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## Safety and efficacy of Availa<sup>®</sup>Cr (chromium chelate of DL-methionine) as a feed additive for dairy cows

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### Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of Availa<sup>®</sup>Cr (active compound: chromium chelate of DL-methionine) as a feed additive for dairy cows. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel was assigned to this mandate. The tolerance study did not show adverse effects with respect to body weight, milk yield, haematology and blood biochemistry up to the 10-fold overdose of chromium from Availa<sup>®</sup>Cr; however, owing to deficiencies in design and reporting of the study, it provided only supportive evidence on the safety of the additive. A safe level for dairy cows could be derived from a toxicological study with laboratory rodents. Taking all together, the FEEDAP Panel considers the maximum recommended use level (8 mg Cr from Availa<sup>®</sup>Cr/cow per day; about 0.4 mg Cr/kg complete feed) as safe. Chromium DL-Met shows a genotoxic activity *in vitro* that is not expressed *in vivo* after systemic exposure; although no data on genotoxicity at the site of contact are available, the Panel notes that chromium(III) is not carcinogenic and concludes that chromium DL-Met is unlikely to pose a carcinogenic risk at levels occurring in the diet. The use of Availa<sup>®</sup>Cr in feed of dairy cows up to the recommended supplementation level would not measurably increase consumer exposure to chromium(III), and therefore is safe for consumers. The additive poses a risk to users by inhalation, it is not an irritant to skin and eyes and should be considered a sensitiser. The use of the additive is not expected to pose a risk to the environment. Since only one study could be considered for the efficacy assessment, and three studies are required, the FEEDAP Panel cannot conclude on the efficacy of Availa<sup>®</sup>Cr for dairy cows.

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## Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on Safety and efficacy of Availa®Cr (chromium chelate of DL-methionine) as a feed additive for dairy cows.

The FEEDAP Panel used the data provided by the applicant and data from other previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' elicitation knowledge, to deliver the present output. This scientific opinion also included the summary of the European Union Reference Laboratory (EURL) report which relates to the methods used for the control of the additive in the feed. The FEEDAP Panel performed this assessment following the requirements of the Regulation (EC) No 429/2008 and the relevant EFSA guidance documents.

The additive, brand name Availa®Cr, is intended to be used as a zootechnical feed additive for dairy cows to increase milk yield. 'Chromium methionine chelate' is considered the active compound and contains one molar equivalent of chromium (III) and three molar equivalents of DL-Met; the final product incorporates the active substance dried onto a carrier.

The additive is intended to be used in all types of feed (either mashed or pelleted form) for dairy cows. The applicant proposes its use at a minimum supplementation rate of 0.3 mg Cr/kg complete feed, up to a maximum of 0.5 mg Cr/kg complete feed (standard feed with 88% DM), corresponding to 300 and 500 mg Availa®Cr/kg complete feed, respectively. The FEEDAP Panel notes that the proposed chromium supplementation rate from Availa®Cr is close to the background content of complete feeds.

Although in the tolerance study with dairy cows no adverse effects were seen with respect to body weight, milk yield, haematology and blood biochemistry at use level (8 mg Cr/cow and day) and the 5- and 10-fold overdose of chromium from Availa®Cr, the FEEDAP Panel considers that this study provides only supportive evidence on the safety of the additive, owing to the significant deficiencies in design and reporting.

On the other hand, a safe level in complete feed of dairy cows could be derived from a toxicological study with laboratory rodents showing a no observed adverse effect level (NOAEL) of 34 mg Cr from chromium DL-Met/kg body weight per day; the safe level for dairy cows would be 9.7 mg supplemental Cr/kg complete feed. The maximum recommended use level (0.5 mg Cr/kg complete feed) would provide a margin of safety of about 19.

Including the background chromium in feed (approximately the same magnitude as for the supplemental chromium from Availa®Cr) and presuming the same qualitative and quantitative efficacy as shown for chromium from the additive, the exposure of the animal would double being at least 10 times lower than the safe concentration.

Taking all together, the FEEDAP Panel considers the maximum recommended use level for dairy cows (8 mg Cr from the additive Availa®Cr/cow per day) as safe for the target animal; this would correspond to about 0.4 mg Cr/kg complete feed.

Absorption of chromium is low ranging from 0.5% to 2–3%. Upon absorption, it binds to plasma proteins and is transported to the liver. It is also found in spleen, kidneys, testes and bone. Deposition in muscle does occur to a very variable and low extent. Whereas most unabsorbed chromium is excreted through the faeces, urine is the main excretory route for absorbed chromium, but it is found in milk. Chromium DL-Met shows a genotoxic activity *in vitro* that is not expressed *in vivo* after systemic exposure. Although no data on genotoxicity at the site of contact are available, the Panel notes that chromium(III) is not carcinogenic and concludes that chromium DL-Met is unlikely to pose a carcinogenic risk at levels occurring in the diet.

The use of chromium DL-Met given as Availa®Cr in feed of dairy cows up to the recommended highest supplementation level would not measurably increase consumer exposure to trivalent chromium; therefore, the FEEDAP Panel concludes that the additive up to maximum recommended level of 0.5 mg Cr(III) from Availa®Cr per kg complete feed for dairy cows does not pose any concern to the safety of consumers.

Based on the evaluation of data regarding the effects of the additive on the respiratory system and on the eyes and skin, the FEEDAP Panel conclude that the additive poses a risk to users by inhalation. Availa®Cr is not an irritant for skin and eyes. The additive should be considered a sensitiser.

Chromium is as a natural constituent in many rocks ubiquitous in the environment. The contribution of chlorine from the additive to the total dietary chlorine is negligible. Methionine – a physiological and natural component in animals and plants – is not excreted by cows as such (but as urea, sulfate and carbon dioxide). The use of the additive is not expected to pose a risk to the environment.

From the three studies submitted to support efficacy of the additive only one could be considered; the other two lacked critical data for the efficacy assessment. The efficacy study evaluated showed that supplementation of Availa®Cr before and after calving had a significant positive effect on milk production (more than 3 kg/day), with no changes in milk components and feed efficiency. Owing to the Guidance requirement of at least three studies showing positive effects, the FEEDAP Panel is not in a position to conclude on the efficacy of supplemental chromium from chromium-chelate of DL-Met.

The FEEDAP Panel recommends to indicate a daily dose per dairy cow (8 mg Cr from Availa®Cr) under *Other Provisions* on a potential authorisation of the additive. The Panel added a remark concerning the analytical methods for the determination of supplemental chromium in compound feed.

## Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	6
1.1. Background and Terms of Reference as provided by the requestor.....	6
1.2. Additional information.....	6
2. Data and methodologies.....	7
2.1. Data.....	7
2.2. Methodologies.....	7
3. Assessment.....	8
3.1. Characterisation.....	8
3.1.1. Manufacturing process.....	8
3.1.2. Characterisation of the additive.....	8
3.1.3. Characterisation of the active substance.....	8
3.1.4. Composition of the dried raw product containing the active compound.....	9
3.1.5. Stability and homogeneity.....	9
3.1.6. Physicochemical incompatibilities or interactions.....	10
3.1.7. Conditions of use.....	10
3.2. Safety.....	10
3.2.1. Absorption, distribution, metabolism and excretion (ADME).....	10
3.2.2. Toxicological studies.....	11
3.2.2.1. Toxicological profile of chromium.....	11
3.2.2.2. Genotoxicity studies with chromium DL-Methionine.....	12
3.2.2.3. Subchronic toxicity study with chromium DL-Methionine.....	13
3.2.2.4. Conclusion on the toxicological studies.....	13
3.2.3. Safety for the target species.....	13
3.2.3.1. Tolerance study.....	13
3.2.3.2. Toxicological study with laboratory animals.....	15
3.2.3.3. Conclusion on the safety of Availa®Cr for dairy cows.....	15
3.2.4. Safety for the consumer.....	16
3.2.4.1. Residue studies.....	16
3.2.4.2. Consumer exposure.....	18
3.2.4.3. Conclusions on safety for the consumer.....	18
3.2.5. Safety for user.....	18
3.2.5.1. Effects on the respiratory system.....	18
3.2.5.2. Effects on the eyes and skin.....	18
3.2.5.3. Conclusions on safety for the user.....	19
3.2.6. Safety for the environment.....	19
3.2.6.1. Conclusions on safety for the environment.....	19
3.3. Efficacy.....	19
3.3.1. Study 1.....	19
3.3.2. Study 2.....	19
3.3.3. Study 3.....	20
3.3.4. Conclusions on efficacy.....	20
3.4. Post-market monitoring.....	20
4. Conclusions.....	21
5. Recommendation.....	21
6. Remark.....	21
Documentation as provided to EFSA/Chronology.....	21
References.....	22
Abbreviations.....	24
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Availa®Cr (chromium chelate of DL-methionine).....	26

## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>1</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Zinpro Animal Nutrition (Europe), Inc.<sup>2</sup> for authorisation of the product Availa®Cr (chromium chelate of DL-methionine), when used as a feed additive for dairy cows (category: zootechnical additives; functional group: other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 21 August 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Availa®Cr (chromium chelate of DL-methionine), when used under the proposed conditions of use (see Section 3.1.5).

### 1.2. Additional information

The additive, Availa®Cr (active compound: chromium chelate of DL-methionine), is intended for use as a zootechnical feed additive (functional group: other zootechnical additives) in dairy cows for milk production. This feed additive is not authorised in the European Union (EU).

The FEEDAP Panel delivered a scientific opinion on the safety and efficacy of chromium methionine (Availa®Cr) as a nutritional feed additive for all species (EFSA, 2009a). The Panel could not conclude on the safety for target animals and consumers; concerning efficacy the opinion reported availability of chromium from the additive, but no conclusions could be drawn regarding zootechnical parameters.

Regarding other outputs, EFSA commissioned the University of Gent (Belgium) to carry out a literature review on selected trace and ultratrace elements, including chromium; this activity resulted in a report (Van Paemel et al., 2010).

The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) delivered a scientific opinion on dietary reference values for chromium (EFSA NDA Panel, 2014a); the same Panel delivered an opinion on the substantiation of several health claims related to chromium (EFSA NDA Panel, 2010), and another opinion on a specific health claim (reduction of post-prandial glycaemic responses) related to a combination of various amino acids and chromium picolinate (EFSA NDA Panel, 2014b).

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) delivered a scientific opinion on the risks to public health related to the presence of chromium in food and drinking water (EFSA CONTAM Panel, 2014).

The EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS Panel) has delivered several opinions on chromium sources: on the safety of trivalent chromium as a nutrient added for nutritional purposes to foodstuffs for particular nutritional uses and foods intended for the general population (including food supplements) (EFSA ANS Panel, 2010a), on a mixture of chromium di- and tri-nicotinate as a source of chromium added for nutritional purposes in food supplements and in foods for particular nutritional uses (EFSA, 2008a,b), on the safety of chromium picolinate as a source of chromium added for nutritional purposes to foodstuff for particular nutritional uses and to foods intended for the general population (EFSA ANS Panel, 2010b), on chromium(III) (EFSA, 2009b) and chromium picolinate added for nutritional purposes in food supplements (EFSA, 2009c), on ChromoPrecise® cellular bound chromium yeast added for nutritional purposes as a source of chromium in food supplements and the bioavailability of chromium from this source (EFSA ANS Panel, 2012a), on chromium(III) lactate tri-hydrate as a source of chromium added for nutritional purposes to food supplements (EFSA, 2009d) and to foodstuff (EFSA ANS Panel, 2012b).

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> Zinpro Animal Nutrition (Europe), Inc. Akkerdistel 2E. 5831 PJ. Boxmeer. The Netherlands.

Four chromium compounds (chromium(III) chloride and its hexahydrate, chromium(III) sulfate and its hexahydrate, chromium picolinate and chromium(III) lactate tri-hydrate) are listed as mineral substances which may be added to foods.<sup>3</sup> Five chromium compounds (chromium(III) chloride, chromium(III) lactate trihydrate, chromium nitrate, chromium picolinate and chromium(III) sulfate) are listed as minerals which may be used in the manufacture of food supplements, and four chromium compounds (chromium(III) chloride and its hexahydrate and chromium(III) sulfate and its hexahydrate) as mineral substances which may be added to foods.<sup>4</sup>

Three sources of chromium (chromium(III) chloride and its hexahydrate, chromium(III) sulfate and its hexahydrate and chromium picolinate) are authorised as food for special medical purposes and as total diet replacement for weight control.<sup>5</sup>

Chromium picolinate is authorised as novel food in the EU.<sup>6</sup>

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier<sup>7</sup> in support of the authorisation request for the use of Availa®Cr (chromium chelate of DL-methionine) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the Availa®Cr (chromium chelate of DL-methionine) in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>8</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Availa®Cr (chromium chelate of DL-methionine) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>9</sup> and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on zotechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b).

<sup>3</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. OJ L 404, 30.12.2006, p. 26.

<sup>4</sup> Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.

<sup>5</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.

<sup>6</sup> Commission Implementing Regulation (EU) 2018/1023 of 23 July 2018 correcting Implementing Regulation (EU) 2017/2470 establishing the Union list of novel foods. OJ L 187, 24.7.2018, p. 1.

<sup>7</sup> FEED dossier reference: FAD-2018-0021.

<sup>8</sup> The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0021-availa-cr.pdf>

<sup>9</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

### 3. Assessment

The additive, brand name Availa®Cr, contains chromium chelate of DL-methionine (called in this opinion chromium DL-Met) as active compound, and is intended to be used as a zootechnical feed additive for dairy cows to increase milk yield.<sup>10</sup>

Unless otherwise indicated, chromium in the opinion refers to chromium(III).

#### 3.1. Characterisation

##### 3.1.1. Manufacturing process



##### 3.1.2. Characterisation of the additive

The additive contains 2.0% dry raw product,<sup>11</sup> 97.0% calcium carbonate and 1.0% of vegetable oil (soybean oil). It is specified to contain at least 1,000 mg Cr/kg additive. Five batches of the additive were analysed for chromium and methionine. The average (range) content of chromium was about 1,154 mg/kg (1,004–1,474) and of methionine about 14,900 mg/kg (13,800–17,500).<sup>12</sup>

Five batches of the additive were analysed for undesirable substances. Levels of heavy metals (cadmium: 0.13–0.17, lead: 0.73–0.89, mercury: < 0.01 mg/kg), of arsenic (0.30–0.40 mg/kg) and fluorine (19.0–81.0 mg/kg) were reported.<sup>13</sup> The levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.16 ng WHO-PCDD/F-TEQ per kg and 0.23 ng WHO-PCDD/F-PCB-TEQ per kg, respectively.<sup>14</sup> The concentrations of the undesirable substances analysed comply with those set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern.<sup>15</sup> The average nickel content of three batches was 1.25 mg/kg (range 1.18–1.31).<sup>16</sup>

Microbiological analysis of three batches (aged from 2 to 3 months) of the additive resulted in the following results: counts of *Enterobacteriaceae* and *E. coli* were below the limit of quantification (LOQ) (< 10 cfu/g) and Salmonella was not detected in 25 g sample.<sup>17</sup> Levels of aflatoxin B1 and ochratoxin A analysed in three batches were below the LOQ (< 0.1 and < 0.4 µg/kg, respectively).<sup>18</sup>

Bulk density ranged in three batches from 1,344 to 1,351 kg/m<sup>3</sup>. The same three batches were analysed for particle size distribution (laser-diffraction technique)<sup>19</sup> and dusting potential (Stauber–Heubach method).<sup>20</sup> On average < 2.3% of the particles were below 10 µm and 7.5% below 50 µm; dusting potential (analysed in four or five measurements of each batch) was in the range of 3.5–5.6 g/m<sup>3</sup>. About 57% of the dust particles had a diameter below 10 µm, and about 96% were below 50 µm, based on the analysis of three batches.<sup>16</sup> The chromium content of the dust, determined from three batches, was on average 6,745 mg/kg, with the highest value of 7,372 mg/kg.<sup>16</sup>

##### 3.1.3. Characterisation of the active substance

The chemical name of chromium methionine chelate according to International Union of Pure and Applied Chemistry (IUPAC) is chromium-DL-2-amino-4-(methylthio)butanoic acid, it has no Chemical

<sup>10</sup> Technical dossier/Section II/2.5.1.

<sup>11</sup> Technical Dossier/Supplementary information/November 2019.

<sup>12</sup> Technical dossier/Section II/Annex II-1, 2&3.

<sup>13</sup> Technical dossier/Section II/Annex II-8.

<sup>14</sup> Technical dossier/Section II/Annex II-9&10.

<sup>15</sup> Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.

<sup>16</sup> Technical dossier/Supplementary information/April 2019.

<sup>17</sup> Technical dossier/Section II/Annex II-11.

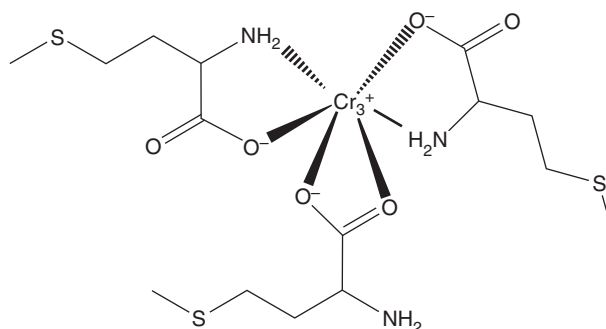
<sup>18</sup> Technical dossier/Section II/Annex II-12.

<sup>19</sup> Technical dossier/Section II/Annex II-14.

<sup>20</sup> Technical dossier/Section II/Annex II-15.



Abstracts Service (CAS) number. Its chemical formula is  $[\text{CH}_3\text{S}(\text{CH}_2)_2\text{CH}(\text{NH}_2)\text{COO}]_3\text{Cr}$ , the structural formula is shown in Figure 1 and the molecular weight 496.61 Da. Chromium methionine chelate contains one molar equivalent of chromium(III) and three molar equivalents of DL-Met. The theoretical chromium content of the active substance is 10.47%, that of the monohydrochloride 9.77%.<sup>21</sup>



**Figure 1:** Structural formula of chromium DL-Methionine

The molecular structure of the active compound was characterised by infrared (IR) spectroscopy and the absorption in  $\text{cm}^{-1}$  was 3,561, 1,662 and 1,511  $\mu\text{m}$ .<sup>22</sup> The IR peaks are a fingerprint of the compound being analysed. When compared to the IR of methionine, they show a certain shift which indicates a change in structure.<sup>16</sup>

### 3.1.4. Composition of the dried raw product containing the active compound

The applicant submitted analytical data on the dried raw product, obtained as described above under manufacturing process. The mean values (range) of five batches were: total chromium 5.3% (5.2–5.4) and free chromium 0.04% (0.02–0.08); methionine 80.4% (79.8–81.2); chlorine 11.4% (11.3–11.5); water 2.3% (2.1–2.5).<sup>16</sup>

Since methionine is added in surplus in the chemical reaction, nearly all chromium (98.5–99.6%; determined by inductively coupled plasma mass spectrometry (ICP-MS) after precipitating free chromium with sodium carbonate<sup>23</sup>) should be bound to methionine as chelate, and some free methionine would remain. The product would contain by calculation about 51% chromium methionine chelate (54% chromium methionine chelate hydrochloride) and about 32% free methionine.

The dried raw product, containing the chromium methionine chelate, is a grey-tan powder with slight odour. Its melting point is 157–159°C and the pH in solution is 2–3. It is soluble in water (< 20 g/100 mL) and slightly soluble in methyl alcohol and ethyl alcohol. It is practically insoluble in ethyl acetate.

### 3.1.5. Stability and homogeneity

It is commonly known that ligands which can coordinate at two or more positions (polydentate ligands) form more stable complexes with a given ion than ligands which can coordinate at only a single position – a process known as chelation. Chromium(III) complexes are polydentate and, owing to this fact, possess an innate chemical and structural stability.<sup>24</sup>

A stability study was submitted with one batch of the additive. Samples were stored under ambient (25°C/60% relative humidity (RH)) and environmental chamber (40°C/70% RH) conditions. The chromium and methionine content of Availa®Cr were monitored for 36 months. The samples were packaged with the original materials that simulate the packaging of the final product.<sup>23</sup> The chromium content was stable after 36 months storage under ambient conditions and under environmental chamber conditions. The methionine content is also considered to be stable under both conditions although showing small variations (an increase from 1.42% to 1.44% after 36 months storage under ambient conditions and a decrease from 1.45% to 1.40% under environmental chamber conditions).<sup>25</sup>

<sup>21</sup> Technical dossier/Section II/2.2.1.1.

<sup>22</sup> Technical dossier/Section II/2.2.2.

<sup>23</sup> Technical dossier/Supplementary information/October 2019.

<sup>24</sup> [https://chem.libretexts.org/Bookshelves/Inorganic\\_Chemistry/Supplemental\\_Modules\\_\(Inorganic\\_Chemistry\)/Coordination\\_Chemistry/Complex\\_Ion\\_Equilibria/Stability\\_of\\_Metal\\_Complexes\\_and\\_Chelation](https://chem.libretexts.org/Bookshelves/Inorganic_Chemistry/Supplemental_Modules_(Inorganic_Chemistry)/Coordination_Chemistry/Complex_Ion_Equilibria/Stability_of_Metal_Complexes_and_Chelation)

<sup>25</sup> Technical dossier/Section II/Annex II-25-26.

The applicant submitted further data indicative for the stability of the chelate during storage.<sup>16</sup> Two batches of Availa®Cr were mixed mainly with calcium carbonate to provide a premixture containing 400 mg complexed Cr (as chromium DL-Met)/kg. Samples were stored in the dark at 37°C and 75% RH, and packaged with the original materials that simulate the packaging of the final product. Samples were assayed for complexed chromium(III) at the start of the trial and at periods of 3 months thereafter for a total of 24 months. The complexed chromium was determined by ICP-MS after precipitating free chromium by sodium carbonate. The intended chromium(III) concentration was analytically confirmed. Duplicate analysis of each batch at each point did not show a time-dependent change of the chromium(III) concentration over the total experimental period.

The stability of the additive in premixtures and feedingstuffs was not assessed.

The capacity of the additive to homogeneously distribute in feed was investigated analysing the chromium content in a mineral premixture and in a concentrate feed (10 subsamples each) in which Availa®Cr was supplemented. The coefficient of variation (CV) of total chromium concentration (mean 192.2 mg/kg) in a mineral premixture containing 176 mg supplemental Cr from Availa®Cr/kg was 8.8%.<sup>26</sup> The CV of a pelleted concentrate (supplemented with 30 mg Cr from Availa®Cr/kg) for dairy cows (mean chromium content: 28.3 mg/kg) was 6.1%.<sup>27</sup>

### 3.1.6. Physicochemical incompatibilities or interactions

No incompatibilities are expected due to the nature of the product.

### 3.1.7. Conditions of use

The additive is intended to be used in feed for dairy cows.<sup>28</sup> The applicant proposes its use at a minimum supplementation rate of 0.3 mg Cr/kg complete feed, up to a maximum of 0.5 mg Cr/kg complete feed (standard feed with 88% DM), corresponding to 300 and 500 mg Availa®Cr/kg complete feed, respectively.<sup>16</sup>

In practice the additive would be predominantly incorporated in complementary feed for dairy cows. Therefore, the applicant recommends a daily dose of 8 mg Cr from Availa®Cr/cow (derived from 0.06 mg Cr/kg body weight (bw)<sup>0.75</sup>, approximately 7.3 mg Cr per 600 kg cow and 8.2 mg Cr per 700 kg cow).<sup>29</sup>

The FEEDAP Panel notes that the proposed chromium supplementation rate from Availa®Cr is close to the background content of complete feeds (0.3–1.6 mg total Cr/kg complete feed: EFSA, 2009a).

## 3.2. Safety

Chromium chelate of methionine is expected to dissociate in the digestive tract into its two components, chromium under chromium(III) form and methionine under DL form (EFSA, 2009). Therefore, with regard to consumer's safety, the FEEDAP Panel retains chromium as the component of potential toxicological significance. Methionine from DL-Met would represent only a minor fraction of the amino acid present in animal feed; in addition methionine is a nutritional additive, already authorised under No 3c301 for all animal species by Regulation (EU) No 469/2013<sup>30</sup>, therefore, no data for consumer safety are required.

The general features of safety of chromium have been revised previously by the FEEDAP Panel (EFSA, 2009a).

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

The applicant provided the results of a literature search regarding absorption, distribution, metabolism and excretion (ADME) and toxicology of chromium(III) and chromium methionine.<sup>16</sup>

<sup>26</sup> Technical dossier/Section II/Annex II-27.

<sup>27</sup> Technical dossier/Section II/Annex II-28.

<sup>28</sup> Technical dossier/Supplementary information/October 2019. The applicant clarified that the target species is "dairy cows".

<sup>29</sup> Metabolic body weight.  $700^{0.75} = 136$ ;  $136 \text{ (kg metabolic bw)} \times 0.06 \text{ (recommended Cr in mg/kg metabolic bw per day)} = 8.2 \text{ mg Cr per 700 kg cow and day}$ . Considering the default values from the FEEDAP Guidance of safety for target species for body weight and feed intake of dairy cows, 8 mg per cow and day would correspond to 0.4 mg Cr/kg complete feed [= 8 mg Cr per cow and day/(20 kg feed dry matter/0.88 (for 12% moisture))].

<sup>30</sup> Commission Implementing Regulation (EU) No 469/2013 of 22 May 2013 concerning the authorisation of DL-methionine, DL-methionine sodium salt, hydroxy analogue of methionine, calcium salt of hydroxy analogue of methionine, isopropyl ester of hydroxy analogue of methionine, DL-methionine protected with copolymer vinylpyridine/styrene and DL-methionine protected with ethylcellulose as feed additives. OJ L 136, 23.5.2013, p. 1.

Absorption of chromium(III) from inorganic sources as well as from foods (basal content) is low ranging from 0.5% to 2–3% (EFSA, 2009a). In its opinion of 2014, the NDA Panel described 'In humans, absorption efficiency of supplemental chromium was reported to be between 0.1% and 5.2% (Donaldson and Barreras, 1966; Anderson et al., 1983; Offenbacher et al., 1986; Gargas et al., 1994; Kerger et al., 1996) and to vary depending on the chromium complex ingested (Kerger et al., 1996; DiSilvestro and Dy, 2007). Absorption of chromium(III) from food was estimated to range from 0.4% to 2.5% (EC, 2003), depending, among other factors, on the chemical properties of the ingested source and on the presence of other dietary components' (EFSA NDA Panel, 2014a).

According to Laschinsky et al. (2012),<sup>16</sup> the oral bioavailability of a range of organic chromium(III) supplements (Cr-picolinate, Cr-nicotinate, Cr-phenylalaninate, Cr-propionate) is in the same magnitude as for the inorganic compounds. According to the EFSA ANS Panel, the limited available data did not permit a definite conclusion on the bioavailability in humans of chromium from an organic source (chromium-enriched yeast) compared to an inorganic source such as chromium chloride, albeit bioavailability might be up to 10 times higher (EFSA ANS Panel, 2012a).

Upon absorption chromium binds to plasma proteins, such as transferrin, and is transported to the liver. In humans and rodents chromium concentrates in the liver, spleen, kidneys, testes, and bone (IOM, 2001).<sup>31</sup> Kinetics in the body of rats may follow a three-compartment model with half-lives of 0.5, 5.9 and 83 days (Mertz et al., 1965)<sup>32</sup>; tissues making up the deep compartment appear to include bone and testes (Borel and Anderson, 1984).<sup>33</sup> Deposition in muscle does occur as well, to a very variable extent: variations in feed composition and environment, as well as uncertainties due to analytical methods likely account for a large part of its variability (EFSA, 2009a and references therein). Data on chromium content in muscle of cattle fed unsupplemented diets are very limited; according to Chang et al. (1992),<sup>34</sup> the content of chromium in muscle of steers fed a diet containing 1.65 mg Cr/kg feed may be higher than 0.5 mg/kg and more than double than that in liver and kidney. Whereas most unabsorbed chromium is excreted through the faeces, urine is the main excretory route for absorbed chromium in both animals and humans: urinary chromium reflects the dietary chromium intake (EFSA, 2009a; EFSA ANS Panel, 2012a). Chromium is excreted in milk, according to Hayirly et al. (2001)<sup>35</sup>; the average concentration in unsupplemented dairy cows was 55 µg/L.

### 3.2.2. Toxicological studies

#### 3.2.2.1. Toxicological profile of chromium

The available toxicological studies on Cr(III) have been reviewed by the EFSA Panels FEEDAP (EFSA, 2009a) and CONTAM Panel (EFSA CONTAM Panel, 2014), concluding that toxicity of Cr(III) is much lower compared to the highly toxic and carcinogenic chromium(VI) and that chromium(III) is not carcinogenic upon dietary administration in rodent species.

In the opinion of the CONTAM Panel, reproductive and developmental toxicity was observed in some studies; in particular, among relevant studies, it was reported a decreased fertility in males mice at 5,000 mg CrCl<sub>3</sub>/L (246 mg Cr(III)/kg bw per day) (Elbetieha and Al-Hamood, 1997), while an increased number of resorptions and dead fetuses were observed in females mated with treated males. In addition, in female mice at doses ≥ 2,000 mg CrCl<sub>3</sub>/L (98 mg Cr(III)/kg bw per day), a decrease in the number of implantations and of viable fetuses and an increase in the number of resorptions in treated females (Elbetieha and Al-Hamood, 1997) were observed. Although methodological limitations prevented an adequate hazard characterisation, the CONTAM Panel considered that in the absence of adequate data, the results of the studies on reproductive and developmental toxicity must be taken into account as they identify potential adverse effects of oral chromium(III) exposure. From the studies—where effects on reproduction and development were observed—the lowest observed adverse effect levels (LOAELs) identified were in the order of 30 mg/kg bw per day (EFSA CONTAM Panel, 2014).

Chromium(III) is the proximate intracellular agent of chromium(VI) genotoxicity (EFSA 2009a; Macfie et al., 2010).<sup>16</sup> High concentrations of chromium(III) may increase oxidative stress and, consequently, oxidative DNA damage (Figgitt et al., 2010<sup>16</sup>; El-Yamani et al., 2011).<sup>16</sup> Gene mutation and DNA fragmentation are elicited in yeast models (Chatterjee and Luo, 2010<sup>16</sup>; Fang et al., 2014)<sup>16</sup>;

<sup>31</sup> Technical Dossier/Section III/Reference88.

<sup>32</sup> Technical Dossier/Section III/Reference18.

<sup>33</sup> Technical Dossier/Section III/Reference33.

<sup>34</sup> Technical Dossier/Section III/Reference68.

<sup>35</sup> Technical Dossier/Section III/Reference70.

interaction with DNA, rather than oxidative damage, may be the main mechanisms for Chromium(III) genotoxicity in yeast cells (Sobol and Schiestl, 2012).<sup>16</sup> Cytotoxicity and genotoxicity (as detected by comet and micronucleus assays) were elicited by chromium(III) alone and in combination with other trace elements (iron (Fe), molybdenum (Mo), nickel (Ni)) in cell lines derived from mouse fibroblasts (BALB/3T3) or human fetal hepatocytes (HEPG2): the complex interactions observed among the trace elements (Cr-Fe synergism, Cr-Mo and Cr-Ni antagonism) suggest modulation of redox metabolism and/or cell nutrition (Terpilowska and Siwicki, 2018).<sup>16</sup> On the other hand, no increase of genotoxic damage *in vivo* was elicited by chromium(III) propionate in rats given 1,000 mg Cr(III)/kg diet (equivalent to at least 50 mg Cr/kg bw per day) for 4 weeks; genotoxicity was measured by comet assay on peripheral blood lymphocytes; the chromium(III) exposed animals showed no signs of general toxicity (mortality, weight gain, feed consumption, liver and kidney histopathology) (Staniek et al., 2010).<sup>16</sup>

In its opinion on chromium in food and drinking water, the EFSA CONTAM Panel reviewed the available literature on genotoxicity of chromium(III) compounds concluding that the information assessed 'provides conflicting information regarding their genotoxicity but in general they gave largely negative results in bacterial assays and mixed, often positive, results in mammalian cell assays (although often at cytotoxic doses)'. Regarding the *in vivo* genotoxicity studies,<sup>36</sup> the CONTAM Panel pointed out that '*In vivo* tests for genotoxicity were all negative with one exception of a non standard assay (i.e. p(un) reversion assay in mice)'. The genotoxic potential of chromium(III) is exerted, at least in part, through the generation of oxygen radicals as indicated by the counteracting effect of antioxidants as well as the induction of oxidised bases observed in *in vitro* and *in vivo* studies. The CONTAM Panel summarised that chromium(III) compounds have the potential to react with DNA in acellular systems, however, restricted cellular access limits or prevents genotoxicity; the Panel also highlighted that generally chromium(III) compounds are poorly absorbed (EFSA CONTAM Panel, 2014).

The applicant provided more information about the adverse effects of chromium(III) resulting from its biological activities, including endocrine effects.<sup>16</sup> The data were obtained from a literature search, and are summarised as follows. By binding to transferrin, chromium may compete with iron storage and transport; although binding to transferrin may be a protective effect towards intracellular chromium(III) genotoxicity (Levina et al., 2016),<sup>16</sup> adverse interactions on iron have been previously observed *in vivo* in rodents treated with high doses (EFSA, 2009a). In pregnant Wistar rats exposed to 100 mg Cr from chromium propionate/kg diet (corresponding to 7.2 mg/kg bw), no maternal toxicity nor teratogenicity were observed: fetal liver zinc levels increased (by 181%) and fetal kidney copper levels decreased (by 34%); in the dams liver copper and zinc level decreased by 9% and 12%, respectively. The potential for long-term adverse effects of a modulation of the Zn-Cu balance during development cannot be ascertained, although it may not be ruled out completely (Staniek and Krejpcio, 2009). In its assessment on cellular bound chromium yeast as source of chromium(III), the EFSA ANS Panel (2012a) considered that the main biological effects of chromium(III) are endocrine (insulin-mediated) and metabolic modulation of glucose and lipid metabolism; the ANS Panel concluded that the available studies in humans do not allow to identify a safe dose for such effects.

### 3.2.2.2. Genotoxicity studies with chromium DL-Methionine

Four genotoxicity tests were provided by the applicant. Three of them were already reviewed in the previous FEEDAP opinion, namely: bacterial reverse mutation assay (according to OECD Guideline No. 471),<sup>37</sup> *in vitro* mammalian cell gene mutation test (according to OECD Guideline No. 476)<sup>38</sup> and *in vivo* mouse bone marrow erythrocyte micronucleus test (according to OECD Guideline No. 474)<sup>39</sup>; these three tests were considered negative (EFSA, 2009a).

The new test is an *in vitro* mammalian cell micronucleus test (according to OECD Guideline No. 487).<sup>40</sup> Following a preliminary cytotoxicity assay, the test item (called in the study report chromium DL-Met with 5.7% chromium) was tested on human peripheral lymphocytes at concentrations ranging 625–5,000 µg/mL with and without S9-mix; the substance was tested in parallel upon 24-h exposure

<sup>36</sup> The *in vivo* relevant studies were tabled in the EFSA CONTAM Opinion and were the following: a rat micronuclei in bone marrow erythrocytes test with chromium picolinate, a mouse micronuclei in peripheral blood with chromium picolinate monohydrate, a mouse in bone marrow and peripheral blood cells with chromium potassium sulfate, a rat micronuclei in bone marrow cells with chromium picolinate and a mouse DNA deletions (p(un) reversion assay) in developing embryos with chromium chloride.

<sup>37</sup> Technical Dossier/Section III/Annex III-3-2.

<sup>38</sup> Technical Dossier/Section III/Annex III-3-5.

<sup>39</sup> Technical Dossier/Section III/Annex III-3-4.

<sup>40</sup> Technical Dossier/Section III/Annex III-3-3.

and upon 4-h exposure followed by a 24-h recovery. Mitomycin, cyclophosphamide and griseofulvin were used as positive controls. Chromium DL-Met induced statistically significant increases in the number of binucleated micronucleated cells, either in the 24-h treatment without metabolic activation (at 2,500 µg/mL, the highest concentration tested) or in the short-term treatment without metabolic activation (at 2,500 but not at 5,000 µg/mL); the other treatment schedules showed negative results. A confirming assay was performed, using a narrow range of concentrations (2,500, 3,200 and 4,000 µg/mL) and a 24-h exposure without metabolic activation; a significant increase in the number of micronucleated cells was observed at the highest concentration, together with significant cytotoxicity. The FEEDAP Panel concludes that the test item induced chromosomal damage under the experimental conditions applied in this study.

Based on the opinion of the scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment from the EFSA Scientific Committee, to select the appropriate *in vivo* follow-up of positive *in vitro* results, the *in vitro* micronucleus test should be combined with kinetochore staining or fluorescence *in situ* hybridisation (FISH) in order to gain additional mechanistic information and cover with the *in vivo* study the same endpoint that was found to be positive *in vitro* (EFSA Scientific Committee, 2011). Data provided by the applicant lack of these information. Indeed, the *in vivo* mouse bone marrow erythrocyte micronucleus test – evaluated previously by the FEEDAP Panel (EFSA, 2009a) – was conducted before the new *in vitro* micronucleus test. That *in vivo* study was performed with the test item (chromium DL-Met with 5.7% chromium) administered by gavage at 500, 1,000 and 2,000 mg/kg bw. Doses were selected on the basis of a preliminary dose finding study showing no signs of toxicity up to the top dose recommended (based on the OECD Guideline No. 474). Two thousand polychromatic erythrocytes were scored for each animal for the analysis of micronuclei. Positive and negative control values of micronucleus frequency were within the historical control ranges of the laboratory confirming the sensitivity of the assay. No evidence of bone marrow exposure was observed. The frequency of micronuclei was comparable between treated and negative control groups.

The FEEDAP Panel concludes that the genotoxic activity of chromium DL-Met observed *in vitro* is not expressed *in vivo* after systemic exposure up to 2,000 mg/kg bw. However, the usefulness of the micronucleus test in the follow-up of *in vitro* positive results is limited when there is no evidence of bone marrow exposure; in this case the genotoxic effects at other potentially relevant targets, such as the site of first contact, should be evaluated (EFSA Scientific Committee, 2011). Since these data are not available, the potential genotoxic effects of chromium DL-Met at the site of first contact cannot be assessed.

### 3.2.2.3. Subchronic toxicity study with chromium DL-Methionine

The applicant submitted a subchronic toxicity study that was assessed by the FEEDAP Panel in 2009 in the former Availa®Cr application. The FEEDAP Panel confirms the conclusion drawn in the previous opinion: 'the NOAEL is 630 and 715 mg test item/kg bw day for males and females, respectively, corresponding to 34 and 39 mg Cr(III)/kg bw and day' (EFSA, 2009a).

### 3.2.2.4. Conclusion on the toxicological studies

Chromium DL-Met shows a genotoxic activity *in vitro* that is not expressed *in vivo* after systemic exposure. Although no data on genotoxicity at the site of contact are available, the Panel notes that chromium(III) is not carcinogenic and concludes that chromium DL-Met is unlikely to pose a carcinogenic risk at levels occurring in the diet.

## 3.2.3. Safety for the target species

### 3.2.3.1. Tolerance study

The applicant provided a study to determine the tolerance of dairy cows to Availa®Cr at different doses, with a duration of 56 days.<sup>41,42</sup>

A total of 60 dairy cows (Holstein; primiparous and multiparous) were randomly allocated to four groups. The cows were housed in one pen with 15 animals per group. They were given a total mixed ration (TMR) based on corn, barley and soybean meal containing in the dry matter 15% crude protein and 6.6 MJ NE<sub>L</sub>/kg for *ad libitum* access. The control group did not receive supplemental chromium.

<sup>41</sup> Technical dossier/Section II/Annex III\_3\_1.

<sup>42</sup> The study report mentions 56 days. But with study start 18.2.2016 and study completion at 15.4.2016, study duration would be 57 days, the statistical output counts with 58 days.

The use level group was provided with 8 mg Cr from Availa®Cr per cow and day. Multi-fold doses corresponded to the 5× (intermediate level, 40 mg/day) and 10× (high level, 80 mg/day) of the use level. All animals were fed a basal pelleted feed. Study duration was 56 days.

All cows received twice a day 750 g of a concentrate (background content: 0.36 mg Cr/kg) in the milking parlour. The daily dose of chromium was achieved by varying the quantity of supplemented concentrate in the total concentrate and the chromium concentration of the supplemented concentrate as shown in Table 1. The intended chromium doses were analytically confirmed (see Table 1).

Numerical data to characterise the experimental groups and to demonstrate their uniformity at the

**Table 1:** Doses of chromium used in the tolerance study with dairy cows

Group	Supplemented concentrate			Un-supplemented concentrate (g/day)	Daily Cr from Availa®Cr mg/cow	
	(g/day)	mg Cr from Availa®Cr/kg concentrate			Intended	Analysed*
		Intended	Analysed <sup>(*)</sup>			
Control		0		1,500	0	
Use level	400	20	19.2	1,100	8	7.7
Intermediate level	750	53	60.6	750	40	45.5
High level	1,500	53	60.6	0	80	90.9

(\*): Total chromium: chromium from Availa®Cr plus background chromium (0.36 mg Cr/kg).

study start (i.e. milk yield before study start, parity, days in milk) were not given.

Body weight and milk production were measured daily. Data on feed intake (from TMR) and milk composition (fat, protein and lactose) were not provided. Blood was collected from eight cows per treatment on days 0, 28 and 56 for analysis of haematology<sup>43</sup> and biochemistry<sup>44</sup> parameters.

Statistical analyses were performed with a mixed-effects model that accounted for the fixed effects of treatment, day, parity (primiparous or multiparous) and their two- and three-way interactions, plus days in milk at the start as covariate, and the random effect of cow. Day entered the model as a repeated measure assuming a compound symmetry structure for the variance-covariance matrix. The three-way interaction was not significant for any of the variables and was removed from the final model. The FEEDAP Panel notes that in this statistical analysis, particularly for body weight and milk yield, although the cow was taken as experimental unit, all the daily records of each cow are used for the calculation of the denominator degrees of freedom. Since this analysis would increase the probability of an error 1 (a difference to reach significance, i.e. falsely positive results; in case of a tolerance study falsely adverse effects), it could be accepted for a tolerance study (where equivalence is to be demonstrated), when no significant adverse effects were seen.

Daily veterinary inspections did not identify any major finding regarding health condition; only few incidences were reported (mainly mastitis) and two animals of the control group were removed from the study (one due to mastitis and the other one for reproductive problems).

Overall body weight was unaffected by the treatment. The mean body weight per cow for each experimental group at start/end was: 647/649 kg for the unsupplemented control group, 660/666 kg for the 8 mg Cr/cow and day group, 656/654 kg for the 40 mg Cr/cow and day group and 641/640 kg for the 80 mg Cr/cow and day group.

Milk yield was not significantly affected by the treatment (mean values of 31.2, 30.4, 29.4 and 29.9 kg/day for the control, use level, intermediate and high chromium level groups, respectively). The starting yield (3-day average) of the groups was 31.2, 30.6, 29.7 and 29.9, yield at the end 32.1, 30.9, 29.2 and 29.9 kg/day for the control, use level, intermediate and high chromium level groups, respectively. The comparison shows that there was a slight increase in milk yield over time with the exception of the intermediate level group, confirming no significant treatment effects.

<sup>43</sup> Red blood cell count (RBC), platelets, mean platelet volume (MPV), mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), leukocytes, lymphocytes, eosinophils, basophils, neutrophils, monocytes.

<sup>44</sup> Alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine transferase (ALAT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), calcium, phosphorus, magnesium, sodium, potassium, chloride, cholesterol, lactic acid, albumin, total protein, urea, creatinine, non-essential fatty acids (NEFA), chromium, glucose, insulin.

The tabulated values for haematology and blood biochemistry are averages from three time points, at study start, after 28 and 56 days. They do not clearly reflect a potential influence of the treatment since they all are influenced by the starting point which should be expectedly equal in all groups, but may (as was shown only for some endpoints) be different between the groups (as it could be derived from figures of the study report given for some endpoints). However, most of the mean endpoints were not different among the groups; significant differences were detected for leucocytes, albumin and chlorine, all not dose-related and therefore not considered treatment related.

A dose-related increase was reported for cholesterol and creatinine (mean values for cholesterol 147.6, 165.9, 168.4 and 187.9 mg/dL and mean values for creatinine 0.75, 0.79, 0.85 and 0.83 mg/dL for the control group, the groups with 8, 40 and 80 mg Cr/cow and day, respectively). Cows supplemented with 80 mg Cr/d had higher ( $p < 0.05$ ) serum cholesterol than cows supplemented 8 and 40 mg Cr/day. Feeding dairy cows at the recommended level (8 mg Cr/day) did not affect ( $p > 0.05$ ) serum creatinine concentration. The intermediate and high-level groups (40 or 80 mg Cr/day) were reported to show increased ( $p < 0.05$ ) serum creatinine as compared to the control cows or cows fed 8 mg Cr/day. The applicant was requested to explain the biological significance of these increases, supported by literature. A literature review was performed, the results are summarised as footnotes.<sup>16,45</sup> The outcome for both endpoints, cholesterol and creatinine, was that all values were within the physiological range reported in the literature for dairy cows.

The FEEDAP Panel notes that the initial values of the four experimental groups of eight cows showed the same tendency for differences as the mean values reported.<sup>46</sup> It can therefore be concluded that cholesterol and creatinine were not influenced by chromium from Availa®Cr in dairy cows.

The study presents significant weaknesses in design (e.g. measurement of individual feed intake not foreseen, no replicates in the treatments) and reporting (e.g. milk composition).

### 3.2.3.2. Toxicological study with laboratory animals

The Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a,b,c), currently in force, offers alternatives to the tolerance study. It is stated there that for all additives with the exception of viable microorganisms, safety for target animals can be derived from toxicological studies with oral administration in laboratory animals. These data should allow establishing a lowest no observed adverse effect level (NOAEL).

Taking 34 mg Cr/kg bw per day as the NOAEL (see Section 3.2.2.3 Subchronic toxicity study with Chromium DL-Met), the highest safe chromium concentration in complete feed for dairy cows would be 9.7 mg Cr/kg.<sup>47</sup> The maximum recommended use level (0.5 mg Cr/kg complete feed) is about 19 times lower, indicating a margin of safety of about 19.

### 3.2.3.3. Conclusion on the safety of Availa®Cr for dairy cows

Although in the tolerance study with dairy cows no adverse effects were seen with respect to body weight, milk yield, haematology and blood biochemistry at use level (8 mg Cr/cow and day) and the 5- and 10-fold overdose of chromium from Availa®Cr, the FEEDAP Panel considers that this study provides only supportive evidence on the safety of the additive, owing to the significant deficiencies in design and reporting.

<sup>45</sup> Human and animals responded to supplementing chromium in diets on plasma/serum cholesterol in three different ways: decrease (1 human study, 12 poultry studies, 2 pig studies, and 2 cattle studies), no response (2 human studies, 5 poultry studies, 4 pig studies and 5 cattle studies), or increase (4 pig studies). It implicates that the effect of supplementation of chromium may interact with other factors, such as stress, environmental conditions etc. The serum cholesterol concentrations of dairy cows in the tolerance study are all within the normal range (35.90–230 mg/dL for dairy cows) as indicated by the literature.

Three studies (1 poultry study and 2 pig studies) showed that supplemental chromium increased plasma/serum creatinine concentration. Other five studies (2 poultry studies, 1 pig study, and 2 dairy cow studies) did not observe a response on plasma/serum creatinine concentration with chromium supplementation. Chromium supplementation at tolerance levels may increase chromium accumulation in kidney and further cause functional dysregulation and/or altered structure. Serum creatinine levels are commonly used as a fairly reliable indicator of renal function. However, serum creatinine concentrations of treated dairy cows in the tolerance study are in the normal range (0.70–1.11 mg/dL) as reported in the literature for dairy cows.

<sup>46</sup> The initial values for cholesterol in the control, the use level and the 5- and 10-fold chromium level groups were for cholesterol: 149.7, 165.2, 171.8 and 190.6 mg/dL, the values after 56 days were 146.0, 166.5, 165.2 and 179.1. The values for creatinine were 0.711, 0.766, 0.844 and 0.808 mg/dL at start and after 56 days 0.788, 0.821, 0.865 and 0.854, respectively.

<sup>47</sup> 9.7 mg Cr/kg complete feed; calculated according to FEEDAP Guidance on Safety for Target Animals (2017) and applying an uncertainty factor of 100 [ $34/100/31 \times 1,000 \times 0.88$ ].

On the other hand, a safe level in complete feed of dairy cows could be derived from a toxicological study with laboratory rodents showing an NOAEL of 34 mg Cr from Chromium DL-Met/kg bw per day; the safe level for dairy cows would be 9.7 mg supplemental Cr/kg complete feed. The maximum recommended use level (0.5 mg Cr/kg complete feed) would provide a margin of safety of about 19.

Including the background chromium in feed (approximately the same magnitude as for the supplemental chromium from Availa®Cr) and presuming the same qualitative and quantitative bioavailability as shown for the additive, the exposure of the animal would increase by about 100%, being at least 10 times lower than the safe concentration.

Taking all together, the FEEDAP Panel considers the maximum recommended use level for dairy cows (8 mg Cr from the additive Availa®Cr/cow per day) as safe for the target animal. This would correspond to about 0.4 mg Cr/kg complete feed.<sup>48</sup>

### 3.2.4. Safety for the consumer

Absorption, distribution, metabolism and excretion (ADME) and toxicological studies have been considered in Sections 3.2.1 and 3.2.2, respectively.

#### 3.2.4.1. Residue studies

##### *Experimental study in dairy cows*

A residue study in milk and tissues conducted with dairy cows was provided in the technical dossier.<sup>49</sup>

The test article, called in the report 'Chromium DL methionine', consisted of chromium DL methionine. It contained 3.55% chromium (analysed by high resolution ICP-MS). It was applied after dilutions with maltodextrin in a gelatine capsule by gavage directly into the rumen.

A total of 107 Holstein cows, with parity between 2 and 5 and a body weight between 612 and 816 kg were obtained from one commercial dairy farm. At assignment to study, all cows were pregnant with a single fetus; they had four functional quarters of the mammary gland with no recent history of chronic or repeated mastitis.

Cows were assigned to one of four groups: the control group without the test article and three groups treated with 0.03, 0.06 and 0.12 mg chromium (Cr)/kg bw<sup>0.75</sup> per day; these doses corresponded to 3.45–4.55, 6.90–9.10 and 13.80–18.20 mg Cr per cow and day. The daily dose of chromium was confirmed by analysis of the gelatine capsules.

The study was blinded and performed in a randomised block design with the cow as the experimental unit. Since only 40 individual pens were available at the same time in the experimental facility, the study was conducted in three consecutive runs. Nine blocks of four animals each were used for each run. Within the runs, the cows were blocked by expected calving date and location.

Cows received treatments from 28 ± 2 days prior to expected calving through 28 ± 2 days postpartum. In the late gestation period, the amount of test article given to cows was based upon the cow's body weight at treatment start. Dosing in the lactation period was based upon the cow's body weight on day 1 ± 1 postpartum. The test article was administered to cows about one hour prior to morning feeding.

Diets fed during late gestation and early lactation were formulated to meet or exceed the nutrient requirements of the cows for the respective life cycle stage, with the exception that the diet fed during early lactation did not meet the energy requirements of the early lactation cow. It was expected that cows in early lactation would be in negative energy balance as they are typically in most commercial settings. Cows received the dry cow diet from 28 ± 2 days prepartum through calving and then began receiving the lactation diet the day of parturition. Throughout the study, cows had ad libitum access to feed and water.

Chromium content in milk (samples collected from the PM milking at 7, 14, 21 and 28 day postpartum) and in tissues<sup>50</sup> from the first 20 cows (and their progeny killed immediately after parturition) that completed the study from the control group and the two groups treated with the intermediate and the high doses were measured.

<sup>48</sup> Considering the default values from the FEEDAP Guidance of safety for target species for body weight and feed intake of dairy cows, 8 mg per cow and day would correspond to 0.4 mg Cr/kg complete feed [= 8 mg Cr per cow and day/(20 kg feed dry matter/0.88 (for 12% moisture))].

<sup>49</sup> Technical Dossier/Supplementary information/April 2019/Annex 9.

<sup>50</sup> Kidney, liver, abdominal fat, semitendinosus muscle.



No treatment group-related differences were observed in dry matter intake, milk yield and quality.

No treatment group-related differences were observed in milk chromium concentration. The LOQ for tissues and milk was 0.025 mg/kg. All chromium concentrations in milk were below LOQ.

Concerning chromium deposition in tissues of newborn calves, treatment-related differences were not seen. However, equality of chromium in liver was rejected ( $p < 0.03$ ), chromium in the control livers being higher than in the chromium treated groups. All results were expressed as proportions of findings above the LOQ, most of the results (about 90%) were below LOQ. The results are not considered in more detail since they are not relevant for food of animal origin and therefore for consumer exposure.

No significant differences between the groups were observed in the proportions of chromium concentrations above the LOQ and chromium concentration in cow fat, liver and muscle tissues; significance of equality between the three groups was for fat 1.00, for liver  $< 0.15$  and for muscle  $< 0.35$ . Concerning fat, only one sample of the intermediate dose group showed a chromium deposition above LOQ (0.026 mg/kg). For liver, the proportions of chromium concentrations above the LOQ were 2/20, 4/20 and 0/20 for the control, the intermediate and the high-level group, respectively; mean chromium concentration of the two control livers was 0.030 mg/kg, of the four intermediate group samples 0.054 mg/kg. For muscle tissues, the proportions of chromium concentrations above the LOQ were 2/20, 3/20 and 0/20 for the control, the intermediate and the high-level group, respectively; mean chromium concentration of the two control muscle tissues was 0.144 mg/kg, of the three intermediate group samples 0.083 mg/kg.

Treatment group-related differences were observed in proportions of chromium concentrations above the LOQ in cow kidney. The proportions of chromium concentrations above LOQ were 0/20, 4/20 and 7/20 for control, intermediate and high-level groups, respectively; equality was rejected. The means of values of chromium concentration above LOQ were 0.076 and 0.035 mg/kg for the intermediate and high-level groups, respectively; the two means were not significantly different.

Among the tissues and products of dairy cows examined, only kidneys showed a dose-dependent increase in chromium deposition. For muscle, liver and fat, no chromium concentrations above LOQ was found in the high-dose group; in a few cases, chromium levels above the LOQ were detected in liver and muscle of the unsupplemented control and the intermediate dose group without a significant difference. All milk samples throughout the study groups were below the LOQ (0.025 mg/kg).

It is concluded that the use of chromium DL-Met in dairy cows at the recommended level (8 mg Cr/cow and day, corresponding to the intermediate dose level of the above study: 0.06 mg Cr/kg  $bw^{0.75}$ ) will not essentially increase the chromium content of milk and edible tissues, except kidney. A quantification of any potential increase is not possible due to the relatively high LOQ, preventing a sufficient number of absolute residue figures.

#### *Literature studies*

The applicant provided some literature studies on chromium residues; those relevant to the current application are summarised below.

In the study of Chang et al. (1992), steers were fed diets with supplemental chromium at the level of 0 or 0.2 mg (from chromium yeast)/kg DM, during 138 days; the authors did not find an increase in chromium in any of the tissues and organs (liver, kidney, fat and muscle) analysed. However, the chromium background of the diet was already one order of magnitude higher than the supplementation level (1.87 mg/kg DM).

Spears et al. (2004)<sup>51</sup> performed three experiments in which residues of chromium in bovine were measured following a chromium supplementation. In the first experiment, steers were supplemented with 0, 0.4 or 0.8 mg Cr (from chromium nicotinic acid or chromium chloride)/kg DM feed during 146 days; chromium content in liver (average 11.4  $\mu\text{g}/\text{kg DM}$ ) and kidney (average 20.6  $\mu\text{g}/\text{kg DM}$ ) was not affected by treatment. In the second experiment, steers were fed diets containing 0–0.8 mg Cr (from Chromium-L-Methionine) during 125 days; control steers had higher chromium concentration in muscle than supplemented animals. In the third experiment, the authors daily supplemented cows starting at 35 days pre-partum with 0 or 0.6 mg Cr (from chromium-Methionine) via a gelatine capsule/day; new-born calves from supplemented cows had higher chromium in semitendinosus muscle than those from control, while the chromium content in the organs of calves (liver, heart, kidney) was unaffected, as it was the chromium content of the tissues of the same cows. Chromium background of the diet was not reported in any of the experiments described.

<sup>51</sup> Technical Dossier/Section III/Reference69.

Hayirli et al. (2001) studied the effect of supplemental chromium methionine on milk deposition. Cows were 0, 0.03, 0.06 and 0.12 mg of Cr as Cr-Met/kg of  $bw^{0.75}$  from 28 d before expected calving date through 28 days of lactation; the supplementation did not influence milk chromium concentration. The average concentration in unsupplemented dairy cows was 55  $\mu\text{g/L}$ . Chromium background of the diet was not reported.

These literature studies support that feed supplementation of chromium methionine at levels up to 0.8 mg/kg DM to cows does not exert an effect in deposition of chromium in milk and tissues of cows and in the organs of new-born calves; an increase was only seen in muscle of new-born calves from cows supplemented with 0.6 mg/kg DM in the pre-partum period. The FEEDAP Panel notes that the absence of information of the chromium background in two of the studies reviewed and the use of a very low chromium supplementation compared to the background content of the diet in the other study, introduce a high uncertainty in the evaluation of these studies.

### 3.2.4.2. Consumer exposure

The EFSA CONTAM Panel estimated the chronic dietary exposure to chromium(III) using dietary surveys from 17 EU countries (EFSA CONTAM Panel, 2014). Overall mean human chronic dietary exposure ranged from a minimum lower bound (LB) of 0.6 to a maximum upper bound (UB) of 5.9  $\mu\text{g/kg}$  bw per day. The 95<sup>th</sup> percentile dietary exposure values ranged from 1.1 (minimum LB) to 9.0 (maximum UB)  $\mu\text{g/kg}$  bw per day, with higher values in the lower age groups (EFSA CONTAM Panel, 2014).

Based on the results of an appropriate residue study in milk and tissues conducted with dairy cows, the FEEDAP Panel concludes that the use of chromium DL-Met in feed of dairy cows up to maximum recommended level of 0.5 mg Cr(III) from Availa®Cr/kg feed would not measurably increase consumer exposure to trivalent chromium.

### 3.2.4.3. Conclusions on safety for the consumer

The use of chromium DL-Met given as Availa®Cr in feed of dairy cows up to the recommended highest supplementation level would not measurably increase consumer exposure to trivalent chromium; therefore, the FEEDAP Panel concludes that the additive up to maximum recommended level of 0.5 mg Cr(III) from Availa®Cr per kg complete feed for dairy cows does not pose any concern to the safety of consumers.

## 3.2.5. Safety for user

### 3.2.5.1. Effects on the respiratory system

Occupational studies indicate that the occupational exposure to inorganic chromium(III) can be associated with increased genotoxic damage in the upper respiratory tract (EFSA, 2009a). Very recently, the American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a new threshold limit value (TLV) of 0.003  $\text{mg/m}^3$  for inhalable inorganic chromium(III) compounds (ACGIH, 2019). No specific limit is set for chromium DL methionine.

The dusting potential of the additive is high (up to 5.6  $\text{g/m}^3$ ) with about 96% of particles below 50  $\mu\text{m}$ ; the chromium content of the dust averages 6.7  $\text{mg/kg}$  dust. Therefore, the FEEDAP Panel considers that the thoracic fraction (< 50  $\mu\text{m}$ ) of the additive should be considered for risk assessment.

Based on dusting potential, the chromium content of the dust, the percentage of particles of inhalable size, the estimated exposure of users would be 0.04  $\text{mg Cr/m}^3$ , one order of magnitude greater than the TLV. TLVs are aimed to industrial exposure scenarios where the exposure is expected to be more prolonged and intense than that of feed additives. Nevertheless, the FEEDAP Panel considers that exceedance of the TLV by one order of magnitude or more indicates a risk by inhalation.

The average nickel content of three batches was 1.25  $\text{mg/kg}$  (range 1.18–1.31). Assuming that these values reflect the nickel content of the dust, the resulting inhalable nickel in the dust would be 0.007  $\text{mg/m}^3$ , lower than the proposed occupational exposure limit (OEL) for the inhalable fraction of water soluble nickel (0.01  $\text{mg Ni/m}^3$ ; European Commission, 2011).

### 3.2.5.2. Effects on the eyes and skin

The potential of skin irritancy of Availa®Cr was tested in a good laboratory practice (GLP) study performed according to OECD Guideline 404 with New Zealand White rabbit<sup>52</sup>; the additive was found to be non-irritant for the skin.

<sup>52</sup> Technical dossier/Section II/Annex III\_3\_7.

The potential of eye irritancy of Availa®Cr was tested in a GLP study performed according to OECD Guideline 405 with New Zealand White rabbit<sup>53</sup>; the additive was found to be non-irritant for the eye.

The skin sensitisation potential of Availa®Cr was tested in a GLP study performed according to OECD Guideline 406 in guinea pigs (Magnusson and Kligman maximisation test)<sup>54</sup>; the test did not provide evidence of skin sensitisation by the additive. However, in particular due to its nickel content, the additive should be considered as a potential skin sensitiser in humans.

### 3.2.5.3. Conclusions on safety for the user

The additive poses a risk to users by inhalation. It is not an irritant for skin and eyes. The additive should be considered a skin sensitiser.

### 3.2.6. Safety for the environment

Chromium, as a natural element, is ubiquitous in the environment, occurring in many oxidation states. Chromium(III) is the predominant naturally occurring form. It is a natural constituent of many rocks, soils, sediments and waters, and the atmosphere. A concern for the environment is not expected from the use of Chromium(III) as a feed additive as already highlighted in a previous opinion of FEEDAP Panel (EFSA, 2009a). The chlorine content of the additive does not require any consideration when comparing its contribution to the total chlorine content of a complete feed.

Methionine as amino acid is a physiological and natural component in animals and plants. After dissociation of the ingested additive, methionine is not excreted as such (but as urea/uric acid, sulfate and carbon dioxide). Therefore, the use of Availa®Cr in animal nutrition would not lead to any localised increase in the concentration of methionine in the environment.

#### 3.2.6.1. Conclusions on safety for the environment

The use of the additive in animal nutrition is not expected to pose a risk to the environment.

### 3.3. Efficacy

The applicant provided three studies to support efficacy of the additive (study 1, 2 and 3 described below): studies 1 and 2 were conducted in the EU, and study 3 in a non-EU country.

#### 3.3.1. Study 1

The study evaluated the efficacy of Availa®Cr in dairy cows at a commercial dose by measuring milk yield.<sup>55</sup> The administration of Availa®Cr started at least 18 days before expected calving and was maintained during the first 96 days of lactation.<sup>56</sup>

In this study, feed intake was not measured. Owing to the lack of these data, the actual supplementation of AvailaCr could not be calculated for its compliance with the minimum recommended dose (0.3 mg/kg complete feed). Consequently, the study was not further considered for efficacy assessment.

#### 3.3.2. Study 2

The study evaluated the efficacy of Availa®Cr in dairy cows at a commercial dose by measuring milk yield. The administration of Availa®Cr started at least 18 days before expected calving and was maintained during the first 90 days of lactation.<sup>57</sup>

Fifty Holstein multiparous cows were distributed in two experimental groups. The study followed a randomised design with two treatment groups: Control (no additive) and Availa®Cr (8 mg of Cr/cow per day).<sup>58</sup> This dose would correspond to an estimated concentration of additive in complete feed of 300 mg Availa®Cr/kg (0.3 mg Cr/kg complete feed) when given to a cow of 680 kg bw assuming a daily feed intake of 23 kg DM. The cows were randomly allocated to one of the experimental groups. Information was provided on the average performance of the cows during the previous lactation (7,820 vs. 7,910 kg of total milk production in 305 day lactation and 29.3 vs. 29.3 kg/day milk yield on

<sup>53</sup> Technical dossier/Section II/Annex III\_3\_8.

<sup>54</sup> Technical Dossier/Supplementary information/April 2019/Annex 10.

<sup>55</sup> Technical dossier/Section II/Annex IV\_4\_1.

<sup>56</sup> Obtained from a premix containing 27 mg Cr from Availa®Cr/kg; the Availa®Cr used contained 0.1% chromium.

<sup>57</sup> Technical dossier/Section II/Annex IV\_4\_2.

<sup>58</sup> Obtained from a premix containing 17.8–19.2 mg Cr from Availa®Cr/kg; the Availa®Cr used contained 0.1% chromium.

average for the control and treated groups, respectively). There were no significant differences in the milk production during the previous lactation, although these data were not used for the allocation of cows to treatments. All cows received a TMR formulated for high-yielding dairy cows offered ad libitum. Cows had electronic ear tags to monitor individual feed consumption. Cows were milked twice daily in a rotary parlour, where the cows included in the study received individually 200 g of a premix at each milking (400 g/day). The premix was ground wheat with no additive (analysed concentration of 0.25–0.36 mg Cr/kg) for the Control cows, or with Availa®Cr added at 20 g per kg wheat for the treated cows, to reach a concentration of 20 mg Cr/kg premix. The analysed concentrations of chromium in the additive and in the premix were 1162 mg Cr/kg and 18–19 mg Cr/kg, respectively.

During the 90 days of lactation, individual feed intake, body weight, milk yield and composition (fat and protein) were recorded daily after calving and health status was monitored. Body condition scores were assessed at days –21, 30, 60 and 84 relative to calving, although these data were not reported.

**Table 2:** Effect of Availa®Cr on milk yield, composition and cow's body weight (first 90 days of lactation)

Treatment	Initial BW (kg)	Final BW (kg)	Dry matter intake (kg DMI/day)	Milk yield (kg/day)	Milk fat (%)	Milk protein (%)	Milk/kg DMI <sup>(2)</sup>
Control	737	632	20.5	39.8	3.89	3.13	2.00
Availa®Cr <sup>(1)</sup>	708	628	21.7	43.1*	3.86	3.14	2.04

(1): 8 mg Cr/cow per day.

(2): DMI: Dry matter intake.

(\*): significantly different from control at  $p \leq 0.05$ .

Main results are shown in Table 2. Body weight, overall, was not affected by treatment. Milk yield was significantly greater in cows supplemented with Availa®Cr compared with Control and milk components were unaffected. Feed efficiency was also not affected by treatments.

Supplementation of Availa®Cr before and after calving had a significant positive effect on milk production (more than 3 kg/day) with no changes in milk components and feed efficiency.

### 3.3.3. Study 3

The objective of this study was to determine the effect of Availa®Cr during the dry period and early lactation on milk production, milk composition (fat, protein and SCC) and health parameters of a cohort group of dairy cows.<sup>59</sup> The administration of Availa®Cr started between 18 and 28 days before expected calving and was maintained during at least 85 days of lactation.

Milk production and composition were recorded only for 78 days (from 7 to 85 DIM). Additionally, feed intake was not measure in this study. Therefore, this study was not further considered for the assessment of efficacy.

### 3.3.4. Conclusions on efficacy

Only one study could be considered for the assessment of efficacy. This study showed that supplementation of Availa®Cr before and after calving had a significant positive effect on milk production with no changes in milk components and feed efficiency. Since a total of three studies are required indicating a positive effect on efficacy, the FEEDAP Panel is not in a position to conclude on the efficacy of chromium DL-Met from Availa®Cr.

## 3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>60</sup> and Good Manufacturing Practice.

<sup>59</sup> Technical dossier/Section II/Annex IV\_4\_3.

<sup>60</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

## 4. Conclusions

Although in the tolerance study with dairy cows no adverse effects were seen with respect to body weight, milk yield, haematology and blood biochemistry at use level (8 mg Cr/cow and day) and the 5- and 10-fold overdose of chromium from Availa®Cr, the FEEDAP Panel considers that this study provides only supportive evidence on the safety of the additive, owing to the significant deficiencies in design and reporting.

On the other hand, a safe level in complete feed for dairy cows of 9.7 mg supplemental Cr/kg feed could be derived from a toxicological study with laboratory rodents showing a NOAEL of 34 mg Cr from chromium DL-Met/kg body weight per day; considering also the background chromium in feed the exposure of the animal would be at least ten times lower than the safe dietary chromium concentration.

Taking all together, the FEEDAP Panel considers the maximum recommended use level for dairy cows (8 mg Cr from Availa®Cr/cow per day) as safe for the target animal. This would correspond to about 0.4 mg Cr/kg complete feed.

Absorption of chromium is low ranging from 0.5% to 2–3%. Upon absorption, it binds to plasma proteins, and is transported to the liver. It is also found in spleen, kidneys, testes and bone. Deposition in muscle does occur to a very variable and low extent. Whereas most unabsorbed chromium is excreted through the faeces, urine is the main excretory route for absorbed chromium, but it is also found in milk.

Chromium DL-Met shows a genotoxic activity *in vitro* that is not expressed *in vivo* after systemic exposure. Although no data on genotoxicity at the site of contact are available, the Panel notes that chromium(III) is not carcinogenic and concludes that chromium DL-Met is unlikely to pose a carcinogenic risk at levels occurring in the diet.

The use of chromium DL-Met given as Availa®Cr in feed of dairy cows up to the recommended highest supplementation level would not measurably increase consumer exposure to trivalent chromium; therefore, the FEEDAP Panel concludes that the additive up to maximum recommended level of 0.5 mg Cr(III) from Availa®Cr per kg complete feed for dairy cows does not pose any concern to the safety of consumers.

The additive poses a risk to users by inhalation. It is not an irritant to skin and eyes. The additive should be considered a sensitiser.

Chromium is as a natural constituent in many rocks ubiquitous in the environment. The contribution of chlorine from the additive to the total dietary chlorine is negligible. Methionine, a physiological and natural component in animals and plants, is not excreted by cows as such (but as urea, sulfate and CO<sub>2</sub>). The use of the additive is not expected to pose a risk to the environment.

Only one study could be considered for the assessment of efficacy. This study showed that supplementation of Availa®Cr before and after calving had a significant positive effect on milk production with no changes in milk components and feed efficiency. Since a total of three studies are required indicating a positive effect on efficacy, the FEEDAP Panel is not in a position to conclude on the efficacy of chromium DL-Met from Availa®Cr.

## 5. Recommendation

The FEEDAP Panel recommends to indicate a daily dose per dairy cow (8 mg Cr from Availa®Cr) under *Other Provisions* on a potential authorisation of the additive. Consequently, the Panel recommends characterising the additive as Availa®Cr containing 1,000 mg Cr from chromium DL-Met/kg.

## 6. Remark

The Panel notes that no analytical method is available to control the presence and supplementation rate of the zootechnical additive Availa®Cr in compound feed.

## Documentation as provided to EFSA/Chronology

Date	Event
23/04/2018	Dossier received by EFSA. Availa® Cr (chromium chelate of DL-methionine) as a feed additive for dairy cows. Submitted by Zinpro Animal Nutrition (Europe), Inc.
06/06/2018	Reception mandate from the European Commission

Date	Event
21/08/2018	Application validated by EFSA – Start of the scientific assessment
26/10/2018	Request of supplementary information (I) to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, safety for target species, safety for the consumer, safety for the user and efficacy</i>
21/11/2018	Comments received from Member States
07/12/2018	Addendum to the request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended Addendum. <i>Issues: characterisation (EURL)</i>
29/04/2019	Reception of supplementary information from the applicant (I)
01/10/2019	Request of supplementary information (II) to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation and efficacy</i>
03/10/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
21/10/2019	Reception of supplementary information from the applicant (II) - Scientific assessment re-started
22/10/2019	Clarification request by e-mail. <i>Issue: characterisation</i>
24/10/2019	Reception of the e-mail from the applicant clarifying the issue requested
30/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

ADME	absorption, distribution, metabolism and excretion
ANS	Food Additives and Nutrient Sources
Bw	body weight
CAS	Chemical Abstracts Service
CV	Coefficient of variation
FEEDAP	Additives and Products or Substances used in Animal Feed
FISH	fluorescence <i>in situ</i> hybridisation
GLP	good laboratory practice



ICP-MS	Inductively coupled plasma mass spectrometry
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
LB	lower bound
LOAELs	lowest observed adverse effect levels
LOQ	Limit of quantification
NOAEL	No observed adverse effect level
NDA	Dietetic Products, Nutrition and Allergies
OEL	occupational exposure limitRH Relative humidity
TLV	threshold limit value
TMR	Total mixed ration
UB	upper bound

## Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Availa®Cr (chromium chelate of DL-methionine)

In the current application, authorisation is sought under Article 4(1) for *chromium chelate of DL-methionine* under the category/functional group (4d) 'zootechnical additives/'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for lactating cows.

The *feed additive* is to be marketed as a grey-tan powder preparation (*Availa-Cr*) with a content of *chromium chelate of DL-methionine* of 3.4% (w/w), including calcium carbonate and vegetable oil as carriers. The content of *chromium* in *Availa-Cr* is ranging from 1004 to 1474 mg/kg and the content of *methionine* in the preparation is in the range from 13800 to 17500 mg/kg. According to the Applicant, the active substance of the *feed additive* is *chromium DL-methionine*.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant proposed minimum and maximum levels of *chromium* ranging from 0.3 to 0.5 mg/kg *feedingstuffs*.

For the quantification of the *chromium DL-methionine* content in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant did not submit any method. Instead, the Applicant proposed the separate determination of the *chromium* and *methionine* contents in the above-mentioned matrices and submitted the corresponding methods.

For the quantification of the *chromium* content in the *feed additive (Availa-Cr)*, the Applicant submitted a single-laboratory validated and further verified method based on inductively coupled plasma-mass spectrometry (ICP-MS). The following performance characteristics were reported in the frame of the validation and verification studies: a relative standard deviation for *repeatability* (RSDr) ranging from 1.0 to 4.6%; a relative standard deviation for *intermediate precision* (RSDip) ranging from 1.3 to 5.9%; and a *recovery rate* (Rrec) ranging from 90 to 116%.

Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on ICP-MS for the quantification of the *chromium* content in the *feed additive (Availa-Cr)*.

For the quantification of *chromium* in *feedingstuffs*, the Applicant proposed the AOAC 2006.03 method and an in-house method based on ICP-MS. However, no experimental proof of the applicability of both methods to quantify *chromium* content in feed, at the proposed added levels of 0.3–0.5 mg/kg *feedingstuffs*, was submitted.

Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the proposed added *chromium* content in *feedingstuffs*.

For the characterisation of the *feed additive*, the Applicant proposed to quantify the *methionine* content by the ring-trial validated AOAC 999.13 method based on ion-exchange chromatography coupled to post-column derivatisation and colorimetric or fluorescence detection. The EURL instead identified the ring-trial validated EU and EN ISO 13903 methods based on ion-exchange high-performance liquid chromatography coupled to post-column derivatisation and photometric detection (IEC-VIS), already evaluated and recommended by the EURL in the frame of a previous *methionine chelate* dossier.

Based on the performance characteristics available, the EURL recommends for official control the above-mentioned EU and EN ISO 13903 methods based on IEC-VIS to quantify *methionine* in the *feed additive*.

Furthermore, for proving the chelated structure of the *feed additive*, the Applicant has proposed an additional experiment, namely the measurement of the product (*Availa-Cr*) by mid-infrared (IR) spectrometry.

Based on the available data, the EURL recommends for official control the measurement by mid-IR spectrometry together with the determination of the content of *chromium* and *methionine* in the product, for proving the chelated structure of the *feed additive*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.