



**APPLICATION FOR THE APPROVAL OF
1- METHYLNICOTINAMIDE CHLORIDE (1 - MNA)
AS A NOVEL FOOD INGREDIENT FOR USE IN THE
MANUFACTURE OF FOOD SUPPLEMENTS PURSUANT TO
EU NOVEL FOODS REGULATION (EC) 258/97**

May, 2013

Dossier prepared and submitted on behalf of
Pharmena S. A., Wólczańska 178, 90-530 Łódź, Poland
for evaluation pursuant to EU Novel Foods Regulation (EC) 258/97
by the UK Food Standards Agency

Author:

Foodie - G. Osęka & I. Kowalczyk-Andrzejewska Sp. j.

OFFICE: Żurawia 6/12, 439, 00-503 Warsaw, Poland

Phone/Fax: (+48) 22 664 27 45, e-mail: foodie@foodie.pl, <http://www.foodie.pl>

NON-CONFIDENTIAL VERSION

Table of Contents:	Page
1. ADMINISTRATIVE DATA	7
2. EXECUTIVE SUMMARY	7
3. INTRODUCTION	9
I. SPECIFICATION OF THE NOVEL FOOD	10
I.1. GENERAL DESCRIPTION	10
I.2. PRODUCT SPECIFICATION	13
I.3. SPECIFICATION COMPLIANCE	14
I.4. ANALYTICAL PROCEDURES FOR 1-MNA	14
I.5. ANALYTICAL PROCEDURES FOR THE 1-MNA IN A FINAL PRODUCT	16
II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD	16
II.1. DESCRIPTION OF THE MANUFACTURING PROCESS	16
II.1.1. FLOWCHART OF THE 1-MNA MANUFACTURING PROCESS	18
II.2. STABILITY OF 1-MNA	18
II.3. STABILITY, REACTION AND FATE OF 1-MNA IN A FINAL PRODUCT	22
III. HISTORY OF THE SOURCE OF THE NOVEL FOOD	22
III.1. 1-MNA SOURCE	22
IV – VIII. NOT APPLICABLE, FOR GENETICALLY MODIFIED FOODS ONLY	
IX. ANTICIPATED INTAKE/ EXTENT OF USE OF THE NOVEL FOOD	23
IX.1. PROPOSED GROUPS FOR THE USE OF 1-MNA	24
IX.2. INDIVIDUAL PROPOSED FOOD USES AND USE LEVELS	24
IX.3. PREDICTED INTAKE OF 1- MNA FROM PROPOSED SUPPLEMENT DOSE	24
IX.4. PREDICTED INTAKES FOR RISK GROUPS	24
X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD	24

X.1. PREVIOUS 1-MNA INTAKE- INTAKE ESTIMATE FROM ALL SOURCES	24
X.2. PREVIOUS EXPOSURE TO 1-MNA PREPARATIONS	25
X.3. INFORMATION ON EXISTING AUTHORISATIONS AND EVALUATIONS	27
XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD	28
XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD	28
XIII. BIOLOGICAL AND TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD	29
XIII.1. BIOAVAILABILITY OF 1-MNA FOLLOWING ORAL CONSUMPTION	30
XIII.1.1 METABOLIC FATE AND BIOLOGICAL DISTRIBUTION	30
XIII.1.1.1 GENERAL METABOLISM OF NIACIN FROM ORGANIC AND INORGANIC SOURCES	30
XIII.1.1.2 METABOLIC FATE OF 1-MNA	30
XIII.1.2 INTERACTIONS WITH OTHER COMPONENTS IN THE DIET	31
XIII.1.3 IMPACT ON THE INTESTINAL MILIEU AND ON THE ABSORPTION OF OTHER NUTRIENTS	31
XIII.1.4 PHARMACOKINETICS AND METABOLISM	31
XIII.1.4.1 PUBLISHED PHARMACOKINETIC STUDIES	31
XIII.1.4.2 UNPUBLISHED PHARMACOKINETIC STUDIES IN ANIMALS	31
XIII.1.4.3. UNPUBLISHED PHARMACOKINETIC STUDIES IN HUMANS	36
XIII.2. TOXICOLOGICAL ASSESSMENT	39
XIII.3. TOXICITY STUDIES PERFORMED WITH 1-MNA	39
XIII.3.1. ACUTE ORAL TOXICITY	39
XIII. 3.2 ACUTE TOXICITY AFTER INTRAPERITONEAL INJECTION	39
XIII.3.3. SHORT-TERM AND SUBCHRONIC TOXICITY	39
XIII.3.4. GENOTOXICITY	42
XIII.3.5. CHRONIC TOXICITY AND CARCINOGENICITY	43
XIII.3.6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY	43

XIII.4. PUBLISHED IN VITRO AND ANIMAL TOXICITY STUDIES	43
XIII.5. OTHER ANIMAL STUDIES WITH 1-MNA	47
XIII.6. HUMAN STUDIES	48
XIII.7. TOXICITY OF 1-MNA	51
XIV. REFERENCES	52

ANNEXES

Annex A – Solubility data

Annex B – Melting point

Annex C – Spectroscopic characterisation

Annex D – Biodegradation in water

Annex E - Analytical data of three batches of 1-MNA

Annex F - Analytical methods

Annex G- Flowchart of the 1-MNA manufacturing process with equipment used for reaction of Step I/Step II and description of procedures

Annex H - Stability data

Annex I – Impurities

Annex J – Stability of 1-MNA in final product

Annex K- Animal toxicity and pharmacokinetic study performed with 1-MNA

Annex K-1 - Expert review of toxicity study with 1-MNA

Annexes L-N Animal pharmacokinetic studies performed with 1-MNA

Annex O- Human pharmacokinetic study performed with 1-MNA (MNA-002)

Annexes P - Z Animal toxicity studies performed with 1-MNA

Annex ZZ - Human study performed with 1-MNA (MNA-001)

LIST OF TABLES

Table 1. Theoretical elemental analysis

Table 2. Solubility

Table 3. Specification of the 1-MNA

Table 4. Potential impurities related to chemical pathway of synthesis/destruction

Table 5. Results of reviewing 3 batches of 1-Methylnicotinamide Chloride manufactured in April and June, 2011 towards impurity contents

Table 6. Food products with a highest content of 1-MNA

Table 7: Marketed products containing 1-MNA

Table 8. Patents owned by PHARMENA and its subsidiaries

Table 9. Summary of pharmacokinetic parameters for the subjects obtaining during the study period 90 mg/d of 1-MNA

Table 10. Summary of pharmacokinetic parameters for the subjects obtaining during the study period 270 mg/d of 1-MNA

Table 11: Safety aspects of 1-MNA studied in human trials

LIST OF FIGURES

Figure I.1.1. 1-methylnicotinamide chloride molecular structure

Figure I.1.2. 1-methylnicotinamide molecular structure

Figure II.1.1 Chemical pathway and in process control

Figure XIII.1.1.1. The scheme of nicotinamide metabolism

Figure XIII.4.1. Incorporation of [¹⁴C]nicotinamide into NAD and 1-methylnicotinamide in liver slices as a function of [¹⁴C]nicotinamide concentration

Figure XIII.4.2. Effect of exogenous 1-methylnicotinamide on the incorporation of [¹⁴C]nicotinamide into NAD in liver slices

Figure XIII.4.3. Effect of exogenous 1-methylnicotinamide on the incorporation of [¹⁴C]nicotinamide into 1-methylnicotinamide in liver slices

Figure XIII.4.4. Increased cell growth of MELCs cultured with 1-methylnicotinamide

NOTE ABOUT CONFIDENTIALITY

Information for which the applicant claims confidentiality because it pertains to production or marketing know-how, future plans and details about manufacturers, is marked [REDACTED] Annexes F, G, K-V and X-ZZ which contain details about the production process and the unpublished studies, are marked "Confidential" in their entirety.

1. ADMINISTRATIVE DATA

NAME AND ADDRESS OF THE APPLICANT

PHARMENA S.A.
Wólczańska 178, 90-530 Łódź, Poland
Phone: +48 42 291 33 70
Fax: +48 42 291 33 71
<http://www.pharmena.com.pl>

Contact person:

Dr Marzena Wieczorkowska

NAME AND ADDRESS OF PERSON(S) RESPONSIBLE FOR DOSSIER

Mrs. Grażyna Oseka
Adviser
Mobile: +48 501 295 443
Phone/Fax: +48 22 664 27 45
grazyna.oseka@foodie.pl

Mrs. Iwona Kowalczyk - Andrzejewska
Adviser
Mobile: +48 695 659 705
Phone/Fax: +48 22 664 27 45
iwona.kowalczyk@foodie.pl

Foodie - G. Oseka & I. Kowalczyk-Andrzejewska Sp. j.
OFFICE: Żurawia 6/12, 439, 00-503 Warsaw, Poland
Phone/Fax: (+48) 22 664 27 45, , e-mail: foodie@foodie.pl
<http://www.foodie.pl>

2. EXECUTIVE SUMMARY

Introduction of synthetic 1-Methylnicotinamide (1-MNA) on the market as a novel food ingredient in the EU as a physiologically active ingredient in food supplements is proposed by Pharmena S.A.

1-MNA is a vitamin-based substance which is formed in the body from vitamin PP, as a result of the nicotinamide N-methyltransferase activity and then it is metabolized to the corresponding pyridines. 1-MNA is present naturally in human body as a normal product of niacin metabolism. It is excreted from the body in urine.

1-MNA is manufactured in two main steps: step I reaction of nicotinamide [REDACTED] [REDACTED] allows to obtain crystalline crude 1-MNA and in step II crude 1-MNA is recrystallized. The manufacturing process is based in the Good Manufacturing Practices. Stability tests, batch-to-batch variability, evaluation of heavy metals residues level and microbiological impurities were performed showing good quality and safety of the product. Analytical methods

used for quality assessment consisted of two basic groups: general methods (including appearance and solubility, identification-IR, solution colouration, loss on drying, water content, residue on ignition, heavy metals content, residual solvents, bioburden) and analytical methods (including identification by PHLC, assay by HPLC, purity by HPLC).

1-MNA is intended for consumption in the form of a powder in several different final formats (gelatine capsules, tablets and possible other). It is supposed to be consumed as dietary supplement, either in a short-term or in a prolonged manner. Proposed use levels 125 mg/single dose and 250 mg/day.

It is expected that the product would be consumed by adult population and elderly people. The final product containing 1-MNA will be designated to maintain circulatory system health because this substance protects normal function of endothelium, lowers risk of vascular impairment induced by postprandial hypertriglyceridemia and stimulates endothelium-dependent repair response or/and organ protection. Positive effect may be expected also in different situations with involvement of endothelial vasoprotective mechanisms not related to cardiovascular system. 1-MNA is already used topically in cosmetology as an anti-irritation component and also as compound which stimulates the processes of repair and regeneration of skin.

Pharmacokinetic studies performed in animal and human models are discussed in details in the dossier. Toxicity of 1-MNA was studied in several animal studies assessing acute toxicity after oral administration, acute skin and eye irritation, allergenic effects on skin, subacute toxicity and genotoxicity (including OECD471 and OECD487 tests). 1-MNA has demonstrated very low toxicity in mice and rats models at doses exceeding those expected to be used in humans. Based on 28-day toxicity study in rats the dose of 1000 mg/kg/day 1-MNA can be considered a no-observed-adverse-effect level (NOAEL) following 1-MNA oral administration. Safety and efficacy aspects of 1-MNA were also studied in randomized placebo-controlled double-blind trial (phase II) in humans.

Since it is produced at a commercial scale from nicotinamide by a methylation and crystallization, 1-MNA complies with the definition of a novel food according to article 1 (2)(c) of the Novel Food Regulation ("foods and food ingredients with a new or intentionally modified primary structure").

The present application for authorization of 1-MNA as a novel food was prepared according to the EU Commission's Guidelines on the scientific information necessary to support applications for the placing on the market of novel foods or novel food ingredients - Commission Recommendation (97/618/EC). 1-MNA was identified as belonging to class 1.1 ("pure chemicals or simple mixtures from non-GM sources with no history of food use in the Community").

3. INTRODUCTION

1-Methylnicotinamide chloride (1-MNA) is a vitamin-based substance which is formed in the body from vitamin PP, as a result of the nicotinamide N-methyltransferase activity and then it is metabolized to the corresponding pyridines. 1-MNA is excreted from the body in urine. In recent years, as a result of research and commercialization carried out by PHARMENA S.A., this substance in the form of the chloride salt was used in cosmetology as an anti-irritation component and also as a compound which can stimulate the processes of repair and regeneration of skin. Recent data suggest also that 1-MNA has an impact on the maintenance of normal endothelial function and cardiovascular system, thus can be used as dietary supplement by adults, especially in elderly, because of its potential properties to reduce the progression of atherosclerosis. It should also be mentioned that vitamin PP conversion into 1-MNA is decreasing with age.

Pharmena S.A. proposes introduction synthetic 1-MNA on the market as a novel food ingredient in the EU as a physiologically active ingredient in food supplements.

1-MNA has not been consumed so far in significant amounts in the European Union. However, 1-MNA has been found in brown seaweed (*Undaria pinnatifida*) extract, in leaves of green tea, and in several other dietary products (see Table 6). Therefore, it should be regarded as a novel food.

1-MNA naturally occurs in human body being the main metabolite of niacin metabolism.

Since it is produced at a commercial scale from nicotinamide by a methylation and crystallization, it complies with the definition of a novel food according to article 1 (2)(c) of the Novel Food Regulation ("foods and food ingredients with a new or intentionally modified primary structure").

The present application for authorization of 1-MNA as a novel food was prepared according to the EU Commission's Guidelines on the scientific information necessary to support applications for the placing on the market of novel foods or novel food ingredients - Commission Recommendation (97/618/EC). 1-MNA was identified as belonging to class 1.1 ("pure chemicals or simple mixtures from non-GM sources with no history of food use in the Community").

For evaluation of a class 1.1 product, the following information is required according to the Commission Guideline, Table II.

I. Specification of the novel food

II. Effect of the production process applied to the novel food

III. History of the organism used as the source of the novel food

IV-VIII. Not applicable as 1-MNA is not a GM food.

IX. Anticipated intake of use of the novel food

X. Information from previous human exposure to the novel food or its source

XI. Nutritional information on the novel food

XII. Microbiological information on the novel food

XIII. Toxicological information on the novel food

I. SPECIFICATIONS OF THE NOVEL FOOD

I.1. GENERAL DESCRIPTION

This section contains a general description of 1-Methylnicotinamide chloride, the product specifications and the analytical controls.

International name:

1-Methylnicotinamide chloride (1- MNA)

Chemical formula:

$C_7H_9N_2OCl$

Structural formula:



Figure I.1.1. 1-methylnicotinamide chloride molecular structure.

Synonyms:

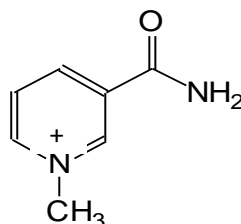
1-Methyl-3carbamoylpyridinium
1-Methyl-3carbamoylpyridinium cation
1-Methylnicotinamide
3-Amido-N-methylpyridinium
1-Methyl-3-Pyridinecarboxamide
3-carbomoyl-1-methyl-Pyridinium
1-methylnicotinamide
N-Methyl-3carbamidopyridinium

MDL number: MFCD00060042

Chemical Abstracts Service (CAS) Registry number: [1005-24-9]

WE number: 463-670-7

RTECS number: UU1925000



Molecular weight: 172.61

Figure I.1.2.1-methylnicotinamide molecular structure.

Table 1. Theoretical elemental analysis:

Element	%
Carbon	48,71
Hydrogen	5,25
Nitrogen	16,23

Kingdom: Organic compounds

Super class: aromatic heteromonocyclic compounds

Class: pyridines and derivatives

Subclass: not available

Other descriptors: primary carboxylic acid amides

State:

1-MNA is white or almost white, fine crystalline, hygroscopic, powder.

Solubility:

Table 2. Solubility

Solvent		Solubility*
Water	very soluble	0,667 g/mL
Methanol	soluble	33 mg/mL
Dichloromethane	practically insoluble	Less than 0.1 mg/mL
2-propanol	practically insoluble	Approx. 1,3 mg/mL

*solubility data obtained in-house at room temperature

Legend:

Very soluble - 1 g in less than 1mL of solvent

Freely soluble - 1 g in 1 ÷ 10 mL of solvent

Soluble - 1 g in 10 ÷ 30 mL of solvent

Sparingly soluble - 1 g in 30 ÷ 100 mL of solvent

Slightly soluble - 1 g in 100 ÷ 1000 mL of solvent
Very slightly soluble - 1 g in 1000 ÷ 10000 mL of solvent
Practically insoluble - 1 g in more than 10 000 mL of solvent
(see Annex A “Determination of solubility in water with pH effect by shaking in the flask”).

Melting point:

Melting point equal to 235.7°C with decomposition for compound recrystallized from methanol was reported in “1-MNA determination of the melting point with the use of a device with metal block”(see Annex B).

Spectroscopic characterisation:

1-Methylnicotinamide chloride was characterized by the following spectroscopic techniques:

1. ¹H- and ¹³C-Nuclear Magnetic Resonance (NMR)
2. IR spectroscopy
3. UV-VIS spectroscopy
4. Mass spectroscopy
5. Differential scanning calorimetry

See Annex C for spectroscopic characterization.

Polymorphic form:

To the best of our knowledge there is no information on polymorphic forms of 1-Methylnicotinamide chloride.

Biodegradation in water:

1-MNA is a substance easily biodegraded in water. In the study performed in [REDACTED] in 2006 according to OECD 302B (UE C.9. method) after 21 days of observation 76.89% of 1- MNA was degraded (see Annex D).

Storage conditions:

The product should be stored in a storage room at temperature up to 25°C and relative humidity up to 60%. The container should be tightly closed.

Proposed shelf-time: (24 months).

I.2. PRODUCT SPECIFICATION

The proposed chemical specification for 1-MNA is listed in table below.

Table 3. Specification of the 1-MNA.

No.	Specification parameter/ Analysis method	Specification value
1	Appearance, solubility test/USP	White or off white, crystalline solid, very soluble in water, soluble in methanol, practically insoluble in 2-propanol and dichloromethane
2	Identification: a) IR spectroscopy/USP <197k> b) HPLC test	a) Spectrum must comply with reference IR spectrum b) The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation as obtained in the Assay
3	Degree of colouration / EP 2.2.2., Method I	Colouration of 10 % sample solution not more intense than reference solution Y ₇
4	Loss of drying/ USP <731>	Not more than 1.0 %
5	Water content/ Karl Fisher method	Not more than 0.3 %
6	Residue on ignition/USP <281>	Not more than 0.1 %
7	Heavy metals USP Method 1 <231>	Not more than 20 ppm
8	HPLC purity Trigonelline Nicotinic Acid Nicotinamide Largest unknown impurity Sum of unknown impurities Sum of all impurities	Not more than 0.05 % Not more than 0.10 % Not more than 0.10 % Not more than 0.05 % Not more than 0.20 % Not more than 0.50 %
9	Residual solvents: Headspace/GC / USP<467> Methanol	Not more than 3000 ppm
10	Assay by HPLC (on dry substance basis)	Not less than 98.5 % and not more than 101.5 % (as anhydrous substance)
11	Bioburden/ EP 2.6.12, 2.6.13 Total Aerobic Microbial Count Mold and Yeasts Count <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Enterobacteriaceae</i>	Not more than 100 cfu/g Not more than 10 cfu/g Not present in 1 g Not present in 1 g Not present in 1 g

I.3. SPECIFICATION COMPLIANCE

All batch.

Analytical data of three batches of 1-MNA are presented in Annex E.

I.4. ANALYTICAL PROCEDURES FOR THE 1-MNA

Analytical methods used for quality assessment of 1-MNA consisted of two basic groups:

A. the pharmacopeia (compendium) General methods, either applied for testing as prescribed or after minor adjustments and/or modifications to meet instruments prerequisite, if applicable.

B. analytical methods developed during the R&D phase in order to meet analytical requirements imposed for API testing.

A. To the group of the General Methods and tests applied as prescribed (or insignificantly modified) the following tests belong:

1. Appearance and Solubility as per USP (MNA 001);
2. Identification-IR as per USP <197K> (MNA 002);
3. Solution Colouration per E.P. 2.2.2 (MNA 003);
4. Loss on Drying as per USP <731> (MNA 004);
5. Water Content as per USP method 1 <921> (MNA 005);
6. Residue on Ignition as per USP <281> (MNA 006)*;
7. Heavy Metals as per USP Method 1 <281> (MNA 007);
8. Residual Solvents as per USP <467> (MNA 009);
9. Bioburden as per E.P. 2.6.12 and E.P. 2.6.13 (MNA 011)*.

* - Tests MNA 006 and MNA 011 are performed by outside contracted laboratories: [REDACTED], respectively.

B. To the group of analytical methods developed in-house to meet specification requirements belong the following tests:

1. Purity by HPLC, Ifotam method (MNA 008);
2. Identification by HPLC, method (MNA 010);
3. Assay by HPLC, method (MNA 010).

Short description of the analytical methods is listed below.

IR spectroscopy (test MNA 002)

The IR spectroscopy method in KBr tablets was selected as the commonly recognized test for chemicals identification after comparison with authentic sample (Standard). The second independent 1-MNA identification is based on comparison of retention time of product peak obtained in the Assay test (MNA 010) with the one obtained for peak of 1-MNA standard. These two tests give unquestionable certainty of identification of substance as 1-MNA.

Loss on drying (test MNA 004)

Loss on drying is also commonly used test for the determination of total content of volatile solvents including water. The test, with the limit set for 1,0 %, is performed according to USP <731>.

Water content by KF (test MNA 005)

Water content tested by Karl Fisher titration is the complementary method to test residual solvents. Karl Fisher method determines only the water content excluding organic solvent content. The limit of water content is set for 0,3 %.

Residue on ignition (test MNA 006)

A chance of contamination of product by silicates and iron salts exists due to the fact of using glass lined reactors to manufacture of 1-MNA. Both kinds of impurities can be determined as the residue on ignition. Also other potential contaminations in form of alkali metals salts, calcium and magnesium salts could be present in product as impurities transferred from raw material (Nicotinamide) and also can be determined as the residue on ignition. The limit for this test is set for 0,1 %.

Heavy metals (test MNA 007)

Heavy metals determination is the analytical standard in API testing and the limit is also established on compendial accepted level or 20 ppm (0,0002 %). For heavy metals determination UPS Method I <231> is applied.

Purity by HPLC (method MNA 008)

To control purity of 1-MNA a sensitive HPLC method for determination of known impurities: Trigonelline, Nicotinic Acid and Nicotinamide with the limits of 0,05 %, 0,10 % and 0,1 %, respectively, was developed. This method was validated within a range of 0,01 to 0,12 %. The limits for each unknown impurity, sum of unknown impurities and sum of all impurities were set for 0,05 %, 0,20 % and 0,50% respectively. The Limits of Quantification for impurities mentioned above are equal to 0,00044 %, 0,00049% and 0,0012 %, respectively. These impurities are detected at following levels: 0,00015 %, 0,00016% and 0,00041 %, respectively. Calculation of unknown impurities is done in relation to peak of 1-MNA in Reference solution.

Residual Solvents by static head Space/GC (test MNA 009)

Due to fact that no organic solvents class I were used in 1-MNA manufacturing there was no need to perform testing for these solvents. However, sample containing set of Class I solvents, (including benzene at level 1,8 ppm), is incorporated into each analytical set for residual solvents. By this means any Class I solvent (e.g. benzene), if present in APL should be easily detected.

For sole organic solvent used in manufacture i.e. methanol the limit was set for 3000 ppm. The method was validated within a range of 5 to 120 % (60 - 3450 ppm). The Limits of Quantification and Detection for methanol are equal to 46,7 ppm and 4,3 ppm, respectively.

Assay by HPLC (method MNA 0010)

Adopted HPLC method for 1-MNA assay requires 1-MNA Standard for calibration. For this purpose a 1-MNA Working Standard was prepared in-house and tested. The structure and purity of the 1-MNA WS were confirmed by testing according to Written Specification and additionally by C NMR, UY-Vis and Mas spectroscopy. Method for Assay determination was validated and found to meet criteria.

Microbiological quality (test MNA 011)

Bioburden is determined by testing of 1-MNA (test MNA 011 by [REDACTED] contract laboratory) according to EP methods 2.6.12 and 2.6.13. The level of microbial contamination is adequate for requirement of use of 1-MNA for manufacture of cosmetics and oral drug and is established at the level as follows: Total Aerobic Microbial Count - not more than 100 cfu/g; Mold and Yeasts Count - not more than 10 cfu/g. The following bacterial strains should not be present in two samples 1 g each: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacteriaceae*.

See Annex F for analytical methods detailed description.

I.5. ANALYTICAL PROCEDURES FOR THE 1-MNA IN A FINAL PRODUCT

For the determination of the presence of 1-MNA in a dietary supplement HPLC method is used. This method is described in Annex F.

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

II.1. DESCRIPTION OF THE MANUFACTURING PROCESS

Manufacturer, manufacturing and testing site address:

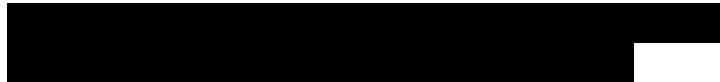
[REDACTED]

[REDACTED]

Contract Laboratory for Biological Tests.

Every batch of 1-MNA is tested for microbiological quality (test MNA 011) by formal contract laboratory:

[REDACTED]



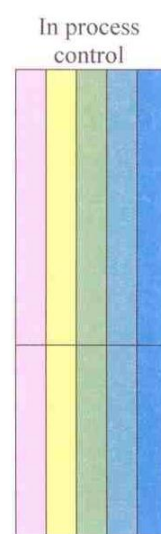
Every batch of 1-MNA is examined for residue on ignition (test MNA 006) by formal contract laboratory:



Step I Methylation



Step II Crystallization



In-process control:






-  Check cleanness of equipment
-  Quality control of raw materials, solvents, reagents
-  Weight (volume) of raw materials, solvents, reagents
-  Temperature and time of reactions
-  Stirring speed (rpm)

Figure II.1.1 Chemical pathway and process control

List of chemicals used in the process

List of starting materials:

[REDACTED]

List of reagents:

[REDACTED]

List of solvents:

[REDACTED]

List of isolated substances:

[REDACTED]

Brief description of 1-Methylnicotinamide Chloride manufacture:

[REDACTED]

[REDACTED]

II.1.1. FLOWCHART OF THE 1-MNA MANUFACTURING PROCESS

Flowchart of the 1-MNA manufacturing process with equipment used for reaction of Step I/Step II and description of can be found in Annex G.

II.2. STABILITY OF 1-MNA

Stability test was performed in real life conditions of temperature (25°C) and humidity and showed good stability of 1-MNA under normal storage conditions.

Stability test is ongoing. The results of the test are going to be updated.

Stability test performed by the former manufacturer of the 1-MNA showed good stability of 1-MNA under normal storage condition during 36 months period.

During storage in real conditions of temperature and humidity, results of the stability test are consistent with specifications of the product. The parameters referring to the active ingredient

(i.e. identification, HPLC purity, content of the active ingredient, level of the impurities) remain unchanged and consistent with specification.

Based on the obtained stability results, the shelf life is proposed as 24 months, with the possibility of extension to 36 months after completion of the full stability test.

The details are given in Annex H.

Batch-to batch control

Batch-to-batch analytical controls are performed in order to make sure that 1-MNA fulfills the specifications. Section I.3. Specifications compliance contains the results from the analytical controls performed in different batches.

Examples of certificates of several standard batches are presented in Annex E.

Residues / Impurities

Every batch of 1-MNA is examined for residues on ignitron (test MNA 006) by formal contract laboratory:



IMPURITIES

Sources of potential impurities

- Potential impurities originate from starting materials;
- Impurities formed during synthesis as by-products;
- Potential impurities as degradation products in various water solutions;
- Residual solvents.

Impurities due to starting materials


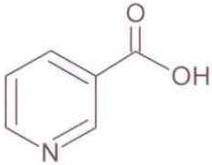
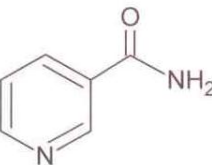
One possible impurity of 1-MNA represents starting material for the synthesis: Nicotinamide. The presence of this impurity was observed on HPLC chromatogram (see FIGURE 11 in Annex I) of concentrated mother liquor after isolation of Crude 1-Methylnicotinamide Chloride. However this impurity was not isolated from filtrate. Nicotinamide was identified based on spiking experiment - FIGURE 13 in Annex I.

Impurities formed during synthesis/degradation

Trigonelline hydrochloride and nicotinic acid are formed in small amounts as a result of hydrolysis/degradation side reaction during 1-MNA synthesis. The presence of these impurities in

mother liquor after isolation of crude 1-MNA was confirmed by spiking experiments - FIGURES 14 and 15 in Annex I. However, none of these compounds was isolated from filtrates.

Table 4. Potential impurities related to chemical pathway of synthesis/destruction.

SN	Name	Structure/Formula	Technique of detection	Analytical test	LoQ [%]	The origin of impurity
1	Trigonelline (hydrochloride)		HPLC	<i>MNA 008</i>	0.00044	By-product, product of destruction
2	Nicotinic Acid		HPLC	<i>MNA 008</i>	0.00049	By-product, product of destruction
3	Nicotinamide		HPLC	<i>MNA 008</i>	0.0012	Starting material

Residual solvents

There are only two solvents used for the production of 1-MNA namely improved water and methanol. Thus, only methanol can be considered as residual solvent and - Headspace/GC method was elaborated for quantification of this solvent. Contents of methanol determined in first three commercial batches are listed in table below.

Potential impurities due to degradation at normal storage condition

The stability data from the present manufacturer of 1-MNA showed good stability of 1-MNA under normal storage condition during 12 months period ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}/60\%$ relative humidity $\pm 5\%$).

At 12 months, all known and unknown impurities remained at an acceptable level and 1-MNA purity is 99% or greater. In fact, only nicotinamide (starting material) and trigonelline (a minor niacin metabolite, also found in coffee, barley, cantaloupe, corn, onions, peas, soybeans, and tomatoes) was found at the impurity level not exceeding 0,0085% for nicotinamide and 0,002% for trigonelline, respectively. The level of those impurities remained unchanged during storage of 1-MNA under normal conditions. Level of largest unknown impurity (0,002% - 0,0046%) also remained unchanged during 12-months storage under normal conditions. HPLC method used for determining purity of 1-MNA is capable to detect trigonelline, nicotinic acid and nicotinamide with

the Limit of Detection (LoD) as low as 0,00015 %, 0,00016 % and 0,00041 %, respectively. Thus, small quantities of these impurities would be easily detected, if formed.

The stability data from the former manufacturer of 1-MNA showed good stability of 1-MNA under normal storage condition during 36 months period (25°C ±2°C/60% relative humidity ± 5%). Any changes in the level of impurities were not observed and 1-MNA content remained unchanged. Only small change in appearance of the crystals was observed.

Impurities found in 1-MNA (Impurity profile)

Table 5. Results of reviewing 3 batches of 1-MNA manufactured in April and June, 2011 towards impurity contents.

SN	Impurity/parameter	limits	MNA batch number			
			3090-110404	3090-110608	3090-110615	
1.	Production date	NA	April 2011	June 2011	June 2011	
2.	Batch size [kg]	34.2<and <37.8	37.10	36.73	35.61	
3.	MNA 008	Trigonelline [%]	<0.05	Below LoD	Below LoD	Below LoD
4.		Nicotinic Acid [%]	<0.1	Below LoD	Below LoD	Below LoD
5.		Nicotinamide [%]	<0.1	0.001	0.004	0.001
6.		Largest unknown impurity [%]	<0.05	Below LoD	0.003	0.002
7.		Sum of unknown impurities [%]	<0.3	0	0.007	0.003
8.	MNA 009	Methanol [ppm]	<3000	551	1057	557

HPLC chromatograms (Tests MNA 008) and HeadSpace/GC of 1-MNA, batch No. 3090-110404 with corresponding chromatograms of impurity standards at limit concentrations are shown in FIGURES 16 ÷ 20 in Annex I, as examples for impurity profile.

For detailed information about impurities and HPLC data see Annex I.

Heavy metals

Determination is the analytical standard in API testing and the limit is also established on compendia accepted level of 20 ppm (0,0002%). For heavy metals determination UPS Method I <231> is applied.

Microbiological impurities

Every batch of 1-MNA is examined for microbiological quality (according to EP methods 2.6.12 and 2.6.13) by formal contract laboratory:



The following requirements for microbiological purity were used:

Total number of bacteria in 1 g of the product: not more than 100

Mould and yeasts in 1 g of the product: not more than 10

Pseudomonas aeruginosa – not present

Staphylococcus aureus – nor present

Enterobacteriaceae – not present

Examples of certificates of several standard batches are presented in Annex E.

II.3. STABILITY, REACTION AND FATE OF 1-MNA IN A FINAL PRODUCT

Stability tests confirm that 1-MNA (in the form of powder) is stable under the temperature and pH-conditions typically encountered in food storage and processing. Stability tests for final products were performed (form: hard gelatin capsule, 1-MNA contents in capsule: 30 mg).

Stability tests showed good stability of final product under normal storage conditions during 24 months period.

The details are given in Annex J.

III. HISTORY OF THE SOURCE OF THE NOVEL FOOD

The novel food ingredient is obtained from nicotinamide in a process of nicotinamide methylation.

III.1. 1-MNA SOURCE

Nicotinamide (niacinamide), the substance representing 1-MNA source, is very well-known substance, having, together with nicotinic acid, the biological activity of niacin (vitamin B3). As in the case for the other B vitamins, meat, poultry, and fish are excellent food sources, followed by dairy and grain products. Certain grains such a maize, and whole highly polished rice can be very poor sources and may be associated with clinical deficiency in the diets are otherwise poor and monotonous. Niacin can be also synthesized from tryptophan in the process of endogenous synthesis. Niacin deficiency is associated with characteristic disease in humans known as pellagra. The best defined role of niacin is in the metabolism of metabolic fuels, as the functional nicotinamide part of the coenzymes NAD and NADP, which play a major role in oxidation and reduction reactions. In general, NAD⁺ is involved as an electron acceptor in energy-yielding metabolism, being oxidized by the mitochondrial electron transport chain, while the major

coenzyme for reductive synthetic reactions is NADPH. In addition to its coenzyme role, NAD has a function as the source of ADP-ribose for the ADP-ribosylation of a variety of proteins and hence activation of nucleoproteins in the DNA repair mechanism.

It was suggested in recent years that nicotinamide has got possibly useful pharmacological properties at high intake levels. Nicotinamide, contrary to nicotinic acid, has not been shown to have antihyperlipemic properties or the associated toxicity. Some studies suggest possible positive effect of nicotinamide on the predicted incidence of insulin-dependent diabetes (through inhibition of poly ADP ribose synthetase in pancreatic β cells), on some abnormalities associated with schizophrenia, Down's syndrome, hyperactivity in children, etc. But it should be underlined that those positive effects are not yet generally accepted.

IV – VIII. NOT APPLICABLE, FOR GENETICALLY MODIFIED FOODS ONLY

IX. ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

Intake of 1-MNA may result from three sources:

- 1) normal dietary intake from food,
- 2) intake from cosmetics,
- 3) intake from proposed use in food [REDACTED].

Intake of 1-MNA from normal dietary sources

The information about dietary intake of 1-MNA is limited.

Taguchi et al. (1986) measured 1-MNA content in 105 food products in Japan. 1-MNA was present in 45 products of both animal and plant origin in a concentration at least 0,1 mg/100 g of edible parts of the product (lowest threshold of the method used – fluorescence Huff method modified by Nose).

In eight products the level of 1-MNA exceeded 1 mg/100 g of the product. The food products with the highest level of 1-MNA are listed in the table 6.

Table 6. Food products with a highest content of 1-MNA (Taguchi et al., 1986).

Food	1-MNA content (mg/100 g of edible product)
Wakame seaweed	3,2
Leaves of green tea	3,0
Poultry stomachs	2,4
Celery	1,6
Chinese black mushroom	1,3
Sea urchin	1,1
Greater sand eel	1,1
Natto (fermented soybeans)	1,0

It should be stressed out that 1-MNA is naturally present in human organism but dietary intake is responsible only for very small proportion of its total pool in human body. The main source of 1-MNA is endogenous metabolism of niacin, which is metabolised in human body mainly (about 60%) to 1-MNA (Menon et al., 2007b).

IX.1. PROPOSED GROUPS FOR THE USE OF 1-MNA

1-MNA is proposed for the use for the general population with the exception of pregnant women and children.

It is expected that the product would be consumed by adult population and elderly people. The final product containing 1-MNA will be designated to maintain circulatory system health owing to protection of normal function of endothelium.

IX.2. INDIVIDUAL PROPOSED FOOD USES AND USE LEVELS

1-MNA is intended for consumption as a food supplement in the form of a powder in several different final formats (gelatin capsules, tablets and possible other). It is supposed to be consumed either in a short-term manner or in a prolonged manner. Proposed use levels 125 mg/single dose.

IX.3. PREDICTED INTAKE OF 1- MNA FROM PROPOSED SUPPLEMENT DOSE

The intended intake is 250 mg/day, split into two doses, taken with some liquid to help it swallow.

IX.4. PREDICTED INTAKES FOR RISK GROUPS

It can be deduced from human data we already have that no potentially toxicity risk should be expected when 1-MNA is administered to pregnant women and children. However, no toxicity studies are yet available for these groups of the population. Due to that, the final product will be labeled for adult consumers and will contain warnings for pregnant women and children.

No allergic episodes have been described in the human and animal studies as a result of the 1-MNA supplementation. It is not planned to use specific warnings related to the allergenicity of the product.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD

X.1. PREVIOUS 1-MNA INTAKE- INTAKE ESTIMATE FROM ALL SOURCES

There are no accessible data estimating intake of 1-MNA from all sources. It can be only roughly estimated, based on the results of study measuring the content of 1-MNA in different food products, that daily intake from normal dietary sources not exceeds several milligrams. Under these conditions, the intake from proposed dietary supplement use will be many times higher than intake from normal food sources and the intake from all sources will be only slightly higher than intake from proposed food supplement use.

X.2. PREVIOUS EXPOSURE TO 1-MNA PREPARATIONS

1-MNA preparation were used up to 0,4 % now only in products designed for topical use (on skin).

Table 7: Marketed products containing 1-MNA.

Product name	Product type	Indications	Registry date in Poland	Registry no.†	Other countries
Accos anti-acne gel	cosmetic	Oily and acneous skin	05/19/2003	RK/77049/2003	Lithuania, Latvia, Slovakia
Accos mattifying cream	cosmetic	Oily and acneous skin	03/12/2009	RK/240381/2009	-
Accos tonic	cosmetic	Oily and acneous skin	03/12/2009	RK/240380/2009	-
Accos washing lotion	cosmetic	Oily and acneous skin	10/09/2007	RK/196743/2007	-
Accos washing bar	cosmetic	Oily and acneous skin	02/18/2008	RK/209604/2008	-
Allerco emollient oily cream	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy	02/03/2005	RK/126078/2005	Lithuania, Latvia, Slovakia
Allerco emollient moisturising cream	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy	03/12/2009	RK/240379/2009	-
Allerco body lotion	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy	10/09/2007	RK/196741/2007	-
Allerco bath lotion	cosmetic	Dry skin, skin sensitive and	06/29/2009	RK/249511/2009	-

		susceptible to irritation and allergy			
Allerco washing gel	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy, contact allergy	10/09/2007	RK/196742/2007	-
Allerco washing bar	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy,	02/18/2008	RK/209603/2008	-
Allerco shampoo	cosmetic	Dry skin, skin sensitive and susceptible to irritation and allergy,	23/03/2012	RK/339662/2012	-
Dermena shampoo	cosmetic	Excessive hair loss	05/19/2003	RK/77051/2003	Lithuania, Latvia, Slovakia
Dermena gel	cosmetic	Excessive hair loss	05/18/2004	RK/106093/2004	Lithuania, Latvia, Slovakia
Dermena conditioner	cosmetic	Excessive hair loss, dry and damaged hair	03/12/2009	RK/240382/2009	-
Dermena REPAIR shampoo	cosmetic	Excessive hair loss, dry and damaged hair	23/03/2012	RK/339656/2012	
Dermena MEN shampoo	cosmetic	Excessive hair loss in men	23/03/2012	RK/339657/2012	
Dermena MEN lotion	cosmetic	Excessive hair loss in men	23/03/2012	RK/339658/2012	
Dermena MEN treatment in ampoules	cosmetic	Excessive hair loss in men	23/03/2012	RK/339659/2012	
Dermena LASH mascara	cosmetic	weak, thin and delicate eyelashes and eyebrows,	23/03/2012	RK/339660/2012	
Dermena LASH conditioner	cosmetic	weak, thin and delicate eyelashes and eyebrows	23/03/2012	RK/339661/2012	
Dermena PLUS shampoo	cosmetic	Dandruff, excessive hair loss	05/18/2004	RK/106094/2004	Lithuania, Latvia,

					Slovakia
Thermi gel	cosmetic	sunburn, skin susceptible to irritations	05/19/2003	RK/77050/2003	Lithuania, Latvia, Slovakia
ReviuM revitalizing day cream	cosmetic	Anti-wrinkle care for mature, dry, sensitive skin	06/29/2009	RK/249512/2009	-
ReviuM regenerating night cream	cosmetic	Anti-wrinkle care for mature, dry, sensitive skin	06/29/2009	RK/249513/2009	-
ReviuM eye cream	cosmetic	Anti-wrinkle care for mature, dry, sensitive skin	06/29/2009	RK/249514/2009	-

X.3. INFORMATION ON EXISTING AUTHORISATIONS AND EVALUATIONS

Beauty or make-up preparations indicated in point X.2.



Table 8. Patents owned by PHARMENA and its subsidiaries.

No.	Patent no.	Country	Refers
1.	190755	Poland	dermatological use of 1-MNA
2.	2199319	Russian Federation	dermatological use of 1-MNA
3.	EP 1147086	United Kingdom, Switzerland France, Germany, Austria, Spain, Italy, the Netherlands, Belgium	dermatological use of 1-MNA
4.	73491	Ukraine	dermatological use of 1-MNA
5.	225 475	Hungary	dermatological use of 1-MNA
6.	285 869	Slovakia	dermatological use of 1-MNA
7.	299 662	Czech Republic	dermatological use of 1-MNA
8.	211459	Poland	dermatological use of 1-MNA
9.	2366420	Russian Federation	vasoprotective use of 1-MNA
10.	267501	Mexico	vasoprotective use of 1-MNA
11.	2,547,234	Canada	vasoprotective use of 1-MNA
12.	2005205066	Australia	vasoprotective use of 1-MNA
13.	CN 1905875	Republic of China	vasoprotective use of 1-MNA
14.	EP 1 713 480	United Kingdom, Ireland, Switzerland / Liechtenstein, France, Germany, Poland,	vasoprotective use of 1-MNA

		Austria, Bulgaria, Greece, Czech Republic, Slovakia, Spain, Finland, Italy, Portugal, Romania, Turkey, Denmark, the Netherlands, Belgium, Sweden, Hungary	
15.	5052138	Japan	vasoprotective use of 1-MNA
16.	US 7,935,717	USA	Method for Treating Hypertriglyceridemia, Dyslipidemia and Hypercholesterolemia with a 1-MNA
17.	US 8124631	USA	vasoprotective use of 1-MNA - enhancing prostacycline level
18.	US 8,211,922	USA	vasoprotective use of 1-MNA in combination with statins
19.	EP 1 919 466	United Kingdom, Ireland, Switzerland / Liechtenstein, France, Germany, Poland, Austria, Bulgaria, Greece, Czech Republic, Slovakia, Spain, Finland, Italy, Portugal, Romania, Turkey, Denmark, the Netherlands, Belgium, Sweden, Hungary	vasoprotective use of 1-MNA in combination with statins

XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD

The product being the subject of this application contains only one chemical substance – 1-MNA. All main nutrients (proteins, fats and carbohydrates), vitamin and mineral compounds are absent. Biological value of the product depends on the properties of 1-MNA described in Section XIII.

XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

There is no presence of any microorganisms or their metabolites due to the novelty of the novel food ingredient.

The manufacturing process of the 1-MNA is based in the Good Manufacturing Practices and the presence of any microorganism (virus and bacteria) which could alter the quality and safety of the product is controlled.

Table 3 of Section I.2 Product Specification, summarizes all parameters controlled and the methods used. Appropriate limits for total viable aerobic count and reference pathogen microorganisms are established and complied with.

Microbiological controls are conducted on a batch-to-batch basis in order to guarantee that the manufacturing process is capable to eliminate any potential bacterial contamination present in the raw material and to prevent bacterial growth during production.

XIII. BIOLOGICAL AND TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

1-MNA is a metabolite of nicotinamide. It is a product of nicotinamide N-methyltransferase in the pathway of nicotinate and nicotinamide metabolism. It is produced primarily in the liver.

1-MNA, long considered as biologically inactive metabolite of nicotinamide, possess anti-thrombotic, anti-inflammatory, gastroprotective and vasoprotective properties, as it has been apparent from the results of studies performed during few last years (Gebicki et al, 2003; Bryniarski et al., 2008; Domagala et al., 2012). It is used already in topical treatment of some dermatologic diseases with inflammatory component (Wozniacka et al., 2005).

Probably the most important physiological effect of 1-MNA is related to vascular system, and especially endothelium, the internal layer of the vascular wall. Endothelial cells produce several vasoactive chemical factors, among them prostacyclin (PGI₂), which induce vasodilatation of blood vessels and inhibit smooth muscle cell proliferation and platelet aggregation. The peptide endothelin is also produced by the endothelium, and is a potent constrictor of blood vessels and promotes cell proliferation. In a normal healthy state, prostacyclin helps counter-balance the actions of endothelin. In certain disease conditions, however, production of prostacyclin by the endothelium is impaired, allowing the deleterious effects of excessive levels of endothelin to predominate.

Dysfunction of the prostacyclin system occurs in several cardiovascular disorders, including thrombosis, myocardial infarction, stroke, myocardial ischemia, atherosclerosis and pulmonary arterial hypertension (PAH). Restoration of prostacyclin function may have potentially beneficial effect in a wide spectrum of physiological situations and diseases.

1-MNA is an endogenous activator of prostacyclin production and thus may regulate thrombolytic as well as inflammatory processes in the cardiovascular system. 1-MNA inhibits platelet-dependent thrombosis by a mechanism involving cyclooxygenase-2 and prostacyclin (Bartus et al, 2008; Chlopicki et al., 2007; Mogielnicki et al., 2007) and increases nitric oxide bioavailability in the endothelium (Domagala et al., 2012).

It was also proved in rat model that 1-MNA inhibits gastric acid secretion and attenuates gastric lesions induced by water resistant stress in animal model (Brzozowski et al., 2008), may have protective properties for brain degenerative changes (Kuchmerovska et al., 2010), and displays lowering effect on fasting glucose concentration in experimentally induced diabetes in rats (Watala et al., 2009).

Taking into account all those effects 1-MNA has potential applicability in several conditions related to human health including:

- protecting normal function of endothelium,
- lowering risk of vascular impairment induced by postprandial hypertriglyceridemia,
- stimulation of endothelium-dependent repair response or/and organ protection.

Positive effect may be expected also in different situations with involvement of endothelial vasoprotective mechanisms not related to cardiovascular system.

XIII.1. BIOAVAILABILITY OF 1-MNA FOLLOWING ORAL CONSUMPTION

XIII.1.1 METABOLIC FATE AND BIOLOGICAL DISTRIBUTION

XIII.1.1.1 GENERAL METABOLISM OF NIACIN FROM ORGANIC AND INORGANIC SOURCES

Niacin undergoes extensive, saturable metabolism *via* two major pathways. One pathway is *via* glycine conjugation with niacin to form nicotinuric acid (NUA). The other major metabolic pathway contributes to the formation of nicotinamide adenine dinucleotide (NAD). Although the specific metabolic steps in this pathway have not been completely elucidated, niacin appears to be first converted to nicotinamide which is, in turn, rapidly methylated to N-methyl nicotinamide (MNA) or conjugated to form NAD (Menon et al., 2007a and 2007b). This enzymatic reaction takes place in liver with active assistance of nicotinamide N-methyltransferase (NNMT) (Felsted and Chaykin, 1967). 1-MNA does not cross the blood– brain barrier (Erb et al., 1999).

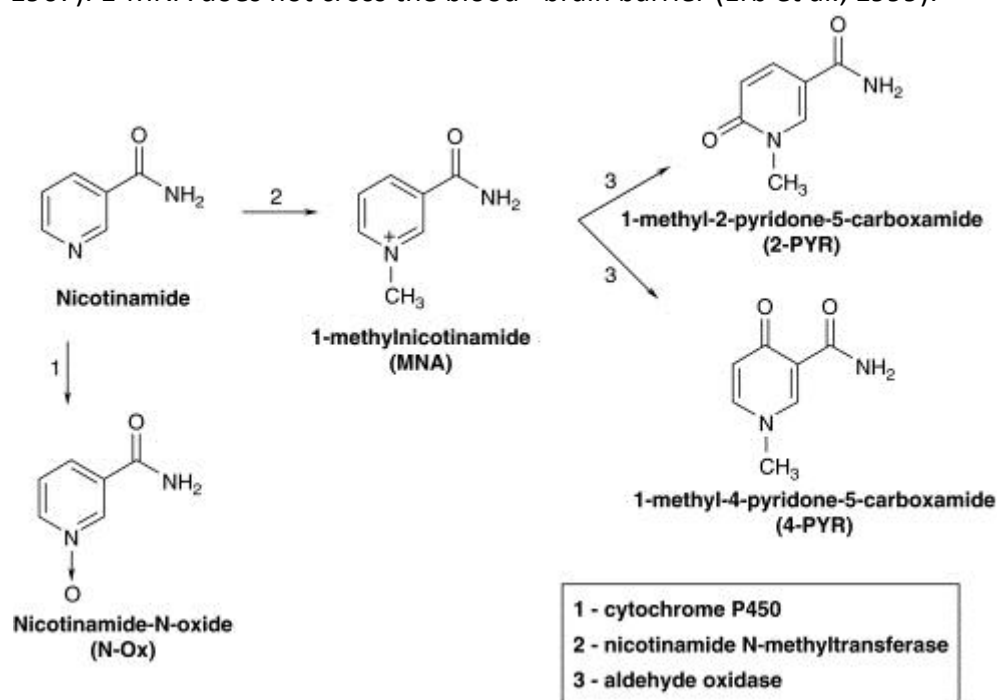


Figure XIII.1.1.1. The scheme of nicotinamide metabolism (Chlopicki et al. 2007).

XIII.1.1.2 METABOLIC FATE OF 1-MNA

1-MNA is oxidized to N-methyl-2-pyridone-5-carbox-amide (2PY) and to the minor metabolite N-methyl-4-pyridone-3-carboxamide (4PY). Nicotinamide-N-oxide (N-Ox) also appears to be a minor niacin metabolite, although it is unclear whether N-Ox is formed directly from niacin or indirectly from nicotinamide. Only very small quantities of N-Ox and 4PY have been found in humans, while niacin, 1-MNA, 2PY and NUA were the predominant compounds excreted following oral dosing with niacin (Felsted and Chaykin, 1967).

1-MNA and its metabolites are excreted by kidneys. 1-MNA measurement in plasma and urine was used to diagnose niacin deficiency (Vivian et al., 1958; Jacob et al., 1989) and then to monitor renal tubular excretion (Maiza et al., 1992) or peroxisome proliferation in the liver (Delaney et al.,

2005). An increased concentration of urine 1-MNA was found in patients with Parkinson's disease (Aoyama et al., 2000) and liver cirrhosis (Pumpo et al., 2001).

XIII.1.2 INTERACTIONS WITH OTHER COMPONENTS IN THE DIET

There is no information about possible interaction of 1-MNA with other components of the diet.

XIII.1.3 IMPACT ON THE INTESTINAL MILIEU AND ON THE ABSORPTION OF OTHER NUTRIENTS

There is no information about possible impact of 1-MNA on the absorption of other nutrients and intestinal milieu.

XIII.1.4 PHARMACOKINETICS AND METABOLISM

XIII.1.4.1 PUBLISHED PHARMACOKINETIC STUDIES

When evaluating the pharmacokinetics of 1-MNA, it is important to consider the 1-MNA and 2PY concentrations achieved in humans following administration of nicotinic acid formulations. These data are available in Table 1 of the US Food and Drug Administration biopharmaceutics review of the Niaspan NDA submitted by Kos available through FOI Services, Inc. 11 Firstfield Road, Gaithersburg, MD 20878-1704. The mean 1-MNA and 2PY C_{max} determined from a single, 1500 mg immediate-release dose of nicotinic acid were 0,72 µg/mL and 4,7 µg/mL respectively and for the same dose of Niaspan were 0,73 µg/mL and 5,10 µg/mL respectively.

The content of 1-MNA in the liver and urine of weaning rats was determined following dietary administration of a large dose of 1-MNA (0,5 g/100 g of control diet) for 15 days in a study by Shibata and Taguchi (1987). The content of 1-MNA in the liver was 158,4±32,1 nmol/g in the 1-MNA containing diet group compared with 111,3±15,3 nmol/g in the control diet group (n=5-6 rats for each group). The content of 1-MNA in the urine was 188,4±28,3 µmol/day in the 1-MNA treated group compared with 1,6±1,0 µmol/day in the control diet group (n=5-6 rats for each group; urine collected on the 13th-15th day).

XIII.1.4.2 UNPUBLISHED PHARMACOKINETIC STUDIES IN ANIMALS

Plasma samples were collected for determination of 1-MNA and 2PY in its animal studies. In one study, plasma concentrations of 4PY were also determined. All plasma samples from the animal studies were analyzed for 1-MNA and its metabolites using a proprietary, fully validated human plasma high performance liquid chromatographic method with tandem mass spectroscopy detection, by [REDACTED]. Standards and quality control samples as well as unknown plasma samples were quantified from the human assay standard curves. Relative exposure could not be determined from the data as the number and sampling times of the plasma data are insufficient for the calculation of area under the plasma curve. Likewise, area under the 2PY and 4PY plasma curves could not be determined.

28-day oral toxicity study in rats (Annex K)

In randomized, placebo-controlled, parallel toxicokinetic 28-day study 1-MNA was administered orally every 12 hours by oral gavage in Wistar rats (36 male and 36 female).

Three groups of six male animals plus one stand-by male animal and six female animals plus one stand-by female animal were randomized to receive 125, 250, or 500 mg/kg body weight of 1-MNA every 12 hours and provide plasma samples for toxicokinetic analysis. The reversal phase of the study also included satellite toxicokinetic animals consisting of an additional group of six male animals plus one male stand-by animal and six female animals plus one stand-by female animal that received 500 mg/kg of 1-MNA every 12 hours and provided plasma samples for toxicokinetic analysis. Two additional groups of three male animals plus one stand-by male animal and three female animals plus one stand-by animal received control treatment every 12 hours and provided plasma samples for toxicokinetic analysis; one group represented the control group and the other acted as the control satellite group. All animals in the toxicokinetic portion of the study survived to termination and therefore the stand-by animals were not used in the toxicokinetic analysis. Therefore the toxicokinetic results are based on exposure of 30 both male and female animals (24 received active treatments, six received control treatments). Blood samples for analysis of plasma 1-methylnicotinamide (1-MNA), N-methyl-2-pyridone-5-carboxamide (2PY), and N-methyl-4-pyridone-5-carboxamide (4PY) were collected on days 1 and 28 of the study. Plasma sample was taken at 3 hours after the morning dose of the sampling days. Animals designated for toxicokinetic analysis that received 1-MNA were separated into two groups of three male and three female animals each for each dose level (six animals/sampling dose group). One group of three male and three female animals provided samples just prior to the morning dose ($t = 0$) and 3 hours after the morning dose on the sampling days. The other group of three male and three female animals provided samples 2 and 6 hours after the morning dose on each sampling day.

The administration of 1-MNA resulted in substantial and dose-dependent exposures of 1-MNA in plasma.

The Day 1-MNA toxicokinetic results indicate that the 1-MNA exposure following 1-MNA was at least 20-fold greater than endogenous 1-MNA levels. Steady-state (Day 28) peak plasma 1-MNA concentrations occurred (T_{max}) at 2 hours following dose administration, regardless of dose. Steady-state 1-MNA C_{max} values also suggested a dose-proportional relationship of exposure regardless of gender as the highest values for individual animals were approximately 5, 13 (11.7 $\mu\text{g}/\text{mL}$ in female animals), and 25 $\mu\text{g}/\text{mL}$ at 1-MNA doses of 125, 250, and 500 mg/kg twice daily. There was also a dose-proportional increase in steady-state 1-MNA AUC_{ave6} with increasing dose, regardless of gender. The steady-state 1-MNA AUC_{ave6} approximately doubled regardless of gender from about 16 $\mu\text{g}\cdot\text{hr}/\text{mL}$, to 37 $\mu\text{g}\cdot\text{hr}/\text{mL}$, and then to 66 $\mu\text{g}\cdot\text{hr}/\text{mL}$ as the dose doubled from 125 mg/kg twice daily, to 250 mg/kg twice daily, and 500 mg/kg twice daily. The Day 28 1-MNA AUC_{ave6} values were nearly twice those from Day 1, indicating accumulation of the parent drug, 1-MNA, from Day 1 to Day 28.

As 1-MNA is metabolized to 2PY and 4PY, the animals in this study were exposed to substantial levels of these metabolites following absorption of 1-MNA. The Day 1 2PY toxicokinetic parameters indicated 2PY exposure following 1-MNA dosing was greater than 40-fold higher than endogenous 2PY levels at all dose levels and the exposure was dose-related. Steady-state 2PY C_{max} concentrations usually occurred close to 3 hours following dose administration, regardless of dose. Steady-state 2PY C_{max} was dose related in the female animals, but less so in the male animals; the C_{max} of the male animals at 250 and 500 mg/kg twice daily were more similar than those of the female animals. There was a dose-related increase in steady-state 2PY AUC_{ave6} with increasing dose, but the relationship was less than linear across the doses, particularly in male animals suggestive of saturable metabolism of 1-MNA to 2PY. Comparison of the Day 1 2PY toxicokinetic parameters to the Day 28 2PY toxicokinetic parameters suggests similar values between Day 1 and Day 28 suggestive of little 2PY accumulation.

The exposure to 4PY following 1-MNA administration appeared to be the same regardless of dose. The Day 1 4PY toxicokinetic parameters indicated that 4PY exposure following 1-MNA dosing was greater than 7-fold higher than endogenous 4PY levels at all 1-MNA dose levels. Steady-state peak plasma 4PY concentrations occurred (T_{max}) commonly 3 hours following 1-MNA dose administration, regardless of dose. Steady-state 4PY C_{max} values for individual animals were very similar across dose and gender (3 – 4 ug/mL). No dose-relationship was evident between 1-MNA dose and steady-state 4PY AUC_{ave6}. Comparison of the Day 1 plasma 4PY toxicokinetic parameters to the Day 28 plasma 4PY toxicokinetic parameters indicates that the values were lower on Day 28 relative to Day 1 suggestive of reduced metabolism of 1-MNA to 4PY or increased elimination of 4PY by Day 28. Therefore the metabolism of 1-MNA to 4PY appears to be saturated to a greater extent than the metabolism of 1-MNA to 2PY at the 1-MNA doses administered in this study based on the absence of dose dependent 4PY exposure.

No substantial gender-related differences in 1-MNA, 2PY, or 4PY exposure were observed in this study.

Study #1762-002 in Fructose-induced Hypertriglyceridemia Rat Model (Annex L): Male Wistar rats were placed on a 60% fructose diet for the study. Dosing was initiated in animals randomized to the PK/PD cohort on Day 15. The results of the PK/PD cohort were then used to set doses for the Pharmacology Cohort which started dosing on Day 37. On Day 15, six animals each were randomized into one of the seven treatments which included by gavage: vehicle 0 mg/kg/day three times daily by gavage, 50 mg/kg/day fenofibrate, 200 mg/kg/day 1-MNA, 400 mg/kg/day 1-MNA; and by drinking water: 200 mg/kg/day nicotinic acid, 100 mg/kg/day 1-MNA, 200 mg/kg/day 1-MNA. Treatments administered via oral gavage were dosed three times daily, approximately 8 hours apart for 42 consecutive days. The control and test articles administered via drinking water were available *ad libitum*, for 42 consecutive days. On Day 37, six animals each in the Pharmacology Cohort were randomized to one of the seven treatments which included by gavage: vehicle 0 mg/kg/day, 50 mg/kg/day fenofibrate, and by drinking water: 200 mg/kg/day nicotinuric acid, 200 mg/kg/day nicotinic acid, 200 mg/kg/day 1-MNA, 400 mg/kg/day 1-MNA, 800 mg/kg/day 1-MNA. Treatments administered via gavage were dosed three times daily, approximately 8 hours apart for 26 consecutive days. Treatments administered via drinking water were available *ad libitum* beginning on Day 37 for 26 consecutive days. Dosing in Group 10 (nicotinuric acid 200 mg/kg/day) began on Day 44, and was available *ad libitum* via drinking water for 26 consecutive days.

The 1-MNA and 2PY plasma concentration data were well-behaved. 1-MNA administration resulted in dose-related increases in mean 1-MNA and 2PY concentrations at all doses. In nearly all cases, the mean 1-MNA and 2PY concentrations were greater than the vehicle and nicotinic acid controls. No obvious evidence of 1-MNA or 2PY accumulation was observed between Day 56 and Day 21. No difference in 1-MNA and 2PY mean plasma concentrations were observed between morning and evening doses.

Median concentration of 1-MNA PK/PD Cohort on Day 21, 1,5 hour after dosing (6:00 Dose) were respectively <0,100 ug/mL (Vehicle), 1,88 ug/mL (1-MNA 200 mg/kg/day), 4,090 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 1-MNA PK/PD Cohort on Day 21, 1,5 hour after dosing (22:00 Dose) were respectively <0,100 ug/mL (Vehicle), 1,798 ug/mL (1-MNA 200 mg/kg/day), 5,187 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 1-MNA PK/PD Cohort on Day 56, 1,5 hour after dosing (6:00 Dose) were respectively <0,100 ug/mL (Vehicle), 3,217 ug/mL (1-MNA 200 mg/kg/day), 5,297 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 1-MNA PK/PD Cohort on Day 56, 1,5 hour after dosing (22:00 Dose) were respectively <0,100 ug/mL (Vehicle), 2,860 ug/mL (1-MNA 200 mg/kg/day), 1,573 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 2-PY PK/PD Cohort on Day 21, 1,5 hour after dosing (6:00 Dose) were respectively <0,200 ug/mL (Vehicle), 0,999 ug/mL (1-MNA 200 mg/kg/day), 1,833 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 2-PY PK/PD Cohort on Day 21, 1,5 hour after dosing (22:00 Dose) were respectively <0,200 ug/mL (Vehicle), 0,758 ug/mL (1-MNA 200 mg/kg/day), 1,016 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 2-PY PK/PD Cohort on Day 56, 1,5 hour after dosing (6:00 Dose) were respectively <0,200 ug/mL (Vehicle), 2,260 ug/mL (1-MNA 200 mg/kg/day), 3,263 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 2-PY PK/PD Cohort on Day 56, 1,5 hour after dosing (22:00 Dose) were respectively <0,200 ug/mL (Vehicle), 2,173 ug/mL (1-MNA 200 mg/kg/day), 3,713 ug/mL (1-MNA 400 mg/kg/day).

Median concentration of 1-MNA Pharmacology Cohort on Day 62, 6 hours after dosing (Midnight Dose) were respectively 2,077 ug/mL (1-MNA 200 mg/kg/day), 3,302 ug/mL (1-MNA 400 mg/kg/day), 8,733 ug/mL (1-MNA 800 mg/kg/day).

Median concentration of 2-PY Pharmacology Cohort on Day 62, 6 hours after dosing (Midnight Dose) were respectively 1,336 ug/mL (1-MNA 200 mg/kg/day), 2,235 ug/mL (1-MNA 400 mg/kg/day), 3,755 ug/mL (1-MNA 800 mg/kg/day).

Study #1762-004 in fructose-induced hypertriglyceridemia rat model (Annex M): Male Wistar rats received a 60% fructose diet for the duration of the study. On Day 28, animals were randomized into one of six treatment groups consisting of placebo, 600, 800, or 1000 mg/kg/day 1-MNA, or 50 mg/kg/day fenofibrate, 200 mg/kg/day nicotinic acid. All treatments were administered three times daily from Day 29 to Day 43 during the study via oral gavage. Blood samples (approximately 0.4 mL) for pharmacokinetic analysis were taken from the sublingual vein of randomized animals at 10:00 on Day 35 and at 10:00 and 22:30 on Day 43.

Nicotinic acid administration resulted in median 1-MNA and 2PY concentrations that were greater than the vehicle control, but lower than the TRIA treatments. This is to be expected as nicotinic acid must first be metabolized via several steps before resulting in 1-MNA concentrations. 1-MNA administration resulted in dose-related increases in 1-MNA concentrations at all three doses. The Application for the approval of 1- MNA as a novel food ingredient | Page 34 of 54

median 10:00, Day 43 plasma sample value was greater than the median 10:00, Day 35 value for all TRIA treatments suggesting that steady state was not achieved by day 7 of dosing. Therefore the apparent 1-MNA half life for Wistar rats must be greater than 24 hours. TRIA treatments resulted in dose-related increases in 2PY plasma concentrations for the 600 mg/kg/day and 800 mg/kg/day treatments only. The 1000 mg/kg/day treatment resulted in median 2PY plasma concentrations that were similar to those from the 800 mg/kg/day treatment.

Median concentration of 1-MNA on Day 43 were respectively 0 ug/mL (Vehicle), 5,95 ug/mL (1-MNA 600 mg/kg/day), 9,465 ug/mL (1-MNA 800 mg/kg/day), 14,550 ug/mL (1-MNA 1000 mg/kg/day).

Median concentration of 2-PY on Day 43 were respectively 0 ug/mL (Vehicle), 3,53 ug/mL (1-MNA 600 mg/kg/day), 5,215 ug/mL (1-MNA 800 mg/kg/day), 4,815 ug/mL (1-MNA 1000 mg/kg/day).

██████████ #C117111 in ZSF1 rats (ZSF1-*Leprfa Leprcp/Crl*) obese rat model (Annex N):

Male ZSF1 rats (ZSF1-*Leprfa Leprcp/Crl*) obese rats were randomized to the following treatments: vehicle, 200 mg/kg/day nicotinic acid, 2,5 mg/kg/day rilmenidine, 400 mg/kg/day 1-MNA, 800 mg/kg/day 1-MNA, 1200 mg/kg/day 1-MNA. Treatments were administered three times a day at 7 AM, 3 PM, and 10 PM by oral gavage for 60 days. Approximately 500 µl of blood to yield 200 ul of plasma was sampled after a 4 hr fast on days 0, 28, 56, and 63 for assay of 1-MNA, 2PY, and 4PY. Samples were collected from unanaesthetized animals through lateral coccigeal (tail) vein or if necessary through the saphenous vein into collection tubes containing lithium heparin. Samples on day 0, 28 and 56 were taken.

Median plasma concentrations of 1-MNA, 2PY, and 4PY are depicted in the following figures, respectively. While 1-MNA and 2PY concentrations were absent in most of the Day 0 samples and throughout the study in the Vehicle treatment group, appreciable 4PY concentrations were evident in all treatment groups. 1-MNA administration resulted in median 1-MNA, 2PY, and 4PY concentrations that were greater than those from the vehicle and nicotinic acid 200 mg/kg/day treatments. 1-MNA administration resulted in dose-related increases in 1-MNA concentrations at all four doses. 1-MNA administration also resulted in dose-related increases in 2PY plasma concentrations. However plasma 2PY concentrations appeared to plateau on Day 56 at the 1200 mg/kg/day dose and failed to rise above the Day 63 median plasma 2PY concentration for the 800 mg/kg/day treatment, suggesting saturation of the conversion of 1-MNA to 2PY above 800 mg/kg/day of 1-MNA. Median plasma concentrations of 4PY demonstrated the least dose-related differences following 1-MNA administration, with the 4PY concentrations following 800 mg/kg/day and 1200 mg/kg/day being nearly superimposable, suggesting saturation of metabolism from 1-MNA to 4PY at 1-MNA doses greater than 400 mg/kg/day.

Median concentrations of 1-MNA on Day 63 were respectively 0 ug/mL (Vehicle), 4,400 ug/mL (1-MNA 400 mg/kg/day), 11,000 ug/mL (1-MNA 800 mg/kg/day), 14,900 ug/mL (1-MNA 1200 mg/kg/day).

Median concentrations of 2-PY on Day 63 were respectively 0 ug/mL (Vehicle), 4,78 ug/mL (1-MNA 400 mg/kg/day), 8,54 ug/mL (1-MNA 800 mg/kg/day), 8,17 ug/mL (1-MNA 1200 mg/kg/day).

Median concentrations of 4-PYon Day 63 were respectively 0,324 ug/mL (Vehicle), 2,21 ug/mL (1-MNA 400 mg/kg/day), 2,78 ug/mL (1-MNA 800 mg/kg/day), 2,74 ug/mL (1-MNA 1200 mg/kg/day).

XIII.1.4.3. UNPUBLISHED PHARMACOKINETIC STUDIES IN HUMANS

Study MNA-002

The safety, pharmacokinetics and pharmacodynamics of 1-MNA were evaluated in a randomized, double-blind, placebo-controlled, single-dose, crossover study, with gender matched dose cohorts. Twenty normal volunteers were exposed on two separate dosing occasions separated by at least 5 days following each dose exposure. Four of the 20 subjects (2 males and 2 females) received placebo on both occasions, and 16 subjects (8 males and 8 females) received active component at each dosing session. Subjects randomized to the active treatment received either the low dose of 1-MNA (90 mg) or the high dose 1-MNA (270 mg) during the first treatment period, followed by the alternate dose during the second treatment period. Subjects randomized to the placebo group received the placebo treatment during both treatment periods. The plasma 1-MNA concentration was measured at the following time points: at pre-dosing, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 8 hours, 12 hours, 18 hours, and 24 hours.

Table 9 provides a summary of pharmacokinetic parameters for the subjects obtaining during the study period 90 mg/d of 1-MNA. The summary includes the mean, standard deviation, CV, median and range of each pharmacokinetic parameter for all subjects, and for male and female separately. P values for the comparison between genders are also provided. In this group no statistically significant difference was indicated between genders for any of the pharmacokinetic parameter.

[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]					
[REDACTED]					
[REDACTED]					

Table 10 provides a summary of pharmacokinetic parameters for the subjects obtaining during the study period 270 mg/d of 1-MNA. The summary includes the mean, standard deviation, CV, median and range of each pharmacokinetic parameter for all subjects, and for male and female separately. P values for the comparison between genders are also provided. For those subjects, female subjects exhibited statistically significantly higher mean values of C_{min} and AUC(T) compared to male subjects, while male subjects exhibited statistically significantly higher mean values off Cl_{app}, compared to female subjects. No other statistically significant difference was found between genders.

[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

Following oral administration of 90 mg 1-MNA to 16 healthy subjects in a placebo-controlled, cross-over design trial (Study MNA-002), mean 1-MNA plasma C_{max} was 114,48 ng/ml, mean T_{max} was 2,06 hours, and mean AUC_t was 842,50 h x ng/ml. Following oral administration of 270 mg 1-MNA to the same 16 healthy subjects, mean 1-MNA plasma C_{max} was 236,94 ng/ml, mean T_{max} was 2,27 hours, and mean AUC_t was 1523,15 h x ng/ml.

There appears to be a dose-dependant gender based difference in plasma concentrations following single oral dosages, with females exhibiting higher concentrations of 1-MNA than males at dosages of 270 mg, but not at dosages of 90 mg orally. Endogenous levels of 1-MNA in plasma before dosing were in the range of 8-27 ng/ml with a mean of 16,4 ng/ml.

The whole Study Report related to study MNA-002 is attached as Annex O.

XIII.2. TOXICOLOGICAL ASSESSMENT

XIII.3. TOXICITY STUDIES PERFORMED WITH 1-MNA

Toxicity of 1-MNA was studied in several animal studies assessing acute toxicity after oral administration, acute skin and eye irritation, allergenic effects on skin, subacute toxicity and genotoxicity. Biodegradation in water was also determined. 1-MNA has demonstrated very low toxicity in mice and rats models at doses exceeding those expected to be used in humans.

XIII.3.1. ACUTE ORAL TOXICITY

Acute oral toxicity study of 1-MNA was performed in [REDACTED] in 2006 according to OECD420 (method B.1.BIS) on rat model. 1-MNA was given orally (by oral gavage) once to 5 female rats in a dose 2000 mg/kg b.w. During 14 days of observation no signs or symptoms of toxic reactions were seen. Macroscopical inspection of internal organs revealed no pathological abnormalities. Therefore, oral median lethal dose (LD50) for 1-MNA was established as more than 2000 mg/kg b.w. In line with guiding principles related to OECD420 studies higher doses were not administered. The result confirms that 1-MNA should be classified as non classified substance (LD50 > 2000 mg/kg b.w.) in regards to low toxicity (see Annex P).

XIII. 3.2. ACUTE TOXICITY AFTER INTRAPERITONEAL INJECTION

A non-GLP acute, single-dose toxicity study was conducted by the [REDACTED]. In this study, 11,5 g/kg 1-MNA was administered by single intraperitoneal injection to 23 male and female Balb/c mice weighing approximately 20 g each. All animals survived the 1-MNA dose. Autopsy of the animals revealed distended, flatulent, abdomens, with livid groins, stomachs containing yellow pulp with gases, duodenum and small intestines filled with mucoid, dirty-brown magma with bubbles. The livers were enlarged, congested, with blurred structure and lungs were dark-pink. There were no visible changes in the hearts. The investigators recommended an LD 50 of 11,5 g/kg as using larger doses for a better estimate of the LD50 was not possible due to the large dosing volumes needed to deliver higher doses. This result indicates that 1-MNA is practically not toxic according to Hodge-Sterner toxicity classification (5th class of toxicity) and non classified as toxic using classical classification (LD50 > 2000 mg/kg b.w.) (see Annex R).

XIII.3.3. SHORT-TERM AND SUBCHRONIC TOXICITY

Subacute toxicity of 1-MNA was assessed in three studies: repeated dose 28-day oral toxicity study in rats, in APOE*3Leiden. CETP mice model and in fructose-induced hypertriglyceridemia rat model.

Repeated dose 28-day oral toxicity study in rats: This study was performed in [REDACTED] according to OECD407 model. The study was carried out in male and female WISTAR rats (Cri: WI (Han); outbred). 1-MNA was administered by oral gavage to groups of 10 rats/sex/dose group at twice daily (*b.i.d.*, 12 hours apart) dose levels of 0 (distilled water vehicle), 125, 250, and 500 mg/kg (0, 250, 500, and 1000 mg/kg/day, respectively)

for 28 consecutive days. These main study groups were identified as groups 0, 1, 2, and 3, respectively. An additional 5/sex rats received the vehicle (controls) and high dose (500 mg/kg *b.i.d.*) for 28 days and were then maintained for 14 days without treatment to assess reversibility, persistence, or delay in potential treatment-related effects (referred to in the report as satellite animal groups 0 SAT and 3 SAT, respectively). After the end of the 28-day treatment period, the animals from the satellite groups were observed for the next 14 days to evaluate the reversibility, persistence, or delay in the occurrence of potential toxic changes.

There was no test item-related mortality. During the experiment, one female from group 3 SAT died accidentally; however, its death was not caused by the test item. There were no significant differences in appearance and behaviour between the animals from groups 1, 2, 3, and 3 SAT and the ones from the control groups (0 and 0 SAT). The ophthalmic examination did not reveal any changes in the eyes of the control and treated animals. The administration of the test item did not lead to any changes in the animals' body weight gain and food consumption. There were no treatment-related findings seen during the detailed behavioural evaluations. Based on the detailed clinical observations made during and after the treatment, as well as the open field observations, no muscarinic symptoms (weakness, lacrimation, salivation, anxiety), nicotine symptoms (diarrhea, motor coordination difficulties), or central symptoms were noticed. There was also no delayed neurotoxicity in the form of paralysis of the hindlimbs, clumsiness, sensory disturbances, and general paralysis. The evaluation of the animals' responses to stimuli showed no effect of the test item. On the basis of the fore- and hindlimb grip strength measurement, and the detailed clinical and open field observations (body posture, gait, locomotor activity), it can be concluded that the test item does not cause any mitotoxic changes. The clinical studies did not show any adverse effects of the test item on the test animals.

The statistical analysis of the results of biochemical markers showed a few statistically significant changes. An increase in the concentration of calcium in males in group 3, a decrease in the urine pH in males and females, and an increase in the number of leukocytes in males discovered during the general urine examination was observed. For the satellite group, no statistically significant changes in the investigated parameters were observed after the 14-day break in the treatment. There were no treatment-related hematologic, bone marrow, coagulation, or clinical chemistry (except calcium) changes.

In autopsy no pathological changes in tissues and organs of most rats euthanized after the experiment were found with a few exceptions. These included: one male from group 3 which had some inflammatory changes in the lungs, one male from the satellite group 3 whose kidneys and liver were enlarged, and one female from group 1, one male from group 2, and two animals (1 male and 1 female) from group 3 which had a necrotic focus in the liver.

When it comes to absolute and relative weights of internal organs, the statistically significant differences related to the brain, adrenal glands, and testicles in groups 3 and 3 SAT. As for males from the former one, the absolute testicle weight was increased, and as for females from the latter one, the absolute and relative brain weights increased, whereas the absolute adrenal gland weight decreased. The remaining organs such as the pituitary gland, thymus, heart, liver, spleen, kidneys, ovaries, epididymides, and prostate with the seminal vesicles and coagulating glands did not show any statistically significant differences in terms of absolute and relative weights in any of the groups. The statistically significant changes revealed when determining weights of internal organs can be regarded as accidental and not test item – related since they were not confirmed histopathologically.

Histopathological examinations of internal organs and tissues of the animals from all the groups showed some changes in the brain, kidneys, liver, lungs, adrenals, intestines pituitary gland,

prostate, and Harderian gland. These changes usually took the form of disturbances in blood circulation (hyperemia, erythrocytorrhagia, effusions, and edema), inflammatory changes (lymphocytic and microcellular infiltrations, and hyperplasia of the lymphoid follicles), and regressive changes (atrophy of the glomeruli and hyaline casts). These changes did not have a specific nature and therefore they should not be associated with the test item. They may be regarded as accidental since they also appeared in the control group, and their frequency in particular treated groups was not dose-dependent. The post mortem examination of the liver revealed the presence of a necrotic focus in the left liver lobe of six animals (one female in group 1, one male in group 2, one male and two females in group 3, and one male in group 3 SAT). To conduct more detailed analyses, the scope of histopathological examinations was expanded by conducting the additional evaluation of the right liver lobe from all animals taking part in the experiment (treated and untreated). The additional histopathological examination did not reveal any necrotic foci in the control group, or in any of the treated groups.

The post mortem examinations of the test animals revealed a wide range of changes in internal organs. Most of them should not be related to the test item. Their frequency in individual groups was not dose-dependent. The only exception was the presence of a necrotic focus in the left liver lobe of a few animals. Histopathological analyses focused on the left and right liver lobe of the test rats showed that a necrotic foci appeared only in the left lobe. No necrotic foci were found in the livers of the control animals (groups 0 and 0 SAT).

The clinical and biochemical examination proved that 1-MNA had no impact on the immune system. The evaluation of the immune system based on histopathological observations of the thymus, spleen, and lymph nodes, and the statistical analysis of absolute and relative weights of the spleen and thymus revealed no pathological changes, or statistically significant differences in any group. The evaluation of the immune system did not show any harmful effects of the test item at the applied doses on this particular system (full study report is placed in the application as Annex K).

The results of this study were consulted by CanBioPharma Consulting Inc.. The purpose of this consultation was to provide an expert opinion whether the liver lesions found in subacute toxicity study in some animals are related to 1-MNA administration (full opinion can be found as Annex K-1). Expert opinion stated that the generally greater relatively higher ALT activities compared to AST activities in the control and 1-MNA treated animals were not accompanied by corresponding pattern of other biomarkers, which would usually be indicators of a potential cholestatic effect. The activities of ALT in the treated and control groups were generally higher and more variable than expected. In six animals with gross and/or microscopic liver lesions no correlation between the anatomic and clinical pathology findings for most of the animals can be seen. The highest ALT activities in each group occurred in animals without grossly or microscopically observed liver lesions. The elevated aminotransferases activities seen in some 1-MNA treated animals cannot be considered as related to 1-MNA administration because the abnormalities were not dose-dependent. The solitary large chronic liver lesions look more like those associated with mechanical trauma, bacterial infection, or an hepatic embolus. The origin of injury may be external or from the peritoneal cavity but it is unlikely related to oral 1-MNA administration. The cause of the elevated aminotransferases activities and focal liver lesions remain unknown. In the absence of significant treatment-related findings for other study parameters and since the liver lesions are considered unlikely to be related to 1-MNA treatment, the dose of 1000 mg/kg/day can be considered a no-observed-adverse-effect level (NOAEL) following 1-MNA oral administration to rats for 28 days.

██████████ in APOE*3Leiden.CETP Mice Model: Thirty female APOE*3Leiden.CETP mice were put on a semi-synthetic Western type diet (Diet T + 0,1% cholesterol) for 4 weeks. With this diet, the mice reached elevated plasma cholesterol levels of approximately 13 mM/dL and triglycerides levels of approximately 5 mM/dL. After exclusion of six low responding mice, the remaining 24 mice were sub-divided into three groups of 8 mice each and treated during 4 weeks with the following compounds as admix to the Western type diet: control (no additions), niacin (1 E%, approximately 1080 mg/kg bw/d) and 1-MNA (1,3 E%, approximately 1402 mg/kg bw/d). No specific clinical signs were observed during the study. At sacrifice, no macroscopic aberrations were observed. Body weights and food intake of both treatment groups were similar as compared to the control group (see Annex S).

██████████ Study #1762-004 in Fructose-induced Hypertriglyceridemia Rat Model: Male Wistar rats received a 60% fructose diet for the duration of the study. On Day 28, animals were randomized into one of six treatment groups consisting of placebo, 600, 800, or 1000 mg/kg/day 1-MNA, or 50 mg/kg/day fenofibrate, and 200 mg/kg/day nicotinic acid. All treatments were administered three times daily from Day 29 to Day 43 via oral gavage. No macroscopic abnormalities were observed in any animal on autopsy performed after finishing of the intervention. Tissues of all animals participated in the study were within normal limits. No biologically significant differences in organ weight parameters were observed across groups, with the possible exception of higher liver and kidney weights in the rats exposed to fenofibrate. Other numerical differences in organ weight parameters across groups were attributed to biological variability or slight differences in the amount harvested (aorta and skeletal muscle) (see Annex T).

XIII.3.4. GENOTOXICITY

Genotoxicity of 1-MNA was tested in two studies: according to OECD guideline 471 and OECD guideline 487.

The first study was performed as Ames test (bacterial reverse mutation assay) according to OECD guideline 471 in a study performed in ██████████ in 2006. Bacterial strains *Salmonella typhimurium* (TA 97, TA 98, TA 100, TA 102 and TA 1535) with and without additional activation of the system S9 in a range of 0,3125 – 5,0 mg/plate) were used. Adequate positive and negative controls were performed. 1-MNA was not toxic up to a maximum dose 5.0 mg/plate. 1-MNA did not show any mutagenic activity under the conditions of the test. 1-MNA is not a mutagenic substance in the settings evaluated (see Annex U).

In another study 1-MNA was tested for potential mutagenic properties on human peripheral blood lymphocytes in Micronucleus test in vitro (MN test). The test was carried out on lymphocytes isolated from two healthy, non-smoking donors. In the preliminary study, lymphocyte cultures were exposed to 1-MNA at 4 concentrations where the highest concentration of 1,72 mg/ml (10 mM) was applied according to OECD TG 487. It was the lowest among the suggested by the TG, i.e. 5 mg/ml, 5 µl/ml or 10 mM (whichever is the lowest). The concentration range (1,72 mg/ml, 0,55 mg/ml, 0,172 mg/ml, 0,055 mg/ml) was prepared using dilution factor of ca. 3.16 ($\sqrt{10}$). The cells were exposed for 3 hrs, with or without metabolic activation system (+S9 and -S9), and for 24-hrs, without metabolic activation system (-S9). As a result of the study, in lymphocytes from donor 1 and donor 2 exposed to 1-MNA at concentrations of 1,72-0,055 mg/ml for 3 and 24 hours no significant changes in Cytokinesis-Block Proliferation Index (CBPI), Replicative Index (RI) and the percentage of the cytostasis were observed. In the positive control cultures (Mitomycin C, 0,15 µg/ml (3 h -S9) or 0,05 µg/ml (24 h -S9); Vinblastine, 10 ng/ml (3 h -S9) or 0,6 ng/ml (24 h -S9);

Cyclophosphamide, 2,5 µg/ml (3 h +S9)) an increase in % cytostasis was observed, however, the values were within an acceptable range (i.e. less than 55%). In the main study, 1-MNA used at 3 concentrations of 1,72 mg/ml, 0,55 mg/ml, and 0,172 mg/ml, did not induce any statistically significant increase in micronuclei frequency in the exposed cell cultures compared to control cultures (Student's t-test at $p < 0,05$) neither after 3-hr exposure (with or without metabolic activation system) nor after 24-hr exposure (without metabolic activation system). Reference mutagens without S9-mix, i.e. Mitomycin C and Vinblastin, as well as with S9-mix, i.e. Cyclophosphamide, induced statistically significant increases in MN frequency comparing to the control values. These results met the acceptance criteria of the test. The results obtained indicate that under the experimental conditions used, 1-MNA does not induce mutagenic effect in Micronucleus test on human peripheral blood lymphocytes (see Annex V).

XIII.3.5. CHRONIC TOXICITY AND CARCINOGENICITY

Chronic toxicity and carcinogenicity of 1-MNA were not studied.

XIII.3.6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity were not studied in studies specially designed to evaluate this effect.

However, some information on the absence of developmental toxicity of 1-MNA can be found in Shibata and Taguchi, (1987) study (see Annex W).

XIII.4. PUBLISHED IN VITRO AND ANIMAL TOXICITY STUDIES

A 1987 study evaluated the effect of dietary 1-MNA on growth in weanling rats (Shibata and Taguchi, 1987). In this study, no growth retardation was observed following the administration of large amounts of 1-MNA to the diet for 31 days. Body weight gain and food intake were not significantly different between those rats fed 10wt% 1-MNA (n=5) or 50wt% 1-MNA (n=5), and those fed the basal diet.

In a 1947 study evaluating the lipotropic effect of 1-MNA, 16 male rats (3-4 weeks old) were fed a diet supplemented with 4% 1-MNA for 7 days (n=5), 14 days (n=5) or 28 days (n=6) (Najar and Ratcliffe, 1947). Compared with control rats, rats fed 1-MNA were active and behaved normally. Rats in the 7-day and 14-day groups gained significantly less weight (91% and 85% less, respectively) compared with control rats, however, this difference was attenuated in the 28-day group (41%). No deaths occurred during this study.

In an *in vitro* genotoxicity study, human polymorphonuclear leukocytes were exposed to 1-MNA in doses of 0.1 and 1.0 mg/mL and examined for chromatid gaps, chromatid breaks, chromosome gaps, chromosome breaks and tetraploidy, after the methodology of Ferenc et al. (Ferenc et al., 1999). There was no evidence of clastogenicity.

In *in vitro* study the neuroprotective potential of 1-MNA was evaluated. Preincubation of cultured rat cerebellar granule cells with 250 and 500 µM 1-MNA resulted in a partial, statistically significant, neuroprotective effect against homocysteine-induced toxicity (Slomka et al., 2005; Slomka et al., 2008a). The exact mechanism of this neuroprotective effect remains unclear, and

appears not to be related to the direct inhibition of NMDA receptor/channel activity (Slomka et al., 2008b). The authors suggest that the neuroprotective effect of 1-MNA may be explained by stabilization of the plasma membrane potential, resulting in inhibition of voltage-dependent activation of NMDA receptors (Slomka et al., 2008b).

Although pharmacologic compounds for the treatment of the dyslipidemias have generally been shown to be safe in both clinical trials and post-marketing surveillance studies, sporadic reports of hepatotoxicity with statins and niacin still arise. A study by Hoshino et al. (1982a) demonstrated that, unlike nicotinamide, the addition of 1-MNA at concentrations higher than 20 μ M increased the rate of DNA synthesis of rat liver cells in culture by approximately 70% over controls. This occurred concomitantly with a decrease in the cellular NAD content. In addition, the proliferation of rat liver cells with 1-MNA at 100 μ M to 1 mM was stimulated by 20% to 30% over control values. 1-MNA at 10 mM did not show any stimulation, probably because of its toxic effect at such high concentration (Hoshino et al., 1982a). Nicotinamide, on the other hand, was found by Hoshino and al.(1982a) to be a potent inhibitor of DNA synthesis and proliferation of cultured rat liver cells, with these effects occurring concomitant with an increase in cellular NAD content. The authors conclude that 1-MNA may act as a regulatory factor for cell growth, and that the close inverse relationship that they observed between the changes in the rate of DNA synthesis and NAD concentration caused by nicotinamide and 1-MNA points to a regulatory role of nicotinamide and its metabolites in cell proliferation via a change in NAD metabolism.

This concept was supported by findings from another study, also by Hoshino's group (Hoshino et al., 1982b), which examined the activity of nicotinamide methylase in regenerating liver from thioacetamide-treated rats (a treatment which induces hepatocellular proliferation). They found that the activity of nicotinamide methylase, an enzyme responsible for the conversion of nicotinamide to 1-MNA, increased in actively proliferating liver 2-4 fold compared with basal levels or levels in control animals. These results support the hypothesis that nicotinamide methylase and its product, 1-MNA, are involved in the control of hepatocellular DNA synthesis and proliferation.

Subsequently, Hoshino et al. (1984) demonstrated that, at physiological levels (11-500 μ M), nicotinamide is preferentially converted to 1-MNA, rather than NAD (Figure XIII.4.1.). Upon increasing nicotinamide concentration (1-10 mM or higher), the rate of NAD synthesis dramatically increased, reaching a level 6-fold higher than 1-MNA. The addition of exogenous 1-MNA resulted in a dose-dependent inhibition of NAD synthesis (up to 60%; Figure 36). However, the degree of inhibition was not only dependent on the concentration of 1-MNA but was also dependent on the concentration of nicotinamide present as substrate, and at low nicotinamide concentrations (11 μ M) a small increase in the synthesis of NAD was observed with increasing concentrations of 1-MNA (Figure XIII.4.2.).

Interestingly, the rate of nicotinamide to 1-MNA synthesis was reduced with increasing concentrations of nicotinamide, and with increasing concentrations of 1-MNA (Figure XIII.4.3.). Since the decrease in NAD synthesis observed with increasing 1-MNA concentrations was accompanied with an increase in cellular ATP levels, and vice-versa, the authors concluded that the previously observed growth stimulation of hepatic cells by 1-MNA can be explained by its ATP-sparing effect due to the inhibition of NAD synthesis, a reaction that requires ATP.

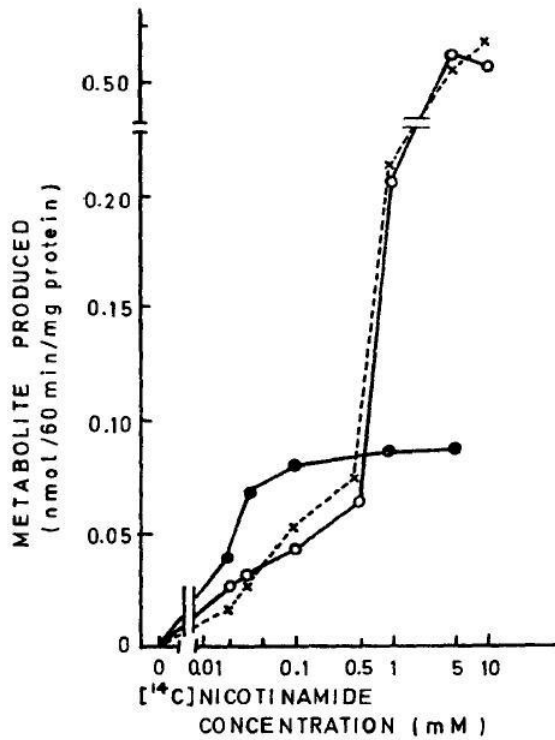


Figure XIII.4.1. Incorporation of [¹⁴C]nicotinamide into NAD and 1-methylnicotinamide in liver slices as a function of [¹⁴C]nicotinamide concentration. o—o, NAD; ●—●, 1-methylnicotinamide, x—x, nicotinamide.

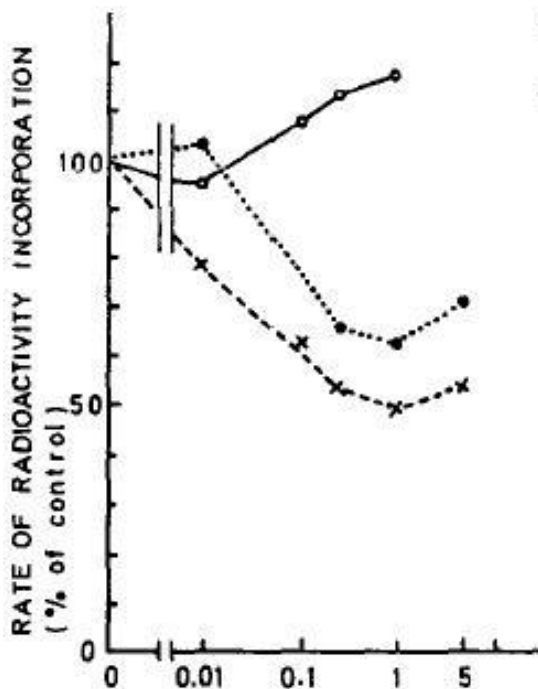


Figure XIII.4.2. Effect of exogenous 1-methylnicotinamide on the incorporation of [¹⁴C]nicotinamide into NAD in liver slices. o—o, 0.011 mM [¹⁴C]nicotinamide; ●—●, 0.53 mM [¹⁴C]nicotinamide, x—x, 5.02 mM [¹⁴C]nicotinamide.

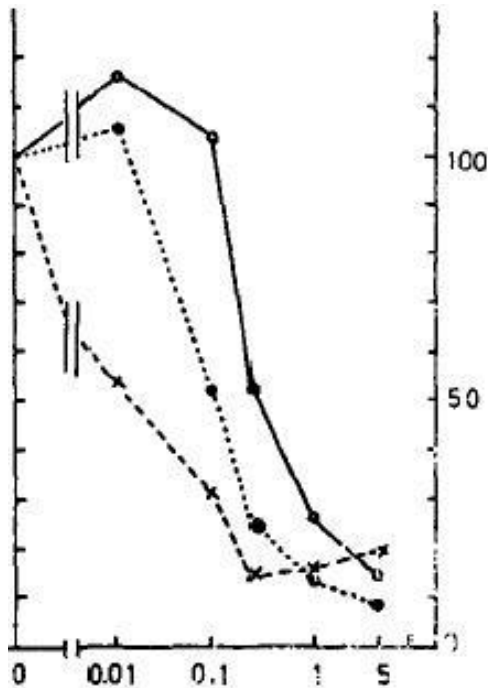


Figure XIII.4.3. Effect of exogenous 1-methylnicotinamide on the incorporation of [¹⁴C]nicotinamide into 1-methylnicotinamide in liver slices. o—o, 0.011 mM [¹⁴C]nicotinamide; ●—●, 0.53 mM [¹⁴C]nicotinamide, x—x, 5.02 mM [¹⁴C]nicotinamide.

More recent study by Kuykendall et al. (2007), found that nicotinamide and 1-MNA have opposing effects on cell growth and differentiation of murine erythroleukemia cells (MELCs). When cultured for 96 hrs in the presence 1-MNA, cell growth of MELCs cells was stimulated at all doses of 1-MNA tested (2,5-10 mM), with up to a 1,5 fold increase in cell density observed in MELCs exposed to 5 mM 1-MNA (Figure 38). Although cell density was higher with 10 mM 1-MNA, higher levels of cytotoxicity being observed compared with control cells. In contrast, nicotinamide inhibited growth of MELCs in a dose-dependent manner, with a 34%, 60%, and 72% reduction in density of cells cultured with 5, 10, and 20 mM nicotinamide for 5 days. Commitment to differentiate post-induction with nicotinamide was also inhibited by 1-MNA (2,5 mM), which reduced the appearance of heme-producing cells and inhibited heme accumulation (Kuykendall et al., 2007). The clinical significance of these *in vitro* results using hyper-physiological concentrations of 1-MNA is unknown.

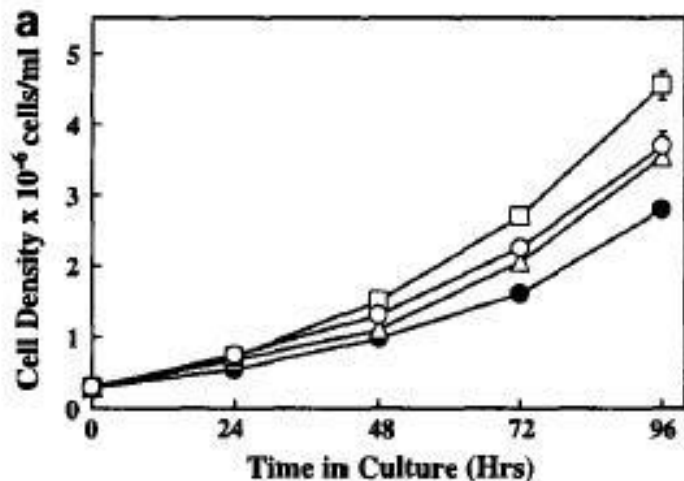


Figure XIII.4.4. Increased cell growth of MELCs cultured with 1-methylnicotinamide. Log phase MELCs were incubated for 4 days with cell growth (a) determined daily. (●) control; (Δ) 2.5 mM 1-methylnicotinamide; (□) 5 mM 1-methylnicotinamide (○) 10 mM 1-methylnicotinamide. All data points represent mean \pm SE of triplicate counts of over 100 cells each.

XIII.5. OTHER ANIMAL STUDIES WITH 1-MNA

Acute skin irritation

Acute skin irritation was studied according to OECD 404 (B4 method) in [REDACTED] in 2006 on rabbit model. 1-MNA dissolved in distilled water was used topically on shaved skin of 3 rabbits in a dose of 0,5 g for 4 hours. The exposure was evaluated 1, 24, 48 and 72 hours after administration of the studied substance. No pathological changes were seen. The study shows that 1-MNA does not irritate the skin of rabbits (see Annex X).

Skin irritation was also assessed in another study in which 1-MNA was given topically in the form of 1 and % salve everyday for 7 days in 5 rabbits (Draize method). 1-MNA did not cause skin redness or skin oedema (see Annex R).

Acute eye irritation

The study of acute irritation/injury of eye was performed on rabbit model using OECD405 B5 method in [REDACTED] in 2006. 1-MNA was administrated to conjunctival sac of one eye of two rabbits in a dose of 0.1 g. Cornea, iris and conjunctivas were examined after 1, 24, 48, 72 hours and 7 days after application of the substance. Erythema and oedema was observed in conjunctiva of both rabbits but those changes were not present at the checkpoint of 7 days after 1-MNA application. 1-MNA is a substance irritating eyes of rabbits (see Annex Y).

In another study 0,01% water solution of 1-MNA was instilled every day for 5 days in 5 rabbits causing only slight redness of conjunctiva disappearing during several hours (see Annex R).

Allergenic effect on skin

Allergenic effect on skin was measured using OECD406 (B.6. method) on guinea pigs model. The study was done in [REDACTED] in 2006. 1-MNA was given twice – firstly

intradermally in 8 % concentration together with complete Freund adjuvant (allergy induction) and secondly topically in 50% concentration in the same place as intradermal injections (allergy induction). In control group at the allergy induction phase water was used instead of the studied substance. The study group consisted of 20 animals and control group of 10 guinea pigs. Skin reaction was assessed after 24, 48 and 723 hours after finishing of exposure. Allergenic reactions were not observed, both in study and control groups. 1-MNA is not allergenic in relation to skin (see Annex Z).

XIII.6. HUMAN STUDIES

To the best knowledge of the applicant, toxicological studies of 1-MNA on humans were not performed.

However, safety aspects of 1-MNA use were taken under consideration in two clinical studies.

Study MNA-002

The safety, pharmacokinetics and pharmacodynamics of 1-MNA were evaluated in a randomized, double-blind, placebo-controlled, single-dose, crossover study, with gender matched dose cohorts. Twenty normal volunteers were exposed on two separate dosing occasions separated by at least 5 days following each dose exposure. Four of the 20 subjects (2 males and 2 females) received placebo on both occasions, and 16 subjects (8 males and 8 females) received active component at each dosing session. No serious adverse events occurred during the course of the study, and no subjects discontinued the study due to adverse events. Adverse events were reported by eight subjects and treatment-related adverse events were reported by three subjects. The total number of adverse events was 12. There was one adverse event in the placebo group (vaso-vagal syncope, n=1), eight adverse events in the 90 mg group (headache, n=4; productive cough, n=1; limb discomfort, n=1; vomiting, n=1; back pain, n=1), and three adverse events in the 270 mg group (headache, n=1; fatigue, n=1; diarrhea, n=1). The total number of treatment-related adverse events was three, with two occurring in the 270 mg group (headache, n=1; diarrhea, n=1 subject) and one occurring in the 90 mg group (headache, n=1). All adverse events classified as mild to moderate in relation to severity. There were no clinically significant changes in vital signs, haematology, blood chemistry, urine analysis, or ECG and no change from baseline in the QT/QTc interval. Overall, no patients withdrew from the study due to AE, SAE, or clinically significant physical, haematological, and biochemical laboratory results. There were no clinically significant hemodynamic changes.

See Annex O.

Study MNA-001

Study Design

This was a randomized, double-blind, placebo-controlled, dose-ranging, multi-center study to estimate the size of the effect of 1-MNA on serum lipid levels. Men and women with hyperlipidemia were allocated in a 1:1:1 ratio to receive either placebo, 30 mg 1-MNA or 90 mg 1-MNA three times daily following a 6-8 week placebo and dietary-controlled lead-in period. The

duration of the treatment period was 12 weeks. Serum lipid levels and a set of secondary parameters were evaluated at screening, during the baseline period, upon randomization and throughout the 12-week active treatment period. Additionally, blood samples were taken at the randomization visit and during following visits at the investigation centre during the active treatment period for sparse sampling population pharmacokinetic assessments. All blood samples for lipid assessments and glucose measurements were collected following a 12-hour fasting period. Safety and tolerability were assessed throughout whole trial period, including physical examination, ECGs, routine hematology and blood chemistry testing, and adverse events.

The primary outcome measure in the trial was serum triglycerides level; secondary lipid measures included (but were not limited to) total cholesterol, LDL-C, VLDL-C, HDL-C total, HDL2, HDL3, TG/HDL-C ratio, apoB and apoA1. The safety and tolerability assessment of 1-MNA comprised physical examination, assessment of vital signs, adverse events, 12-lead electrocardiograms and laboratory evaluations.

Of the 405 patients screened, 339 patients entered the placebo lead-in period. One hundred and twenty-eight patients were considered as baseline screening failures/not randomized and 211 patients completed the running placebo lead-in period, met all inclusion/exclusion criteria and were randomized to receive at least one dose of the studied substance (placebo group n = 73, 1-MNA 90 mg daily n = 71 and 1-MNA 270 mg daily n = 67). Out of the 211 randomized patients, four patients were excluded from the full analysis population (one patient from the 270 mg 1-MNA group, one patient from the 90 mg 1-MNA group, and two patients from the placebo group) due to no post-randomization assessment reported on the CRF. The full analysis population therefore includes 207 patients (placebo group n = 71, 90 mg 1-MNA daily n = 70 and 270 mg 1-MNA daily n = 66). In total, 195 (92,4%) patients completed the study (placebo group n = 67, 90 mg 1-MNA group n = 65 and 270 mg 1-MNA group n = 63). A total of 16 patients from the randomized population discontinued before the end of the study for the following reasons: consent withdrawn, non-serious adverse event, physician/sponsor decision, protocol violation and other reason.

The safety assessment included 211 subjects: 73 subjects in the placebo group, 71 patients in the 90 mg 1-MNA group and 67 patients in the 270 mg 1-MNA group. One hundred and twenty seven subjects (placebo group n = 45, 90 mg 1-MNA group n = 44 and 270 mg 1-MNA group n = 38) experienced at least one adverse event (AE) during the course of the trial. Two hundred and fifty seven AEs (excluding flushing) have been reported and 17 events have been reported as flushing.

All subjects were asked to record the occurrence of SAEs up to 30 days after the last administration dose. No death has been reported during the study and no SAE occurred after the last dose, however six SAEs have been reported by four patients during the course of the study. None of these SAEs were related to the study substance. One subject (90 mg daily 1-MNA group) experienced “syncope, secondary to complete third degree AV Block” and a “cerebral hemorrhage” which resulted discontinuation from the study. One subject experienced “asthma exacerbation” and one subject experienced “urinary retention”; both were in the 270 mg daily 1-MNA group. One patient (placebo group) experienced “motorcycle accident” and “foot fracture”.

A full clinical laboratory profile for each visit included:

- Hematology: RBC, hemoglobin, hematocrit, WBC, platelet count and differential if WBC abnormal;
- Chemistry: fasting glucose, AST, ALT, alkaline phosphatase, LDH, CPK, BUN, creatinine, uric acid, total protein, albumin, total bilirubin, sodium, potassium, calcium, amylase, HBA1C, T4 and TSH.

Several laboratory abnormalities were observed for glucose at the end of the active treatment phase (placebo n=41, 1-MNA 90 mg/d n=33, 1-MNA 270 mg/d n=32). For the other chemistry parameters, laboratory abnormalities were ranging from 0 to 16 cases in the placebo group, from 0 to 17 in the 1-MNA 90 mg/d group and from 0 to 13 cases in the 1-MNA 270 mg/d group at the end of the active treatment phase.

In summary, this study showed that 1-MNA at doses up to 270 mg/d was safe and well tolerated, no treatment related severe adverse events were seen, no liver toxicity and glucose deregulation was shown, and no evidence of flushing was demonstrated.

See Annex ZZ.

Safety aspects of 1-MNA measured in two human studies are summarized in Table 11.

Table 11. Safety aspects of 1-MNA studied in human trials.

Study and objectives	Study description	Patient population and sample size	Safety outcomes	Efficacy outcomes	Completed or ongoing
<u>Protocol -002</u> Assess safety and PK of 1-MNA	Randomized, double-blind, placebo-controlled, single dose crossover study (Phase I)	Healthy adult volunteers (N=20); N=16 exposed to 1-MNA 90 or 270 mg.	12 mild-mod. AEs in 8 (N=8) subjects. # TEAEs with more than one AE: 1 (headache). No SAEs.	N/A	Completed
<u>Protocol -001</u> Assess safety and efficacy	Randomised placebo-controlled double-blind trial (PII/III)	405 subjects screened. 339 subjects entered placebo lead in. 211 dyslipidemic patients enrolled; 71 received 1-MNA 30 mg po tid, 67 received 1-MNA 90 mg po tid, 73 received placebo tid.	4 patients reported SAEs; 1 patient on Placebo, 1 patient on 90 mg 1-MNA 2 patients on 270 mg 1-MNA; all SAEs not related to study drug. No deaths.	No statistically significant difference was observed relative to placebo in any lipid endpoints.	Completed

XIII.7. TOXICITY OF 1-MNA

Toxicity and safety aspects of 1-MNA intake was studied in *in vitro*, animal and human studies described above in this section.

The results of acute toxicity tests (after oral gavage and single intraperitoneal injection) in animal model show that 1-MNA is practically not toxic and should be classified as non toxic according to classical classification (LD50 > 2000 mg/kg b.w.). Also sub-acute toxicity study in animal model (OECD 407) did not show any abnormal changes (except some liver abnormalities not considered as related to 1-MNA administration). Based on this 28-day toxicity study in rats the dose of 1000 mg/kg/day 1-MNA can be considered a no-observed-adverse-effect level (NOAEL) following 1-MNA oral administration.

Two genotoxicity *in vitro* studies were performed following recent EFSA recommendations (1) which recommends the following *in vitro* tests:

- a bacterial reverse mutation test (OECD TG 471),
- an *in vitro* mammalian cell micronucleus test (OECD TG 487).

1-MNA did not show any mutagenic activity in bacterial reverse mutation test. In mammalian cell micronucleus test 1-MNA used at 3 concentrations (1,72; 0,55 and 0,172 mg/ml) did not induce any increase in micronuclei frequency in the exposed cell cultures which indicates that 1-MNA does not induce mutagenic effect on human peripheral blood lymphocytes. According to EFSA recommendations these results are sufficient to recognise 1-MNA as a not genotoxic substance.

Several other *in vitro* and animal studies designed for different purposes did not show and toxic effects of 1-MNA intake.

The results of two human studies (MNA-001 and MNA-002) did not show any relevant safety aspects of 1-MNA intake.

In conclusion, it should be stated that the results of published and non-published studies submitted show that 1-MNA is a substance which can be reasonably expected to be safe and non-toxic.

XIV. REFERENCES

1. Aoyama K, Matsubara K, Okada K, Fukushima S, Shimizu K, Yamaguchi S et al. (2000). N-methylation ability for azaheterocyclic amines is higher in Parkinson's disease: nicotinamide loading test. *J Neural Transm* 107: 985–995.
2. Bartus M, Lomnicka M, Kostogryś RB, Kaźmierczak P, Watała C, Słominska EM, Smoleński RT, Pisulewski PM, Adamus J, Gebicki J, Chłopicki S. „1-Methylnicotinamide (MNA) prevents endothelial dysfunction in hypertriglyceridemic and diabetic rats.” *Pharmacol Rep.* 2008;*60(1)*:127-38.
3. Bender DA, 2000. The Vitamins. Chapter in *Introduction to Human Nutrition*, ed. MJ Gibney, HH Vorster, FJ Kok, Blackwell Science, p.149-153.
4. Bryniarski K, Biedron R, Jakubowski A, Chłopicki S, Marcinkiewicz J. „Anti-inflammatory effect of 1-methylnicotinamide in contact hypersensitivity to oxazolone in mice; involvement of prostacyclin.” *Eur J Pharmacol.* 2008;*578(2-3)*:332-8.
5. Brzozowski T, Konturek PC, Chłopicki S, Sliwoski Z, Pawlik M, Ptak-Belowska A, Kwiecien S, Drozdowicz D, Pajdo R, Slonimska E, Konturek SJ, Pawlik WW, 2008. Therapeutic potential of 1-methylnicotinamide against acute gastrin lesions induced by stress: role of endogenous prostacyclin and sensory nerves. *Journal of Pharmacology and Experimental Therapeutics*, 326, 105-116.
6. Chłopicki S, Swies J, Mogielnicki A, Buczko W, Bartus M, Lomnicka M, Adamus J, Gebicki J. „1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway.” *Br J Pharmacol.* 2007;*152(2)*:230-9.
7. Domagała TB, Szeffler A, Dobrucki LW, Dropinski J, Polanski S, Leszczynska-Wiloch M, Kotula-Horowitz K, Wojciechowski J, Wojnowski L, Szczeklik A, Kalinowski L, 2012. Nitric oxide production and endothelium-dependent vasorelaxation ameliorated by N1-Methylnicotinamide In human blood vessels. *Hypertension* (publication online DOI: 10.1161).
8. Erb C, Seidel A, Frank H, Platt KL, Oesch F, Klein J, 1999. Formation of N-Methylnicotinamide in the brain from a dihydropyridine-type prodrug. *Biochemical Pharmacology*, 57, 681-684.
9. EFSA Scientific Committee, 2011, Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal* 9, 2379.
10. Felsted R, Chaykin S, 1967. N-Metylnicotinamide oxidation in a number of mammals. *J Biol Chem*, 242, 1274-1279.
11. Ferenc T, Janik-Spiechowicz E, Bratkowska W, Lopaczynska D, Strozynski H, Denys A, Mordalska A. Genotoxicity assessment of new synthesized acridine derivative--3,6-diamino-10-methyl-9,10-dihydroacridine. *Mutat Res* 1999;*444(2)*:463-70.
12. Gebicki J, Sysa-Jedrzejowska A, Adamus J, Woźniacka A, Rybak M, Zielonka J. „1-Methylnicotinamide: a potent anti-inflammatory agent of vitamin origin.” *Pol J Pharmacol.* 2003; *55(1)*: 109-12.

13. Hoshino J, Kühne U, Kröger H. Enhancement of DNA synthesis and cell proliferation by 1-methylnicotinamide in rat liver cells in culture: implication for its *in vivo* role. *Biochem Biophys Res Commun* 1982a;105(4):1446-52.
14. Hoshino J, Kühne U, Kröger H. Methylation of nicotinamide in rat liver cytosol and its correlation with hepatocellular proliferation. *Biochim Biophys Acta* 1982b;719(3):518-26.
15. Hoshino J, Schlüter U, Kröger H. Nicotinamide methylation and its relation to NAD synthesis in rat liver tissue culture. Biochemical basis for the physiological activities of 1-methylnicotinamide. *Biochim Biophys Acta* 1984;801(2):250-8.
16. Jacob RA, Swendseid ME, McKee RW, Fu CS, Clemens RA, 1989. Biochemical markers for assessment of niacin status in young men: urinary and blood levels of niacin metabolites. *J Nutr*, 119, 591-598.
17. Kuchmerovska T, Shymanskyi I, Chlopicki S, Klimenko A, 2010. 1-Methylnicotinamide (MNA) In prevention of diabetes-associated brain disorders. *Neurochemistry International*, 56, 221-228.
18. Kuykendall JR, Cox R, Kinder D. 1-Methylnicotinamide stimulates cell growth and inhibits hemoglobin synthesis in differentiating murine erythroleukemia cells. *Toxicol In Vitro*. 2007 Dec;21(8):1656-62. Epub 2007 Jul 18.
19. MacKay D, Hathcock J, Guarneri E., 2012. Niacin: chemical forms, bioavailability, and health effects. *Nutrition Reviews*, 70, 357-366.
20. Maiza A, Waldek S, Ballardie FW, Daley-Yates PT (1992). Estimation of renal tubular secretion in man, in health and disease, using endogenous N-1-methylnicotinamide. *Nephron* 60: 12–16.
21. Menon RM, Adams MH, Gonzalez MA, Tolbert DS, Leu JH, Cefali EA, 2007a. Plasma and urine pharmacokinetics of niacin and its metabolites from an extended-release niacin formulation. *International Journal of Clinical Pharmacology and Therapeutics*, 45, 448-454.
22. Menon RM, Gonzalez MA, Adams MH, Tolbert DS, Leu JH, Cefali EA, 2007b. Effect of the rate of niacin administration on the plasma and urine pharmacokinetics of niacin and its metabolites. *J Clin Pharm* (published online as doi: 10.1177/0091270007300264).
23. Mogielnicki A, Kramkowski K, Pietrzak L, Buczek W, 2007. N-Methylnicotinamide inhibits arterial thrombosis in hypertensive rats. *J Physiol Pharmacol*, 58, 515-527.
24. Najjar, VA., Ratcliffe, I.M. The lipotropic effect of N1-methylnicotinamide. *Bulletin of the Johns Hopkins Hospital* 1947;80:142-8.
25. Parra JL, Reddy KR. Hepatotoxicity of hypolipidemic drugs. *Clin Liver Dis* 2003;7(2):415-33.
26. Pumpo R, Sarnelli G, Spinella A, Budillon G, Cuomo R (2001). The metabolism of nicotinamide in human liver cirrhosis: a study on N-methylnicotinamide and 2-pyridone-5-carboxamide production. *Am J Gastroenterol* 96: 1183–1187.
27. Slomka M, Zieminiska E, Lazarewicz JW. Neuroprotective potential of methylnicotinamide in homocysteine-induced neurotoxicity *in vitro*. *Acta Neurobiologiae Experimentalis* 2005; 65:349 (published as abstract).

28. Slomka M, Zieminska E, Lazarewicz J. Nicotinamide and 1-methylnicotinamide reduce homocysteine neurotoxicity in primary cultures of rat cerebellar granule cells. *Acta Neurobiol Exp (Wars)*. 2008a;68(1):1-9.
29. Slomka M, Zieminska E, Salinska E, Lazarewicz JW. Neuroprotective effects of nicotinamide and 1-methylnicotinamide in acute excitotoxicity in vitro. *Folia Neuropathol*. 2008b;46(1):69-80.
30. Watala C, Kazmierczak P, Dobaczewski M, Przygodzki T, Bartus M, Lomnicka M, Slominska EM, Durackova Z, Chlopicki S, 2009. Anti-diabetic effects of 1-methylnicotinamide (MNA) In streptozocin-induced diabetes in rats. *Pharmacological Reports*, 61, 86-98.
31. Woźniacka A, Wieczorkowska M, Gebicki J, Sysa-Jedrzejowska A. „Topical application of 1-methylnicotinamide in the treatment of rosacea: a pilot study.” *Clin Exp Dermatol*. 2005; 30(6): 632-5.