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Safety of 1-methylnicotinamide chloride (1-MNA) as a novel food pursuant to Regulation (EC) No 258/97

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Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on 1-methylnicotinamide chloride (1-MNA) as a novel food (NF) ingredient submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council, taking into account the comments and objections of a scientific nature raised by Member States. 1-MNA is a substance present naturally in the human body as a normal downstream product of niacin metabolism. The Panel considers that the information provided on the composition, the specification and the batch-to-batch variability of the NF is sufficient. The applicant intends to use 1-MNA in food supplements and proposes a maximum intake of 58 mg/day. 1-MNA is not genotoxic. In a subchronic rat study, epithelium degeneration of the non-glandular stomach was observed at all dose levels with increasing frequency. The Panel notes that the human stomach does not have non-glandular epithelium and considers this finding is toxicologically not relevant for humans. At doses of 500 and 1,000 mg/kg body weight (bw), changes of the urine pH, that did not reverse in the recovery period, were reported. As adversity of this finding cannot be ruled out, the Panel selected 250 mg/kg bw in this rat study as the reference point. The Margin of Exposure to humans weighing 70 kg and consuming 58 mg would be about 300. The Panel notes the upper level for nicotinamide, i.e. 900 mg/day for adults. Taking into account that 1-MNA is a main metabolite from nicotinamide, the Panel considers that it is unlikely that an intake of 58 mg 1-MNA from food supplements would result in adverse health outcomes in humans. The Panel concludes that the NF, 1-MNA, is safe under the proposed uses and use levels.

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on 1-methylnicotinamide chloride (1-MNA) as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. The assessment follows the methodology set in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. The assessment is based on the data supplied in the original application, the initial assessment by the competent authority of the United Kingdom, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant.

The NF ingredient is a synthetic organic compound, classified as a pure chemical compound from a non-genetically modified (GM) source (Class 1.1). 1-MNA is a substance present naturally in the human body as a normal downstream product of niacin metabolism. Niacin is a generic term referring to nicotinamide and nicotinic acid. The Panel notes that the limit proposed for 'heavy metals' (≤ 20 ppm) may not comply with the existing legislation. Provided that the limits for heavy metals are in compliance with existing legislation, the Panel considers that the information provided on the composition, the specification and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

The applicant intends to use 1-MNA in food supplements and proposes a maximum intake of 58 mg/day. 1-MNA is proposed to be used by adults excluding pregnant and breastfeeding women. The applicant proposed to indicate on the label of the product that it should be ingested during or immediately after a meal, and that it should be consumed alongside at least half a glass of water.

Low intakes of 1-MNA may result from the consumption of specific foodstuffs and certain medicinal products. Most of the systemic exposure to 1-MNA however derives endogenously from metabolism of niacin.

Results of bacterial reverse mutation test and *in vitro* mammalian cell micronucleus test indicated that 1-MNA is not genotoxic. Three studies were used to assess the subacute toxicity of 1-MNA. The findings of the two studies did not show any reasons for concern. Nevertheless, some of the results of the third study could not be ruled out as adverse. To address this, the applicant commissioned a 91-day rat feeding study. In this subchronic rat study, epithelium degeneration of the non-glandular stomach was observed at all dose levels with increasing frequency. Since the human stomach does not have non-glandular epithelium, the Panel considers that this finding is toxicologically not relevant for humans. At doses of 500 and 1,000 mg/kg body weight (bw), changes of the urine pH, that did not reverse in the recovery period, were reported. As adversity of this finding cannot be ruled out, the Panel selected 250 mg/kg bw in this rat study as the reference point. The Margin of Exposure to humans weighing 70 kg and consuming 58 mg would be about 300.

One Member State expressed concerns that the intake of 1-MNA would add to endogenously formed 1-MNA from niacin metabolism. The Panel notes the upper level for nicotinamide, i.e. 900 mg/day for adults. Taking into account that 1-MNA is a main metabolite from nicotinamide, the Panel considers that it is unlikely that an intake of 58 mg 1-MNA from food supplements in addition to the niacin intake from other sources diet would result in adverse health outcomes in humans.

The Panel concludes that the NF, 1-MNA, is safe under the proposed uses and use levels.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 18 September 2013, the company Pharmena S.A. submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97¹ to place on the market 1-methylnicotinamide chloride (1-MNA) as a novel food (NF) ingredient.

On 26 November 2015, the competent authority of the United Kingdom forwarded to the Commission its initial assessment report, which came to the conclusion that 1-MNA meets the criteria for acceptance of a novel food ingredient defined in Article (3)1 of Regulation (EC) No 258/97.

On 11 December 2015, the Commission forwarded the initial assessment report to the other Member States (MS). Several of the MS submitted comments or raised objections.

The concerns of a scientific nature raised by the MS can be summarised as follows:

- It is not clear whether some of the enrolled laboratories are accredited in accordance with an internationally recognised system.
- Methanol level of the product is indicated to be 'not more than 3,000 ppm'. However, such content is considered to be too high.
- Data regarding storage stability for a period up to 36 months, as intended by the applicant, were not available.
- Additional information is required on the possible interaction of 1-MNA with other food components or on their intake.
- Lack of documentation regarding distribution of 1-MNA after oral intake.
- Changes to urine parameters in the 28-day and 90-day toxicity studies could not be ruled out as adverse.
- There is an uncertainty regarding the anticipated intake/extent of use. In the initial application, a daily dose of 250 mg (corresponding to two single 125 mg doses) is recommended. Nevertheless, conclusions of ACNFP concern an intake of 58 mg/day.
- Additionally to children and pregnant women, breastfeeding mothers are to be excluded from the target population of the specific product, as a precautionary health protection measure.
- There is a lack of information on the long-term effect of 1-MNA, especially when consumed alongside other foods and with food supplements containing niacin. Consumption of the NF would lead to an increment of 1-MNA, which is also endogenously produced from niacin degradation.
- Concerns were raised regarding the chronic consumption of 1-MNA alongside antidiabetic, antithrombotic and antiplatelet drugs, and anticoagulants.
- Study records from a randomised, placebo-controlled, double-blind human study reported 17 flushing events but it was not clear in which study group the events took place. Flushing symptoms observed during this study may represent typical reactions due to overdose with nicotinic acid.
- The human studies provided may be inadequate for the safety evaluation of 1-MNA consumption by humans, since the initial study design was not aiming towards that scope.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002², the European Food Safety Authority (EFSA) is asked to carry out the additional assessment for 1-MNA as a NF ingredient in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other MS.

¹ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

² Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

2. Data and methodologies

2.1. Data

The assessment of the safety of this NF is based on data supplied in the original application, the initial assessment by the competent authority of the United Kingdom, the concerns and objections of scientific nature of the other MS and the responses of the applicant.

In accordance with Commission Recommendation 97/618/EC³, 1-methylnicotinamide chloride (1-MNA) is allocated to Class 1.1, i.e. foods or food ingredients that are 'Pure chemicals or simple mixtures from non-GM sources'. The data are required to comply with the information required for novel foods of Class 1.1, i.e. structured schemes I, II, III, IX, X, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current scientific opinion, these structured schemes are listed in Sections 3.1–3.9.

The intention is to use the NF as an ingredient for food supplements. Following concerns expressed by Member States, the applicant proposed that 1-MNA is to be used by the general population, excluding children, pregnant women and breastfeeding mothers. The applicant presumes that 1-MNA may reduce risk factors of atherosclerosis. The Panel notes that this assessment concerns only risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of 1-MNA with regard to any claimed benefit.

2.2. Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council.

3. Assessment

3.1. Specification of the Novel Food

The structural formula of 1-methylnicotinamide chloride (IUPAC name: 3-carbamoyl-1-methylpyridinium chloride; chemical formula: C₇H₉N₂OCl; CAS registry number: 1005-24-9; molecular weight: 172.61 Da) is presented in Figure 1. Synonyms for this compound are 1-methyl-3-carbamoylpyridinium, 1-methyl-3-carbamoylpyridinium cation, 1-methylnicotinamide, 3-amido-*N*-methylpyridinium, 1-methyl-3-pyridinecarboxamide, N1-methylnicotinamide and *N*-methyl-3-carbamidopyridinium.

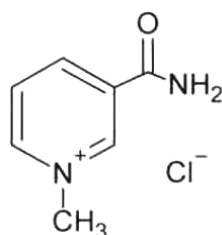


Figure 1: Structural formula of 1-methylnicotinamide chloride

The chemically synthesised NF has been classified as a pure chemical compound from a non-genetically modified (GM) source. 1-MNA is a substance present naturally in the human body as a normal downstream product of niacin metabolism. Niacin (also called vitamin B3) is a generic term referring to nicotinamide and nicotinic acid.

The NF is a fine crystalline, white or off-white, hygroscopic powder. At room temperature, it is soluble in water (667 mg/mL), soluble in methanol (33 mg/mL), and practically insoluble in dichloromethane (< 0.1 mg/mL) and 2-propanol (~ 1.3 mg/mL).

³ Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

On the table below, the proposed specification for 1-MNA is listed. Methods employed to measure each of the parameters are also included (Table 1).

Table 1: Specification of 1-MNA

Specification parameter	Analytical method	Specification value
Appearance	USP	White or off-white, crystalline solid
Solubility test	USP	Soluble in water, soluble in methanol, practically insoluble in 2-propanol and dichloromethane
Identification	a) IR spectroscopy/USP <197k> b) HPLC test	a) Spectrum must comply with reference IR spectrum b) Retention time of the major peak in the chromatogram of the assay preparation corresponds to that of the standard
Degree of coloration	EP 2.2.2., Method I	Colouration of 10% sample solution not more intense than reference solution
Loss on drying	USP <731>	≤ 1.0%
Water content	Karl Fisher method	≤ 0.3%
Residue on ignition	USP <281>	≤ 0.1%
Heavy metals	USP Method 1 <231>	≤ 20 ppm
Purity	Assay by HPLC (on dry substance basis) Anhydrous substance	≥ 98.5% ≤ 101.5%
Impurities Trigonelline ^(a) Nicotinic Acid ^(b) Nicotinamide ^(c) Largest unknown impurity Sum of unknown impurities Sum of all impurities	HPLC	< 0.05% < 0.10% < 0.10% < 0.05% < 0.20% < 0.50%
Residual solvents Methanol	Headspace/GC/USP<467>	≤ 3,000 ppm
Microbiological specification Total Aerobic Microbial Count Mould and yeasts Count <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Enterobacteriaceae</i>	Bioburden/EP 2.6.12, 2.6.13	≤ 100 cfu/g ≤ 10 cfu/g Not present in 1 g Not present in 1 g Not present in 1 g

HPLC: high-performance liquid chromatography; cfu: colony forming unit.

(a): LOD = 0.00015%, LOQ = 0.00044%.

(b): LOD = 0.00016%, LOQ = 0.00049%.

(c): LOD = 0.00041%, LOQ = 0.0012%.

Analysis of three independently produced batches indicated high purity levels of 1-MNA of ≥ 99.8%. Two high-performance liquid chromatography (HPLC) methods were implemented to verify the chemical purity of the product. 1-MNA was identified and quantified by an in-house-developed HPLC method. The levels of nicotinamide, nicotinic acid and trigonelline (known impurities) were determined by another in-house-developed HPLC method. Limit of detection (LOD), limit of quantification (LOQ) and detected levels of the above-mentioned compounds are presented in the Table 2. Unknown impurities were detected and quantified with the same HPLC method, and were found to be below the specification limits (< 0.20%). Batch analysis results showed compliance with the proposed specifications. The Panel notes that the limit proposed for 'heavy metals' (≤ 20 ppm) may not comply with the existing legislation.

Provided that the limits for heavy metals are in compliance with existing legislation, the Panel considers that the information provided on the composition, the specification and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

Table 2: Batch analysis results

Batch no. Manufacturing date (day/month/year)	3090-110404 4/4/2011	3090-110608 8/6/2011	3090-110615 15/6/2011	
Characters:				
Appearance and solubility	White or almost white, crystalline powder, very soluble in water, soluble in methanol, practically insoluble in dichloromethane and 2-propanol	Conformed	Conformed	Conformed
Identity:				
IR	IR spectrum identical with spectrum of primary standards	Conformed	Conformed	Conformed
HPLC	Retention time of the main peak consistent with retention time of the main peak of standard	Conformed	Conformed	Conformed
Quantity: HPLC	Not less than 98.5% and not more than 101.5%	100.9%	100.9%	99.8%
Appearance of 10% solution:				
Degree of colouration	Colouration of 10% sample solution not more intense than reference solution Y7	Conformed	Conformed	Conformed
Properties:				
Loss on drying	Not more than 1.0%	0.1%	0.11%	0.17%
Water content	Not more than 0.5%	0.073%	0.13%	0.089%
Impurities:				
Residue on ignition	Not more than 0.1%	0.02%	0.02%	0.02%
Heavy metals	Not more than 0.002%	Below 0.002%	Below 0.002%	Below 0.002%
Trigonelline^(a)	Not more than 0.05%	Not detected	Below detection limit	Below detection limit
Nicotinic acid^(b)	Not more than 0.10%	Below detection limit	Below detection limit	Below detection limit
Nicotinamide^(c)	Not more than 0.10%	0.001%	0.004%	0.001%
Largest unidentified impurity	Not more than 0.05%	Below detection limit	0.003%	0.002%
Sum of unidentified impurities	Not more than 0.20%	0%	0.003%	0.002%
Sum of all impurities	Not more than 0.50%	0.001%	0.007%	0.003%
Residual methanol	Not more than 3,000 ppm	551 ppm	1,057 ppm	557 ppm

Batch no.	3090-110404	3090-110608	3090-110615	
Manufacturing date (day/month/year)	4/4/2011	8/6/2011	15/6/2011	
Microbiological parameters				
Total aerobic microbial count	Not more than 10 ² cfu/g	1 cfu/g	3 cfu/g	1 cfu/g
Total combined yeasts/moulds count	Not more than 10 cfu/g	1 cfu/g	0 cfu/g	0 cfu/g
<i>Pseudomonas aeruginosa</i>	Not present in 1 g	Not present in 1 g	Not present in 1 g	Not present in 1 g
<i>Staphylococcus aureus</i>	Not present in 1 g	Not present in 1 g	Not present in 1 g	Not present in 1 g
<i>Enterobacteriaceae</i>	Not present in 1 g	Not present in 1 g	Not present in 1 g	Not present in 1 g

IR: infrared; HPLC: high-performance liquid chromatography; cfu: colony forming units.

(a): LOD = 0.00015%, LOQ = 0.00044%.

(b): LOD = 0.00016%, LOQ = 0.00049%.

(c): LOD = 0.00041%, LOQ = 0.0012%.

3.1.1. Stability of the NF

The stability of the NF was investigated under normal storage conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}/60\%$ relative humidity $\pm 5\%$) during a 36-month period.

During the above-mentioned time span, the impurities remained within the specification limits. The purity of the product was found to be at each time point equal or greater than 99%. Nicotinamide and trigonelline were detected at levels less than 0.0085% and 0.002%, respectively. The levels of these compounds in the NF remained unaltered during the storage period. Furthermore, stability data met also the rest of the proposed specifications presented in Table 1. Based on the obtained results, the applicant proposes to consider that 1-MNA is stable for at least 36 months under the above mentioned storage conditions.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.2. Effect of the production process applied to the NF

The applicant has provided details of the production process in the confidential version of the dossier. 1-MNA is chemically synthesised in two main steps. During the first step, chemically synthesised nicotinamide is methylated, resulting in crystalline crude 1-MNA. In the second step, after dissolution of crude 1-MNA in the proper solvent and recrystallisation, high purity 1-MNA crystals are obtained. The manufacturing process is performed according to Good Manufacturing Practice.

Residual methanol from the production process may exist in the final product, as indicated in the specifications ($< 3,000$ ppm). The Panel notes that the maximum amount of methanol that might be consumed daily is calculated to be 0.174 mg/day, when considering the proposed specification limit and the intended use levels of 58 mg of the NF. The Panel also notes that methanol occurs naturally in various foodstuffs such as fruits, vegetables and their juices (Velisek, 2013). Methanol levels in fruit juices can range from 1 up to 640 mg/L (WHO, 1997). Thus, compared with consumption of certain fruit juices, the maximum intake resulting from consumption of the NF is negligible and not of concern.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.3. History of use of the NF and its source

1-MNA is produced from nicotinamide in a process of nicotinamide methylation.

The main food sources of nicotinamide and nicotinic acid are meat, poultry, liver, fish, eggs, peanuts, rice bran and wheat bran. Significant amounts of nicotinamide can also be obtained from legumes, nuts, mushrooms and sunflower seeds.

3.4. Anticipated intake/extent of use of the NF

The applicant intends to use 1-MNA in food supplements only (in the form of gelatine capsules, tablets and 'possibly others'), and following MSs' comments proposes a maximum intake of 58 mg/day. 1-MNA is proposed to be used by adults excluding pregnant and breastfeeding women.

The applicant proposed to indicate on the label of the product that it should be ingested during or immediately after meal, and that it should be consumed alongside at least half a glass of water.

3.5. Information from previous exposure to the NF or its source

The applicant reported that 1-MNA can be obtained from dietary sources, such as wakame (edible seaweed), leaves of green tea, poultry stomach and celery (3.2, 3.0, 2.4 and 1.6 mg/g, respectively) (Taguchi et al., 1986). The Panel considers that the intake from dietary sources is low and that most of the systemic exposure to 1-MNA derives endogenously from metabolism of niacin.

3.6. Nutritional information on the NF

1-MNA is a metabolite of nicotinamide without vitamin function. The Panel considers that the NF is not nutritionally disadvantageous.

3.7. Microbiological information on the NF

The applicant states that every batch of 1-MNA is tested for the presence of a range of microorganisms. Appropriate limits for total aerobic counts and pathogenic microorganisms are

detailed in the specification. The applicant has presented microbiological analyses illustrating that the levels of microbial contamination in the product are low.

The Panel considers that the microbiological information provided does not raise safety concerns.

3.8. Toxicological information on the NF

3.8.1. Absorption, distribution, metabolism and excretion

1-MNA is an intermediate product of niacin metabolism which is further converted to *N*-methyl-2-pyridone-5-carboxamide (2PY) and *N*-methyl-4-pyridone-3-carboxamide (4PY) via aldehyde oxidase (Felsted and Chaykin, 1967). Kinetic data were obtained from a 28-day oral toxicity study in rats (Przybyła, 2013; see Section 8.3.8), in which the animals were administered 1-MNA by gavage at dose levels of 0 (vehicle control), 125, 250 or 500 mg/kg body weight (bw) per day every 12 h. Blood samples were taken on the first and 28th day of treatment, and plasma was analysed for the presence of 1-MNA and the metabolites 2PY and 4PY. On day 1, plasma levels of 1-MNA increased proportional to dose and reached maximum concentrations (C_{max}) 2–3 h after administration (t_{max}). The plasma levels reached were at least 20-fold greater than those in the control animals (i.e. the endogenous levels). The area under the curve six hours after treatment (AUC_{6h}) also increased with dose. On day 28, maximum plasma concentrations increased with dose and were observed 2–3 h following administration. The plasma 1-MNA concentrations before administration on day 28 were much higher than those on day 1 indicating accumulation of plasma 1-MNA over the study period at all dose levels. Plasma levels of the metabolite 2PY on day one increased with dose and reached a maximum approximately 3 h after administration. The AUC_{6h} values on day 28 (steady state) indicate a saturable metabolism of 1-MNA to 2PY. On day 1, plasma concentrations of 4PY also increased but appeared to be the same at all 1-MNA dose levels. The AUC_{6h} showed no relation to dose. Comparison of the kinetic parameters on day 1 and 28 suggest a reduced metabolism of 1-MNA to 4PY or increased elimination. The metabolism of 1-MNA to 4PY appears to be saturated to a greater extent than the metabolism to 2PY at the doses administered in this study.

In a study by Shibata and Taguchi (1987) in weanling Sprague–Dawley rats receiving 1-MNA in feed (0.5% w/w), about 10% of the administered compound was metabolised to 2PY and 70% of it was excreted unaltered in the urine.

2PY is the predominant 1-MNA metabolite in humans (Felsted and Chaykin, 1967). Between 11.9% and 20.0% of orally administered niacin was excreted in the urine as 1-MNA after administration at a single dose of 2,000 mg to healthy men ($n = 12$) and between 30.8% and 44.3% of the dose was excreted as 2PY (Menon et al., 2007). According to de Lange and Joubert (1964) about 20–30% and 40–60% of ingested niacin is excreted as 1-MNA and 2PY, respectively. This pattern however *varies* with the amount and form of niacin ingested and the niacin status of the subject (Gibson, 2005).

Kinetics and tolerability of 1-MNA were studied in a randomised, double-blind, placebo-controlled, single-dose study, with gender matched dose cohorts (Proskin, 2008). Twenty volunteers were exposed on two separate dosing occasions separated by at least 5 days following each dose exposure. Four (2 males and 2 females) of the 20 subjects received placebo on both occasions, and 16 subjects (8 males and 8 females) received orally either 90 or 270 mg of 1-MNA at each dosing session. Plasma samples were collected before and 1, 2, 3, 4, 5, 8, 12, 18 and 24 h after the dosing. A dose-dependent gender difference was observed 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. For both dosages, the maximum concentration (C_{max}) was reached after about 2 h.

3.8.2. Genotoxicity

The potential genotoxicity of 1-MNA was studied in accordance with the EFSA recommendations on genotoxicity testing (EFSA Scientific Committee, 2011).

Tests on gene mutations using *Salmonella* Typhimurium strains TA100, TA98, TA97, TA1535 and TA102 (Ames test) were conducted according to OECD guideline 471 and in compliance with good laboratory practice (GLP) principles (Muckova, 2006). In a range-finding experiment, 1-MNA (purity 99.6%) was not toxic up to the highest tested dose of 5.0 mg/plate. Both in the absence and presence of a metabolic activation system (S9-mix), 1-MNA did not induce a biologically relevant increase in the number of revertant colonies per plate when compared with the negative control.

An *in vitro* micronucleus (MN) test using human peripheral blood lymphocytes was conducted in accordance with OECD TG 487 and in compliance with GLP principles (Stepnik, 2012). In a preliminary study, cells were exposed to 1-MNA at concentrations up to 1.72 mg/mL for 3 h (with or without S9-mix) or for 24 h (without S9-mix). There were no relevant changes in the Cytokinesis-Block Proliferation Index (CBPI), Replication Index (RI) and the percentage of cytohalation when compared with the controls. Based on these results, cells were exposed to 1-MNA at concentrations of 0 (negative control), 1.72, 0.55 and 0.172 mg/mL in the main study. No statistically significant increase in MN frequency in the exposed cells compared to the control was observed, neither after 3-h exposure (with or without metabolic activation system) nor after 24-h exposure (without metabolic activation system). The positive control substances induced statistically significant increases in MN frequency compared to the negative control.

Based on the results of these two studies, the Panel concludes that there is no concern with respect to genotoxicity of 1-MNA.

3.8.3. Acute and subacute toxicity studies

In an acute oral toxicity study in rats, which was conducted according to OECD TG 420 and in compliance with the principles of GLP, 1-MNA (99.6% purity) did not induce adverse effects after administration at a dose of 2,000 mg/kg bw (Sornat, 2006).

A 28-day oral toxicity study was carried out according to OECD TG 407 and in compliance with GLP principles (Przybyła, 2013). Groups of 10 male and 10 female Wistar Han [CrI:WI(Han)] rats were administered 1-MNA (100.3% purity) by gavage at dose levels of 0 (vehicle control: distilled water) 125, 250 or 500 mg/kg bw every 12 h. Five additional animals per sex were allocated to the control group and the high-dose group in order to assess the reversibility or progression of any test item-related changes after a 14-day treatment-free recovery period.

One female animal in the high-dose (recovery) group died due to mechanical trauma caused by gavage administration. Apart from this, no clinically relevant signs or ophthalmic changes were identified during the treatment period. Body weights and feed consumption of animals administered 1-MNA showed no statistically significant differences compared with the control group. Behavioural and functional examinations revealed no effects related to administration of the test material. Haematology, clinical chemistry and urine analyses at the end of the treatment period showed statistically significant differences between the high-dose group and the control group. Blood calcium levels were higher in males; urine pH was lower in males and females; leucocyte count in urine was lower in males (not significantly also in females). After the recovery period, no significant differences with respect to these parameters were noted. Organ weight determinations at necropsy after 4 weeks identified no significant differences in relative weights compared with the control group. Microscopic examinations of selected organs and tissues revealed no findings related to treatment with the test material except for a necrotic focus in the left lobe of the liver in several animals administered 1-MNA (low-dose group one female; mid-dose group one male; high-dose group one male and two females; high-dose recovery group one male). This lesion was not identified in the control group.

There was no difference in body weight gain and feed intake of weanling rats given either 1-MNA (0.5% w/w) or a control diet for 15 days (Shibata and Taguchi, 1987).

The applicant also made reference to two other rat studies, which were designed to characterise the effects of 1-MNA on blood lipid parameters. These studies are considered not pertinent for the safety of the NF.

3.8.4. Subchronic/chronic toxicity studies

A subchronic (90-day) oral toxicity study was conducted according to OECD TG 408 and in compliance with GLP principles, with the exception of formulation analysis. Groups of 10 male and 10 female Wistar Han [CrI:WI(Han)] rats, were administered 1-MNA (100.5% purity) by gavage for up to 91 days at dose levels of 0 (vehicle control: distilled water) 125, 250, 500 or 1,000 mg/kg bw per day (Ford, 2014). Six additional animals per sex were allocated to the control group and the high-dose group in order to assess the reversibility or progression of any test item-related changes after a 28-day treatment-free recovery period.

All animals survived the treatment period, and regular daily observations as well as weekly detailed observations did not identify clinical signs attributable to treatment with 1-MNA. Body weights in the test groups showed no statistically significant differences compared with the control group, and there were no relevant differences in feed consumption. Functional observation battery (FOB) evaluations

and motor activity measurements as well as ophthalmoscopic examinations (week 13) revealed no effects related to administration of the test material. Haematology analyses (week 13) showed a statistically significantly lower eosinophil count in females administered 1,000 mg/kg bw per day compared with the control group. In the absence of changes in related parameters, this difference is not considered toxicologically relevant. Urine analyses (week 13) showed a statistically significantly lower pH value in males and females administered 500 mg/kg bw (6.38, SD \pm 0.64 and 6.33, SD \pm 0.58, respectively) and females administered 1,000 mg/kg bw (6.38, SD \pm 0.75) compared with the control groups (7.22, SD \pm 0.565 and 7.44, SD \pm 0.417, respectively). In the female 1,000 mg group, the difference was still significant after the 28-day recovery period (6.83, SD \pm 0.289 vs 8.25, SD \pm 0.289), and also males showed a lower mean value (7.33 vs, 8.3). The adversity of this effect is unclear. Organ weight determinations at necropsy showed a statistically significantly higher absolute, but not relative (to body weight) adrenal gland weight in males (0.071 vs 0.055 g). In the absence of histopathological changes in this organ, the difference is not considered toxicological relevant. Macroscopic examinations at necropsy revealed no gross pathological findings related to treatment with 1-MNA.

Microscopic examinations did not identify necrotic liver lesions as observed in the 28-day study. An increase in the frequency of epithelial degeneration of the non-glandular stomach (males: 2, 2, 3 and 4 animals in the test groups; females: 0, 1, 1 and 2 animals in the test groups compared to 0 in the control group) was reported. After the 28-day recovery period, none of the animals administered 1,000 mg/kg bw showed this lesion, while it was identified in one control female. No changes were observed in the glandular stomach of the rodents.

On request of EFSA the applicant provided historical control data, which showed that epithelial degeneration of the non-glandular stomach was a rare finding in control animals, even in studies with gavage administration. The NDA Panel notes that one female in the recovery control group also showed this lesion, and the severity of this effect did not increase with dose. The frequency of occurrence increased with dose. The NDA Panel considers that the observed effect at the site of contact may be related to treatment with the test material.

However, forestomach effects in rat studies are not considered a relevant endpoint for humans since humans lack this organ. The human stomach does not have non-glandular (but only glandular) epithelium (e.g. Wester and Kroes, 1988; Proctor et al., 2007; Nolte et al., 2016). The human oesophagus, however, has non-glandular epithelium (Greaves, 2012), but its exposure to 1-MNA would be low in terms of duration and concentration and unlikely to cause adverse effects.

3.8.5. Developmental and reproductive toxicity studies

Studies on reproductive and developmental toxicity performed in accordance with OECD Guidance protocols were not provided.

3.8.6. Human studies

The applicant has provided two unpublished reports (Proskin, 2008; Cossette, 2009).

Besides the kinetics (Section 3.8.1), also tolerability of 1-MNA was studied in a randomised, double-blind, placebo-controlled, single-dose study, with gender matched dose cohorts (Proskin, 2008). A total of 20 volunteers were exposed on two separate dosing occasions separated by at least 5 days following each dose exposure. Four (2 males and 2 females) of the 20 subjects received placebo on both occasions and 16 subjects (8 males and 8 females) received either 90 or 270 mg of 1-MNA at each dosing session. No serious adverse events (AEs) occurred during the course of the study, and no subjects discontinued the study due to AEs. The Panel notes that this trial studied the administration of only two single doses and the low number of subjects. No conclusions with regard to the safety of the NF can be drawn from this study.

The second randomised, double-blind, placebo-controlled, multi-centre study with 211 subjects (mean age 53 years) aimed to estimate the size of the effect of 1-MNA on serum lipid levels (Cossette, 2009). About 70 subjects (about 60% male subjects) per group received placebo, 90 or 270 mg 1-MNA for 12 weeks. Safety relevant endpoints included physical examination, ECGs, routine haematology and blood chemistry testing, and AEs. A total of 127 subjects (45 in the placebo group, 44 in the 90 mg 1-MNA group, and 38 in the 270 mg 1-MNA group) experienced at least one AE during the course of the trial. A total of 257 AEs (excluding flushing) have been reported and 17 events have been reported as flushing (7 in the placebo group; 4 in the 90 mg and 6 in the 270 mg group). The Panel considers that no safety concerns can be deduced from this study.

One MS expressed concerns that the flushing reported in this study may represent typical reactions to an overdose reported for nicotinic acid. The Panel notes that a tolerable upper intake level (UL) of 10 mg nicotinic acid was established by the Scientific Committee on Food (SCF, 2002) on the basis of reports of occasional flushing at 30 mg/day and by applying an uncertainty factor of 3 to allow for the fact that a slight effect was reported. However, the Panel also notes that the SCF established an UL of 900 mg for nicotinamide (SCF, 2002). Considering the kinetics of nicotinamide and of 1-MNA, the Panel considers it unlikely that 58 mg of 1-MNA would cause flushing symptoms.

3.9. Allergenicity

Taking into account the production process and the nature of the NF, the Panel considers it unlikely that the NF exhibits allergenic properties.

4. Discussion

The NF ingredient is a synthetic organic compound, classified as a pure chemical compound from a non-GM source (Class 1.1). 1-MNA is naturally present in the human body as a normal downstream product of niacin metabolism. The applicant intends to use 1-MNA only in food supplements (58 mg/day), in the form of powder, in several different final formats. 1-MNA is intended to be used by the general population, excluding children, pregnant women and breastfeeding mothers.

Information provided on the production process, composition, specifications, batch-to-batch variability and stability of the NF is sufficient and does not raise concerns about the safety of the NF. The results of bacterial reverse mutation test and *in vitro* mammalian cell MN test indicated that 1-MNA is not genotoxic. The metabolic pathway of 1-MNA was demonstrated by four studies, provided by the applicant, mainly in the context of niacin metabolism. Dose-dependent levels for 1-MNA and one of its metabolites were indicated by three rat studies.

The Panel concludes that there is no concern with respect to genotoxicity of 1-MNA.

In a subchronic rat study, epithelium degeneration of the non-glandular stomach was observed at all dose levels with increasing frequency. Since the human stomach does not have non-glandular epithelium, the Panel considers that this finding is toxicologically not relevant for humans. At doses of 500 and 1,000 mg/kg bw, changes of the urine pH, maintained in the recovery period, were reported. As adversity of this finding cannot be ruled out, the Panel selected 250 mg/kg bw in this rat study as the reference point. The Margin of Exposure to humans weighing 70 kg and consuming daily 58 mg would be about 300.

One MS expressed concerns that the intake of 1-MNA would add to endogenously formed 1-MNA from niacin metabolism. The Panel notes the upper level for nicotinamide, i.e. 900 mg/day for adults (SCF, 2002). Taking into account that 1-MNA is a main metabolite from nicotinamide, the Panel considers it unlikely that an intake of 58 mg 1-MNA from food supplements in addition to the niacin intake (EFSA NDA Panel, 2014) from other sources would result in adverse health outcomes in humans.

5. Conclusions

The Panel concludes that the NF, 1-methylnicotinamide chloride, is safe under the proposed uses and use levels.

Documentation provided to EFSA

- 1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of 1-methylnicotinamide chloride (1-MNA) as a novel food ingredient. Ref. Ares(2016)41677369, dated 5 August 2016.
- 2) On 11 August 2016, EFSA received the following documentation: mandate and technical dossier, which was submitted by Pharmena S.A.; initial assessment report carried out by the Food Safety Authority of United Kingdom, Member States' comments and objections; response by the applicant to the initial assessment report and Member States comments.
- 3) Upon a request by EFSA for missing information, on 25 October 2016, EFSA received the missing information as submitted by the applicant. On 22 March, 8 June and 29 June 2017, EFSA sent requests to the applicant to provide additional information to accompany the application. Additional data were provided by the applicant on 13 April, 13 June and 15 July 2017.

- 4) During its meeting on 19–21 September 2017, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of 1-methylnicotinamide chloride as a novel food pursuant to Regulation (EC) No 258/97.

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Abbreviations

1-MNA	1-methylnicotinamide chloride
2PY	N-methyl-2-pyridone-5-carboxamide
4PY	N-methyl-4-pyridone-3-carboxamide
AE	adverse event
AUC	area under the curve
cfu	colony forming unit
CAS	Chemical Abstracts Service
CBPI	Cytokinesis-Block Proliferation Index
FOB	functional observation battery

GM	genetically modified
HPLC	high-performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MN	micronucleus
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
OECD	Organisation for Economic Co-operation and Development
RI	Replication Index
SAE	severe adverse effects
SCF	Scientific Committee on Food
UL	tolerable upper intake level