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**Evaluation Report on the Analytical Methods submitted  
in connection with the Application for Authorisation of a  
Feed Additive according to Regulation (EC) No 1831/2003**

**Maxiban® G160**

*(FAD-2014-0036; CRL/140030)*

*(FAD-2014-0045; CRL/140036)*





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Feed Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FAD-2014-0036 - CRL/140030**  
**FAD-2014-0045 - CRL/140036**

Name of Product: ***Maxiban® G160***

Active Agent (s): **Narasin and Nicarbazin**

Rapporteur Laboratory: **European Union Reference Laboratory for  
Feed Additives (EURL-FA)  
Geel, Belgium**

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Date: **12/11/2015**

Report approved by: **Christoph von Holst**  
Date: **13/11/2015**

## EXECUTIVE SUMMARY

*Maxiban® G160* is a *feed additive* – belonging to the group "Coccidiostats and histomonostats" listed in Regulation (EC) No 1831/2003 – initially authorized for chickens for fattening by Regulation (EC) 2430/1999 and further re-authorized by Commission Regulation (EC) No 885/2010. In the current applications authorisation is sought under article 13(3)<sup>1,2</sup> of the Regulation (EC) No 1831/2003 for chickens for fattening. *Maxiban® G160* consists of 80g/kg of *narasin* and 80g/kg of *nicarbazin* (as active substances) antidusting oil, anticaking agent, microtrazer-F-Red and rice hulls. It is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposes (1) the inclusion of a lower amount of the microtrazer-F-Red<sup>1</sup> and (2) to increase the maximum level in the complete *feedingstuffs*<sup>2</sup>. Consequently the Applicant proposed a concentration of *narasin+nicarbazin* in *feedingstuffs* ranging from 40+40 mg/kg to 70+70 mg/kg for chickens for fattening. Furthermore maximum residue limits (MRLs) in chicken *tissues* of 50 µg/kg for *narasin*<sup>2</sup> and ranging from 4000 to 15000 µg/kg (depending on the *tissue*) for 4,4-dinitrocarbanilide (*DNC*) – marker residue for *nicarbazin*<sup>1,2</sup> have been already established by Commission Regulation (EC) No 885/2010.

For the quantification of *narasin* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted two single-laboratory validated and further verified methods based on EN ISO 14183 using High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.). For the quantification of *nicarbazin* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted two single-laboratory validated and further verified methods based on EN 15782 using HPLC-UV.

Based on the performance characteristics available the EURL recommends for official control the two single-laboratory validated methods for the quantification of *narasin* and *nicarbazin* in the *feed additive* together with the EN methods for the quantification of the two active substances in *premixtures* and *feedingstuffs*.

For the quantification of *narasin* and *nicarbazin* in chicken *tissues* the Applicant submitted methods based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode validated according to the requirements set by Commission Decision 2002/657/EC. Based on the performance characteristics available the EURL recommends for official control these methods or any equivalent methods complying with the requirements set by Commission

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<sup>1</sup> FAD 2014-0036; <sup>2</sup> FAD 2014-0045

Decision 2002/657/EC, to enforce the *narasin* and 4-4'-*dinitrocarbanilide (DNC)*-marker residue for *nicarbazin* MRLs in the relevant *tissues*.

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) is not considered necessary.

## KEYWORDS

*Narasin*, *nicarbazin*, *Maxiban® G160*, coccidiostat, chickens for fattening

## 1. BACKGROUND

*Maxiban® G160* is a *feed additive* - belonging to the group "Coccidiostats and histomonostats" listed in Regulation (EC) No 1831/2003 – initially authorized for chickens for fattening by Regulation (EC) 2430/1999 [1] and further re-authorised by Commission Regulation (EC) No 885/2010 [2]. In the current applications authorisation is sought under article 13(3)<sup>1,2</sup> (modification, suspension and revocation authorisations) of the Regulation (EC) No 1831/2003 for chickens for fattening [3][4][5][6].

*Maxiban® G160* is a *feed additive* containing two active substances namely *narasin* and *nicarbazin* [5],[6],[7]. The *feed additive* consists of 80g/kg of *narasin* and 80g/kg of *nicarbazin*, soybean or mineral oil as antidusting oil, vermiculite as anticaking agent, microtrazer-F-Red and rice hulls [5],[6],[7]. *Maxiban® G160* is currently authorised to be incorporated directly into *feedingstuffs* or through *premixtures* for chickens for fattening at a concentration of *narasin* + *nicarbazin* ranging from 40+40 to 50+50 mg/kg *feedingstuffs* [1].

In the frame of the current applications the Applicant proposes (1) the inclusion of a lower amount of the microtrazer-F-Red<sup>1</sup>, and (2) to include a new range for for (*narasin* + *nicarbazin*) in the complete *feedingstuffs* from 40+40 to 70+70 mg/kg *feedingstuffs*<sup>2</sup> [6].

Maximum residue limits (MRLs) in chicken for fattening *tissues* (i.e. muscle, kidney, skin/fat and liver) of 50 µg/kg for *narasin*<sup>2</sup> and ranging from 4000 to 15000 µg/kg (4000 µg/kg for muscle and skin/fat; 6000 µg/kg for kidney and 15000 µg/kg for liver) for 4,4-*dinitrocarbanilide (DNC)* – marker residue for *nicarbazin*<sup>1,2</sup> have been already established by Commission Regulation (EC) No 885/2010 [2]. These MRLs are not covered by the Commission Regulation (EC) No 37/2010 [8], and therefore corresponding methods of analysis are evaluated by the EURL.

<sup>1</sup> FAD 2014-0036; <sup>2</sup> FAD 2014-0045

Note: The EURL has evaluated the analytical methods for the determination of *narasin* and/or *nicarbazin* in the frame of the dossiers FAD 2008-0037, FAD 2013-0041 and FAD 2012-0027 [9].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Maxiban® G160* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

## 3. EVALUATION

### *Identification /Characterisation of the feed additive*

#### *Qualitative and quantitative composition of impurities in the additive*

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [10].

### *Description of the analytical methods for the determination of the active substances in feed additive, premixtures and feedingstuffs*

#### *Narasin*

For the quantification of *narasin* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated methods [11][12] based on EN ISO 14183 [13] using High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis).

*Narasin* is extracted using methanol:water (90:10) with mechanical shaking for 1 h, filtered and subjected to analysis without further clean-up. The target analyte is determined by reverse-phase HPLC using post-column derivatisation with vanillin and detection at 520 nm. According to *Campbell & Nayeri*, potential interferences in the determination of *narasin* cannot be expected [14].

This method was ring-trial validated for broiler *feedingstuffs* at a mean *narasin* content of 66.2 mg/kg leading to the following performance characteristics [13]:

- a relative standard deviation for *repeatability* ( $RSD_r$ ) of 4.5 %;
- a relative standard deviation for *reproducibility* ( $RSD_R$ ) of 6.5 %; and
- a limit of quantification (LOQ) of 2 mg/kg.

The Applicant applied this EN ISO method to the analysis of the *feed additive* using different sample intakes and extraction volumes and reported experimental data within the frame of the validation study [11]. These data were used by the EURL to calculate a  $RSD_r$  of 3.4 % and a relative standard deviation for *intermediate precision* ( $RSD_{ip}$ ) of 4.1 % [15].

Based on the provided performance characteristics the EURL recommends for official control the HPLC-PCD-UV-Vis methods for the quantification of *narasin* in the *feed additive* [11], *premixtures* and *feedingstuffs* [13].

### *Nicarbazin*

For the quantification of *nicarbazin* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted two single-laboratory validated methods [16][17] based on EN 15782 [19] using High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV).

*Nicarbazin* is extracted using acetonitrile:methanol (50:50) with manual shaking, heated in a water bath at 50°C for 15 min and further mixing, sonicated for another 15 min. After appropriate dilution with the eluent, an aliquot is filtered and subjected to analysis without further clean-up. The target analyte is determined by reverse-phase HPLC and the *4,4'-dinitrocarbanilide (DNC)* moiety is detected at 350 nm. According to *Jacob de Jong et al.* [20], potential interferences in the determination of *nicarbazin* cannot be expected. This method was ring-trial validated for broiler *feedingstuffs* and *premixtures* at a mean *nicarbazin* content ranging from 22 to 7308 mg/kg leading to the following performance characteristics [19]: -  $RSD_r$  ranging from 2.6 to 10.2 %; -  $RSD_R$  ranging from 4.8 to 12.3 %; and - a limit of detection (LOD) of 0.5 mg/kg

The Applicant applied the EN method, using different extraction solvent (dimethylformamide instead 50% acetonitrile:methanol) to analyse the *feed additive (Maxiban G160)*. Based on the experimental data provided in the frame of the validation studies [16][17], the EURL calculated a  $RSD_r$  of 0.4 % and a  $RSD_{ip}$  of 0.9 % [18]

Based on the performance characteristics available the EURL recommends for official control the HPLC-UV methods for the quantification of *nicarbazin* in the *feed additive* [16][17] *premixtures* and *feedingstuffs* [19].

***Methods of analysis for the determination of the residues of the additive in food.***

**Narasin**

For the quantification of *narasin* in chicken *tissues* the Applicant submitted a method based on reversed phase high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards validated (in muscle, kidney, skin/fat and liver) according to the Commission Decision 2002/657/EC [21][22][23]. Additionally the Applicant provided a verification study in muscle *tissue* and reported a recovery rate ( $R_{rec}$ ) of 91.6 % and a detection limit (LOD) of 3.0 µg /kg [24][25]. Four identification points were set for *narasin* using one parent and two daughter ions. Quantification is based on the transition  $m/z$  787.5 > 431.2 while confirmation is based on the transition  $m/z$  787.5 > 531.4.

In the frame of previous dossiers the EURL already evaluated and recommended similar methods, fulfilling the requirements of the Commission Decision 2002/657/EC [23] for the determination of *narasin* in chicken *tissues* [9].

Table 1 presents the performance characteristics reported in the frame of the validation and verification studies together with those reported by the European Union Reference Laboratory Pharmacologically Active Substances (BVL).

**Table 2.** Performance characteristics for the quantification of *narasin* residues in chicken tissues obtained in the frame of the validation (Val.) and verification (Ver.) studies, compared to those reported by BVL.

Tissue		Conc. (µg /kg)	RSD <sub>r</sub> (%)	RSD <sub>ip</sub> (%)
Muscle	BVL	0.75-2.75	10-18	13-18
	Val.	7.5	1.9-2.7	4.2
		15	3.6-5.3	4.2
		22.5	4.5-5.0	5.8
Ver.	50	3.9	8.5	
Liver	BVL	0.75-2.75	10-18	13-18
	Val.	25	2.5-3.9	4.5
		50	2.8-4.5	4.3
		75	2.7-4.5	3.9
Kidney	Val.	7.5	1.8-4.8	9.0
		15	1.8-3.7	5.6
		22.5	2.6-3.8	4.6
Skin/Fat	Val.	25	2.8-7.9	9.4
		50	3.5-6.2	6.2
		75	3.8-6.9	6.1

RSD<sub>r</sub>; RSD<sub>ip</sub>: relative standard deviation for *repeatability* and *intermediate precision*



The satisfactory performance characteristics provided by the Applicant for muscle and liver *tissues* demonstrate that the BVL method was equivalent to the one proposed by the Applicant. Additionally the satisfactory results provided by the Applicant for kidney and skin/fat further demonstrate the applicability - and therefore the extension of scope - of the Applicant method to these two additional *tissues*.

Consequently, the EURL recommends for official control the RP-HPLC-MS/MS method, validated according Commission Decision 2002/657/EC, for the determination of *narasin* in chicken *tissues*.

### **Nicarbazin**

For the quantification of *DNC* (marker residue for *nicarbazin*) in target *tissues* (skin/fat, muscle, liver and kidney) the Applicant submitted a published AOAC method (AOAC 2013-07) [26] based on RP-HPLC-MS/MS in electrospray ionisation (ESI) mode validated (in muscle, kidney, skin/fat and liver) according to the Commission Decision 2002/657/EC [23]. Additionally the Applicant verified this method in the frame of the ion ratio assay [27]. Four identification points were set for *DNC* using one parent and two daughter ions. Quantification is based on the transition  $m/z$  301.0 > 136.9 while confirmation is based on the transition  $m/z$  301.0 > 106.7.

In the frame of previous dossiers the EURL already evaluated and recommended similar methods, fulfilling the requirements of the Commission Decision 2002/657/EC [23] for the determination of *nicarbazin* (as *DNC*) in chicken *tissues* [9].

The method performance characteristics of the AOAC method are presented in Table 3. Furthermore LOQ of 20 µg/kg was reported for muscle, liver, kidney and skin/fat *tissues* [26].

The satisfactory performance characteristics provided by the AOAC method for muscle and liver *tissues* demonstrate that the BVL method was equivalent to the AOAC method. Additionally the results provided by the AOAC method for kidney and skin/fat further demonstrate the applicability - and therefore the extension of scope - of the AOAC method to these two additional *tissues*.

Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS AOAC method or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs for *nicarbazin* (as *DNC*) in the target *tissues*.

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) is not considered necessary.

**Table 3.** Performance characteristics for the quantification of *DNC* residues in chicken tissues obtained with the AOAC method, compared to those reported by the European Union reference Laboratory Pharmacologically Active Substances (BVL).

Tissue		Conc. ( $\mu\text{g}/\text{kg}$ )	RSD <sub>r</sub> (%)	RSD <sub>ip</sub> (%)
Muscle	BVL	0.75-2.75	3.4-8.7	8.0-11.6
	AOAC	100	0.81-5.3	10.0
		200	1.6-7.0	11.3
		400	1.9-4.9	4.9
		2000	1.8-5.0	4.5
		4000	1.4-4.5	5.7
		8000	1.6-2.2	3.0
Liver	BVL	0.75-2.75	3.4-8.7	8.0-11.6
	AOAC	100	2.2-10.4	8.2
		200	3.6-7.2	8.6
		400	2.2-4.8	4.8
		2000	3.2-5.4	4.8
		4000	3.2-6.8	5.5
		8000	2.1-2.6	2.5
Kidney	AOAC	100	1.3-10.8	6.6
		200	1.1-4.7	8.3
		400	1.2-1.9	6.9
		2000	1.9-4.0	5.4
		4000	0.7-5.7	4.4
		8000	1.2-6.2	8.4
Skin/Fat	AOAC	100	2.1-5.6	6.8
		200	2.0-11.6	8.2
		400	1.7-11.5	7.8
		2000	2.0-10.2	6.9
		4000	1.5-8.3	5.8
		8000	1.6-8.1	6.1

RSD<sub>r</sub>; RSD<sub>ip</sub>: relative standard deviation for *repeatability* and *intermediate precision*

#### 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified methods based on HPLC-PCD-UV-Vis and on HPLC-UV for the quantification of *narasin* and *nicarbazin* in the *feed additive*, *premixtures* and *feedingstuffs* together with the single-laboratory validated and further verified methods based on RP-HPLC-MS/MS - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the quantification of *narasin* and *nicarbazin* in chicken *tissues*.

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***Recommended text for the register entry (analytical method)***

For the quantification of *narasin* in the *feed additive*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis)

For the quantification of *narasin* in *premixtures* and *feedingstuffs*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis) - EN ISO 14183

For the quantification of *nicarbazin* in the *feed additive*:

- High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV)

For the quantification of *nicarbazin* in *premixtures* and *feedingstuffs*:

- High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV) - EN ISO 15782

For the quantification of *narasin* in chicken *tissues*:

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

For the quantification of *nicarbazin* (as *4,4-dinitrocarbanilide (DNC)*) in chicken *tissues*:

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) - AOAC 2013-07 or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

## **5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL**

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Maxiban® G160* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

## 6. REFERENCES

- [1] Commission Regulation (EC) No 2430/1999 of 16 November 1999 linking the authorisation of certain additives belonging to the group of coccidiostats and other medicinal substances in feedingstuffs to persons responsible for putting them into circulation (Text with EEA relevance)
- [2] Commission Regulation (EU) No 885/2010 of 7 October 2010 concerning the authorisation of the preparation of narasin and nicarbazin as a feed additive for chickens for fattening (holder of authorisation Eli Lilli and Company Ltd) and amending regulation (EC) No 2430/1999.
- [3] \*Application, Reference SANCO/G1: Forw. Appl. 1831/0052-2014
- [4] +Application, Reference SANCO/G1: Forw. Appl. 1831/0003-2015
- [5] \*Application, Proposal of Registry Entry – Annex A
- [6] +Application, Proposal of Registry Entry – Annex A
- [7] \*+Technical dossier, Section II: II.1 Identity of the additive
- [8] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
- [9] EURL Evaluation Reports FAD 2013-0041, FAD 2012-0027 and FAD 2008-0037  
[https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0041-monteban\\_0.pdf](https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0041-monteban_0.pdf)  
<https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2012-0027-monimax.pdf>  
<https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2008-0037.pdf>
- [10] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
- [11] Technical dossier, Section II: +Annex II.24 & \*Annex II.25
- [12] Technical dossier, Section II: +Annex II.27 & \*Annex II.28
- [13] EN ISO 14183:2008 Animal feedingstuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatography method using post-column derivatisation (ISO 14183:2005)
- [14] Harold Campbell et Gita Nayeri, Determination of Monensin, Narasin and Salinomycin in mineral premixes, supplements and animal feeds by liquid chromatography and post-column derivatization: collaborative study, J. of AOAC Int., 89, 5, 1229 – 1242, 2006
- [15] \*+Supplementary Information, eurl\_anova\_nar\_fa.pdf
- [16] \*Technical dossier, Section II: Annex II.26; Annex II.27 & Annex II.29
- [17] +Technical dossier, Section II: Annex II.25; Annex II.26 & Annex II.28
- [18] \*+Supplementary Information, eurl\_anova\_nic\_fa.pdf
- [19] EN ISO 15782:2009 Animal feedingstuffs – Determination of nicarbazin – High performance liquid chromatography method

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- [20] Jacob de Jong et al. Liquid Chromatographic Method for Nicarbazin in Broiler Feeds and Premixtures: Development, Validation, and Interlaboratory Study, *J. of AOAC Int.*, 87, 6, 1269 – 1277, 2004
- [21] <sup>+</sup>Technical dossier, Section II: Annex II.29 & Annex II.30
- [22] \*Technical dossier, Section II: Annex II.30 & Annex II.31
- [23] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results
- [24] <sup>+</sup>Technical dossier, Section II: Annex II.31
- [25] \*Technical dossier, Section II: Annex II.32
- [26] \*<sup>+</sup>Supplementary Information, Annex 1
- [27] \*<sup>+</sup>Supplementary Information, Annex 2

\*Refers to Dossier no: FAD-2014-0036

<sup>+</sup> Refers to Dossier no: FAD-2014-0045

## **7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES**

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

## **8. ACKNOWLEDGEMENTS**

The following National Reference Laboratories contributed to this report:

- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)
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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT –FAVV) (BE)
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