Studies of NMN and NR
CONFIDENTIAL

To identify other data and information relevant to the safety of NMN, a comprehensive search of published scientific literature was conducted through November 2021. Published studies identified during the literature search consisted of efficacy studies relating to glucose and lipid metabolism of NMN, endothelial dysfunction, oxidative stress, and other parameters. Although these studies were designed to investigate the efficacy of NMN on various health parameters, several safety-related endpoints were obtained during the experiments. Therefore, these studies are reviewed below as additional supporting information. None of the animal efficacy studies reported adverse effects of NMN. For these 'pivotal' studies, the dose levels represent the maximum doses administered, rather than the absolute safety endpoints. Our review does not include studies that evaluated ameliorating efficacy after chemical induced toxicity since such studies are not relevant when evaluating the safety of a substance. In addition, our review is limited to the studies of orally given NMN. No other routes of administration are included.

3.4.1. Animal Efficacy Studies of NMN
CONFIDENTIAL

A literature search identified a few mouse studies evaluating the effects of oral NMN supplementation (Table 20: de Picciotto et al., 2016; Mills et al., 2016; Stromsdorfer et al., 2016; Youngson et al., 2019). Although these studies were designed to investigate the efficacy of NMN on various health parameters, several safety-related endpoints were obtained during the experiments. Therefore, these studies are reviewed below as additional supporting information. These efficacy studies did not observe AEs with NMN doses up to 300 mg/kg bw/day for up to 12 months or 500 mg/kg bw/day for 2 months.

Yu et al. (2021) examined the combined effects of NMN (GeneHarbor, Hong Kong) and treadmill exercise in female C57BL/6J mice with established obesity after 10 weeks of diet. Five-week-old female C57BL/6J mice were exposed to a control diet or high-fat diet (HFD). Mice fed a HFD received one of the following 4 treatments for 8 weeks: 1) untreated (HFD), 2) NMN in drinking water (400 mg/kg bw), 3) treadmill exercise 6 days/week, or 4) both exercise and NMN. Measurements included glucose tolerance, glucose-stimulated insulin secretion, and gene expression (*Tnfa* and *Tlr4* for mitochondrial function, *Gpx4* for glutathione peroxidase, *Nox4* for nicotinamide adenine dinucleotide phosphate hydrogen [NADPH] oxidation) in islets, anthropometric measures, hepatic concentrations of TG, NAD^+, and nicotinamide adenine dinucleotide hydrogen (NADH) as well as mitochondrial deoxyribonucleic acid (DNA) content, gene expression (*Pgc1a* for expression of antioxidants, *Ppara* for fat metabolism, *Sod2* and *Nox4*

for antioxidative capacity, and *Nrf2* for the thioredoxin pathway) and lipid peroxidation in skeletal muscle. No adverse effects of NMN were reported.

de Picciotto et al. (2016) determined whether NMN supplement could increase arterial sirtuin-1 (SIRT1) activity and reverse age-associated arterial dysfunction and oxidative stress in old mice. Young (aged 4-8 months old) and old (aged 26-28 months old) C57Bl/6 mice, fed a normal rodent chow *ad libitum*, consumed either control or 300 mg/kg bw/day NMN (Sigma-Aldrich Corp., US) in drinking water for 8 weeks. The effects of NMN supplement were determined on endothelial dysfunction (measured by carotid artery endothelium-dependent dilation [EDD], nitric oxide [NO]-mediated EDD, and endothelium-independent EDD), large elastic arterial stiffness (measured by aortic pulse wave velocity, total collagen-1, elastin, and elastic modulus), vascular oxidative stress (measured by aortic superoxide production and nitrotyrosine abundance), and SIRT1 activity in the aorta. No adverse effects were reported on the measured outcomes.

In the study by Stromsdorfer et al. (2016), adipocyte-specific Nampt knockout (ANKO) mice, generated by using *adiponectin*-Cre transgenic mice and floxed-*Nampt* mice, received one of the following treatments: 0 or 500 mg/kg bw/day of NMN (Oriental Yeast) in the drinking water from weaning to up to 2 months of age (or for 4-6 weeks total). For the rosiglitazone rescue model, 20 mg/kg bw/day of rosiglitazone (peroxisome proliferator-activated receptor gamma [PPARγ] agonist to improve insulin sensitivity) was administered for 7 weeks from weaning. The effects of NMN consumption were evaluated on glucose metabolism (as measured by intraperitoneal glucose tolerance tests and insulin tolerance tests of insulin resistance in multiple metabolic organs), adipose tissue dysfunction (characterized by insulin-stimulated glucose uptake in visceral adipose tissue (VAT), hepatic and plasma TG, plasma free fatty acid, plasma concentrations of adiponectin and adipsin, and VAT gene expression of inflammatory markers), phosphorylation of cyclin-dependent kinase 5 and PPARγ, gene expression of obesity-like phosphorylated PPARγ targets, and lysine acetylation of nuclear proteins in VAT. No adverse effects were reported on the measured outcomes.

Mills et al. (2016) examined whether long-term administration of NMN had preventive effects on age-associated pathophysiological changes. Male C57BL/6N mice (aged 5 months), fed a regular chow diet *ad libitum*, consumed either control or 100, or 300 mg/kg bw/day NMN (Oriental Yeast Co., 96-97% pure) in drinking water until 17 months old (or for 12 months total) in mice. Plasma NMN and NAD^+ levels were determined in the liver, skeletal muscle, and adipose tissue. Pathophysiological assessments included plasma NMN and NAD^+ levels in peripheral tissues (skeletal muscle, white adipose tissue, and liver); age-associated body weight gain; energy metabolism, physical activity, insulin sensitivity, plasma lipid profile, and eye function; age-associated gene expression changes in peripheral tissues, mitochondrial oxidative

metabolism and mitonuclear protein imbalance in skeletal muscle, and bone density. No adverse effects were reported on the measured outcomes.

Overall, daily NMN doses up to 500 mg/kg bw/day were well tolerated with no side effects in mice. In particular, daily NMN dose of 300 mg/kg bw/day for 12 months had no adverse effects on indicators of glucose and lipid metabolism, age-associated body weight gain, eye function, age-associated gene expression changes in peripheral tissues, mitochondrial respiratory capacity in the skeletal muscle, bone mineral density, and myeloid-lymphoid composition in mice. The data indicate that daily doses of 300 mg/person/day would be well tolerated in humans after considering a safety margin of 100.

Table 20. Animal Efficacy Studies of Orally Administered NMN

Objective (to test NMN effects on)	Animal	Dose	Duration	Measurement Endpoints	Reference
To test effects of NMN and treadmill exercise on metabolic parameters associated with diet-induced obesity.	Female C57BL/6J mice; control or obese mice after consuming standard chow or HFD, respectively, for 10 weeks	5 groups: standard chow control and 4 HFD groups; Untreated, 2) NMN in drinking water, 3) exercise, 4) both NMN and exercise. NMN=400 mg/kg bw	8 wk	Body composition; respiratory quotient, resting energy expenditure; glucose tolerance, glucose-stimulated insulin secretion and gene expression in islets, anthropometric measures, hepatic concentrations of TG, NAD, and NADH as well as mitochondrial DNA content, gene expression, and lipid peroxidation in skeletal muscle	Yu et al., 2021
Age-associated arterial dysfunction and oxidative stress, and arterial SIRT1 activity	Young (4-8 mo) and old (26-28 mo) C57Bl/6 male mice fed normal rodent chow *ad libitum*	4 groups: 0 (control young and old animals) or 300 mg/kg bw/d NMN (young and old animals) (Sigma-Aldrich Corp., USA) in drinking water	8 wk	Endothelial dysfunction (measured by carotid artery endothelium-dependent dilation [EDD], nitric oxide-mediated EDD, and endothelium-independent EDD), large elastic arterial stiffness, vascular oxidative stress, and SIRT1 activity in the aorta	de Picciotto et al., 2016
To test if defects in Nampt-mediated NAD^+ biosynthesis in adipocytes play a role in the pathogenesis of obesity-associated systemic	Adipocyte-specific nicotinamide phosphoribosyl-transferase (Nampt) knockout (ANKO) mice	0 or 500 mg/kg bw/d NMN (Oriental Yeast) in drinking water to ANKO mice in a NAD^+ precursor experiment. In a RGS rescue model, rosiglitazone at 20	NMN from weaning to up to 2 mo of age (4-6 wk)	Insulin resistance in multiple metabolic organs, adipose tissue dysfunction (characterized by Insulin-stimulated glucose uptake in VAT, hepatic and plasma TG, plasma free fatty acid, plasma adiponectin and adipsin, and VAT gene expression of inflammatory markers), phosphorylation of CDK5 and PPARγ, gene expression of obesity-like phosphorylated PPARγ	Stromsdorfer et al., 2016

metabolic complications		mg/kg bw/d for 7 wk from weaning		targets, and lysine acetylation of nuclear proteins in VAT	
Age associated pathophysiological changes	Male C57BL/6N mice (5 mo old) fed regular chow diet ad libitum	Control; 100 or 300 mg/kg bw/d NMN (Oriental Yeast Co., 96-97% pure) in drinking water	12 mo (age 5 mo until 17 mo old)	Plasma NMN and NAD^+ levels in peripheral tissues (skeletal muscle, white adipose tissue, and liver); age-associated body weight gain; energy metabolism, physical activity, insulin sensitivity, plasma lipid profile, and eye function; age-associated gene expression changes in peripheral tissues, and mitochondrial oxidative metabolism and mitonuclear protein imbalance in skeletal muscle, bone density	Mills et al., 2016

CDK5 = cyclin-dependent kinase 5; d = days; EDD=endothelium-dependent dilation; mo = months; IL = interleukin; NAD= nicotinamide adenine dinucleotide; PPARγ = peroxisome proliferator-activated receptor gamma; SIRT = sirtuin; TG = triglyceride; TNF = tumor necrosis factor; VAT = visceral adipose tissue; wk = weeks.

3.4.2 Animal Efficacy Studies of NR

CONFIDENTIAL

Zong et al. (2021) examined the effect of elevating NAD^+ levels in models with reduced hematopoietic stem cell (HSC) potential, ATM-deficient, and aged wild type mice. After weaning, $Atm^{-/-}$ mice and $Atm^{+/+}$ littermates were given NR (12 mM) in drinking water. In addition, mice used in the aging studies were C57BL/6 males: young (3–4 months) and old (24–29 months). NR was supplemented for 4–6 weeks. Lymphoid-progenitor cell frequencies were measured in bone marrow of $Atm^{-/-}$ mice and littermate $Atm^{+/+}$ controls (wild type). The lymphoid potential of HSCs and inflammation, as measured by neutrophils and monocytes, were assessed in young and old mice. No adverse effects were reported on the measured outcomes.

de Castro et al. (2021) investigated the effects of calorie restriction on skeletal muscle tissue and hypothalamic inflammatory biomarkers in obese adult male Wistar rats, and whether NR supplementation alone or in combination with calorie restriction affects these parameters. Obesity was induced in rats through a cafeteria diet for 6 weeks. After that, a group of obese rats was exposed to calorie restriction, with or without NR supplementation (400 mg/kg), for another 4 weeks. Measurements included body weights, absolute and relative skeletal muscle tissue weight, and hypothalamic levels of tumor necrosis factor (TNF)-α. No adverse effects of NR were observed.

Cartwright et al. (2021) studied the effects of NR supplementation on whole-body energy metabolism and mitochondrial function in mildly obese C57BL/6N and C57BL/6J mice, two commonly used strains to investigate metabolism. Male C57BL/6N and C57BL/6J mice were fed a HFD or standard chow with or without NR supplementation for 8 weeks. Body and organ weights, glucose tolerance, and metabolic parameters (body and fat depot weight, fasting blood glucose, hepatic lipid accumulation, and energy expenditure), as well as mitochondrial O_2 flux in liver and muscle fibers were assessed. No adverse effects were reported on the measured outcomes.

Gariani et al. (2016) examined the effect of NR administration on non-alcoholic fatty liver disease (NAFLD) in male C57BL/6J mice (8 weeks old) for up to 18 weeks. In experiment 1, mice were randomized into 4 groups (n=10): high-fat, high-sucrose diet (44.6% kcal from fat) + 400 mg/kg/day NR; normal chow diet + 400 mg/kg/day NR; high-fat, high-sucrose diet alone; or normal chow diet alone. In experiment 2, $Sirt1^{hep-/-}$ and $Sirt1^{1,2/\ 1,2}$ mice were fed with high-fat, high-sucrose pellets containing water or NR (400 mg/kg/day) for 14 weeks. In experiment 3, *Apoe*-/- mice were placed on either low-fat (4% fat by weight) or high-fat and cholesterol (22% fat, 0.2% cholesterol by weight) diets for 3 weeks to induce aberrant lipid metabolism. At 10 weeks of age, 500 mg/kg/day of NR was supplemented to the assigned high-fat and cholesterol diet group for 7 weeks. The high-fat, high-sucrose diet induced NAFLD. In experiment 1, the measurements included hepatic NAD^+ levels, glucose tolerance, insulin sensitivity, relative

expression of genes associated with fibrosis, lipogenesis, lipid metabolism, and inflammation, liver tissue respiratory capacity *ex vivo* and b-oxidation gene expression, and mitochondrial complex content and activity. Because genetically modified animal model studies are not relevant when evaluating safety, the measurements tested in experiments 2 and 3 are not presented in the summary of this study. No adverse effects from NR administration were observed on the measured outcomes.

Zhou et al. (2016) investigated the influences of NAD^+ decline on steatosis and steatohepatitis in wild-type and H247A dominant-negative, enzymically-inactive NAMPT transgenic (DN-NAMPT) mice given a normal or HFD. Induced NAFLD, 8-week-old mice were fed a HFD (60% kcal from fat) for 16 weeks. Then, wild-type male C57BL/6J mice and DN-NAMPT mice received either the normal HFD or HFD supplemented with NR at daily dose of 200 mg/kg bw for 4 weeks. Measurements in wild type mice included hepatic NAD^+ levels, lipid homeostasis (plasma and hepatic lipid profile), plasma non-esterified fatty acid levels, steatohepatitis, liver fibrosis and insulin resistance, hepatic steatosis, liver weight, and body weight. Because the tests in DN-NAMPT mice are not relevant when evaluating the safety of NR, they are not summarized in this review. No adverse effects of NR were reported on the measured outcomes.

Pham et al. (2019) investigated NR supplementation on liver fibrosis in male C57BL/6 J mice (7 weeks old). Mice were assigned to one of three groups (n=14-15) for 20 weeks: low-fat control (6% fat by weight), high-fat and cholesterol control (35% fat by weight), or a high-fat and cholesterol diet supplemented with 400 mg/kg bw/day NR. Measurements included body weight, blood lipid profile, glucose tolerance, liver lipid accumulation and inflammation (ALT activity), hepatic expression of inflammatory genes (including *Ccl2, Emr1, Il1b,* and *Tnfa*), and liver fibrosis indicators [hepatic collagen accumulation and mRNA expression of fibrotic genes]), liver NAD^+ metabolism, whole body energy utilization, and expression of genes responsible for energy utilization in soleus muscle and brown adipose tissue. No adverse effects were reported on the measured outcomes.

Taken together, long term daily consumption of NR at 400 mg/kg bw for up to 20 weeks did not result in any adverse effects on indicators of glucose and lipid metabolism and hepatic functions in rodents.

Table 21. Animal Efficacy Studies of Orally Administered NR

Objective; to test	Animal	Dose of NR	Duration	Measurement Endpoints	Reference
If NR improves lymphoid lineage potential (hematopoietic system)	Expt 1, C57BL/6 males, young (3–4 mo) and old (24–29 mo); Expt 2, $Atm^{-/-}$ mice and $Atm^{+/+}$ control (wild type)	0 or 12 mM in drinking water	4 to 6 wk	Lymphoid-progenitor cell frequencies in bone marrow Atm−/− mice and littermate Atm+/+ controls (wild type); lymphoid potential of HSCs and inflammation, as measured by neutrophils and monocytes, in young and old mice	Zong et al., 2021
If NR can ameliorate skeletal muscle tissue weight loss and increased level of hypothalamic TNF-α	Normal wt control Wistar rats; obese rats (induced by cafeteria diet for 6 wk) with or without calorie restriction (60 d old)	Each of 3 groups was treated with or without 400 mg/kg bw/d NR	4 wk	Body weights, absolute and relative skeletal muscle tissue weight, hypothalamic levels of TNF-α	de Castro et al., 2021
The NR's effects on whole-body energy metabolism and mitochondrial function	Mildly obese male C57BL/6N and C57BL/6J mice	0 or 3 mg/mL NR chloride in drinking water	8 wk	Body and organ weights, glucose tolerance, and metabolic parameters as well as mitochondrial O_2 flux in liver and muscle fibers	Cartwright et al., 2021
if NR can reduce the effects of NAFLD in mice induced by a high-fat, high-sucrose diet	Expt 1, Male C57BL/6J mice (8 weeks old)	Normal chow with or without 400 mg/kg bw/d NR	Expt 1, up to 18 wk NMN or HFC diet	Severity of NAFLD, hepatic NAD levels, glucose tolerance, insulin sensitivity, liver tissue respiratory capacity *ex vivo* and b-oxidation gene expression, mitochondrial complex content and activity.	Gariani et al., 2016
Reduction of NAFLD in mice induced by HFD	Male C57BL/6J mice (initially 8 weeks old), Pre-administration of	High fat diet; High fat diet + 200 mg/kg bw/d NR	4 wk	Hepatic NAD levels, lipid homeostasis (plasma and hepatic lipid profile), plasma NEFA levels, steatohepatitis, liver fibrosis and insulin resistance,	Zhou et al., 2016

	HFD for 16 wk to induce NAFLD			hepatic steatosis, liver weight, and body weight	
Liver fibrosis in mice	Male C57BL/6 J mice (7 weeks old)	Low fat diet; High fat diet; High fat diet + 400 mg/kg bw/d NR	20 wk	body weight, blood lipid profile, glucose tolerance, liver lipid accumulation and inflammation, liver fibrosis indicators, liver NAD^+ metabolism, whole body energy utilization and expression of genes responsible for energy utilization in soleus muscle and brown adipose tissue	Pham et al., 2019

ALT = alanine aminotransferase; AST = aspartate transaminase; d = days; HFD = high fat diet; HSC= hematopoietic stem cell; NAD= nicotinamide adenine dinucleotide; NAFLD = non-alcoholic fatty liver disease; TC = total cholesterol; TG = triglyceride; TNF = tumor necrosis factor; wk = weeks

References for 3.4.

Cartwright DM, Oakey LA, Fletcher RS, Doig CL, Heising S, Larner DP, Nasteska D, Berry CE, Heaselgrave SR, Ludwig C, Hodson DJ, Lavery GG, Garten A. Nicotinamide riboside has minimal impact on energy metabolism in mouse models of mild obesity. J Endocrinol. 2021;251:111-23.

de Castro JM, Stein DJ, Medeiros HR, de Oliveira C, Torres ILS. Nicotinamide riboside neutralizes hypothalamic inflammation and increases weight loss without altering muscle mass in obese rats under calorie restriction: A preliminary investigation. Front Nutr. 2021;8:648893.

de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. Aging Cell. 2016;15:522-30.

Gariani K, Menzies KJ, Ryu D, Wegner CJ, Wang X, Ropelle ER, Moullan N, Zhang H, Perino A, Lemos V, Kim B, Park YK, Piersigilli A, Pham TX, Yang Y, Ku CS, Koo SI, Fomitchova A, Cantó C, Schoonjans K, Sauve AA, Lee JY, Auwerx J. Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. Hepatology. 2016;63:1190-204.

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 2016;24:795-806.

Pham TX, Bae M, Kim MB, Lee Y, Hu S, Kang H, Park YK, Lee JY. Nicotinamide riboside, an NAD^+ precursor, attenuates the development of liver fibrosis in a diet-induced mouse model of liver fibrosis. Biochim Biophys Acta Mol Basis Dis. 2019;1865:2451-63.

Stromsdorfer KL, Yamaguchi S, Yoon MJ, Moseley AC, Franczyk MP, Kelly SC, Qi N, Imai S, Yoshino J. NAMPT-mediated NAD(+) biosynthesis in adipocytes regulates adipose tissue function and multi-organ insulin sensitivity in mice. Cell Rep. 2016;16:1851-60.

Yu J, Laybutt DR, Kim LJ, Quek LE, Wu LE, Morris MJ, Youngson NA. Exercise-induced benefits on glucose handling in a model of diet-induced obesity are reduced by concurrent nicotinamide mononucleotide. Am J Physiol Endocrinol Metab. 2021;321:E176-E189.

Zhou CC, Yang X, Hua X, Liu J, Fan MB, Li GQ, Song J, Xu TY, Li ZY, Guan YF, Wang P, Miao CY. Hepatic NAD(+) deficiency as a therapeutic target for non-alcoholic fatty liver disease in ageing. Br J Pharmacol. 2016;173:2352-68.

Zong L, Tanaka-Yano M, Park B, Yanai H, Turhan FT, Croteau DL, Tian J, Fang EF, Bohr VA, Beerman I. NAD^+ augmentation with nicotinamide riboside improves lymphoid potential of $Atm^{-/-}$ and old mice HSCs. NPJ Aging Mech Dis. 2021;7:25.

3.5. Human Clinical Studies
CONFIDENTIAL

3.5.1. Human Clinical Studies of NMN
CONFIDENTIAL

In a study by Irie et al. (2020), a single-arm, non-randomized intervention was conducted by single oral administration of 100, 250, and 500 mg NMN in 10 healthy men. These doses were well tolerated without causing severe AEs, including flush and gastrointestinal symptoms. Measurement endpoints included heart rate, blood pressure, oxygen saturation, body temperature, neurological system, ocular fundus, ophthalmic parameters, sleep quality score by Pittsburgh Sleep Quality Index, and clinical chemistry data of blood and urine. While serum levels of bilirubin significantly increased by 51.3%, the levels of glucose, creatinine, and chloride significantly decreased by 11.7, 5.1, and 2.3%, respectively, at 300 minutes at all doses from baseline. However, all of these values stayed within normal ranges, independent of the NMN dose. Thus, they were not considered of toxicological concern.

Yoshino et al. (2021) evaluated the effect of NMN supplementation in overweight or obese postmenopausal women with prediabetes in a randomized, placebo-controlled, double-blind trial. Twenty-five overweight or obese postmenopausal women (mean age of 61.5 years; mean body mass index [BMI] of 35.5 kg/m^2) were randomized to receive placebo (n=12) or 250 mg/day NMN (n=13) for 10 weeks. All participants were prediabetic based on the criteria proposed by the American Diabetes Association. Measurements included insulin-stimulated glucose disposal assessed by using the hyperinsulinemic-euglycemic clamp, and skeletal muscle insulin signaling (phosphorylation of protein kinase AKT and mechanistic target of rapamycin), expression of platelet-derived growth factor receptor b and other genes related to muscle remodeling, body composition (fat mass, fat-free mass, intra-abdominal adipose tissue volume, and intrahepatic TG content), blood pressure, clinical chemistry (plasma concentrations of glucose, insulin, glycosylated hemoglobin, free fatty acid, TG, high-density lipoprotein cholesterol, high molecular weight adiponectin, and leptin), and both basal glucose and fatty acid kinetics In addition, NMN metabolites and NAD^+ in plasma, PBMCs, and skeletal muscle were measured 4 hours post-administration during the 10-week intervention period. No AEs were reported, and no abnormalities were detected on the measured outcomes.

Liao et al. (2021) investigated the effect of a combination of exercise training with NMN supplementation on the cardiovascular fitness in healthy amateur runners in a randomized, double-blind, placebo-controlled trial. Forty-eight men and women (40 men, 8 women; 27-50 years old; regular exercise for 1-5 years; China) were randomized to receive 0 (placebo), 300 mg/day NMN, 600 mg/day NMN, or 1,200 mg/day NMN for 6 weeks (split over 2 doses/day). All participants adhered to an aerobic exercise program during the study with each exercise session comprised of 40-60 minutes of running or cycling for 5-6 sessions per week.

Cardiopulmonary exercise testing was performed at baseline and after the intervention at 6 weeks to assess the aerobic capacity of the runners. The following parameters were determined by subjects completing an incremental ramp exercise until exhaustion was reached: anthropometric parameters, cardiopulmonary endurance performance, including cardiovascular parameters (oxygen uptake [VO_2], O_2-pulse, and VO^2-related to work rate), ventilatory parameter, metabolic parameters (respiratory exchange ratio, and first and second ventilatory threshold), exercise capacity parameters (workload and power), and AEs. The authors concluded that NMN at daily doses up to 1,200 mg for 6 weeks did not result in any obvious adverse symptoms.

Kim et al. (2022) investigated the effects of the time dependent intake of NMN on sleep quality, fatigue, and physical performance in older adults. A total of 108 older adults (mean age of 72.2-73.0 years; Japan) consumed 1,500 mg/day NMN or placebo at either antemeridian (A.M.; after waking up until 12:00) or post-meridian (P.M.; from 18:00 until bedtime) for 12 weeks. Sleep quality was evaluated with the Pittsburgh Sleep Quality Index. Fatigue was determined by the "Jikaku-sho shirabe" questionnaire. Physical performance included grip strength, 5-times sit-to-stand, timed up and go, and 5-m habitual walk. No adverse events were reported.

Pencina et al. (2022) reported the pharmacokinetics and pharmacodynamics of a microcrystalline NMN (source, NA). Overweight or obese, middle-aged or older men and postmenopausal women (16 men, 16 women; mean age of 63.9 years; mean BMI 29.1 kg/m^2) consumed placebo, 1,000 mg NMN (once a day), 2,000 mg NMN (divided into 2 doses) for 14 days. No serious adverse events were reported. The frequency of adverse events was similar between the groups. In the 1,000-mg twice daily group, one participant discontinued on days 8-14 due to diarrhea. Changes from baseline in clinical laboratory analytes in test groups did not differ from placebo (Supplementary Figure 2). Two participants (one in the low-dose and one in the placebo group) experienced mild AST and ALT elevations on day 14. However, AST and ALT levels in both participants returned towards baseline after discontinuation of study product. There was no clinically significant change from baseline in vital signs, change in fasting glucose levels, serum lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and free fatty acids) and serum uric acid concentrations in any group. The data indicate that daily consumption of NMN at doses up to 2,000 mg for 14 days was safe.

Summary of Human Studies of NMN

It is reasonable to conclude that NMN intake at daily doses of up to 1,500 mg for 12 weeks is safe in humans.

References for 3.5.1.

Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, Yamaguchi S, Yamashita K, Shigaki S, Ono T, Yukioka H, Okano H, Nabeshima YI, Imai SI, Yasui M, Tsubota K, Itoh H. Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocr J. 2020;67:153-60.

Kim M, Seol J, Sato T, Fukamizu Y, Sakurai T, Okura T. Effect of 12-Week Intake of Nicotinamide Mononucleotide on Sleep Quality, Fatigue, and Physical Performance in Older Japanese Adults: A Randomized, Double-Blind Placebo-Controlled Study. Nutrients. 2022;14:755.

Liao B, Zhao Y, Wang D, Zhang X, Hao X, Hu M. Nicotinamide mononucleotide supplementation enhances aerobic capacity in amateur runners: A randomized, double-blind study. J Int Soc Sports Nutr. 2021;18:54.

Pencina K, Lavu S, Dos Santos M, Beleva YM, Cheng M, Livingston D, Bhasin S. MIB-626, an Oral Formulation of a Microcrystalline Unique Polymorph of β-Nicotinamide Mononucleotide, Increases Circulating Nicotinamide Adenine Dinucleotide and its Metabolome in Middle-aged and Older Adults. J Gerontol A Biol Sci Med Sci. 2022:glac049.

Yoshino M, Yoshino J, Kayser BD, Patti GJ, Franczyk MP, Mills KF, Sindelar M, Pietka T, Patterson BW, Imai SI, Klein S. Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science. 2021;372:1224-29.

Table 22. Human Clinical Trials of NMN

Objective	Subjects	Dose	Duration/ Design	Outcome/Measurements	References
To evaluate the safety and PK of NMN	10 healthy males	0, 100, 250, or 500 mg/d	Single dose	Heart rate; blood pressure; oxygen saturation; body temperature; examination results of the neurological system, ocular fundus, and ophthalmic parameters; sleep quality score by Pittsburgh Sleep Quality Index; and clinical chemistry of blood and urine	Irie et al., 2020
To evaluate the effect of NMN supplementation in postmenopausal, overweight or obese, women with prediabetes	25 postmenopausal, overweight or obese, women with prediabetes (mean age, 61.5 years; mean BMI, 35.5 kg/m^2)	0 or 250 mg/d	10 wk/ Parallel	Insulin-stimulated glucose disposal; skeletal muscle insulin signaling; expression of platelet-derived growth factor receptor b and other genes related to muscle remodeling; body composition; blood pressure; clinical chemistry; both basal glucose and fatty acid kinetics; and NMN metabolites and NAD^+ in plasma, PBMCs, and skeletal muscle	Yoshino et al., 2021
To examine the effect of a combination of exercise training and NMN on cardiovascular fitness	48 healthy amateur runners (40 men, 8 women; 27-50 years old; regular exercise 1-5 years)	0, 300, 600, or 1,200 mg/d (split over 2 doses/d)	6 wk/ Parallel	Anthropometric parameters; cardiopulmonary endurance performance including cardiovascular parameters (VO_2, O_2-pulse, VO2-related to work rate); ventilatory parameter (VE); metabolic parameters (respiratory exchange ratio, VT1, and VT2); exercise capacity parameters; and adverse events	Liao et al., 2021
To investigate the effects of the time-dependent intake of NMN on sleep quality,	108 older adults (mean age 72.2-73.0 years)	1500 mg/d NMN at A.M. or P.M.; placebo at	12 weeks/ Parallel	Sleep quality; fatigue; physical performance (grip strength, 5-times sit-to-stand, timed up and go, 5-m habitual walk)	Kim et al., 2022

fatigue, and physical performance		A.M. or P.M.			
To report the pharmacokinetics and pharmaco-dynamics of NMN	32 overweight or obese, middle-aged and older men and postmenopausal women (16 men, 16 women; mean age 63.9 years; mean BMI 29.1 kg/m^2)	1,000 or 2,000 mg NMN, or placebo	14 d/ Parallel	Blood and urinary NMN, NAD^+, and NAD^+ metabolome; pharmacokinetics and pharmacodynamics; safety; blood lipid profile and changes in fasting glucose concentrations	Pencina et al., 2022

d = days; BMI = body mass index; NA = not available; PK = pharmacokinetic; VO_2 = oxygen uptake; VT = ventilatory threshold; wk = weeks.

3.5.2. Human Clinical Studies of NR

CONFIDENTIAL

Human clinical studies evaluated NR at doses of 100 to 2,000 mg/day for a duration up to 12 weeks.

Dolopikou et al. (2020) investigated the effect of acute NR supplementation on redox homeostasis and physical performance in young and elderly individuals in a double-blinded, cross-over design. Before and 2 hours after NR or placebo supplementation, blood and urine samples were collected, while physical performance (maximal oxygen uptake, muscle strength, and fatigue) was assessed after the second blood sample collection. Measurements included physical performance (as measured by isometric knee extensor peak torque at 90° knee flexion, concentric peak torque at angular velocity 60°/s, and VO_{2max}), and clinical chemistry (erythrocyte glutathione concentration, glutathione reductase, glutathione peroxidase, catalase, superoxide dismutase, NADH, and NADPH), and urinary concentration of F_2-isoprostanes.

In a study by Trammell et al. (2016), time-dependent PBMC NAD^+ metabolomes from 12 healthy female subjects were quantified after three different oral doses of NR (100, 300, or 1,000 mg/day NR for 3 days) in a cross-over design. Two individuals self-reported flushing at the 300 mg NR dose, but not at the 100 mg or 1,000 mg dose, and two individuals self-reported feeling hot at the 1,000 mg dose, but not at the lower dose. Over the total of 36 days of observation of the study participants, there were no serious AEs and no events that were dose-dependent. Due to the fact this study did not employ a validated flushing symptom questionnaire, the authors suggested that future studies may need to include such a questionnaire.

Airhart et al. (2017) conducted a pharmacokinetics study to determine the effect of NR on whole blood NAD^+ levels. Healthy volunteers consumed 250 mg NR (ChromaDex, Irvine, US; ~99% purity) on days 1 and 2, and then up-titrated to peak doses of 1,000 mg twice daily on days 7 and 8. In other words, participants took incremental doses of NR from 250 mg to 2,000 mg/day. Measurement endpoints were safety, NR and NAD^+ in whole blood, pharmacokinetics in whole blood, hematology (WBC counts, hematocrit, hemoglobin, and platelet counts), and clinical chemistry related to liver and kidney functions (potassium, glucose, uric acid, creatine kinase, ALT, AST, and lactate dehydrogenase). NR was well tolerated with no AEs.

Martens et al. (2018) conducted a small randomized, placebo-controlled, cross-over clinical trial of NR supplementation (500 mg twice daily; a total of 1,000 mg/day for 6 weeks) to assess its overall tolerability and efficacy in raising levels of NAD^+-related metabolites in healthy middle-aged and older men and women (average age of 65 years). Measurement included NAD^+ and related metabolite levels in the PBMCs, blood pressure, arterial functions (mean carotid-femoral pulse wave velocity, ultrasound-determined carotid artery compliance, or brachial artery flow-mediated dilation, a measure of vascular endothelial function), overall motor

function (maximal exercise capacity as assessed by VO_2 max and treadmill time to exhaustion), and side effects. A total of 14 treatment-emergent AEs were reported by 7 of the 30 participants enrolled in the study, of which 9 and 5 were reported in placebo and test groups, respectively. The other 23 subjects reported no AEs. All self-reported AEs were mild in severity. The reported symptoms included nausea, flushing, leg cramps, and increased bruising during the NR condition, and headache, skin rash, flushing, fainting, and drowsiness during the placebo condition. Two subjects in the placebo group, but no one in the NR group, reported flushing. The authors provided evidence that supplementation with NR is well tolerated with no side effects in healthy middle-aged and older adults.

Dellinger et al. (2017) assessed the safety and efficacy of a repeated dose of a combination of NR and pterostilbene, NRPT, in a randomized, double-blinded, placebo-controlled study. One hundred twenty healthy adults between the ages of 60 and 80 years were randomized to receive one of three treatments: placebo, a recommended dose of NRPT (NRPT 1X; 250 mg of NR plus 50 mg of pterostilbene), or a double dose of NRPT (NRPT 2X; 500 mg of NR plus 100 mg of pterostilbene) for 8 weeks. NRPT significantly increased the concentration of NAD^+ in a dose-dependent manner (approximately 40% in NRPT 1X and 90% in NRPT 2X) after 4 weeks as compared to placebo and baseline, and this significant increase in NAD^+ levels was sustained throughout the entire 8-week trial. Insulin sensitivity, endogenous glucose production, and glucose disposal and oxidation were not significantly different among the groups. TC and LDL-C levels were increased in the NRPT-treated groups (control vs. NRPT 1X vs. NRPT 2X: TC, 5.15 vs. 5.89 vs. 5.70 mmol/L, $P<0.05$; LDL-C, 2.93 vs. 3.65 vs. 3.47 mmol/L, $P<0.05$). When subjects were stratified by BMI, changes in cholesterol in the NRPT 1X group were not significantly different in the normal BMI subgroup and were confined to the overweight subgroup. One confounding factor in interpreting the cholesterol data is that TC and LDL-C levels at baseline were statistically significantly higher in the test groups (control vs. NRPT 1X vs. NRPT 2X: TC, 5.09 vs. 5.58 vs. 5.21 mmol/L, $P<0.05$; LDL-C, 2.93 vs. 3.65 vs. 3.47 mmol/L, $P<0.05$), possibly due to the vagaries in the randomization of subjects. Considering the fact that normal biological variation for LDL for an individual was estimated to be 9% and the study employed test subjects having higher cholesterol levels than the control group, changes in LDL-C may be from natural variations instead of treatment-related. A total of 66 AEs were reported by 45 participants. Of these, 18 AEs were reported by 13 participants in the placebo group, 25 reported by 15 participants in the NRPT 1X group, and 23 reported by 17 participants in the NRPT 2X group. There were no significant differences in the incidence of AEs among the groups. All participants reporting AEs recovered and no serious AEs were reported in this study.

Dollerup et al. (2018) examined the safety of dietary NR supplementation over a 12-week period and the potential to improve insulin sensitivity and other metabolic parameters in obese, insulin-resistant men (BMI >30 kg/m^2; aged 40-70 years old) in a randomized, placebo-

controlled, double-blinded, and parallel-group designed clinical trial. The otherwise healthy men were randomly assigned to 12 weeks of NR (1,000 mg twice daily, a total of 2,000 mg/day) or placebo. Measurements included safety, insulin sensitivity, glucose turnover parameters (endogenous glucose production and nonoxidative glucose disposal), body composition (total body mass, lean mass, total fat mass, or fat percentage), hepatic lipid content, substrate metabolism (resting energy expenditure), clinical chemistry including fasting glucose, glycosylate hemoglobin, lipid profile, ALT, and urinary NR metabolites. No treatment-related adverse effects or AEs were observed. From these results, 12 weeks of NR supplementation at 2,000 mg/day is safe in obese adult men.

Dollerup et al. (2020) studied the effects of NR on skeletal muscle mitochondrial function, content, and morphology in obese and insulin resistant men. Forty obese, insulin resistant, but otherwise healthy, male Caucasian volunteers (aged 40-70 years old, BMI >30) received placebo or 2,000 mg NR (1,000 mg 2/day) in a 12-week randomized, placebo-controlled clinical trial. Skeletal muscle biopsies were collected before and after the study. Parameters included NAD^+ metabolite levels, Nampt protein abundance, mitochondrial respiration, mitochondrial fractional area, mitochondrial network morphology, and lipid deposition in skeletal muscle. The Nampt protein levels in skeletal muscle were 14% lower in the NR group compared to placebo ($P<0.05$), while Nampt mRNA expression and cellular levels of NAD^+ metabolites were not changed. The decrease in Nampt protein observed in this study may represent an adaptive response following exposure to supraphysiological levels of an exogenous NAD^+ precursor. NR may diminish the muscle's reliance on the NAD^+ salvage pathway by exploiting Nampt-independent pathways to maintain NAD^+ homeostasis. There were no significant differences between the groups in other parameters. No AEs were reported; therefore, this study implies NR supplementation is safe at doses of up to 2,000 mg/day in obese adult men.

Conze et al. (2019) investigated the safety of NR (NIAGEN) in 140 overweight, but otherwise healthy, men and women (40-60 years old; BMI of 25-30) in an 8-week randomized, double-blind, placebo-controlled clinical trial. Subjects received one of four treatments: 0 (placebo), 100 mg, 300 mg, or 1,000 mg NR per day in addition to additional dietary restriction counseling. Subjects returned to the clinic on days 7, 14, 28, and 56 for safety assessments and blood and urine collection. Primary endpoint was the difference in urinary 1-methylnicotinamide levels between the placebo and NR groups. The secondary endpoints included the rate of increase in urinary 1-methylnicotinamide levels, the difference and rate of increase in NR metabolites levels in blood including clinical chemistry, hematology, concentrations of branched-chain amino acids, homocysteine, or high-sensitivity C-reactive protein, and AEs. There were no reports of flushing or significant differences in AEs between the groups. It did not elevate LDL-C or negatively affect 1-carbon metabolism. Of the 26, 27, and 22 AEs reported in the low-dose, mid-dose, and high-dose NR groups, respectively, 24, 25, and 19, respectively, were reported as being unlikely or not related to the study product. All AEs reported as being

possibly related were all mild in intensity. Of the 20 AEs reported in the placebo group, 16 were reported as being unlikely related to the study product. Of the 4 AEs reported as being possibly related, 3 were mild in intensity (rash, raised liver function tests, and nausea) and 1 was moderate in intensity (upset stomach). Importantly, all AEs were resolved by the end-of-study. The authors concluded that NR was safe to supplement at doses up to 1,000 mg/day in overweight adults.

Elhassan et al. (2019) studied whether oral NR chloride (Niagen) supplementation in aged men would increase NAD^+ metabolome in skeletal muscle and whether it could change muscle mitochondrial bioenergetics. Twelve healthy elderly men (median age of 75 years, median BMI of 26.6) were given 1,000 mg NR/day for 3 weeks (500 mg twice per day) in a placebo-controlled, randomized, double-blind, cross-over design with 3 weeks washout between phases. Outcomes measured included AEs, NAD^+ metabolome in skeletal muscle, whole venous blood, and urine, skeletal muscle mitochondrial bioenergetics or hand-grip strength, NR-mediated transcriptional changes in skeletal muscle, angiogenesis and muscle blood flow, body weight, blood pressure, fasting glucose and insulin, lipid profile, inflammatory cytokines, and a homeostatic model assessment of insulin resistance. No clinical AEs were reported during the intervention in either phase. Of note, 4 of 12 participants (33.3%) reported increased libido during the intervention period, but this was not considered an AE. There were no such reports with the placebo. This study demonstrated that up to 1,000 mg NR/day was safe and well tolerated in elderly men.

Remie et al. (2020) examined 6-week NR supplementation on insulin sensitivity, mitochondrial function, and other metabolic health parameters in 13 overweight and obese men and women (45-65 years old; BMI of 27-35) in a randomized, double-blinded, placebo-controlled, cross-over intervention study. Participants received either placebo or 1,000 mg/day NR for each 6-week intervention period, with a 4-7-week washout period. Parameters included compliance and side effects, skeletal NAD^+ metabolites, mitochondrial respiration and protein and acylcarnitine concentrations in skeletal muscle, insulin sensitivity and substrate kinetics, plasma biochemistry and inflammatory markers, body composition, sleeping metabolic rate, intrahepatic and intramuscular lipid content, blood pressure, and cardiac function as measured by cardiac phosphocreatine:ATP ratios. No adverse effects were noted. Thus, it was concluded that the dose of 1,000 mg/day NR was safe and well-tolerated.

Summary of Human Studies of NR

Overall, human studies of NR alone showed that daily doses up to 2,000 mg did not result in adverse effects on the measured outcomes, including glucose and lipid metabolism indicators, NAD homeostasis, skeletal muscle NAD^+ metabolome, inflammatory biomarkers, exercise performance, clinical chemistry, hematology, and body composition. Although a human study testing the combination of NR and pterostilbene significantly elevated TC and LDL-C (Dellinger et al., 2017), it is hard to conclude that it was treatment-related. This is due to the fact that there

was a major confounding factor: TC and LDL-C levels in the test groups were significantly higher than the control group at baseline. All other studies testing NR alone did not report any adverse effects on the measured outcomes. No studies reported treatment-related AEs. Thus, it is concluded that NR is safe at up to 2,000 mg/person/day.

References for 3.5.2.

Airhart SE, Shireman LM, Risler LJ, Anderson GD, Nagana Gowda GA, Raftery D, Tian R, Shen DD, O'Brien KD. An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside (NR) and its effects on blood NAD^+ levels in healthy volunteers. PLoS One. 2017;12:e0186459.

Conze D, Brenner C, Kruger CL. Safety and metabolism of long-term administration of NIAGEN (nicotinamide riboside chloride) in a randomized, double-blind, placebo-controlled clinical trial of healthy overweight adults. Sci Rep. 2019;9:9772.

Dellinger RW, Santos SR, Morris M, Evans M, Alminana D, Guarente L, Marcotulli E. Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD(+) levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. NPJ Aging Mech Dis. 2017;3:17.

Dollerup OL, Christensen B, Svart M, Schmidt MS, Sulek K, Ringgaard S, Stødkilde-Jørgensen H, Møller N, Brenner C, Treebak JT, Jessen N. A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am J Clin Nutr. 2018;108:343-53.

Dollerup OL, Chubanava S, Agerholm M, Søndergård SD, Altıntaş A, Møller AB, Høyer KF, Ringgaard S, Stødkilde-Jørgensen H, Lavery GG, Barrès R, Larsen S, Prats C, Jessen N, Treebak JT. Nicotinamide riboside does not alter mitochondrial respiration, content or morphology in skeletal muscle from obese and insulin-resistant men. J Physiol. 2020;598:731-54.

Dolopikou CF, Kourtzidis IA, Margaritelis NV, Vrabas IS, Koidou I, Kyparos A, Theodorou AA, Paschalis V, Nikolaidis MG. Acute nicotinamide riboside supplementation improves redox homeostasis and exercise performance in old individuals: A double-blind cross-over study. Eur J Nutr. 2020;59:505-15.

Elhassan YS, Kluckova K, Fletcher RS, Schmidt MS, Garten A, Doig CL, Cartwright DM, Oakey L, Burley CV, Jenkinson N, Wilson M, Lucas SJE, Akerman I, Seabright A, Lai YC, Tennant DA, Nightingale P, Wallis GA, Manolopoulos KN, Brenner C, Philp A, Lavery GG. Nicotinamide riboside augments the aged human skeletal muscle NAD^+ metabolome and induces transcriptomic and anti-inflammatory signatures. Cell Rep. 2019;28:1717-28.e6.

Martens CR, Denman BA, Mazzo MR, Armstrong ML, Reisdorph N, McQueen MB, Chonchol M, Seals DR. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults. Nat Commun. 2018;9:1286.

Remie CME, Roumans KHM, Moonen MPB, Connell NJ, Havekes B, Mevenkamp J, Lindeboom L, de Wit VHW, van de Weijer T, Aarts SABM, Lutgens E, Schomakers BV, Elfrink HL, Zapata-Pérez R, Houtkooper RH, Auwerx J, Hoeks J, Schrauwen-Hinderling VB, Phielix E, Schrauwen P. Nicotinamide riboside supplementation alters body composition and skeletal muscle acetylcarnitine concentrations in healthy obese humans. Am J Clin Nutr. 2020;112:413-26.

Table 23. Human Clinical Trials of NR

Objective	Subjects	Dose	Duration/Design	Outcome/Measurements	References
Kinetics and efficacy in exercise performance in elderly people	Healthy young volunteers (n=12, 22.9 ± 1.0 y) and elderly (n=12, 71.5 ± 1.0 y)	Placebo or 500 mg NR	Single/randomized, double-blinded, cross-over study	Physical performance, and clinical chemistry (erythrocyte glutathione concentration, glutathione reductase, glutathione peroxidase, catalase, superoxide dismutase, NADH, and NADPH), and urinary concentration of F_2-isoprostanes.	Dolopikou et al., 2020
Pharmacokinetics and safety in healthy volunteer	Healthy man aged 52 y old	1 ,000 mg/d NR (source, NA)	7 d (just 1 subject study)	Blood NAD^+ and NAAD levels in PBMC	Trammell et al., 2016
	Healthy adult females, 30-55 y, BMI 18.5 to 29.9	100, 300, or 1,000 mg/d NR (source, NA)	3 d each with 7 d washout; cross-over design	Blood NAD^+ metabolome; two individuals self-reported flushing at the 300 mg dose but not at the 100 mg or 1,000 mg dose, and two individuals self-reported feeling hot at the 1,000 mg dose but not at lower dose. No serious adverse events and no events that were dose-dependent.	
Pharmacokinetics and safety in healthy volunteer	Healthy volunteers (n=8), 21 to 50 y	Dose-escalation at 250 mg (day 1, 2), 500 mg (day 3, 4), 1,000 mg (day 5, 6), and 2,000 mg NR (day 7, 8)	8 d/non-randomized, open-label, non-placebo-controlled study	Safety, NR and NAD^+ in whole blood, pharmacokinetics in whole blood, hematology, and clinical chemistry related to liver and kidney functions (potassium, glucose, uric acid, creatine kinase, ALT, AST, and lactate dehydrogenase). No side effects.	Airhart et al., 2017
Safety, bioavailability, and efficacy in lowering blood pressure and arterial stiffness in elderly people	Healthy volunteers (n=30), 55 to 79 y, BMI= 24 ± 4	Placebo or 1,000 mg/d NR (500 mg twice daily)	6 wk/non-randomized, open-label, cross-over study	NAD and related metabolite levels in PBMC, blood pressure, arterial functions, overall motor function, and side effects. Two cases of flushing were reported in the placebo group, but not in the test group.	Martens et al., 2018

Safety and efficacy against NAD sustainability in elderly people	Healthy volunteers (n=120), age from 60 to 80 y	Placebo, NRPT 1X (250 mg NR and 50 mg PT), or NRPT 2X (500 mg NR and 100 mg PT) daily	8 wk/ randomized, placebo-controlled, double-blinded study	NAD level in whole blood; total and LDL cholesterol levels. TC and LDL-C levels were higher in the test groups, but it is hard to conclude that it was treatment-related due to a confounding factor and natural variations in LDL-C. No adverse side effects.	Dellinger et al., 2017
Safety and efficacy against insulin sensitivity in obese men	Healthy, sedentary, obese men (n=40), 40 to 70 y, BMI >30	Placebo or 2,000 mg/d NR (1,000 mg twice daily)	12 wk/randomized, placebo-controlled, double-blinded study	Safety, insulin sensitivity, glucose turnover parameters, body composition, hepatic lipid content, substrate metabolism (resting energy expenditure), clinical chemistry, and urinary NR metabolites. No side effects.	Dollerup et al., 2018
Safety and metabolism in healthy, overweight, adults	Healthy, overweight men and women (n=140), 40 to 60 y, BMI 25-30	Placebo or 100, 300, or 1,000 mg/d NR	8 wk/randomized, placebo-controlled, double-blinded study	Urinary 1-methylnicotinamide levels, the difference and rate of increase in NR metabolites levels in blood, clinical chemistry, hematology, and adverse events. No adverse side effects.	Conze et al., 2019
Skeletal muscle mitochondrial function, content, and morphology in obese and insulin resistant men	Healthy, obese and insulin resistant Caucasian men (n=40), 40 to 70 y, BMI >30	Placebo or 2,000 mg/d NR (1,000 mg twice daily)	12 wk/randomized, placebo-controlled, double-blinded study	Mitochondrial respiratory capacity; mitochondrial fractional area and morphology; steady-state NAD^+ levels; gene expression and protein abundance of NAD^+ biosynthetic enzymes, including NAMPT; No adverse side effects.	Dollerup et al., 2020
NAD^+ metabolism in skeletal muscle and muscle	Healthy, aged, men (n=12), median age 75	Placebo or 1,000 mg/d NR (500 mg twice daily)	21 d/ placebo-controlled, randomized, double-blind	Adverse events, NAD^+ metabolome in skeletal muscle, whole venous blood, and urine, skeletal muscle mitochondrial bioenergetics or hand-	Elhassan et al., 2019

mitochondrial bioenergetics	y, median BMI 26.6		crossover study with 21 d washout period	grip strength, NR-mediated transcriptional changes in skeletal muscle, angiogenesis and muscle blood flow, body weight, blood pressure, fasting glucose and insulin, lipid profile, and inflammatory cytokines, and HOMA-IR No adverse side effects.	
Insulin sensitivity, mitochondrial function, and other metabolic health parameters	Overweight and obese men and women (n=13), 45 - 65 y, BMI, 27 - 35	Placebo or 1,000 mg/d NR	6 wk/ placebo-controlled, randomized, double-blind crossover study with 4-7 wk washout period	Compliance and side effects, skeletal NAD^+ metabolites, mitochondrial respiration and protein and acylcarnitine concentrations in skeletal muscle, insulin sensitivity and substrate kinetics, plasma biochemistry and inflammatory markers, body composition, sleeping metabolic rate, intrahepatic and intramuscular lipid content, blood pressure, and cardiac function. No side effects.	Remie et al., 2020

BMI = body mass index; d = days; homeostatic model assessment of insulin resistance (HOMA-IR; LDL = low density lipoprotein; MNA = N-methylnicotinamide; NA = not applicable; NAAD = nicotinic acid adenine dinucleotide; NAD^+ = nicotinamide adenine dinucleotide; NAMPT = nicotinamide phosphoribosyltransferase; NR = nicotinamide riboside; NRPT = a combination of NR and PT; PBMC = peripheral blood mononuclear cell; PK = pharmacokinetic; PT = pterostilbene; wk = weeks; y = years.

3.6. Potential Adverse Effects

CONFIDENTIAL

NMN shares similar properties with other NAD^+ precursors (e.g., NR, nicotinic acid, and NAM; Sauve, 2008). Unlike NMN, nicotinic acid and NAM have several disadvantages in their therapeutic application. NAM may cause hepatotoxicity or flushing, while a recent preclinical study suggested that it resides in the rat body for a shorter period of time compared to NMN (Kawamura et al., 2016; Knip et al., 2000). Niacin or nicotinic acid is associated with adverse effects, such as cutaneous flushing, when administered as an immediate release formulation. The the sustained release formulations may cause hepatotoxicity (Pieper, 2003). Among the NAD^+ precursors, NR and NMN are exceptions as fewer unfavorable side effects have been reported for these two metabolites (Cantó et al., 2015).

In a study by Trammell et al. (2016), two healthy individuals self-reported flushing at the 300 mg NR dose, but not at the 100 mg or 1,000 mg dose, and two individuals self-reported feeling hot at the 1,000 mg dose, but not at the lower doses. Over the total of 36 days of observation of the study participants, there were no serious AEs and no events that were dose-dependent. Due to the fact that this study did not employ a validated flushing symptom questionnaire, the authors suggested that future studies may need to include such a questionnaire.

On the other hand, the study by Martens et al. (2018) reported two cases of flushing in healthy individuals in the placebo group, but not in the test group consuming 1,000 mg NR a day. The data indicated that the two cases of flushing reported in the 300 mg/day group with no dose dependency (Trammell et al., 2016) was incidental, and may not be related to NR.

In addition, it is noteworthy that no human studies of NMN reported flushing with daily doses up to 1,200 mg per person. Thus, it is reasonable to conclude that daily intake of NMN at 300 mg a day will not cause side effects, such as flushing.

References for 3.6.

Cantó C, Menzies KJ, Auwerx J. NAD(+) metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. Cell Metab. 2015;22:31-53.

Kawamura T, Mori N, Shibata K. β-Nicotinamide mononucleotide, an anti-aging candidate compound, is retained in the body for longer than nicotinamide in rats. J Nutr Sci Vitaminol (Tokyo). 2016;62:272-76.

Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, Gale EA; European Nicotinamide Diabetes Intervention Trial Group. Safety of high-dose nicotinamide: A review. Diabetologia. 2000;43:1337-45.

Pieper JA. Overview of niacin formulations: Sifferences in pharmacokinetics, efficacy, and safety. Am J Health Syst Pharm. 2003;60:S9-14.

Sauve AA. NAD^+ and vitamin B3: from metabolism to therapies. J Pharmacol Exp Ther. 2008;324:883-93.

Part 4. Basis for Concluding that the NDI Will Reasonably Be Expected to Be Safe for Use as a Dietary Supplement

CONFIDENTIAL

4.1. Determination of the NOAEL or LOAEL

The LD_{50} value of NMN in rats is over 12 g/kg bw. A 90-day subchronic toxicity study in rats reported the NOAELs for NMN as 1,200 mg/kg bw/day for male and female rats.

A human clinical study showed that daily doses up to 1,000 mg NR per person was well tolerated with no side effects.

4.2. Determination of the Safety Factor

A safety margin of 100 may be applied.

4.3. Determination of the ADI

After considering a safety margin of 100, the ADI may be calculated as 840 mg/person/day for an adult weighing 70 kg. This dose is further supported by a human study that reported no adverse effects of NMN. Thus, ADI is determined as 840 to 1,200 mg per day for adults weighing 70 kg.

4.4. Determination of Estimated Daily Intake (EDI) and the EDI/ADI ratio

The EDI is 300 mg per person per day. Thus, the EDI/ADI ratio ranged from 0.25 to 0.35.

4.5. Determination of the Margin of Safety

The intended use of 300 mg NMN per day is within the safe intake range of up to 1,200 mg a day.

4.6. Safety Narrative and Conclusion

The information/data provided by SyncoZymes and (b) (4) (specifications, manufacturing process, intended use, and safety data) in this report, and supplemented by publicly available literature/safety data on NMN, provide a sufficient basis for an assessment of the safety of NMN for the proposed use as a dietary ingredient prepared according to appropriate specifications.

For the purpose of safety evaluation of NMN, the following 3 factors were considered:

1) Substantial Equivalence of syncoZymes' NMN with Other Sources of NMN: A key concept in evaluating the safety of a substance is related to substantial equivalence. The SyncoZymes' NMN in this NDI notice has similar specifications (≥97% purity) compared to other sources of NMN described in published studies, it is recognized that

the information and data of NMN or β-NMN described in other papers are pertinent to the safety of the SyncoZymes' NMN,

2) The terms NMN and β-NMN are interchangeably used in the literature because only β isomer is biologically active, and
3) NR is a precursor of NMN. In addition, NMN is metabolized extracellularly to NR that is then taken up by the cell and converted back to NMN before being metabolized into NAD^+(Poddar et al., 2019). Thus, NMN and NR are expected to have the overlapping metabolic effects.

Thus, for the purpose of safety evaluation of SyncoZymes' and (b) (4) NMN, the pharmacokinetics and safety studies of other sources of NMN and NR were reviewed in this NDI notice in addition to toxicity studies of SyncoZymes' and (b) (4) NMN.

Key findings are summarized as follows:

1) NMN serves as an intermediate in the metabolism of NAM to NAD. NMN is a metabolite of NR. The FDA had no question on its safety when used up to 300 mg/person/day (NDIN 1062). NAM has been safely used under 21 CFR §184.1535.

2) Mutagenicity, genotoxicity, and animal studies of SyncoZymes' and (b) (4) NMN showed no mutagenicity and genotoxicity as well as no adverse effect of NMN in rats. The LD_{50} of SyncoZymes NMN was over 12 g/kg bw, indicating that NMN was practically non-toxic.

3) Subchronic toxicity studies of NMN with similar purity showed the NOAEL value as 1,200 mg NMN/kg bw/day, the highest dose tested in rats.

4) Studies of other sources of NMN, whose purities are comparable to that of SyncoZymes, have also shown no adverse effects in humans. Daily consumption of 1,500 mg of NMN for 12 weeks did not cause any side effects.

5) NMN is well-characterized and is free from chemical or other microbial contamination. SyncoZymes observe the principles of Hazard Analysis Critical Control Points (HACCP)-controlled manufacturing processes, and rigorously tests its final production batches to verify adherence to quality control specifications.

6) SyncoZymes NMN (300 mg/day) has been legally marketed in Japan with no side effects reported by consumers in the past 2 years.

7) NMN, whose purities are similar to that of SyncoZymes, have been marketed with no major side effects in Japan and USA.

Therefore, it is reasonable to conclude that intended use of NMN up to 300 mg per day, divided into 1 to 2 servings, as a dietary ingredient is safe.

4.7. Alternative Basis for Reasonable Expectation of Safety

NR, a precursor of NMN, has been safely used as a dietary ingredient around the world since 2015. As a result, comprehensive review articles related to the safety of NMN have been published. In addition, the US FDA had no question on a GRAS notice and NDI notices of NR, a precursor of NMN (GRN 635, FDA, 2016; NDINs 882 and 1062, FDA, 2015, 2018).

References in Part 4.

FDA. 2016. GRN 635. Nicotinamide riboside chloride, filed by ChromaDex, Inc.
FDA. 2015. NDIN 882. Nicotinamide riboside chloride, filed by ChromaDex, Inc.
FDA. 2018. NDIN 1062. Nicotinamide riboside chloride, filed by ChromaDex, Inc.

PART 5. REFERENCES
CONFIDENTIAL
(55 FULL ARTICLES + 4 STUDIES CITED IN EFSA, 2019)

Airhart SE, Shireman LM, Risler LJ, Anderson GD, Nagana Gowda GA, Raftery D, Tian R, Shen DD, O'Brien KD. An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside (NR) and its effects on blood NAD^+ levels in healthy volunteers. PLoS One. 2017;12:e0186459.

Altug T. 2003. Introduction to Toxicology and Food. Boca Raton, FL: CRC Press.

Belenky P, Racette FG, Bogan KL, McClure JM, Smith JS, Brenner C. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD^+. Cell. 2007;129:473-84.

Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 2012;15:838-47.

Cantó C, Menzies KJ, Auwerx J. NAD(+) metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. Cell Metab. 2015;22:31-53.

Cartwright DM, Oakey LA, Fletcher RS, Doig CL, Heising S, Larner DP, Nasteska D, Berry CE, Heaselgrave SR, Ludwig C, Hodson DJ, Lavery GG, Garten A. Nicotinamide riboside has minimal impact on energy metabolism in mouse models of mild obesity. J Endocrinol. 2021;251:111-23.

Conze D, Brenner C, Kruger CL. Safety and Metabolism of long-term administration of NIAGEN (nicotinamide riboside chloride) in a randomized, double-blind, placebo-controlled clinical trial of healthy overweight adults. Sci Rep. 2019;9:9772.

Conze DB, Crespo-Barreto J, Kruger CL. Safety assessment of nicotinamide riboside, a form of vitamin B3. Hum Exp Toxicol. 2016;35:1149-60.

Cros C, Cannelle H, Laganier L, Grozio A, Canault M. Safety evaluation after acute and sub-chronic oral administration of high purity nicotinamide mononucleotide (NMN-C®) in Sprague-Dawley rats. Food Chem Toxicol. 2021;150:112060.

de Castro JM, Stein DJ, Medeiros HR, de Oliveira C, Torres ILS. Nicotinamide riboside neutralizes hypothalamic inflammation and increases weight loss without altering Muscle mass in obese rats under calorie restriction: A preliminary investigation. Front Nutr. 2021;8:648893.

de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. Aging Cell. 2016;15:522-30.

Dellinger RW, Santos SR, Morris M, Evans M, Alminana D, Guarente L, Marcotulli E. Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD(+) levels in humans safely and sustainably: A randomized, double-blind, placebo-controlled study. NPJ Aging Mech Dis. 2017;3:17.

Dollerup OL, Christensen B, Svart M, Schmidt MS, Sulek K, Ringgaard S, Stødkilde-Jørgensen H, Møller N, Brenner C, Treebak JT, Jessen N. A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am J Clin Nutr. 2018;108:343-53.

Dollerup OL, Chubanava S, Agerholm M, Søndergård SD, Altıntaş A, Møller AB, Høyer KF, Ringgaard S, Stødkilde-Jørgensen H, Lavery GG, Barrès R, Larsen S, Prats C, Jessen N, Treebak JT. Nicotinamide riboside does not alter mitochondrial respiration, content or morphology in skeletal muscle from obese and insulin-resistant men. J Physiol. 2020;598:731-54.

Dolopikou CF, Kourtzidis IA, Margaritelis NV, Vrabas IS, Koidou I, Kyparos A, Theodorou AA, Paschalis V, Nikolaidis MG. Acute nicotinamide riboside supplementation improves redox homeostasis and exercise performance in old individuals: a double-blind cross-over study. Eur J Nutr. 2020;59:505-15.

EFSA Panel on Nutrition, Novel foods and Food allergens (NDA), Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Engel KH, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Pöting A, Poulsen M, Sanz Y, Schlatter JR, van Loveren Agnès de Sesmaisons-Lecarré H, Germini A, Knutsen HK. Safety of nicotinamide riboside chloride as a novel food pursuant to Regulation (EU) 2015/2283 and bioavailability of nicotinamide from this source, in the context of Directive 2002/46/EC. EFSA J. 2019;17:e05775.

Elhassan YS, Kluckova K, Fletcher RS, Schmidt MS, Garten A, Doig CL, Cartwright DM, Oakey L, Burley CV, Jenkinson N, Wilson M, Lucas SJE, Akerman I, Seabright A, Lai YC, Tennant DA, Nightingale P, Wallis GA, Manolopoulos KN, Brenner C, Philp A, Lavery GG. Nicotinamide riboside augments the aged human skeletal muscle NAD^+ metabolome and induces transcriptomic and anti-inflammatory signatures. Cell Rep. 2019;28:1717-28.e6.

European Medicines Agency (EMA). ICH topic S1C(R2): Step 5. Note for guidance on dose selection for carcinogenicity studies of pharmaceuticals (EMEA/CHMP/ICH/383/1995). 2008.

Expert Group on Vitamins and Minerals (EVM) (2003). Niacin (Nicotinic Acid and Nicotinamide). In: *Safe Upper Levels for Vitamins and Minerals: Report of the Expert Group on Vitamins and Mineral*s. London, UK: Food Standards Agency (FSA), Expert Group on Vitamins and Minerals (EVM), pp. 52-61, 334. Available at: https://cot.food.gov.uk/committee/committee-on-toxicity/cotreports/cotjointreps/evmreport.

FDA. 2016. GRN 635. Nicotinamide riboside chloride, filed by ChromaDex, Inc.

FDA 2015. NDIN 882. Nicotinamide riboside, filed by ChromaDex.

FDA. 2018. NDIN 1062. Nicotinamide riboside, filed by ChromaDex.

Ganiger S (Advinus Therapeutics Limited), 2016. Nicotinamide riboside chloride: One generation reproduction toxicity study through diet in Sprague-Dawley rats. Study No G10959, 313 pp. [Unpublished; cited in EFSA, 2019].

Gariani K, Menzies KJ, Ryu D, Wegner CJ, Wang X, Ropelle ER, Moullan N, Zhang H, Perino A, Lemos V, Kim B, Park YK, Piersigilli A, Pham TX, Yang Y, Ku CS, Koo SI, Fomitchova A, Cantó C, Schoonjans K, Sauve AA, Lee JY, Auwerx J. Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. Hepatology. 2016;63:1190-204.

Geetha Rao G (Advinus Therapeutics Limited), 2016. Nicotinamide riboside chloride: Embryo-fetal developmental toxicity study in Sprague Dawley rats by oral route. Study No G10957. Unpublished Study Report, 319 pp. [Unpublished; cited in EFSA, 2019].

Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A, Küttler K, Malarkey DE, Maronpot RR, Nishikawa A, Nolte T, Schulte A, Strauss V, York MJ. Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol. 2012;40:971-94.

He Q, Su G, Liu K, Zhang F, Jiang Y, Gao J, Liu L, Jiang Z, Jin M, Xie H. Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. PLoS One. 2017;12:e0189837.

Health Canada. 2021. Available at: HC Chemical Substance - Nicotinamide mononucleotide.pdf.

Institute of Medicine (IOM, 1998). Niacin: In: *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B(6), Folate, Vitamin B(12), Pantothenic Acid, Biotin, and Choline*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB, 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). Washington (DC): National Academy Press (NAP), pp. 123-49. Available at: http://www.nap.edu/openbook.php?record_id=6015&page=123.

Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, Yamaguchi S, Yamashita K, Shigaki S, Ono T, Yukioka H, Okano H, Nabeshima YI, Imai SI, Yasui M, Tsubota K, Itoh H. Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocr J. 2020;67:153-60.

Kawamura T, Mori N, Shibata K. β-Nicotinamide mononucleotide, an anti-aging candidate compound, is retained in the body for longer than nicotinamide in rats. J Nutr Sci Vitaminol (Tokyo). 2016;62:272-76.

Kim M, Seol J, Sato T, Fukamizu Y, Sakurai T, Okura T. Effect of 12-Week Intake of Nicotinamide Mononucleotide on Sleep Quality, Fatigue, and Physical Performance in Older Japanese Adults: A Randomized, Double-Blind Placebo-Controlled Study. Nutrients. 2022;14:755.

Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, Gale EA; European Nicotinamide Diabetes Intervention Trial Group. Safety of high-dose nicotinamide: A review. Diabetologia. 2000;43:1337-45.

Liao B, Zhao Y, Wang D, Zhang X, Hao X, Hu M. Nicotinamide mononucleotide supplementation enhances aerobic capacity in amateur runners: A randomized, double-blind study. J Int Soc Sports Nutr. 2021;18:54.

MacKay D, Hathcock J, Guarneri E. Niacin: Chemical forms, bioavailability, and health effects. Nutr Rev. 2012;70:357-66.

Marinescu AG, Chen J, Holmes HE, Guarente L, Mendes O, Morris M, Dellinger RW. Safety assessment of high-purity, synthetic nicotinamide riboside (NR-E) in a 90-day repeated dose oral toxicity study, with a 28-day recovery arm. Int J Toxicol. 2020;39:307-20.

Martens CR, Denman BA, Mazzo MR, Armstrong ML, Reisdorph N, McQueen MB, Chonchol M, Seals DR. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults. Nat Commun. 2018;9:1286.

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 2016;24:795-806.

Pencina K, Lavu S, Dos Santos M, Beleva YM, Cheng M, Livingston D, Bhasin S. MIB-626, an Oral Formulation of a Microcrystalline Unique Polymorph of β-Nicotinamide Mononucleotide, Increases Circulating Nicotinamide Adenine Dinucleotide and its Metabolome in Middle-aged and Older Adults. J Gerontol A Biol Sci Med Sci. 2022:glac049.

Pham TX, Bae M, Kim MB, Lee Y, Hu S, Kang H, Park YK, Lee JY. Nicotinamide riboside, an NAD^+ precursor, attenuates the development of liver fibrosis in a diet-induced mouse model of liver fibrosis. Biochim Biophys Acta Mol Basis Dis. 2019;1865:2451-63.

Pieper JA. Overview of niacin formulations: Differences in pharmacokinetics, efficacy, and safety. Am J Health Syst Pharm. 2003;60:S9-14.

Poddar SK, Sifat AE, Haque S, Nahid NA, Chowdhury S, Mehedi I. Nicotinamide mononucleotide: Exploration of diverse therapeutic applications of a potential molecule. Biomolecules. 2019;9:34.

Remie CME, Roumans KHM, Moonen MPB, Connell NJ, Havekes B, Mevenkamp J, Lindeboom L, de Wit VHW, van de Weijer T, Aarts SABM, Lutgens E, Schomakers BV, Elfrink HL, Zapata-Pérez R, Houtkooper RH, Auwerx J, Hoeks J, Schrauwen-Hinderling VB, Phielix E, Schrauwen P. Nicotinamide riboside supplementation alters body composition and skeletal muscle acetylcarnitine concentrations in healthy obese humans. Am J Clin Nutr. 2020;112:413-26.

Roos J, Zinngrebe J, Fischer-Posovszky P. Nicotinamide mononucleotide: A potential effective natural compound against insulin resistance. Signal Transduct Target Ther. 2021;6:310.

Rutkowski B, Slominska E, Szolkiewicz M, Smolenski RT, Striley C, Rutkowski P, Swierczynski J. N-methyl-2-pyridone-5-carboxamide: A novel uremic toxin? Kidney Int Suppl. 2003:S19-21.

Sauve AA. NAD^+ and vitamin B3: From metabolism to therapies. J Pharmacol Exp Ther. 2008;324:883-93.

Scientific Committee on Food (SCF) (2002). Opinion of the Scientific Committee on food on the tolerable upper intake levels of nicotinic acid and nicotinamide (niacin) (expressed on 17 April 2002). (SCF/CS/NUT/UPPLEV/39 Final). Brussels Belgium: European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (SCF). Available at: https://ec.europa.eu/food/sites/food/files/ safety/docs/sci-com_scf_out80j_en.pdf.

Shen Q, Zhang SJ, Xue YZ, Peng F, Cheng DY, Xue YP, Zheng YG. Biological synthesis of nicotinamide mononucleotide. Biotechnol Lett. 2021;43(12):2199-2208.

Stromsdorfer KL, Yamaguchi S, Yoon MJ, Moseley AC, Franczyk MP, Kelly SC, Qi N, Imai S, Yoshino J. NAMPT-mediated NAD(+) biosynthesis in adipocytes regulates adipose tissue function and multi-organ insulin sensitivity in mice. Cell Rep. 2016;16:1851-60.

Thorsrud B (Experimur), 2017. Oral 7-day dose range finding toxicity study in juvenile dogs with NIAGEN®. Experimur Study No. 17-921. Unpublished Study Report. 172 pp. [cited in EFSA, 2019].

Thorsrud B (Experimur), 2018. Oral 28-day toxicity study in juvenile dogs with NIAGEN®. Experimur Study No. 17-940. Unpublished Study Report. 388 pp. [cited in EFSA, 2019].

Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, Dellinger RW, Li Z, Abel ED, Migaud ME, Brenner C. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat Commun. 2016;7:12948.

Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 2011;14:528-36.

Yoshino J, Baur JA, Imai SI. NAD(+) intermediates: The biology and therapeutic potential of NMN and NR. Cell Metab. 2018;27:513-28.

Yoshino M, Yoshino J, Kayser BD, Patti GJ, Franczyk MP, Mills KF, Sindelar M, Pietka T, Patterson BW, Imai SI, Klein S. Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science. 2021;372:1224-29.

You Y, Gao Y, Wang H, Li J, Zhang X, Zhu Z, Liu N. Subacute toxicity study of nicotinamide mononucleotide via oral administration. Front Pharmacol. 2020;11:604404.

Yu J, Laybutt DR, Kim LJ, Quek LE, Wu LE, Morris MJ, Youngson NA. Exercise-induced benefits on glucose handling in a model of diet-induced obesity are reduced by concurrent nicotinamide mononucleotide. Am J Physiol Endocrinol Metab. 2021;321:E176-E189.

Zhou CC, Yang X, Hua X, Liu J, Fan MB, Li GQ, Song J, Xu TY, Li ZY, Guan YF, Wang P, Miao CY. Hepatic NAD(+) deficiency as a therapeutic target for non-alcoholic fatty liver disease in ageing. Br J Pharmacol. 2016;173:2352-68.

Zong L, Tanaka-Yano M, Park B, Yanai H, Turhan FT, Croteau DL, Tian J, Fang EF, Bohr VA, Beerman I. NAD^+ augmentation with nicotinamide riboside improves lymphoid potential of $Atm^{-/-}$ and old mice HSCs. NPJ Aging Mech Dis. 2021;7:25.

LIST OF ABBREVIATIONS:

ADI = acceptable daily intake
AE = adverse event
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AMP = adenosine monophosphate
ANKO = adipocyte-specific nicotinamide phosphoribosyltransferase knockout
AST = aspartate aminotransferase
ATP = adenosine triphosphate
AUC = area under the curve
BMI = body mass index
BUN = blood urea nitrogen
bw = body weight
CAS = Chemical Abstracts Service
CFR = Code of Federal Regulations
cGMP = current Good Manufacturing Practice
CHL = Chinese hamster lung
CHO = Chinese hamster ovary
COA = certificate of analysis
DN-NAMPT = dominant-negative, enzymically-inactive nicotinamide phosphoribosyltransferase transgenic
DNA = deoxyribonucleic acid
EDD = endothelium-dependent dilation
EDI = estimated dietary intake
FDA = Food and Drug Administration
GMO = genetically modified organism
GRAS = Generally Recognized as Safe
HACCP = Hazard Analysis Critical Control Point
HFD = high-fat diet
HPLC = high-performance liquid chromatography
HSC = hematopoietic stem cell
i.p. = intraperitoneally
IOM = Institute of Medicine
IUPAC = International Union of Pure and Applied Chemistry
LD_{50} = median lethal dose
LOAEL = lowest-observed-adverse-effect-level
MeNAM = methylnicotinamide
MNPCE = micronucleated polychromatic erythrocyte
MTD = maximum tolerate dose
NAAD = nicotinic acid adenine dinucleotide
NAD^+ = nicotinamide adenine dinucleotide
NADH = nicotinamide adenine dinucleotide hydrogen
$NADP^+$ = nicotinamide adenine dinucleotide phosphate
NADPH = nicotinamide adenine dinucleotide phosphate hydrogen
NAFLD = non-alcoholic fatty liver disease

NAM = nicotinamide
Nampt = nicotinamide phosphoribosyltransferase
NCE = normochromatic erythrocyte
NDI = New Dietary Ingredient
NMN = nicotinamide mononucleotide
NOAEL = no-observed-adverse-effect-level
NR = nicotinamide riboside
NRPT = nicotinamide riboside and pterostilbene
OECD = Organisation for Economic Co-operation and Development
PBMC = peripheral blood mononuclear cell
PCE = polychromatic erythrocyte
PPARγ = peroxisome proliferator-activated receptor gamma
RICC = relative increase in cell count
RNA = ribonucleic acid
SD = Sprague-Dawley
SIRT = sirtuin
TC = total cholesterol
TG = triglyceride
TNF = tumor necrosis factor
U.S.C. = United States Code
UL = tolerable upper intake level
US = United States
UV-VIS = ultraviolet-visible
VAT = visceral adipose tissue
VO2 = oxygen uptake
WBC = white blood cell
2-PY = N-methyl-2-pyridone-5-carboxamide
4-PY = N-methyl-4-pyridone-5-carboxamide