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## Extension of use of nicotinamide riboside chloride as a novel food pursuant to Regulation (EU) 2015/2283

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### Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of an extension of use of the novel food (NF) nicotinamide riboside chloride (NRC) pursuant to Regulation (EU) 2015/2283. The assessment addresses the use of NRC in 'meal replacement products' and 'nutritional drink mixes' at levels up to 300 mg/day for the general population, and in food for special medical purposes (FSMP) and total diet replacement for weight control (TDRWC) (as per Regulation (EU) No 609/2013) at levels up to 500 mg/day in adults. Benchmark dose modelling was carried out on data from the 90-day oral toxicity studies in rats relevant to the safety assessment. Considering the lack of tolerable upper intake level (UL) for nicotinamide in infants and the narrow margin of exposure between the estimated intake in infants and the lower confidence bound of the benchmark doses (BMDL<sub>05</sub>) estimated by the models, the Panel concludes that the safety of the NF has not been established for use in 'meal replacement products' and 'nutritional drink mixes' under the proposed conditions of use. For FSMP and TDRWC, the proposed maximum use level corresponds to an intake of 210 mg nicotinamide per day, which is below the current UL for nicotinamide of 900 mg/day for adults. The Panel considers that the NF is as safe as pure nicotinamide for use in FSMP and TDRWC. The Panel, however, notes experimental data which indicate several pathways by which intakes of nicotinamide (or its precursors), at levels that are substantially higher than the physiological requirement, might cause adverse effects. The Panel considers that further investigations are required and that a re-evaluation of the UL for nicotinamide may be warranted.

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**Keywords:** nicotinamide, nicotinamide riboside chloride, niacin, novel food, nutrient source, extension of use

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## 1. Introduction

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

The European Union legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The relevant Union legislative measures are:

- Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods.<sup>1</sup>
- Directive 2002/46/EC of the European Parliament and of the Council lays down requirements on food supplements.<sup>2</sup>
- Regulation (EU) No 609/2013 of the European Parliament and of the Council on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control.<sup>3</sup>
- Regulation (EC) 1925/2006 on the addition of vitamins and mineral and of certain other substances to foods.<sup>4</sup>

Nicotinamide riboside chloride has been authorised for placing on the Union market as a novel food by Commission Implementing Regulation (EU) 2020/16 for use as a source of niacin in food supplements for the general adult population.<sup>5</sup> On 2 March 2020, the company ChromaDex Inc. submitted a request to the European Commission to change the conditions of use of the novel food nicotinamide riboside chloride within the meaning of Article 10(1) of Regulation (EU) 2015/2283.

The application requested to extend the use of nicotinamide riboside chloride in additional food categories as follows: food for special medical purposes as defined by Regulation (EU) No 609/2013; total diet replacement for weight control as defined by Regulation (EU) No 609/2013; meal replacement products; and nutritional drink mixes.

The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 29(l)(a) of Regulation (EC) No 178/2002<sup>6</sup>, the European Commission asks EFSA to provide a scientific opinion:

- by carrying out the assessment for an extension of use of nicotinamide riboside chloride as a novel food in accordance with Article 10(3) of Regulation (EU) 2015/2283;
- following the outcome of the novel food assessment, by evaluating the safety and bioavailability of nicotinamide riboside chloride when added for nutritional purposes as a source of niacin to food for special medical purposes, total diet replacement for weight control and food for the general population, in the context of Regulation (EU) No 609/2013 and Regulation (EC) No 1925/2006.

In addition, the European Food Safety Authority is requested to include in its scientific opinion a statement as to if, and if so to what extent, the proprietary data for which the applicant is requesting data protection was used in elaborating the opinion in line with the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283.

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<sup>1</sup> Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.

<sup>2</sup> Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements.

<sup>3</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009.

<sup>4</sup> Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods.

<sup>5</sup> Commission Implementing Regulation (EU) 2020/16 of 10 January 2020 authorising the placing on the market of nicotinamide riboside chloride as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470.

<sup>6</sup> 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.

## 1.2. Additional information

In 2019, the NDA Panel established the safety of nicotinamide riboside chloride as a novel food pursuant to Regulation (EU) 2015/2283 for use in food supplements for the adult population, and the bioavailability of nicotinamide from this source, in the context of Directive 2002/46/EC (EFSA NDA Panel, 2019). The European Commission authorised the placing on the market of nicotinamide riboside chloride on 10 January 2020.<sup>7</sup>

In 2002, the Scientific Committee on Food (SCF) published an opinion on the Tolerable Upper Intake level (UL) for niacin [nicotinic acid (NA) and nicotinamide (NAM)] (EFSA, 2006). An UL of 900 mg/day was established for NAM for adults, excluding pregnant and lactating women in view of the lack of data for these population groups.

In 2014, the NDA Panel published an opinion on dietary reference values for niacin (EFSA NDA Panel, 2014).

## 2. Data and methodologies

### 2.1. Data

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469<sup>8</sup>.

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

The assessment of the safety of the NF at the new proposed uses and use levels is based on the data provided by the applicant and the scientific opinion on the safety of nicotinamide riboside chloride (NRC) as a novel food pursuant to Regulation (EU) 2015/2283 and bioavailability of nicotinamide from this source, in the context of Directive 2002/46/EC (EFSA NDA Panel, 2019).

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application, by means of a literature search following a search strategy and standard operating procedure as described by the University of Chemistry and Technology of Prague (Dibusz and Vejvodova, 2020).

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise a human study evaluating the safety and dose-dependent effects of NRC supplementation in generally healthy adults aged  $\geq 55$  years (Maki et al., 2020).

### 2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by UCT Prague (Dibusz and Vejvodova, 2020).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

<sup>7</sup> Commission Implementing Regulation (EU) 2020/16 of 10 January 2020 authorising the placing on the market of nicotinamide riboside chloride as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470. OJ L7, 13.1.2020.

<sup>8</sup> Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

The evaluation of bioavailability of the nutrient niacin from the source nicotinamide riboside chloride was conducted in line with the principles contained in the 'Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources' (EFSA ANS Panel, 2018).

### 3. Assessment

#### 3.1. Introduction

The NF which is the subject of the application is nicotinamide riboside chloride, a synthetic form of nicotinamide riboside. The NF is proposed to be used as a source of niacin. Niacin is a generic term for nicotinic acid (NA) and nicotinamide (NAM), which are water-soluble organic compounds that belong to the group of B vitamins (EFSA NDA Panel, 2014).

The NF falls under the following category, as defined in Art. 3 of Regulation (EU) 2015/2283: ix) Vitamins, minerals and other substances used in accordance with Directive 2002/46/EC, Regulation (EC) No 1925/2006 and Regulation (EU) No 609/2013.

On 7 August 2019, the EFSA NDA Panel concluded that nicotinamide riboside chloride is safe to be used in food supplements for the healthy adult population, pursuant to Regulation (EU) 2015/2283. In the present application, the applicant seeks to extend the use of the NF to four food categories, i.e. foods for special medical purposes (FSMP) and total diet replacement products for weight control (TDRWC), as defined by Regulation (EU) No 609/2013, as well as 'meal replacement products' and 'nutritional drink mixes'. The target population for these products is the adult population, excluding pregnant and lactating women. The applicant indicates that the products are not intended to be consumed by children.

The NF under assessment is identical to the previously evaluated NF. There is no change regarding the production process and compositional data of the NF.

In its previous evaluation, the Panel concluded that the NF is likely to be absorbed mainly as NAM following hydrolysis in the gut based on the data on absorption, distribution, metabolism and elimination available in mice, rats, dogs and humans. If a fraction of the NF were absorbed intact, it would be expected to be rapidly metabolised to NAM in the blood. Upon absorption, the NF contributes to the NAM body pool, i.e. acts as a precursor of NAD<sup>+</sup> in cells and is primarily metabolised in the liver to 1-methylnicotinamide (1-MNM) through methylation and subsequently to N-methyl-2-pyridone-carboxamide and N-methyl-4-pyridone-carboxamide, following oxidation. These metabolites are then excreted in the urine. The Panel confirmed the bioavailability of nicotinamide, a form of niacin, from that source, in the context of Directive 2002/46/EC (EFSA NDA Panel, 2019).

No concerns regarding genotoxicity of the NF were identified by the Panel in its previous evaluation, considering available genotoxicity studies (Appendix A) and the nature of the NF.

A no observed adverse effect level (NOAEL) of 300 mg/kg bw per day was derived from the available repeated dose toxicity studies with rats and dogs conducted with the NF. Reproductive and developmental toxicity studies in rats were also provided, from which the Panel derived an NOAEL for fertility and reproductive performance of 675 mg/kg bw per day in males and 1,088 mg/kg bw per day in females and an NOAEL for maternal and embryo/fetotoxicity of 325 mg/kg bw per day (Appendix A).

One single-dose pharmacokinetic study and four clinical trials conducted in healthy adult subjects (NRC doses from 100 mg for 1 day up to 2,000 mg/day for up to 12 weeks) were provided. Findings from these studies did not raise safety concerns.

The proposed maximum use level in food supplements was 300 mg/day (i.e. 4.3 mg/kg bw in a 70-kg adult). In the light of the human data available on nicotinamide riboside chloride and nicotinamide, the Panel considered that the margin of exposure (MoE) of 70 was sufficient for the adult population, excluding pregnant and lactating women. An NOAEL of 325 mg/kg bw per day for maternal and embryo/fetotoxicity was identified from reproductive and developmental toxicity studies in rats. In the absence of data which could justify accepting an MoE lower than 100 for pregnant and lactating women, the Panel concluded that an intake of 230 mg/day of the NF was safe for these two population groups. The European Commission authorised the placing on the market of nicotinamide riboside chloride on 10 January 2020.<sup>7</sup>

#### 3.2. Specifications

The specifications proposed by the applicant are indicated in Table 1. In addition to the specifications currently authorised as per the Union List,<sup>7</sup> the specifications include maximum levels for

mercury, cadmium and lead. No maximum levels for heavy metals are established for the food categories which are the subject of this application (Commission Regulation (EC) No 1881/2006<sup>9</sup>).

**Table 1:** Specifications of the NF

Parameter	Specification	Method of analysis
<b>Description: The novel food is a synthetic form of nicotinamide riboside. The novel food contains <math>\geq</math> 90% nicotinamide riboside chloride, predominantly in its <math>\beta</math> form, the remaining components being residual solvents, reaction by-products and degradation products.</b>		
Nicotinamide riboside chloride:		
CAS number: <b>23111-00-4</b>		
EC number: <b>807-820-5</b>		
IUPAC name: <b>1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyridin-1-ium-3-carboxamide;chloride</b>		
Chemical formula: <b>C<sub>11</sub>H<sub>15</sub>N<sub>2</sub>O<sub>5</sub>Cl</b>		
Molecular weight: <b>290.7 g/mol</b>		
<b>Colour</b>	White to Light Brown	Visual
<b>Form</b>	Powder	Visual
<b>Identification</b>	Conforms by NMR	NMR
<b>Nicotinamide riboside chloride</b>	$\geq$ 90 wt %	HPLC-UV*
<b>Water content</b>	$\leq$ 2.0%	Karl Fischer Titration (USP < 921 >)*
<b>Residual solvents</b>		
<b>Acetone</b>	$\leq$ 5,000 mg/kg	GC Headspace (USP < 467 >)
<b>Methanol</b>	$\leq$ 1,000 mg/kg	GC Headspace (USP < 467 >)
<b>Acetonitrile</b>	$\leq$ 50 mg/kg	GC Headspace (USP < 467 >)
<b>Methyl tert-butyl ether</b>	$\leq$ 500 mg/kg	GC Headspace (USP < 467 >)
<b>Reaction by-products</b>		
<b>Methyl acetate</b>	$\leq$ 1,000 mg/kg	GC Headspace (USP < 467 >)
<b>Acetamide</b>	$\leq$ 27 mg/kg	GC-FID*
<b>Acetic acid</b>	$\leq$ 5,000 mg/kg	GC-FID*
<b>Heavy metals</b>		
<b>Arsenic</b>	$\leq$ 1 mg/kg	ICP-MS (USP < 232 >, < 233 >, < 2232 >)
<b>Mercury</b>	$\leq$ 0.1 mg/kg	ICP-MS (USP < 232 >, < 233 >, < 2232 >)
<b>Cadmium</b>	$\leq$ 1 mg/kg	ICP-MS (USP < 232 >, < 233 >, < 2232 >)
<b>Lead</b>	$\leq$ 0.5 mg/kg	ICP-MS (USP < 232 >, < 233 >, < 2232 >)
<b>Microbiological limits</b>		
<b>Total plate count</b>	$\leq$ 1000 CFU/g	AOAC or equivalent
<b>Yeast and mould</b>	$\leq$ 100 CFU/g	AOAC or equivalent
<b><i>Escherichia coli</i></b>	Absent/10 g	AOAC or equivalent

AOAC: Association of Analytical Communities; CFU: colony forming units; GC: gas chromatography; GC-FID: gas chromatography coupled with a flame ionisation detector; HPLC-UV: high-performance liquid chromatography-ultraviolet spectroscopy; ICP-MS: inductively coupled plasma mass spectrometry; NMR: nuclear magnetic resonance; USP: United States Pharmacopeia.

\*: In-house validated analytical methods.

Although the average nicotinamide riboside chloride purity of the NF is approximately 96% at the time of production, a specification of not less than 90% has been set to account for the degradation of nicotinamide riboside chloride over the course of shelf-life. Specifications have also been set to control the amounts of residual solvents, reaction by-products and heavy metals. Forced degradation studies indicate that during shelf-life, NF-containing products will accumulate small amounts of NAM, ribose and chloride.

The Panel previously concluded that the information provided on the specifications of the NF was sufficient and did not raise safety concerns (EFSA NDA Panel, 2019).

<sup>9</sup> Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

### 3.3. History of use of the NF and/or of its source

The NF has a generally recognised as safe (GRAS) status in the USA since 2016 for addition to vitamin waters, protein shakes, nutrition bars, gum and chews, as a source of niacin.<sup>10</sup> The intended maximum use level is 0.027% by weight. It was also filed to the U.S. Food and Drug Administration as a new dietary ingredient (NDI) for use in dietary supplements in 2015 (daily dose: 180 mg), without objection<sup>11</sup>; the NDI status was updated in 2017 with new proposed intake level (daily dose 300 mg) and product specifications.<sup>12</sup>

In 2018, the NF was included in the Licensed Natural Health Products Database (LNHPD) by Health Canada.<sup>13</sup>

The NF is authorised for use in food supplements on the EU market as of January 2020.<sup>7</sup>

### 3.4. Proposed uses and use levels and anticipated intake

#### 3.4.1. Proposed uses, use levels and target populations

The applicant applies for an extension of authorised conditions of use for the NF to the four food categories described below.

##### 3.4.1.1. Food for special medical purposes (FSMP) as defined by Regulation (EU) No 609/2013

The applicant intends to use the NF as a source of niacin in FSMP, as defined in Article 2.2(g) of Regulation (EU) No 609/2013<sup>14</sup> and further regulated by Commission delegated Regulation (EU) No 2016/128.<sup>15</sup> The proposed maximum use level in FSMP is 500 mg of the NF per day. The applicant indicates that the extension of use covers products for adults only. Children, pregnant and lactating women are excluded.

Commission delegated Regulation (EU) 2016/128<sup>16</sup> stipulates a limit of maximum 3 mg Niacin Equivalent (NE) per 100 kcal in products defined as per Art 2(1)(a), i.e. 'nutritionally complete food with a standard nutrient formulation which, used in accordance with the manufacturer's instructions, may constitute the sole source of nourishment for the persons for whom it is intended'. Products defined as per Art 2(1)(b), i.e. 'nutritionally complete food with a nutrient-adapted formulation specific for a disease, disorder or medical condition which, used in accordance with the manufacturer's instructions, may constitute the sole source of nourishment for the persons for whom it is intended' and Art 2(1)(c), i.e. 'nutritionally incomplete food with a standard formulation or a nutrient-adapted formulation specific for a disease, disorder or medical condition which is not suitable to be used as the sole source of nourishment' shall comply with the maximum amounts of vitamins and mineral substances as specified in Annex I, Part B of Regulation (EU) 2016/128, without prejudice to modifications for one or more of these nutrients rendered necessary by the intended use of the product.

<sup>10</sup> GRAS No 635, available at <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=635>

<sup>11</sup> NDIN 882, available at <https://www.regulations.gov/document?D=FDA-2015-S-0023-0087>

<sup>12</sup> NDIN 1062, available at <https://www.regulations.gov/document?D=FDA-2018-S-0023-0032>

<sup>13</sup> NPN 80088977, available at <https://health-products.canada.ca/lnhpd-bdpsnh/info.do?licence=80088977>

<sup>14</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013.

<sup>15</sup> Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016.

<sup>16</sup> Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes [<https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32016R0128>].



### 3.4.1.2. Total Diet Replacement for Weight Control (TDRWC) as defined by Regulation (EU) No 609/2013

The applicant intends to use the NF in TDRWC, as defined in Article 2.2(h) of Regulation (EU) No 609/2013<sup>17</sup> and further regulated by Commission delegated Regulation (EU) No 2017/1798<sup>18</sup>. The proposed maximum use level in TDRWC is 500 mg of the NF per day. As defined by Regulation (EU) No 609/2013, TDRWC are intended for healthy overweight or obese adults who intend to achieve weight reduction.

### 3.4.1.3. 'Meal replacement products' and 'nutritional drink mixes'

The applicant intends to use the NF as an ingredient in 'meal replacement products' and 'nutritional drink mixes' at a proposed maximum use level of 300 mg/day.

The target population proposed by the applicant is adults only, excluding children, pregnant and lactating women. However, as the NF is intended to be used as an ingredient in standard food categories, it cannot be excluded that the NF would also be consumed by other groups of the population. Therefore, the safety data and the exposure assessment shall cover all population groups (Commission Implementing Regulation (EU) 2017/2469, article 5(6)<sup>19</sup>).

The applicant indicates that 'meal replacement products' are foods presented as a replacement for one or more meals of the daily diet. The applicant notes that indication as meal replacement for weight control is subject to the conditions of use of two health claims authorised under the provisions of Regulation (EC) No 1924/2006<sup>20</sup>.

The applicant indicates that 'nutritional drink mixes' are intended primarily as nutritional supplement to the daily diet of elderly people.

### 3.4.2. Anticipated intake of the NF

On the basis of the proposed uses and use levels (Sections 3.4.1), the Panel considers the following maximum anticipated intake of the NF for the safety evaluation:

- 300 mg/day from meal replacement products or nutritional drink mixes for the general population, including infants, children, pregnant and lactating women.
- 500 mg/day from FSMP or TDRWC for adult consumers of these products, excluding pregnant and lactating women.

Estimated maximum intake of nicotinamide riboside chloride in children, relative to body weight (bw), is presented in Table 2.

**Table 2:** Estimated maximum intake of nicotinamide riboside chloride in children, relative to body weight, from meal replacement products or nutritional drink mixes

	Mean body weight <sup>(a)</sup> (kg)	Nicotinamide riboside chloride intake <sup>(b)</sup> (mg/kg bw per day)
<b>Infants, &lt; 1 year</b>	5	60
<b>Toddlers, 1–2 years</b>	12	25
<b>Other children, 3–9 years</b>	23	13
<b>Adolescents, 10–13 years</b>	43	7
<b>Adolescents, 14–18 years</b>	61	5

<sup>17</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013.

<sup>18</sup> Commission Delegated Regulation (EU) 2017/1798 of 2 June 2017 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for total diet replacement for weight control. OJ L 259, 7.10.2017.

<sup>19</sup> Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. C/2017/8874. OJ L 351, 30.12.2017, p. 64–71.

<sup>20</sup> Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

- (a): Mean body weight of children (males and females) for each age group reported in the Scientific Committee Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA Scientific Committee, 2012). EFSA Journal 2012;10(3):2579.
- (b): Considering a maximum intake of 300 mg/day.

### 3.4.3. Combined intake from the NF and other sources

The Panel previously concluded that the contribution of nicotinamide riboside from food sources other than the NF is too small to be relevant for the safety assessment (EFSA NDA Panel, 2019).

Doses of 300 and 500 mg/day of the NF would deliver 126 mg and 210 mg nicotinamide per day, respectively, under the assumption that the NF is fully metabolised (see Section 3.5).

Mean intakes of niacin from the background diet were estimated to range from 42.2 to 50.1 mg niacin equivalents<sup>21</sup> (NE) per day in adult men and 27.5–35.5 mg NE/day in adult women, across EU countries (EFSA NDA Panel, 2014). Estimates of 95th percentile intakes were up to 78.2 mg NE/day in adult men. Mean total niacin intakes ranged from 8 to 11 mg NE/day in infants, from 12 to 20 mg NE/day in toddlers (1–2 years), from 14 to 32 mg NE/day in other children aged 3–9 years and from 25 to 41 mg NE/day in adolescents aged 10–17 years. Estimates of 95th percentile intakes were up to 24 mg NE/day in male infants and 34 mg NE/day in male toddlers. These estimates were calculated considering the food contents of preformed niacin (i.e. NAM and NA) as well as of tryptophan (i.e. tryptophan content divided by a factor of 60).

### 3.4.4. Estimate of exposure to undesirable substances

The applicant provided estimates of the maximum exposure to potential degradants of NRC, i.e. furfural, ribose and chloride, based on the results of stability studies (Table 3).

**Table 3:** Estimated exposure to NRC's degradants

Substance	Conditions	Exposure estimate (mg/day)	
		For 300 mg of the NF	For 500 mg of the NF
Furfural	Assuming 0.16% w/w after 24 months storage (25°C/60% RH)	0.5	0.8
	Assuming 0.32% w/w after storage in accelerated conditions (worst case scenario)	1.9	3.2
	Assuming 7% <sup>(a)</sup> of NRC degraded into equimolar amounts of free nicotinamide, ribose and chloride	10.5	17.5
Chloride	Assuming 7% <sup>(a)</sup> of NRC degraded into equimolar amounts of free nicotinamide, ribose and chloride	2.1	3.5

(a): Considering that the product may contain 90% NRC, 2% water, 1.25% residual solvents and reaction by-products, as per its specifications.

For the proposed maximum intake of the NF of 500 mg/day, maximum intake estimates of 3.2 mg furfural per day and 17.5 mg ribose per day were estimated. These estimates are below the acceptable daily intake (ADI) for furfural of 0.5 mg/kg body weight/day (EFSA, 2004) and the maximum level of ribose of 36 mg/kg bw per day considered as safe (EFSA NDA Panel, 2018). The consumption of the NF under the proposed use levels does not contribute significantly to the overall exposure to chloride through the diet.

The Panel identifies no concern from the information provided on the exposure to undesirable substances under the new proposed conditions of use.

## 3.5. Absorption, distribution, metabolism and excretion (ADME)

A new human study, which assessed metabolic effects of NRC supplementation, was submitted as part of the present application (Remie et al., 2020) (see also Section 3.7.5). In a randomised, double-blind, crossover design, 13 healthy overweight or obese participants (seven women; six men) received

<sup>21</sup> Values relate to niacin in the form of nicotinamide and nicotinic acid. Niacin can be synthesised in the human body from the indispensable amino acid tryptophan. 1 mg niacin equivalent (NE) = 1 mg nicotinamide = 1 mg nicotinic acid = 60 mg dietary tryptophan.

a placebo or the NF at a dose of 1,000 mg/day for two periods of 6 weeks separated by 4–7 weeks of wash out. Skeletal muscle biopsies were taken at the end of each period. NAD<sup>+</sup> was amplified and quantified by a cycling assay involving alcohol dehydrogenase and malic acid (Kato et al., 1973) and related metabolites were quantified by mass spectrometry. No effect was observed on the concentration of NAD<sup>+</sup> of skeletal muscle (n = 8). Compared to the control period, significant increases in the concentration of nicotinic acid adenine dinucleotide (NAAD), a precursor of NAD<sup>+</sup> (+677 ± 155%, p < 0.01, n = 12) and of 1-MNM (+299 ± 62%, p < 0.01, n = 12), were observed at the end of the supplementation period. The concentrations of NADH, NADP, NADPH, nicotinamide adenosine mononucleotide and nicotinamide mononucleotide in muscle samples were not affected. Plasma levels of these metabolites were not measured. The Panel notes that, in contrast to the increase in plasma concentration of NAD<sup>+</sup> observed in human studies with daily supplementation of NRC (Airhart et al., 2017; Conze et al., 2019; EFSA NDA Panel, 2019), no effect was found on the NAD<sup>+</sup> concentration of muscle cells in this experiment in a small group of overweight and obese adults at a daily dose of 1,000 mg NRC for 6 weeks.

### 3.6. Nutritional information

No new information has been submitted by the applicant.

The Panel notes that, at the maximum use level of 300 and 500 mg/day in the respective food categories, the NF would deliver 126 and 210 mg NAM/day, respectively, under the assumption that nicotinamide riboside chloride is fully metabolised to NAM. This largely exceeds the physiological requirement for niacin (population reference intake (PRI) = 1.6 mg NE/MJ per day<sup>22</sup>) for all population groups (EFSA NDA Panel, 2014).

In 2002, the SCF established an UL for NAM of 900 mg/day (12.5 mg/kg bw per day) for the adult population, excluding pregnant and lactating women (EFSA, 2006). ULs for toddlers, children and adolescents were extrapolated from the UL for adults based on body weight (Table 4).

**Table 4:** Tolerable upper intake level for nicotinamide

Age (years)	UL for nicotinamide (mg per day)
1–3	150
4–6	220
7–10	350
11–14	500
15–17	700
18+ (excluding pregnant and lactating women)	900

Source: EFSA (2006).

The Panel notes that 300 mg NRC per day would deliver 126 mg nicotinamide per day, which is close to the UL for nicotinamide in toddlers of 150 mg/day established by the SCF (EFSA, 2006). The Panel also notes that no UL for nicotinamide has been established for infants (< 1 year of age).

### 3.7. Toxicological information

Toxicological studies on the NF evaluated as part of the previous evaluation are summarised in Appendix A (EFSA NDA Panel, 2019).

#### 3.7.1. Genotoxicity

No new data were submitted.

#### 3.7.2. Acute and subacute toxicity studies

No new data were submitted.

<sup>22</sup> Niacin requirement is related to energy requirement and therefore expressed in mg NE/MJ. PRIs for niacin expressed in mg NE/day can be calculated based on the energy requirement of the population group considered, depending on the age, sex and physical activity level. See Appendices G, H, I and J of NDA Panel, 2014; available at: <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3759>

### 3.7.3. Subchronic toxicity

In the previous assessment of the NF (EFSA NDA Panel, 2019), the applicant provided a 90-day repeated dose toxicity study in the rat [Study No. 14022, unpublished, good laboratory practice (GLP), OECD TG 408; Bhoite et al., 2015; Conze et al., 2016]. Briefly, the NF was administered by oral gavage at doses of 0, 300, 1,000 and 3,000 mg/kg bw per day to both male and female Sprague Dawley rats (10/sex per group) (Appendix A). One additional group was given NAM at 1,260 mg/kg bw per day (equimolar to 3,000 mg/kg bw per day of the NF). Lower body weights were noted in all male rats that received the test items compared to the control. At the time, the Panel noted that the effects were related to reduced food consumption and not very pronounced in the low and mid dose and were not considered as being adverse. Substance-related effects that were considered adverse by the Panel involved haematology endpoints, liver, kidneys, genital organs and the hormonal system. Effects were observed at 1,000 and 3,000 mg/kg bw per day, with a steep dose–response relationship. Histopathological examination also revealed adverse effects in several organs at 3,000 mg/kg bw per day. Notably, the same effects were observed in the group which received a dose of NAM of 1,260 mg/kg bw. An NOAEL of 300 mg/kg bw per day was identified from this study.

As part of the request for extension of uses, the applicant provided an additional GLP-, OECD-compliant 90-day subchronic rat toxicity study to the Panel on NRC produced by another company (Marinescu et al., 2020). The test substance used in the study is described as 'a synthetic, nature-identical nicotinamide riboside chloride (CAS #23111-00-4) designated as NR-E manufactured using a proprietary process under the guidelines of good manufacturing practice (GMP) 21 CFR 111/210 (C.F.R. § 111 (2007), C.F.R. § 210 (1978))'. The test substance was not available to the applicant. To ascertain the nature of the test substance, the applicant compared the NF with NRC produced by the other company in its commercially available form, which is a combination with pterostilbene. The commercial combination was analysed by high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). HPLC chromatograms were similar, although the impurities present in traces were not identified. The molecular identity of NRC was confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR and by  $^1\text{H}$ - $^1\text{H}$  COSY (correlated spectroscopy) two-dimensional NMR. Both products are crystalline and of high purity. The Panel considers that the study from Marinescu et al. (2020) is relevant to the safety assessment of the NF.

In this study (Marinescu et al., 2020), Sprague Dawley rats were exposed to 0, 300, 500 or 1,200 mg/kg bw per day of synthetic NRC by daily oral gavage (10 per sex for each dose group) for 90 days, followed by a 28-day recovery for five rats per sex for each dose group.

There was no mortality in the study. Both males and females displayed a treatment-related decrease in body weight gain. The mean body weight was lower (statistically significant) in males from the high-dose group on day 92 (–13%). The body weight difference persisted during the recovery period (not statistically significant). Decreases in food consumption and food efficiency were also observed in the high dose-treated male rats (not statistically significant).

A dose-dependent increase in mean relative brain, liver, kidney, adrenal and testis weights was reported for males on day 94 (statistically significant in the high-dose group for brain, liver, kidneys and adrenals). After recovery, a dose-dependent increase in mean relative testicular weight was reported (statistically significant in the high-dose group), while mean relative heart and kidney weights displayed a statistically significant increase in the high-dose group. In females, a dose-dependent increase in relative liver and kidney weights was observed (significant for liver in the high-dose group), as well as dose-dependent decreases in uterus and spleen weights. Trends were not maintained after recovery. Observations were consistent with those reported by Conze et al. (2016). The Panel, however, notes that the numerical values of the relative organ weights reported in the paper appear to be inflated, probably by a constant factor.

Clinical chemistry analyses revealed a decrease in plasma total cholesterol and triglyceride concentrations in all treated males, both before and after recovery, with statistical significance in the low- and high-dose groups as compared to controls. Although there was no dose response, due to the consistent occurrence in all treated males in combination with the increase in liver weight, the decrease in body weight gain and decreased food consumption and efficiency, the Panel considers these changes in blood lipids as treatment-related.

Haematological analysis revealed a decrease in white blood cell counts in all treated males reaching statistical significance after recovery in the high-dose group (–30.5%), as well as a dose-related decrease in lymphocyte counts after recovery (reaching –27.6% in the high-dose group;  $p < 0.05$ ). In all treated females, before recovery, both neutrophil and monocyte counts were increased, with

stronger, statistically significant effects in the middle-dose group (62.6% and 65% increases, respectively;  $p < 0.05$ ). After recovery, neutrophil counts remained non-significantly increased in all dose groups. Considering the magnitude of the effects in the context of the large variability of the data and the absence of dose–response, the Panel considers these effects non-adverse.

Considering the decreased body weight and body weight gain in the high-dose male group as adverse effects of NRC, the authors identified an NOAEL of 500 mg/kg bw from the study.

The Panel considers that the study by Marinescu et al. (2020) shows similar findings to the study conducted with the NF (Bhoite et al., 2015; Conze et al., 2016), such as decreased body weights, increased relative liver and kidney weights and effects on the haematopoietic system. In light of this, the Panel reconsidered the validity of its conclusions regarding the adversity of the effect on body weight observed in the Conze study.

**Table 5:** Values for terminal body weight, relative kidney weight, relative liver weight from the 90-day toxicity studies in rats on NRC – absolute mean  $\pm$  SD (percent change)

<b>Conze et al. (2016)</b>					
<b>Dose (mg/kg bw per day)</b>		<b>0</b>	<b>300</b>	<b>1,000</b>	<b>3,000</b>
<b>Males</b>	<b>Terminal body weight (g)</b>	395.70 $\pm$ 18.36	363.56 $\pm$ 23.22 (–8%) <sup>(a)</sup>	354.22 $\pm$ 21.86 (–10%) <sup>(a)</sup>	317.21 $\pm$ 25.80 (–20%) <sup>(a)</sup>
	<b>Relative kidney weight</b>	0.715 $\pm$ 0.047	0.701 $\pm$ 0.033 (–2%)	0.777 $\pm$ 0.020 (+9%) <sup>(a)</sup>	0.876 $\pm$ 0.063 (+23%) <sup>(a)</sup>
	<b>Relative liver weight</b>	2.958 $\pm$ 0.143	3.013 $\pm$ 0.163 (+2%)	3.200 $\pm$ 0.180 (+8%) <sup>(a)</sup>	3.600 $\pm$ 0.272 (+22%) <sup>(a)</sup>
<b>Females</b>	<b>Terminal body weight (g)</b>	232.29 $\pm$ 8.10	234.43 $\pm$ 23.28 (+1%)	219.51 $\pm$ 9.92 (–6%)	216.19 $\pm$ 14.75 (–7%)
	<b>Relative kidney weight</b>	0.676 $\pm$ 0.053	0.645 $\pm$ 0.060 (–5%)	0.678 $\pm$ 0.058 (–)	0.822 $\pm$ 0.044 (+22%) <sup>(a)</sup>
	<b>Relative liver weight</b>	2.902 $\pm$ 0.191	3.003 $\pm$ 0.327 (+3%)	3.295 $\pm$ 0.181 (+14%) <sup>(a)</sup>	4.046 $\pm$ 0.174 (+39%) <sup>(a)</sup>
<b>Marinescu et al. (2020)</b>					
<b>Dose (mg/kg bw per day)</b>		<b>0</b>	<b>300</b>	<b>500</b>	<b>1,200</b>
<b>Males</b>	<b>Terminal body weight (g)</b>	534.3 $\pm$ 72.4	518.6 $\pm$ 39.2 (–3%)	506.1 $\pm$ 39.4 (–5%)	463.6 $\pm$ 40.3 (–13%) <sup>(a)</sup>
<b>Females</b>	<b>Terminal body weight (g)</b>	288.3 $\pm$ 28.8	274.9 $\pm$ 29.5 (–5%)	270.5 $\pm$ 29.1 (–6%)	275.4 $\pm$ 35.7 (–4%)

(a): Statistically significant change compared to control group.

The applicant conducted a benchmark dose (BMD) modelling based on combined data from the two above-described 90-day subchronic toxicity studies (Conze et al., 2016; Marinescu et al., 2020), using male body weight as an endpoint. The Panel notes the difference in body weights of the rats involved (i.e. 119–135 g vs. 222–293 g at study start for the respective studies), in spite of the fact that rats of the same strain (Sprague Dawley) and age (i.e. 6–8 weeks at study start) were used. Because of such heterogeneity, the Panel considers that the two data sets cannot be combined and modelled together, and that the BMD modelling provided by the applicant cannot be used to identify a reference point for establishing a safe level of intake for the NF.

EFSA carried out BMD analyses of terminal body weight for the two studies separately, following the EFSA Guidance on the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2017). Upon visual evaluation of the consistency of the trends observed in both sexes in the respective studies (Table 5), the Panel decided to model the data for males and females combined for the study by Conze et al. (2016), and to model the data for males only for the study by Marinescu et al. (2020). In the latter, there was no indication of a dose-dependent effect in female rats on this endpoint.

The modelling was also applied to the changes in kidney-to-body weight and liver-to-body weight observed in male and female rats in the study by Conze et al. (2016). These data sets were selected based on the dose-dependent effects observed on these endpoints in both sexes (Table 5). Because of the inaccuracies in the values for relative organ weights reported in the study by Marinescu et al. (2020), they could not be used for BMD modelling.

A benchmark response (BMR) of 5% was used for all endpoints and model averaging was applied. Results are reported in Table 6 and Appendix D.

**Table 6:** Results of BMD modelling on body weight, relative liver weight and relative kidney weight

Reference	Sex	Body weight	Relative liver weight	Relative kidney weight
		BMDL <sub>05</sub> –BMDU <sub>05</sub>	BMDL <sub>05</sub> –BMDU <sub>05</sub>	BMDL <sub>05</sub> –BMDU <sub>05</sub>
Conze et al. (2016)	Males and females	55–563	226–721	425–1040
Marinescu et al. (2020)	Males <sup>(a)</sup>	104–1110	–	–

BMDL<sub>05</sub>: benchmark dose 95% one-sided lower confidence limit using a benchmark response of 5%; BMDU<sub>05</sub>: benchmark dose 95% one-sided upper confidence limit using a benchmark response of 5%.

(a): There is no dose-dependent effect on body weight in female rats in the study.

The 95% one-sided lower confidence limits of the benchmark dose (BMDL<sub>05</sub>) for body weight were 55 and 104 mg/kg bw per day based on the study by Conze et al. (2016) and Marinescu et al. (2020), respectively. The BMDL<sub>05</sub> for relative liver weight and relative kidney weight were 226 and 425 mg/kg bw per day, respectively, based on the study by Conze et al. (2016).

The Panel notes that BMD modelling of terminal body weight provides the lowest BMDL<sub>05</sub> (55 mg/kg bw based on the data from the study by Conze et al.), but results in wide confidence intervals (BMDU/BMDL ratios > 10) for both studies, indicating high uncertainty. In addition, the central estimate of the BMD of 190 mg/kg bw per day based on the data set from Conze et al. is below the lowest dose of 300 mg/kg bw per day tested in that study (Appendix D). Extrapolation below the lowest dose implies additional uncertainty.

The Panel also notes that, among the two other endpoints, the lowest BMDL<sub>05</sub>, which was obtained for relative liver weight based on Conze et al. (226 mg/kg bw per day), showed a narrower confidence interval (BMDU/BMDL ratio 3.2) compared to the endpoint bw.

### 3.7.4. Reproductive and developmental toxicity

No new data were submitted.

### 3.7.5. Human data

In its previous submission, the applicant provided one single dose pharmacokinetic study (Wilson, 2015; Trammell et al., 2016) and four clinical trials (Airhart et al., 2017; Martens, 2017; Dollerup et al., 2018; Martens et al., 2018; Schacter, 2018; Conze et al.,) in which safety-related parameters following the consumption of the NF were addressed. These studies were conducted with healthy, adult human subjects and doses of the NF from 100 mg for 1 day up to 2,000 mg/day for 12 weeks. The Panel noted that the changes in haematology and clinical chemistry reported in these studies remained within reference ranges and that no dose-dependent adverse effects in the safety parameter examined were observed (EFSA NDA Panel, 2019).

As part of the present evaluation, the applicant provided the results of a post hoc analysis of the study by Conze et al. (2019) and Schacter (2018) to determine if 8 weeks of NRC supplementation at doses of 100, 300 and 1,000 mg/day affected circulating levels of pro- and anti-inflammatory cytokines (IL6, INF $\gamma$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, IL-18, TNF $\alpha$ , hs-CRP). The trial involved 140 healthy men and women (n = 30 per dose group) aged 40–60 years and with BMI 25–30.1 kg/m<sup>2</sup>. No significant changes were observed for any of the parameters.

Two additional human studies on NRC were provided by the applicant as part of the present evaluation (Maki et al., 2020; Remie et al., 2020). They are summarised in Table 7.

The applicant also provided a clinical trial on a combination of nicotinamide riboside and pterostilbene, a polyphenol found in blueberries (Dellinger et al., 2017). The Panel considers that the study cannot be used for the safety assessment of the NF as nicotinamide riboside was combined with another substance.

The Panel concludes that the available human studies on NRC do not raise safety concerns.

**Table 7:** Overview of human studies provided as part of the present request for extension of uses

Reference	Study design	Study population	Duration of study	Doses	Safety-related parameters investigated	Summary of results
Maki et al. (2020)	Randomised, double-blind, placebo controlled, 3-period crossover study	36 healthy adults (BMI of 18.5–34.99 kg/m <sup>2</sup> ) and score of $\geq 80$ on the Executive Function domain of the Central Nervous System Vital Signs (CNS VS) Test Battery Age $\geq 55$ years	Each subject received each of the two treatment or placebo twice daily for 8 weeks  No washout period was included; carryover not expected.	300 mg NRC 1,000 mg NRC Placebo	Anthropometric measures, haematology and clinical chemistry (CBC, Na, K, Cl, creatinine, BUN, AST, ALT, ALP, bilirubin, albumin, globulin, total protein, Ca, carbon dioxide, glucose and eGFR)  Resting BP and heart rate  Monitoring of AEs	<p><u>AEs classified as possibly related to the treatment</u> 1,000 mg NRC group (2 AEs): Difficulty sleeping, headaches (subject withdrawn) 300 mg NRC group: none Placebo group (3 AEs): Brittle nails, dry skin, elevated creatinine (subject withdrawn)</p> <p><u>Haematology and clinical chemistry</u> No significant between-group differences reported in haematology values (including platelet count) or serum chemistry values, except for a small difference for fasting serum glucose between 300 mg and 1,000 mg NRC and placebo [median (IQL) values in mg/dL: placebo, 91.5 (82.0, 102.5); 300 mg/day NR, 94.0 (86.0, 110.0); 1000 mg/day NR, 93.0 (89.0, 106.5)], which reached statistical significance between the placebo and high-dose group. Median fasting glucose concentrations were lower at the end of intervention than at baseline, in all groups and within the normal clinical reference range.</p> <p><u>Vital signs</u> No significant differences between treatments in heart rate, systolic and diastolic BP.</p>

Reference	Study design	Study population	Duration of study	Doses	Safety-related parameters investigated	Summary of results
Remie et al. (2020)	Randomised, double-blind, placebo-controlled crossover intervention study  The sample size was determined based on demonstrating the statistical superiority of NR on insulin-stimulated skeletal muscle glucose disposal compared with placebo	13 healthy overweight or obese participants (7♀; 6♂) Age: 59 ± 5 years BMI: 30.2 ± 2.6 kg/m <sup>2</sup>	6 weeks → 4–7 weeks washout period → 6 weeks of other treatment	Placebo or 1,000 mg/day NRC	Insulin sensitivity via hyperinsulinaemic–euglycaemic clamp; intrahepatic and intramuscular lipid content by MRS  PCr:ATP ratio by P-MRS, left ventricular ejection fraction, ambulatory BP  Plasma concentrations of glucose, FFAs, TG, cholesterol, HDL, inflammatory cytokine concentrations (n = 7 participants)  Monitoring of AEs	No AEs or side effects reported.  No effects on insulin sensitivity, hepatic and intramyocellular lipid content, measures of cardiac function, plasma markers of inflammation, glucose or lipids

AE: adverse event; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; BP: blood pressure; BMI: body mass index; BUN: blood urea nitrogen; Ca: calcium; CBC: complete blood count; Cl: chloride; eGFR: estimated glomerular filtration rate; FFAs: free fatty acids; HDL: high density lipoprotein; K: potassium; P-MRS: proton magnetic resonance spectroscopy; Na: sodium; NR: nicotinamide riboside chloride; PCr:ATP ratio: creatine phosphate:adenosine triphosphate ratio; RBC: red blood cell; TG: triglycerides; WBC: white blood cell.



### 3.7.6. Additional data

EFSA commissioned a literature search (LS) from the University of Chemistry and Technology of Prague (Dibusz and Vejvodova, 2020), with the aim of identifying studies investigating the effect of nicotinamide riboside chloride intake on health outcomes *in vivo* with a focus on hepatic function, haematology parameters, methyl balance and carcinogenicity. These outcomes were prioritised considering the animal toxicity data available on NRC (Section 3.7) and potential adverse effects of high-dose nicotinamide and its precursors discussed in the literature (Knip et al., 2000; Poljsak, 2016; Sun et al., 2017; Zhang et al., 2018; Demarest et al., 2019; Braidy and Liu, 2020; Hwang and Song, 2020).

In view of the metabolism of nicotinamide riboside chloride (Section 3.5), data on nicotinamide and 1-methyl nicotinamide were considered relevant for the risk assessment of nicotinamide riboside chloride. Thus, these compounds were also covered by the search.

Appendix B presents the details of the search strategy, number of hits, workflow and outcome of the LS. Several papers discussed the potential adverse effects of high intake of nicotinamide or its precursors in the light of current knowledge of nicotinamide metabolism. Several routes have been proposed by which high intake of nicotinamide could lead to adverse health effects, including: (i) through affecting methyl group transfers in a variety of metabolic pathways and epigenetic mechanisms; (ii) through modulating NAD<sup>+</sup> metabolism; (iii) through an elevated circulation and renal excretion of nicotinamide metabolites, 1-methyl nicotinamide, 2-PYR (1-methyl-2-pyridone-5-carboxamide), 4-PYR (1-methyl-4-pyridone-5-carboxamide) and the potential toxicity of these compounds. Mechanistic studies and related reviews on these topics are not described here.

No relevant additional data were retrieved through the LS in relation to potential adverse effects of nicotinamide or its precursors on hepatic function.

Cases of thrombocytopaenia have been reported in clinical trials in which supplemental doses of NAM were given to haemodialysis patients as a treatment for hyperphosphataemia. In a systematic review conducted by Zhang et al. (2018), seven randomised controlled trials on nicotinamide supplementation in haemodialysis patients were included (doses ranged between 400 and 1,500 mg NAM/day, for 8–24 weeks). A higher risk of thrombocytopaenia was reported in the supplemented groups compared to placebo (15 cases in the NAM groups, receiving 500 mg NAM/day or more, vs. five cases in the placebo groups) (Zhang et al., 2018). No serious adverse reactions were observed. The Panel notes that cases of thrombocytopenia were observed in patients undergoing haemodialysis treatment upon nicotinamide supplementation at doses of 500 mg/day and above. In an 8-day open-labelled pharmacokinetics study in which increasing doses of NRC were administered orally to eight healthy adults (from 250 mg on days 1 and 2 to 2,000 mg on days 7 and 8), a slight decrease in mean platelet count was observed (220,000/ $\mu$ L on day 1 vs. 200,000/ $\mu$ L on day 9,  $p = 0.031$ ) (Airhart et al., 2017). No effect on platelet counts was observed in the four placebo-controlled supplementation trials available on the NF (dose range: 100–1,000 mg/day NRC) (Martens, 2017; Dollerup et al., 2018; Martens et al., 2018; Schacter, 2018; Conze et al., 2019; EFSA NDA Panel, 2019; Maki et al., 2020).

As discussed in the previous assessment, experimental studies in animal models (Kazgan et al., 2014; Tian et al., 2014) and acute loading studies in humans (Sun et al., 2012; Tian et al., 2013; Sun et al., 2017) suggest that high NAM intake, that undergoes methylation-mediated degradation, could affect the methyl group pool balance. The Panel considered, in 2019, that no conclusions could be drawn on potential adverse effects from the data available (EFSA NDA Panel, 2019). No relevant additional data were retrieved through the LS.

The LS did not retrieve human epidemiological studies on the association between NAM intake and risk of cancer. A role of nicotinamide in carcinogenicity has been subject of numerous experiments in animals.

In one mouse carcinogenicity study, NAM was found not to be carcinogenic when administered to mice as 1% NAM solution (900 mg/kg bw<sup>23</sup>) in drinking water (corresponding to an average daily intake of 66.3 mg/day in female mice and 100.5 mg/day in male mice), during their lifespan (Toth, 1983).

An overview of animal experiments investigating the effect of NAM on chemical- and UVR-induced tumours is provided in Appendix C. Three experiments reported a promoting effect of NAM

<sup>23</sup> Applying a conversion factor of 0.09 to calculate doses in mg/kg bw per day from concentrations in drinking water in mg/L, for chronic studies in mice (EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. <https://doi.org/10.2903/j.efsa.2012.2579>).

administration on chemical-induced tumours (Rakieten et al., 1971; Schoental, 1977; Rosenberg et al., 1985). NAM was administered intraperitoneally in two of these studies and orally in one. NAM doses of 0.082% (41 mg/kg bw<sup>24</sup>) and 0.37% (183 mg/kg bw) administered in drinking water to rats for 20 months increased the incidence of diethylnitrosamine (DEN)-induced kidney tumours to 28% and 59%, respectively, compared to 5% in controls (DEN only). NAM by itself had no effect on tumour formation (Rosenberg et al., 1985). High doses of nicotinamide (350–500 mg/kg bw intraperitoneal, multiple dosing) inhibited DEN-induced liver tumours (34% reduction), but promoted DEN-induced kidney neoplasia (44% increase) in Wistar rats (Schoental, 1977). Intraperitoneal NAM (350 mg/kg) increased the incidence of streptozotocin-induced pancreatic islet cell tumours in F344 rats from 4% in controls (streptozotocin only) to 64% (Rakieten et al., 1971), but it decreased the incidence of renal adenomas from 77% to 18% (Rakieten et al., 1976).

In CBA mice inoculated with an immunogenic mouse sarcoma line (adenotype 12 virus, A12B3) or the sarcoma F line, NAM between 100 and 1,000 mg/kg bw administered i.p. caused a high level of *in vivo* DNA strand breaks in tumours and normal tissues in mice bearing the immunogenic sarcoma, but not in the non-immunogenic sarcoma F line. The DNA repair process was delayed in association with an accumulation of NAM and NAD. No effect was observed at a dose of 10 mg/kg bw (Olsson et al., 1996).

One study was retrieved regarding the NAD<sup>+</sup> precursor nicotinamide mononucleotide (NMN). In a pancreatic cancer mouse model, daily intraperitoneal injections of 500 mg/kg bw NMN administered to mice developing pancreatic intraepithelial neoplasia significantly decreased the proportion of normal acinar area in pancreas compared to controls, indicative of an increase in the amount of precancerous and cancerous lesions. This was accompanied by an increase in the amount of desmoplastic tissue in pancreas compared to controls (Nacarelli et al., 2019).

Overall, while the majority of animal studies report no or even protective effects of the administration of NAM or other NAD<sup>+</sup> precursors on cancer, some studies indicate that nicotinamide administration influences carcinogenesis in a dose-dependent and organ-specific manner, specifically in the presence of carcinogens and/or premalignant conditions.

The Panel notes that these studies were primarily designed to investigate the mechanisms of nicotinamide involved in various physiological and pathophysiological conditions and not to derive safe levels of intake with regard to potential risks for detrimental metabolic and epigenetic changes or tumour promotion. The Panel notes a lack of studies addressing these concerns. In particular, with regard to vulnerable population groups such as children, pregnant and lactating women and individuals with premalignant conditions, the available studies do not provide sufficient evidence to conclude on a safe high level of intake.

### 3.8. Allergenicity

The NF is a synthetic product containing > 90% nicotinamide riboside chloride. Potential process impurities have been well characterised. Since the NF does not contain any protein, the risk of allergenicity is low (EFSA NDA Panel, 2019).

## 4. Discussion

The evaluation addresses a request for an extension of use of nicotinamide riboside chloride. The NDA Panel previously concluded that nicotinamide riboside chloride is safe to be used in food supplements for the healthy adult population at doses up to 300 mg/day and for pregnant and lactating women at doses up to 230 mg/day (EFSA NDA Panel, 2019). The Panel confirmed the bioavailability of nicotinamide, a form of niacin, from that source. The Panel also noted the similar toxicity profiles of nicotinamide riboside chloride and nicotinamide demonstrated in a toxicity study in rats.

The applicant requests to use the NF in 'meal replacement products' and 'nutritional drink mixes' at a maximum use level of 300 mg of NRC per day, which corresponds to an intake of 126 mg nicotinamide per day. The applicant requested to exclude infants, children, pregnant or lactating women from the target population for the consumption of 'meal replacement products' and 'nutritional

<sup>24</sup> Applying a conversion factor of 0.05 to calculate doses in mg/kg bw per day from concentrations in drinking water in mg/L, for chronic studies in rats (EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. <https://doi.org/10.2903/j.efsa.2012.2579>).

drink mixes', but the Panel notes that such consumption cannot be excluded for these population groups. The applicant also requests to use the NF in foods for special medical purposes (FSMP) and total diet replacements products for weight control (TDRWC) at a maximum use level of 500 mg of NRC per day (i.e. 7.1 mg/kg bw in a 70-kg adult), which corresponds to an intake of 210 mg nicotinamide per day. The target population for these products are adults only, excluding pregnant and lactating women.

The Panel notes that the proposed use levels greatly exceed the physiological requirement for niacin for all population groups.

In the previous evaluation, an NOAEL of 300 mg/kg bw per day was identified from the available repeated dose toxicity studies with rats and dogs conducted with the NF. An NOAEL for maternal and embryo/fetotoxicity of 325 mg/kg bw per day was identified from an embryo-fetal developmental toxicity study on the NF.

One additional 90-day toxicity study in rats was provided (Marinescu et al., 2020) as part of the present application. The study was conducted with NRC produced by another company, which the Panel considers representative of the NF. The findings reported in this new study were similar to the findings in the 90-day toxicity study conducted with the NF (Bhoite et al., 2015; Conze et al., 2016).

The Panel applied BMD analyses to terminal body weight, relative kidney weight and relative liver weight of the study by Conze et al. (2016), where dose-dependent effects in male and female rats were observed. The Panel also applied a BMD analysis to terminal body weight of the study by Marinescu et al. (2020), where a dose-dependent effect in male rats was observed. The most sensitive adverse effect was a decrease in body weight, providing a BMDL<sub>05</sub> of 55 mg/kg bw, based on the data from the study by Conze et al. (2016). The Panel noted the high modelling uncertainty for this endpoint, and that the modelling uncertainty was lower for the increase in relative liver weight, providing a BMDL<sub>05</sub> of 226 mg/kg bw per day.

Regarding the request for extension of use in 'meal replacement products' and 'nutritional drink mixes', the Panel notes the lack of an UL for nicotinamide in infants. The Panel also notes that the MoE between the estimated intake of 60 mg NRC/kg bw per day in infants and the BMDL<sub>05</sub> of 55 and 226 mg/kg bw per day estimated for the selected endpoints in the animal toxicity studies would be < 4. In the absence of data that could be used to establish a safe level of intake of NRC in infants, the Panel considers that the safety of use of the NF in 'meal replacement products' and 'nutritional drink mixes' under the proposed conditions of uses is not established.

Regarding the request for extension of use in FSMP and TDRWC, the Panel notes the proposed maximum use level of 500 mg of NRC per day (7.1 mg/kg bw), corresponding to an intake of 210 mg nicotinamide per day, which is below the current UL for nicotinamide of 900 mg/day for adults. The Panel considers that, in the context of this UL, the NF can be considered as safe as pure nicotinamide, which is authorised for use in FSMP and TDRWC (Annex of Regulation (EC) No 609/2013<sup>25</sup>). The Panel notes that the MoE between the estimated intake, based on the proposed maximum use level, and the BMDL<sub>05</sub> of 55 and 226 mg/kg bw per day estimated for the selected endpoints in the animal toxicity studies are 8 and 32, respectively.

The Panel also notes that the scientific evidence on the toxicity of nicotinamide has increased since the SCF established the UL. Experimental data indicate several pathways by which intakes of nicotinamide that are substantially higher than the physiological requirement, or its precursors, might cause adverse effects. The Panel considers that further investigations are required to elucidate the effects of nicotinamide, or its precursors, at doses which are substantially higher than the physiological requirement for niacin, the results of which may necessitate a re-evaluation of the UL for nicotinamide.

## 5. Conclusions

The Panel concludes that the safety of the novel food, nicotinamide riboside chloride, has not been established for use in 'meal replacement products' and 'nutritional drink mixes'.

The Panel concludes that the NF, nicotinamide riboside chloride, is as safe as pure nicotinamide, for use in FSMP and TDRWC (Annex of Regulation (EC) No 609/2013<sup>25</sup>). The maximum use level of 500 mg of NRC per day corresponds to a maximum intake of 210 mg nicotinamide per day.

<sup>25</sup> Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

## 5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant: an *in vitro* study evaluating the metabolism of nicotinamide riboside in blood (Study No. 160312); an oral 7-day dose range finding toxicity study in juvenile dogs (study No. SN17-921); a 28-day repeated-dose oral toxicity study in juvenile dogs (Study No. SN17-940); a 90-day repeated-dose oral toxicity study in Sprague–Dawley rats (Study No. S14022); a reproductive toxicity study (Study No. G10959) and a developmental toxicity study (Study No. G10957) in rats.

### Steps taken by EFSA

- 1) On 8 June 2020, EFSA received a letter from the European Commission with the request for a scientific opinion on the extension of use of nicotinamide riboside chloride as NF (Ref. Ares (2020)2952737).
- 2) On 8 June 2020, a valid application on the extension of use of nicotinamide riboside chloride as NF, which was submitted by ChromaDex Inc., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1613) and the scientific evaluation procedure was initiated.
- 3) On 23 October 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 4) On 22 December 2020, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 5) On 18 January 2021, EFSA requested the applicant to provide further clarifications to the additional information provided.
- 6) On 09 February 2021, additional clarifications were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 7) On 12 February 2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
- 8) On 13 March 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
- 9) During its meeting on 14 September 2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of the extension of use of nicotinamide riboside chloride as a NF pursuant to Regulation (EU) 2015/2283.

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## Abbreviations

1-MNM	1-methylnicotinamide
2-PYR	1-methyl-2-pyridone-5-carboxamide
4-PYR	1-methyl-4-pyridone-5-carboxamide
ADI	acceptable daily intake

ADME	absorption, distribution, metabolism and excretion
AOAC	Association of Official Analytical Chemists
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
bw	body weight
CFU	colony forming units
DEN	diethylnitrosamine
DNA	deoxyribonucleic acid
FID	Flame Ionisation Detector
FSMP	foods for special medical purposes
GC	Gas Chromatography
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	generally recognised as safe
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma mass Spectrometry
LNHPD	Licensed Natural Health Products Database
LS	literature search
MoE	margin of exposure
NA	nicotinic acid
NAAD	nicotinic acid adenine dinucleotide
NAD+	nicotinamide adenine dinucleotide
NAM	nicotinamide
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NDI	new dietary ingredient
NE	niacin equivalent
NF	novel food
NMN	nicotinamide mononucleotide
NMR	Nuclear Magnetic Resonance
NOAEL	no observed adverse effect level
NRC	nicotinamide riboside chloride
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
RP	reference point
SCF	Scientific Committee on Food
TDRWC	total diet replacements products for weight control
UCT	University of Chemistry and Technology (Prague)
UL	tolerable upper intake level
USP	United States Pharmacopeia
UV	ultra violet



## Appendix A – Overview of toxicological studies on the NF

Reference	Type of study	Test system	Dose of NRC	Findings and conclusions <sup>(a)</sup>
Study No. S15004 (Unpublished) (Kamath, 2015; Conze et al., 2016)	Bacterial reverse mutation test (GLP, OECD TG 471)	Salmonella Typhimurium and <i>Escherichia coli</i>	Up to 5 mg/plate (absence and presence of S9 mix)	Not mutagenic
Study No. S15005 (Unpublished) (Conze et al., 2016; Kamath, 2016)	<i>In vitro</i> mammalian chromosome aberration test (GLP, OECD TG 473)	Human peripheral blood lymphocyte	Up to 5 mg/mL	Not clastogenic
Study No. S15006 (Unpublished) (Conze et al., 2016; Pandey, 2016)	<i>In vivo</i> mammalian erythrocyte micronucleus test (GLP, OECD TG 474)	Sprague–Dawley rats	Up to 2,000 mg/kg bw	No bone marrow toxicity; does not induce micronuclei
Study No. S13101 (Unpublished) (Bhoite and Jayachandra, 2014; Conze et al., 2016)	Single dose oral toxicity study (GLP)	Sprague–Dawley rats (5/sex per group)	5,000 mg/kg bw	f: ↓ (3%) cumulative bw gain in vs. control group
Study No. SN17-921 (Unpublished) (Thorsrud, 2017)	7-day dose range finding oral toxicity study (GLP)	Juvenile dogs (2/sex per group)	0, 100, 300 and 1,000 mg/kg bw per day	No toxicologically relevant findings
Study No. S13120 (Unpublished) (Bhoite and Jayachandra, 2014; Conze et al., 2016)	14-day dose range finding oral toxicity study (non-GLP)	Sprague–Dawley rats (5/sex per group)	0, 750, 1,500, 2,500, or 5,000 mg/kg bw per day, by gavage	m: bw ↓ in dose groups 1,500, 2,500 and 5,000 mg/kg bw per day vs. control group at different time points; overall food consumption ↓ in dose group 5,000 mg/kg bw per day
Study No. SN17-940 (Unpublished) (Thorsrud, 2018)	28-day oral toxicity study (GLP)	Juvenile dogs (test animals: 4/sex per group; TK satellite animals: 2/sex per group)	0, 100, 300 or 1,000 mg/kg bw per day, by gavage	m + f: bw ↓ and salivation after dosing, abdominal contractions, diarrhoea and vomiting (high-dose group). After lowering the dose from 1,000 to 500 mg/kg bw per day, salivation and vomiting still occurred. m: prothrombin time ↓ (low-dose group, not clearly dose-related); glucose ↓ (mid-dose group, not clearly dose-related); Na ↓, K ↓, eosinophils ↓, absolute and relative testes and thyroid weights ↓ (high-dose group) f: ↑ AST, ↓ phosphate, ↓ fibrin, ↑ relative ovary weight (high-dose group) NOAEL = 300 mg/kg bw per day

Reference	Type of study	Test system	Dose of NRC	Findings and conclusions <sup>(a)</sup>
Study No. S14022 (Unpublished) (Bhoite et al., 2015; Conze et al., 2016)	90-day repeated dose oral toxicity study (GLP, OECD TG 408)	Sprague–Dawley rats (10/sex per group)	0, 100, 300 or 3,000 mg/kg bw per day	<p>m + f: neutrophils ↑, ALT ↑, triglycerides ↑, rel. liver weight ↑, rel. kidney weight ↑ (mid and high-dose groups); hepatocellular hypertrophy and necrosis, thyroid follicular cell hypertrophy, hypertrophy of cortical zona glomerulosa in adrenals (high-dose group)</p> <p>m: bw ↓ (dose-related), feed consumption ↓ (high-dose group; low and mid dose groups sometimes); leucocytes ↑ (mid and high-dose groups); ALP ↑, bile acids ↑, absolute liver weight ↑, absolute and relative testes weight ↓, absolute and relative epididymis weight ↓, chronic progressive nephropathy, tubular degeneration/atrophy of testes, reduced luminal sperm in epididymis and cellular debris (high-dose group)</p> <p>f: AST ↑, ALP ↑, leucocytes ↑ (mid and high dose group); feed consumption ↓ (sometimes), monocytes ↑, GGT ↑, relative ovary weight ↑, hypertrophy of corpora lutea (high-dose group)</p> <p>NOAEL = 300 mg/kg bw per day</p>
Study No. G10959 (Unpublished) (Ganiger, 2016)	One generation reproduction toxicity study (GLP, OECD TG 415)	Sprague–Dawley rats (25/sex per group)	0, 3,000, 6,000 and 12,000 mg/kg feed in the diet (ad libitum) (corresponding to 169, 334 and 675 mg/kg bw per day in males and 273, 543 and 1,088 mg/kg bw per day in females)	<p>m: bw ↓, feed consumption ↓ (at two time points) (high dose group)</p> <p>No effect on precoital time, gestation length, fertility parameters, pathological and histopathological examinations of reproductive organs of adult rats, survival and abnormalities in life and death at any dose</p> <p>NOAEL = 675 mg/kg bw per day in males and 1,088 mg/kg bw per day in females for fertility and reproductive performance</p>

Reference	Type of study	Test system	Dose of NRC	Findings and conclusions <sup>(a)</sup>
Study No. G10957 (Unpublished) (Geetha Rao, 2016)	Embryo-fetal developmental toxicity study (GLP, OECD TG 414)	Sprague-Dawley pregnant rats (24/group)	0, 325, 750 and 1,500 mg/kg bw per day, by gavage	Maternal feed consumption ↓, maternal bw ↓, maternal bw gain ↓ (mid and high dose groups); gravid uterine weight ↓ (high-dose group); late weight resorption ↑ (dose-related); mean fetal weight ↓ (mid- and high-dose groups) Incidence of fetal anasarca ↑, 2 cases of small fetuses, 1 fetus with moderate flexed right forelimb, 1 fetus with thread-like tail (high-dose group) Delayed, incomplete or poor ossification (dose-related) Embryo/fetotoxicity findings observed at a dose of 750 mg/kg bw per day considered secondary to maternal toxicity NOAEL = 325 mg/kg bw per day for maternal and embryo/fetotoxicity

↓: decrease; ↑: increase; bw: body weight; f: females; GLP: good laboratory practice; m: males; NRC: nicotinamide riboside chloride; OECD TG: Organisation for Economic Co-operation and Development test guidelines.

(a): Studies evaluated in the previous evaluation (EFSA NDA Panel, 2019).

## Appendix B – Literature search on health effects of the intake of nicotinamide riboside chloride, nicotinamide and methyl nicotinamide

An outsourced literature search following a search strategy and standard operating procedure as described by UCT Prague (Dibusz and Vejvodova, 2020) was conducted to investigate four main categories of health outcomes following exposure to nicotinamide riboside chloride: hepatic function, haematology parameters, methyl balance and carcinogenicity.

Four databases were searched on 28 October 2020. Due to the very small number of articles available for nicotinamide riboside chloride, the search string also included three related metabolites. The search terms were: "nicotinamide riboside chloride" or "23111-00-4" or "nicotinamide" or "98-92-0" or "nicotinamide riboside" or "1341-23-7" or "methyl-nicotinamide" or "114-33-0". The number of articles available for nicotinamide being very large, the following restrictions was applied for this compound only:

- Scopus: added the search term "toxic".
- Pubmed: added the search term "toxic actions" [Medical Subject Headings (MeSH®) Major Topic].
- Web of Science: search limited to Toxicology.
- SciFinder: search limited to CAS number and Toxicology.

The number of hits in each database is reported below.

Database	Number of hits in the database
Web of Science	5,989
Scopus	687
Chemical Abstracts (SciFinder)	5,443
Pubmed	1,012
Unique number of articles	10,400

A stepwise abstract evaluation methodology was carried out as follows:

- 1) Articles were sorted by default weighed keywords (predefined for chemicals by the EFSA NF team) as well as custom-weighed keywords applicable to the particular NF (transaminase, aminotransferase, AST, ASAT, ALT, ALAT, alkaline phosphatase, ALP, bilirubin, BR, serum, blood, thrombocytopenia, platelet, homocysteine, S-adenosylmethionine, SAM, S-adenosylhomocysteine, SAH, betaine, SAM/SAH) [10,400 articles].
- 2) A list of articles containing the names 'nicotinamide riboside chloride' or '23111-00-4' OR 'nicotinamide' or '98-92-0' or 'nicotinamide riboside' or '1341-23-7' or 'methyl-nicotinamide' or '114-33-0' in the title or in the abstract excluding patents was created [5,349 articles; referred to as standard list hereafter].
- 3) Several sublists of the standard list were created to focus the search on the desired areas of interest:
  - a) Hepatic function (using liver, hepat, transaminase, aminotransferase, AST, ASAT, ALT, ALAT, alkaline phosphatase, ALP, bilirubin or BR as keywords) [sublist 1; 1,002 articles].
  - b) Haematology (using serum, blood, haemat, heamat, thrombocytopenia or platelet as keywords) [sublist 2; 802 articles].
  - c) Methyl balance (using methyl, homocysteine, S-adenosylmethionine, SAM, S-adenosylhomocysteine, SAH, betaine, SAM/SAH as keywords) [sublist 3; 1337 articles].
  - d) Carcinogenicity (using cancer, carcino, tumo(u)r as keywords) [sublist 4; 681 articles].
- 4) Following a review of the abstracts in the top 100 of the standard list and the abstracts in the four sublists, several additional sublists of the standard list were created to capture relevant articles related to:
  - a) Haematology (using thrombocytopenia or platelet as keywords) [sublist 5; 49 articles].
  - b) Methyl balance (using homocysteine as keyword) [sublist 6; 30 articles].
  - c) Carcinogenicity (using carcino as keyword) [sublist 7; 177 articles].
  - d) Carcinogenicity (using tumour as keyword) [sublist 8; 47 articles].

- e) Sublist 9 was created to capture relevant articles specific to the NF (using nicotinamide riboside chloride as keyword) [7 articles].
  - f) Sublist 10 was created to reduce the noise brought in by the very large nicotinamide data set (using nicotinamide riboside or methyl-nicotinamide as keywords) [1,473 articles].
- 5) Within the sublists, evaluation of titles and abstracts sorted by their relevance (keywords) was carried out by both the contractor (varying number of references for each sublist) and the SO in charge of the dossier (top 100 references for each sublist).
- 6) In total, 51 relevant articles were identified by the contractor, and 27 additional articles were identified by the SO in charge of the dossier, bringing the total to 78 articles of interest, listed hereafter.

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## Appendix C – Animal experiments on nicotinamide effect on chemical- and UV-induced tumours

Adapted from Surjana et al. (2010) and Hwang and Song, (2020).

Authors	Year	Species	Carcinogen	Form of nicotinamide (dose)	Organ	Effect on tumour
Rosenberg et al. (1985)	1985	Rat	Diethylnitrosamine (DEN)	Oral (0.082%; 0.37%)	Kidney	Increase
Kim et al. (2011a,b)	2011	Mouse	N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN)	Oral (0.1%; 0.25%; 0.5%; 1%; drinking water)	Bladder	Inhibition
Roe (1962)	1962	Mouse	DMBA and croton oil	Oral (0.2% diet)	Skin	None
French (1977)	1978	Mouse	Urethane	Oral (0.25%; 0.4% diet)	Lung	Inhibition
				Oral (niacin; 0,25% diet)	Lung	None
Bartleman et al. (2008)	2008	Rat	EthylNitrosourea	Oral (niacin; 0.4% diet)	Bone marrow (haemopoietic cells)	Inhibition
Pamukcu et al. (1981)	1981	Rat	Bracken fern	Oral (0.5% diet)	Intestine	Inhibition
					Bladder	Inhibition
Gensler et al. (1999)	1999	Mouse	UVB	Oral (niacin; 0.5%; 1% diet)	Skin	Inhibition
Gotoh et al. (1988)	1988	Mouse	Urethane	Oral (1%; 2.5% diet)	Lung	Inhibition
Gotoh et al. (1993)	1993	Mouse	Transplanted murine breast adenocarcinoma	Oral (2.5%; 5%)	Recipient subcutaneous tissue	Inhibition
Schmahl and Stackelberg (1968)	1968	Rat	Diethylnitrosamine (DEN)	Oral (200 mg/kg; drinking water)	Liver	None
Pour and Lawson (1984)	1984	Hamster	N-nitrosobis(2-oxopropyl) amine (BOP)	IP (30 mg/kg bw)	Pancreas	Inhibition
Al-Gayyar et al. (2019)	2019	Rats	Thioacetamide	IP (30 mg/kg)	Liver	Inhibition
Rakieten et al. (1971)	1971	Rat	Streptozotocin	IP (350 mg/kg bw)	Pancreas	Increase
Rakieten et al. (1976)	1976	Rat	Streptozotocin	IP (350 mg/kg bw)	Kidney	Inhibition
Schoental (1977)	1977	Rat	Diethylnitrosamine (DEN)	IP (350–500 mg/kg bw)	Kidney	Increase
					Liver	Inhibition
Horsman et al. (1995)	1995	Mouse	Transplanted murine breast adenocarcinoma	IP (1,000 mg/kg bw)	Recipient subcutaneous tissue	Inhibition
Gensler (1997)	1997	Mouse	UV	Topical (200 nM)	Skin	Inhibition
Ludwig et al. (1990)	1990	Mouse	12-O-tetradecanoylphorbol-13-acetate (TPA)	Topical (150 µM)	Skin	Inhibition

## Appendix D – Benchmark dose modelling reports with a 5% BMR

### D.1. Body weight

#### I. Marinescu et al. (2020)

##### A. Data description

The endpoint analysed was terminal body weight in male rats. The BMD analysis was performed using summary data as reported below.

Dose	Mean body weight (males, g)	SD	N
0	534.3	72.4	10
300	518.6	39.2	10
500	506.1	39.4	10
1,200	463.6	40.3	10

##### B. Selection of the BMR

The BMR (benchmark response) used is a 5% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. A 90% confidence interval around the BMD was estimated; the lower bound is reported by BMDL and the upper bound by BMDU.

##### C. Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

##### D. Results

###### Fitted models

Model	Log-likelihood	Number of parameters	AIC
full model	38.76	5	-67.52
null model	33.26	2	-62.52
Expon. m3-	38.76	4	-69.52
Expon. m5-	38.76	5	-67.52
Hill m3-	38.76	4	-69.52
Hill m5-	38.76	5	-67.52
Inv.Expon. m3-	38.76	4	-69.52
Inv.Expon. m5-	38.76	5	-67.52
LN m3-	38.76	4	-69.52
LN m5-	38.76	5	-67.52

###### Estimated model parameters

Estimate	EXP	HILL	INVEXP	LOGN
var-	0.00843	0.00843	0.008429	0.008429
a-	529.7	529.7	529.5	529.6
CED-	534.6	534.5	526.4	529.9
d-	1.217	1.219	0.1921	0.3796

Weights for Model Averaging

EXP	HILL	INVEXP	LOGN
0.25	0.25	0.25	0.25

Final BMD Values

Endpoint	BMDL	BMDU
Absolute body weight (g)	104	1,110

Confidence intervals for the BMD are based on 200 bootstrap data sets.

E. Visualisation

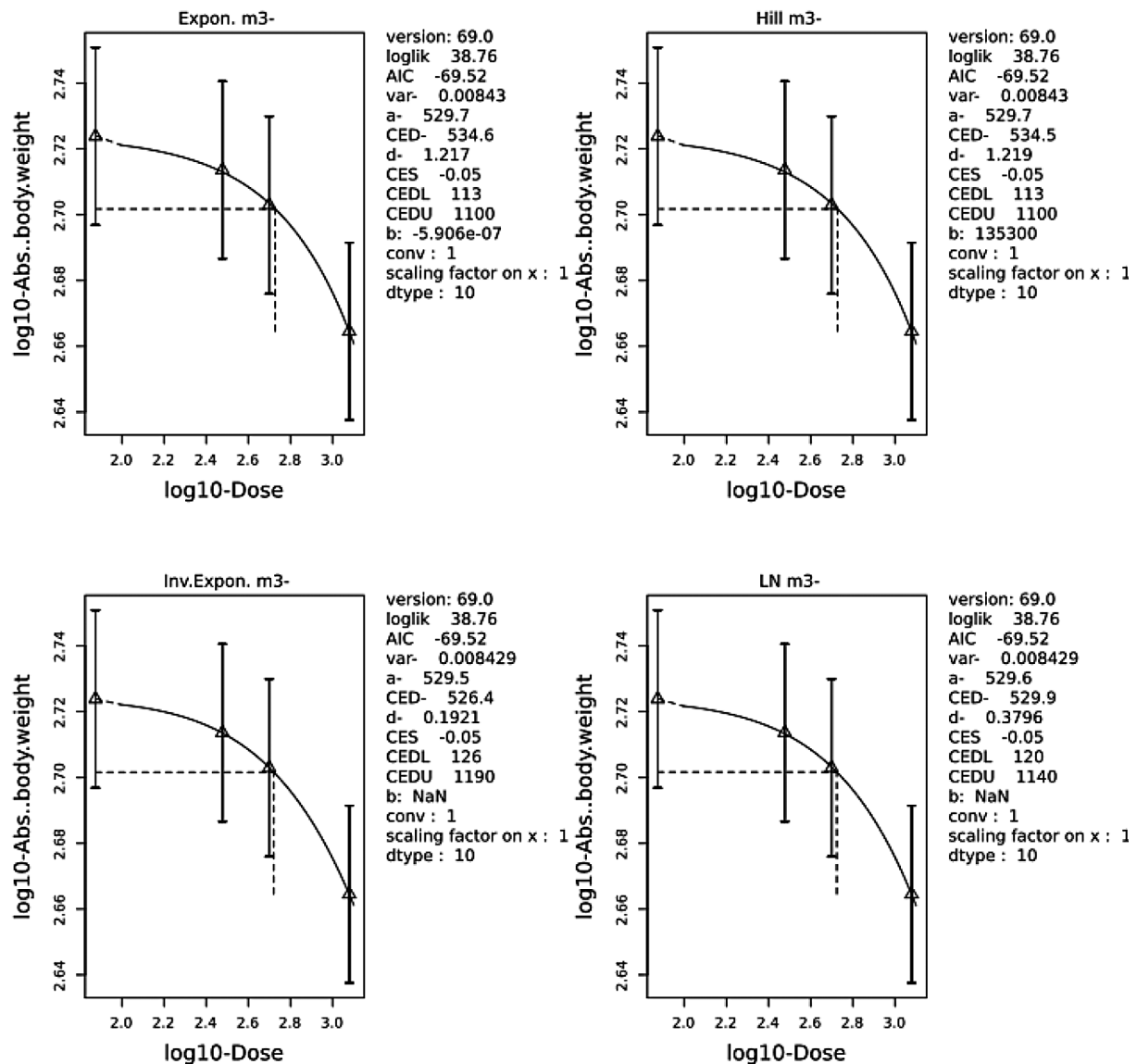
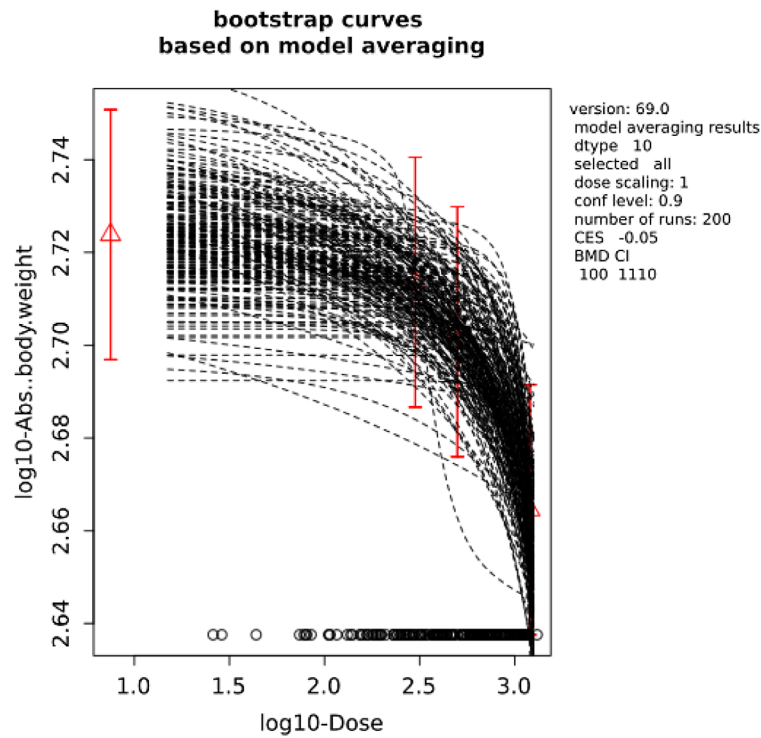


Figure D.1: Visualisation of the individual BMD model curves



**Figure D.2:** Visualisation of bootstrap curves based on BMD model averaging

**F. Conclusions**

Conclusions on the BMD modelling are discussed in the opinion.

**II. Conze et al. (2016)**

**A. Data description**

The endpoint analysed was terminal body weight in male and female rats. The BMD analysis was performed using summary data as reported below.

Dose	Sex	Mean body weight, g	SD	N
0	M	395.70	18.36	10
300	M	363.56	23.22	10
1,000	M	354.22	21.86	10
3,000	M	317.21	25.80	10
0	F	232.29	8.10	10
300	F	234.43	23.28	10
1,000	F	219.51	9.92	10
3,000	F	216.19	14.75	10

**B. Selection of the BMR**

The BMR (benchmark response) used is a 5% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

**C. Software used**

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

## D. Results

### Fitted models

Model	Log-likelihood	Number of parameters	AIC
full model	108.75	9	-199.50
full-v	108.77	10	-197.54
null model	-1.10	2	6.20
null model-a	81.49	3	-156.98
Expon. m3-	0.96	4	6.08
Expon. m3-a	101.70	5	-193.40
Expon. m3-ab	107.00	6	-202.00
Expon. m5-a	101.72	6	-191.44
Expon. m5-ab	107.00	7	-200.00
Hill m3-a	101.70	5	-193.40
Hill m3-ab	107.00	6	-202.00
Hill m5-a	101.72	6	-191.44
Hill m5-ab	107.00	7	-200.00
Inv.Expon. m3-a	101.72	5	-193.44
Inv.Expon. m3-ab	106.98	6	-201.96
Inv.Expon. m5-a	101.72	6	-191.44
Inv.Expon. m5-ab	106.96	7	-199.92
LN m3-a	101.71	5	-193.42
LN m3-ab	106.99	6	-201.98
LN m5-a	101.72	6	-191.44
LN m5-ab	106.99	7	-199.98

### Estimated model parameters

Estimate	EXP	HILL	INVEXP	LOGN
var-	0.004034	0.004034	0.004037	0.004035
a-f	234	234	233.6	233.8
a-m	393.4	393.4	393.9	393.7
CED-f	1,144	1,145	1,238	1,199
CED-m	189.5	189.6	192.2	191.5
d-	0.5198	0.5206	0.08739	0.1681

### Weights for Model Averaging

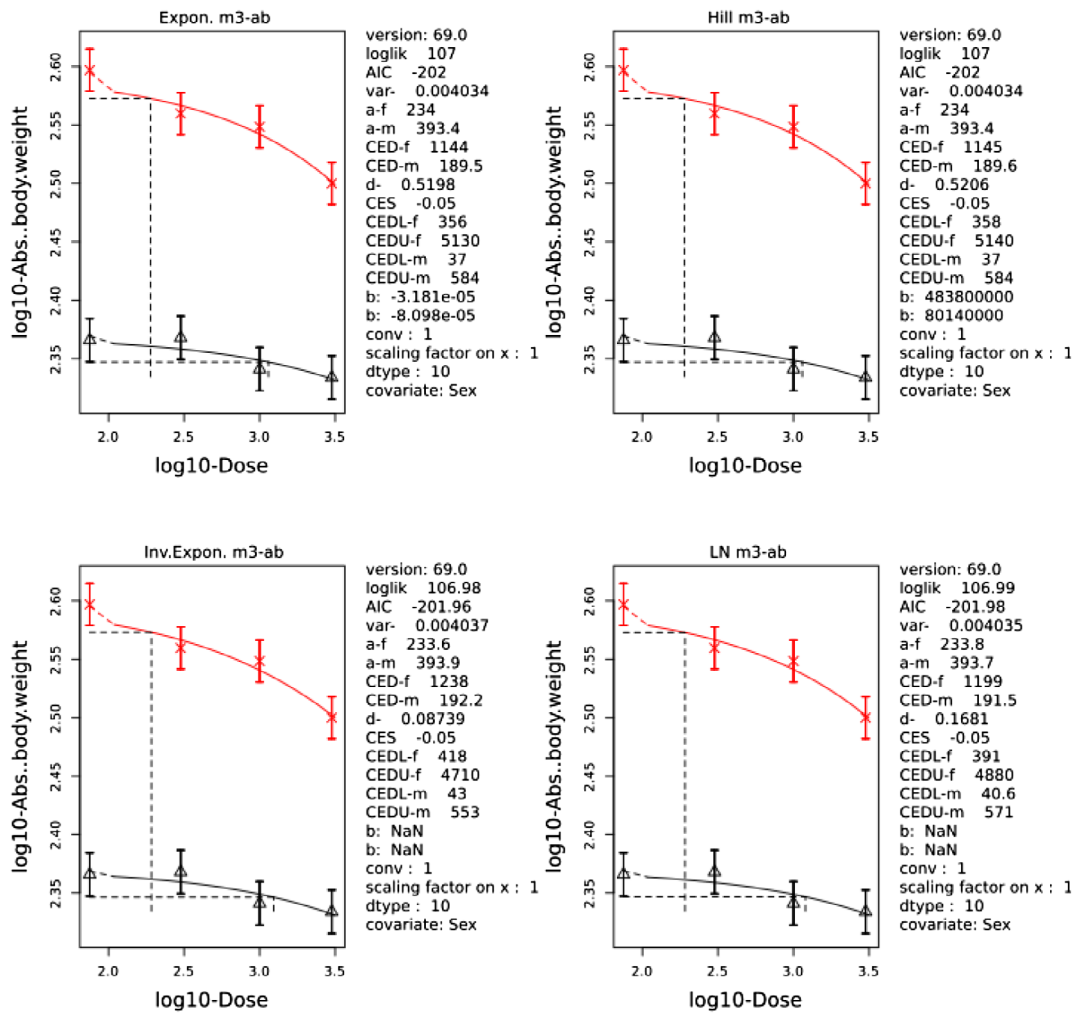
EXP	HILL	INVEXP	LOGN
0.25	0.25	0.25	0.25

### Final BMD Values

Endpoint	Subgroup	BMDL	BMDU
Absolute body weight	M	54.6	563
Absolute body weight	F	510.0	5,400

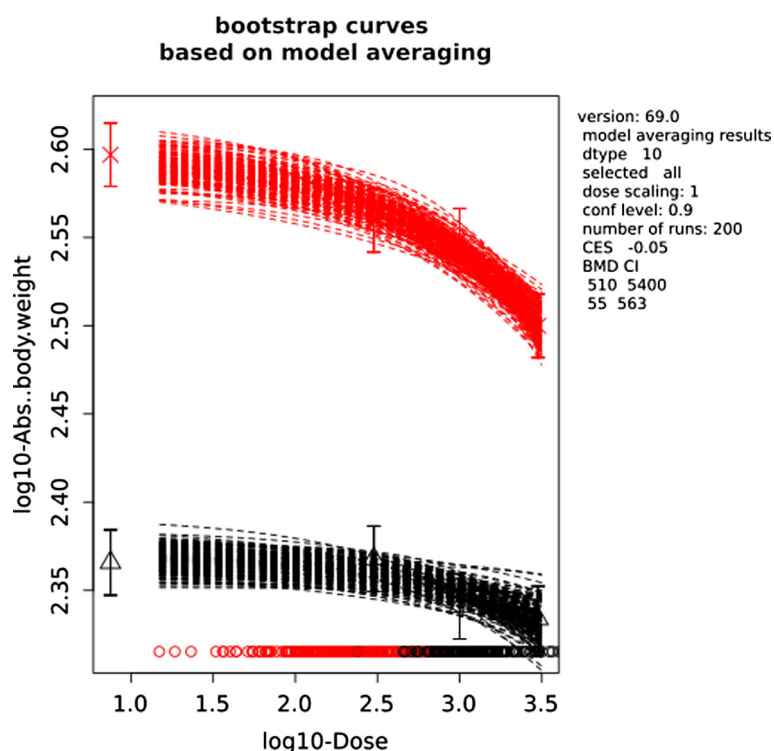
Confidence intervals for the BMD are based on 200 bootstrap data sets.

### E. Visualisation



**Figure D.3:** Visualisation of the individual BMD model curves





**Figure D.4:** Visualisation of bootstrap curves based on BMD model averaging

## F. Conclusions

Conclusions on the BMD modelling are discussed in the opinion.

## D.2. Relative liver weight (Conze et al., 2016)

### A. Data description

The endpoint analysed was terminal liver weight relative to body weight in male and female rats. The BMD analysis was performed using summary data as reported below.

Dose	Sex	Relative liver weight	SD	N
0	M	2.958	0.143	10
300	M	3.013	0.163	10
1,000	M	3.200	0.180	10
3,000	M	3.600	0.272	10
0	F	2.902	0.191	10
300	F	3.003	0.327	10
1,000	F	3.295	0.181	10
3,000	F	4.046	0.174	10

### B. Selection of the BMR

The BMR (benchmark response) used is a 5% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. A 90% confidence interval around the BMD will be estimated; the lower bound is reported by BMDL and the upper bound by BMDU.

### C. Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

## D. Results

### Fitted models

<b>Model</b>	<b>Log-likelihood</b>	<b>Number of parameters</b>	<b>AIC</b>
full model	107.88	9	-197.76
full-v	108.66	10	-197.32
null model	52.96	2	-101.92
null model-a	53.55	3	-101.10
Expon. m3-	98.97	4	-189.94
Expon. m3-a	100.84	5	-191.68
Expon. m3-b	107.00	5	-204.00
Expon. m3-ab	107.65	6	-203.30
Expon. m5-	99.14	5	-188.28
Expon. m5-a	101.03	6	-190.06
Expon. m5-b	107.00	6	-202.00
Expon. m5-ab	107.72	7	-201.44
Hill m3-	98.97	4	-189.94
Hill m3-a	100.84	5	-191.68
Hill m3-b	107.00	5	-204.00
Hill m3-ab	107.65	6	-203.30
Hill m5-	99.14	5	-188.28
Hill m5-a	101.03	6	-190.06
Hill m5-b	107.00	6	-202.00
Hill m5-ab	107.73	7	-201.46
Inv.Expon. m3-	99.11	4	-190.22
Inv.Expon. m3-a	100.99	5	-191.98
Inv.Expon. m3-b	106.95	5	-203.90
Inv.Expon. m3-ab	107.76	6	-203.52
Inv.Expon. m5-	99.14	5	-188.28
Inv.Expon. m5-a	101.03	6	-190.06
Inv.Expon. m5-b	106.90	6	-201.80
Inv.Expon. m5-ab	107.75	7	-201.50
LN m3-	99.06	4	-190.12
LN m3-a	100.94	5	-191.88
LN m3-b	106.99	5	-203.98
LN m3-ab	107.74	6	-203.48
LN m5-	99.14	5	-188.28
LN m5-a	101.03	6	-190.06
LN m5-b	106.98	6	-201.96
LN m5-ab	107.76	7	-201.52

### Estimated model parameters

<b>Estimate</b>	<b>EXP</b>	<b>HILL</b>	<b>INVEXP</b>	<b>LOGN</b>
var-	0.004035	0.004035	0.00404	0.004035
a-	2.922	2.922	2.929	2.926
<b>CED-f</b>	387.3	387.6	424.1	408.3
<b>CED-m</b>	614.6	615.3	692.5	659
d-	0.925	0.927	0.1666	0.3111

Weights for Model Averaging

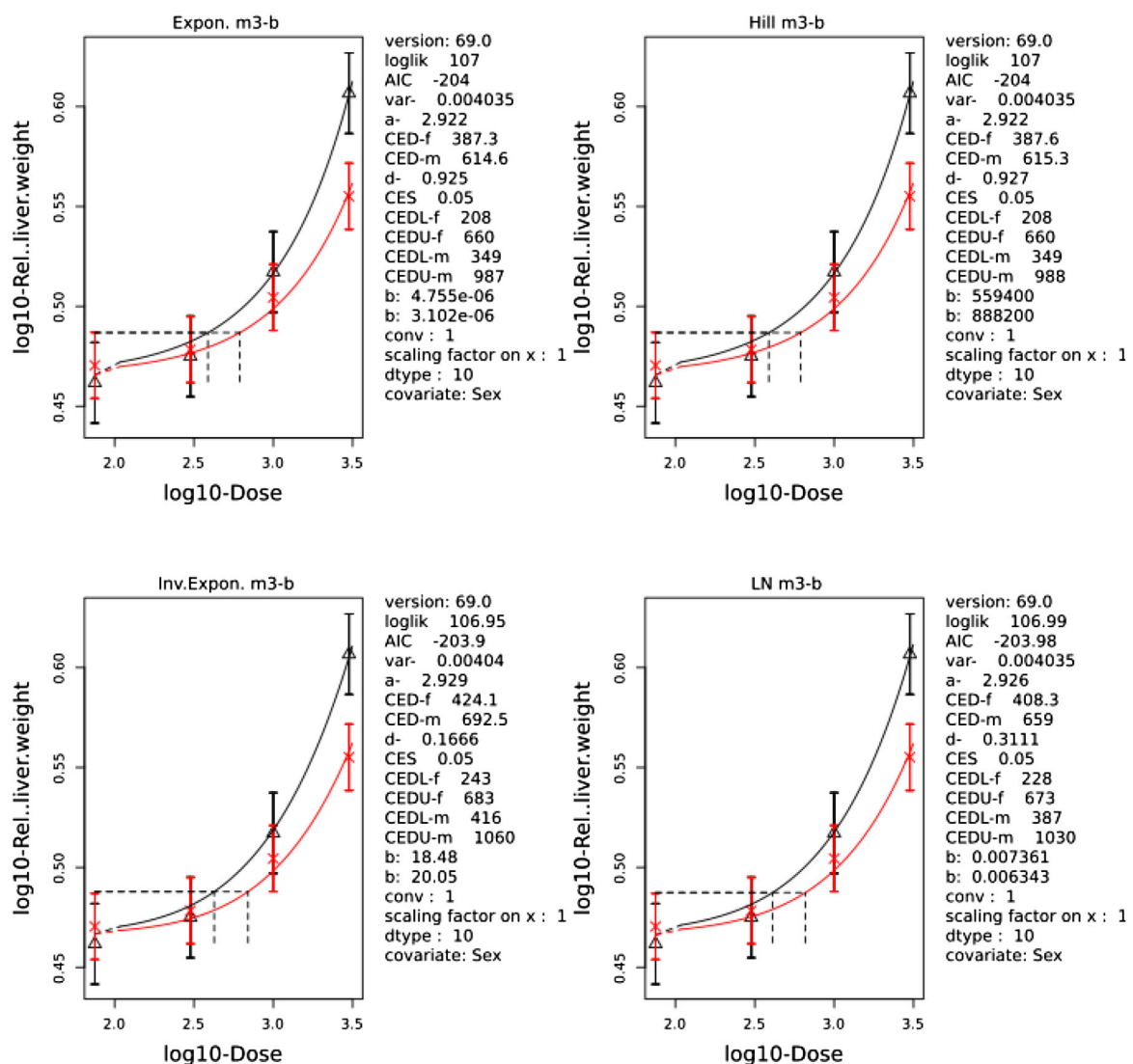
EXP	HILL	INVEXP	LOGN
0.25	0.25	0.24	0.25

Final BMD Values

Endpoint	Subgroup	BMDL	BMDU
Relative liver weight	M	399	1,120
Relative liver weight	F	226	721

Confidence intervals for the BMD are based on 200 bootstrap data sets.

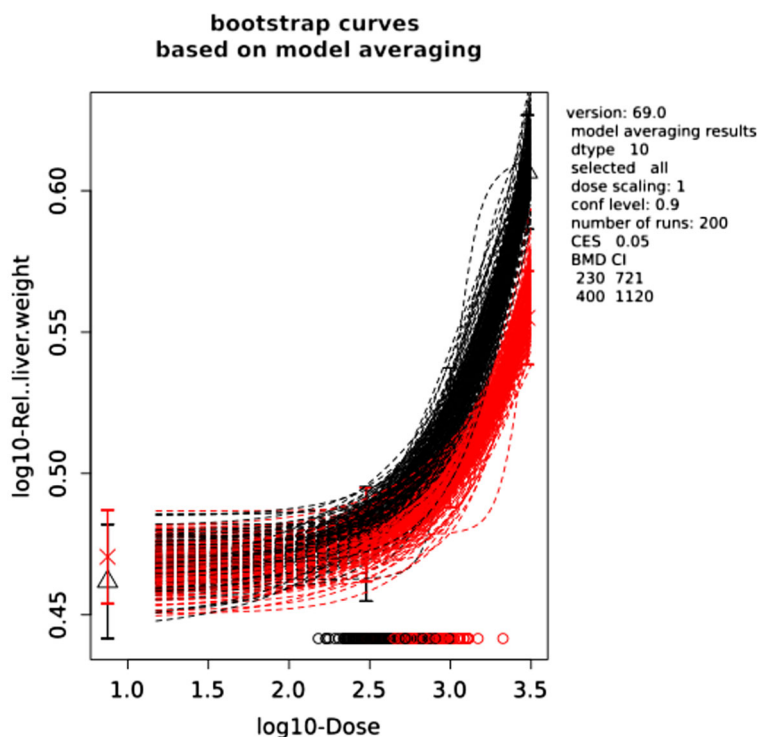
**E. Visualisation**



**Figure D.5:** Visualisation of the individual BMD model curves

**F. Conclusions**

Conclusions on the BMD modelling are discussed in the opinion.



**Figure D.6:** Visualisation of bootstrap curves based on BMD model averaging

### D.3. Relative kidney weight (Conze et al., 2016)

#### A. Data description

The endpoint analysed was terminal kidney weight relative to body weight in male and female rats. The BMD analysis was performed using summary data as reported below.

Dose	Sex	Relative kidney weight	SD	N
0	M	0.715	0.047	10
300	M	0.701	0.033	10
1,000	M	0.777	0.020	10
3,000	M	0.876	0.063	10
0	F	0.676	0.053	10
300	F	0.645	0.060	10
1,000	F	0.678	0.058	10
3,000	F	0.822	0.044	10

#### B. Selection of the BMR

The BMR (benchmark response) used is a 5% change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

#### C. Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

## D. Results

### Fitted models

Model	Log-likelihood	Number of parameters	AIC
full model	105.49	9	-192.98
full-v	107.90	10	-195.80
null model-v	56.90	3	-107.80
null model-a-v	62.48	4	-116.96
Expon. m3-v	89.66	5	-169.32
Expon. m3-av	102.57	6	-193.14
Expon. m3-abv	102.65	7	-191.30
Expon. m5-av	103.39	7	-192.78
Expon. m5-abv	103.60	8	-191.20
Hill m3-av	102.58	6	-193.16
Hill m3-abv	102.65	7	-191.30
Hill m5-av	103.98	7	-193.96
Hill m5-abv	105.00	8	-194.00
Inv.Expon. m3-av	103.12	6	-194.24
Inv.Expon. m3-abv	103.14	7	-192.28
Inv.Expon. m5-av	104.44	7	-194.88
Inv.Expon. m5-abv	105.81	8	-195.62
LN m3-av	102.88	6	-193.76
LN m3-abv	102.93	7	-191.86
LN m5-av	104.22	7	-194.44
LN m5-abv	105.03	8	-194.06

### Estimated model parameters

Estimate	EXP	HILL	INVEXP	LOGN
var-f	0.006255	0.00604	0.005994	0.006303
var-m	0.003247	0.002978	0.002881	0.002967
a-f	0.6508	0.6568	0.6593	0.6508
a-m	0.7095	0.7004	0.7036	0.7095
CED-	855.3	N/A	N/A	821.5
CED-f	N/A	1045	1093	N/A
CED-m	N/A	704.4	763.8	N/A
c-	N/A	1.3	1.3	1.3
d-	1.196	2.67	1.862	1.425

### Weights for Model Averaging

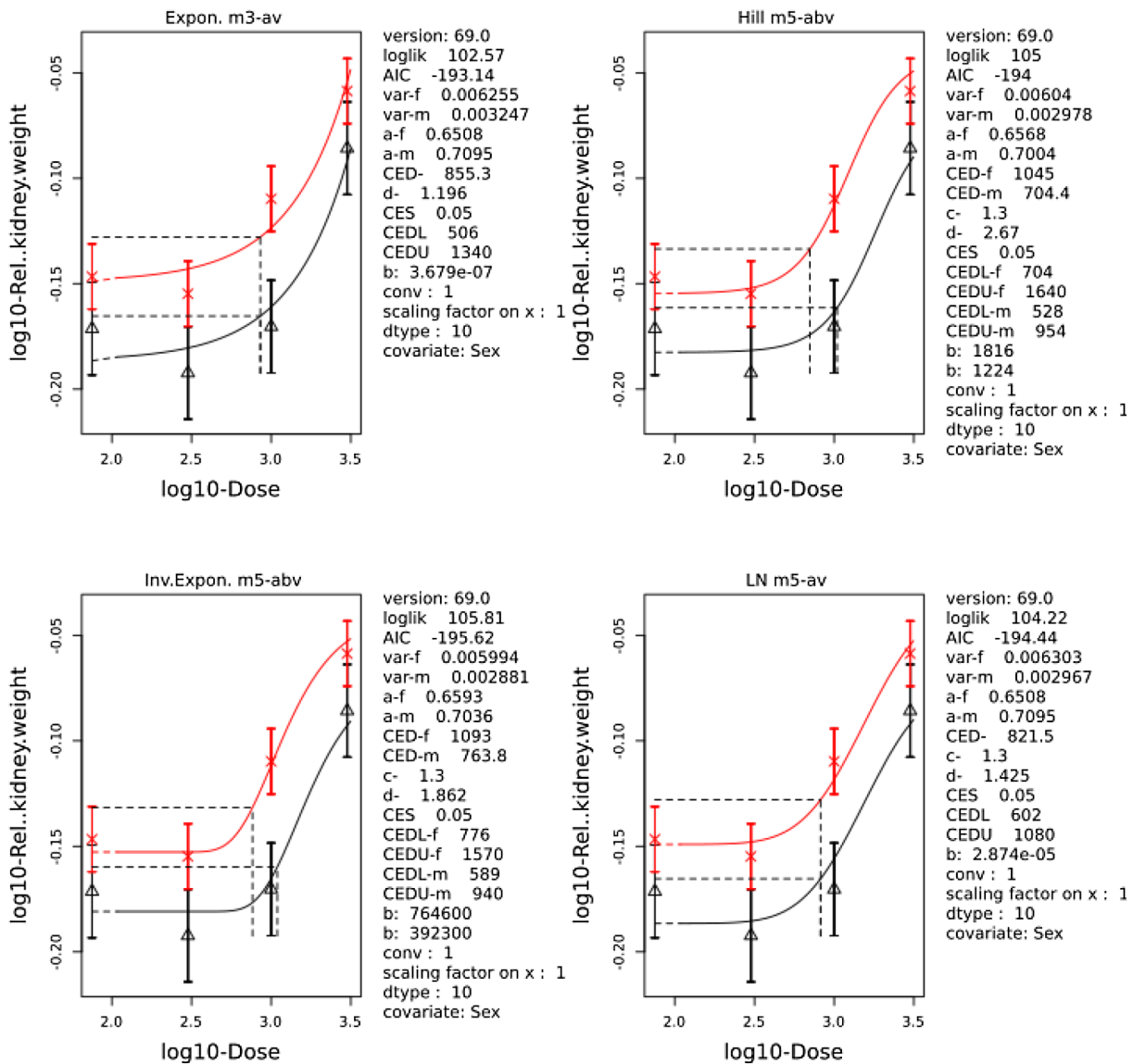
EXP	HILL	INVEXP	LOGN
0.13	0.19	0.44	0.24

### Final BMD Values

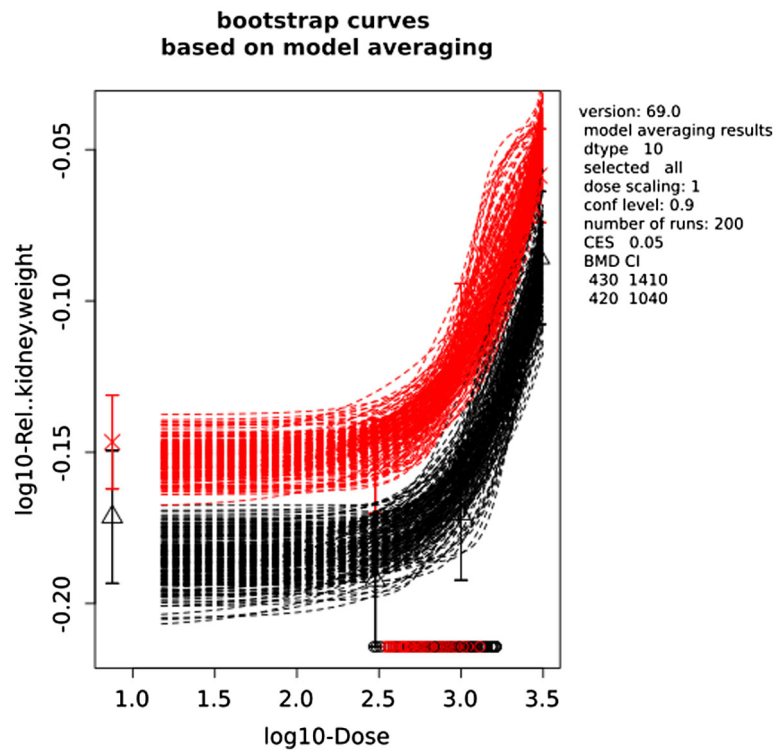
Endpoint	Subgroup	BMDL	BMDU
Relative kidney weight	M	425	1,040
Relative kidney weight	F	427	1,410

Confidence intervals for the BMD are based on 200 bootstrap data sets.

**E. Visualisation**



**Figure D.7:** Visualisation of the individual BMD model curves



**Figure D.8:** Visualisation of bootstrap curves based on BMD model averaging

## F. Conclusions

Conclusions on the BMD modelling are discussed in the opinion.