SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Maxiban® G160 is a coccidiostat for chickens for fattening used at levels ranging from 40–50 mg narasin/kg plus 40–50 mg nicarbazin/kg complete feed. Maxiban® G160 is safe for chickens for fattening and effective in controlling coccidiosis. The metabolism and residue data for narasin and nicarbazin administered separately have already been described and assessed. Additional information concerning Maxiban® G160 indicates that the simultaneous administration of nicarbazin and narasin increases DNC-labelled nicarbazin derived total residues by 20 % (liver) to 50 % (muscle), whereas no modification is observed for HDP-labelled nicarbazin derived residues. There is no evidence resulting from the toxicological studies for any significant interaction between narasin and nicarbazin. The ADI for narasin is 0.005 mg/kg bw, derived from a NOAEL of 0.5 mg/kg bw in a one-year dog toxicity study and applying an uncertainty factor of 100. The ADI for DNC is 0.77 mg DNC/kg bw, based on a NOAEL of 154 mg DNC/kg bw in a two-year dog toxicity study and applying an uncertainty factor of 200. The following MRLs can be applied: 50 μg narasin/kg liver, kidney, muscle and skin/fat; 15 mg DNC/kg liver, 6 mg DNC/kg kidney and 4 mg DNC/kg muscle and skin/fat. A zero-day withdrawal period is considered appropriate. Maxiban® G160 is a slight skin irritant, an eye irritant and a skin sensitiser. The inhalatory risk of users from narasin in Maxiban® G160 dust should be reduced by safety measures normally employed for handling dust-generating products. Maxiban® G160 would not pose a foreseeable risk for the soil compartment, groundwater or secondary poisoning. However, the risk for surface water could not be assessed.

KEY WORDS

Coccidiostats, Maxiban® G160, narasin, nicarbazin, chickens for fattening, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2008-474, adopted on 7 April 2010.

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SUMMARY

Following a request from European Commission, the European Food Safety Authority was asked to deliver an opinion on the safety and efficacy of Maxiban® G160, a coccidiostat for chickens for fattening. The additive is composed of two active substances, narasin and nicarbazin. The intended use ranges from 40–50 mg narasin/kg plus 40–50 mg nicarbazin/kg complete feed.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that Maxiban® G160 up to the highest use level (0.625 g Maxiban® G160/kg feed corresponding to 50+50 mg narasin+nicarbazin) is safe for chickens for fattening. The margin of safety for Maxiban® G160 in chickens for fattening is small but at least equal to 1.25 (62.5+62.5/50+50).

Narasin is toxic to equines, turkeys and rabbits at levels below those used in the prevention of coccidiosis in chickens. Interactions between the polyether ionophore coccidiostats, including narasin, and tiamulin as well as other antimicrobials (mainly macrolides) are demonstrated in the literature.

Nicarbazin does not display any antimicrobial properties and consequently no microbiological safety concern is associated with this compound. However, narasin has an antimicrobial activity against several Gram-positive intestinal bacterial species (0.25–4.0 mg/L).

The metabolism and residue data for narasin and nicarbazin administered separately have already been described and assessed. Additional information concerning Maxiban® G160 indicates that the simultaneous administration of nicarbazin and narasin increases DNC-labelled nicarbazin derived total residues by 20 % (liver) to 50 % (muscle), whereas no modification is observed for HDP-labelled nicarbazin derived residues.

There is no evidence resulting from the toxicological studies for any significant interaction between narasin and nicarbazin.

An ADI for narasin of 0.005 mg/kg bw (equal to 300 µg/person/day for a 60 kg person) was established in 2004 by the FEEDAP Panel, based on the NOAEL of a one-year dog toxicity study (0.5 mg/kg bw/day) and applying an uncertainty factor of 100. The same ADI has been retained by JECFA. In its assessment of nicarbazin in 2010, the FEEDAP Panel concluded that an ADI for DNC would protect the consumers as they are only exposed to DNC. An ADI of 0.77 mg/kg bw for DNC was set, derived from the NOAEL of the two-year dog study (154 mg DNC/kg bw) and applying a safety factor of 200. The ADIs established for narasin and nicarbazin when used alone can be applied to the same compounds in Maxiban® G160 in assessing the safety of consumers exposed to foodstuffs from chickens for fattening treated with Maxiban® G160.

The FEEDAP Panel expects that narasin-related residues following the administration of Maxiban® G160 are not higher than those from Monteban® G100 after a zero-day withdrawal period. The MRLs set for narasin from Monteban® G100 (50 µg narasin/kg liver, kidney, muscle and skin/fat) could consequently be applied to narasin from Maxiban® G160.

Consumer exposure to the whole DNC-derived residues present in chicken tissues (fed with 125 mg nicarbazin /kg) at a one-day withdrawal period complies with the ADI. The MRLs for DNC in liver (15 mg/kg), kidney (6 mg/kg), muscle (4 mg/kg) and skin/fat (4 mg/kg) proposed in the Koffogran opinion would also apply for nicarbazin from Maxiban® G160.

The DNC residues following the administration of Maxiban® G160 at a zero-day withdrawal time comply with the DNC ADI (4 %). Therefore, a zero-day withdrawal period for Maxiban® G160 is considered appropriate.

Maxiban® G160 is considered as a slight skin irritant. Since narasin from Monteban® G100 is an eye irritant and a skin sensitiser, and no (conclusive) studies with Maxiban® G160 were provided, the FEEDAP Panel considers Maxiban® G160 as an eye irritant and a skin sensitiser. The inhalatory risk

from Maxiban® G160 dust for users is considered negligibly small for nicarbazin, but deserves minimisation for narasin by safety measures normally employed for handling dust-generating products (i.e. masks).

Considering the condition of use of Maxiban® G160 and based on the data provided, a safety concern for the soil compartment, groundwater or by secondary poisoning could not be identified. In the absence of raw data for the algae toxicity study with nicarbazin, the risk of Maxiban® G160 for surface water compartment could not be assessed.

The optimum dose range was established to be 80–100 mg active substances from Maxiban® G160/kg with a narasin to nicarbazin ratio of 1:1. The FEEDAP Panel concluded, based on the results of three floor pen studies and four field trials, that Maxiban® G160 used at the lowest proposed dose (40+40 mg nicarbazin+narasin/kg complete feed) is effective in controlling coccidiosis in chickens for fattening.

No effect of Maxiban® G160 used at the highest proposed dose is expected on the quality of the animal product.

The FEEDAP Panel made some recommendations concerning the conditions of use, the instructions for use, the purity of nicarbazin, the monitoring of bacterial resistances to clinically relevant antibiotics and further monitoring of potential *Eimeria* resistance in chickens for fattening.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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. In particular Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Eli Lilly and Company Ltd[5] for authorisation of the product Maxiban® G160, narasin and nicarbazin, to be used as a feed additive for chickens for fattening (category: coccidiostats and histomonostats) under the conditions mentioned in Table 1.

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 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 12 November 2008.

The product Maxiban® G160 is composed of two active ingredients: nicarbazin and narasin. It is currently authorised for chickens for fattening under Regulation (EC) No 2430/1999 until 30 September 2009.

The Scientific Committee on Animal Nutrition (SCAN) issued two opinions on the use of narasin and nicarbazin in feedingstuffs for chickens (provisional opinion adopted in July 1991; opinion adopted 7 July 1995). In July 2004, EFSA published an opinion on the re-evaluation of efficacy and safety of the coccidiostat Monteban® G100 (narasin) in accordance with article 9G of Council Directive 70/524/EEC. In December 2003, EFSA published an opinion on the efficacy and safety of the coccidiostat Koffogran (nicarbazin). EFSA has recently assessed nicarbazin on the basis of a supplementary dossier and adopted an opinion in favour of a zero-day withdrawal period for narasin from Monteban® G100 (maximum dose 70 mg/kg feed). The use of narasin as feed additive has been authorised by Commission Regulation (EC) No 545/2006 until August 2014 (MRLs, 50 µg narasin/kg wet tissues chickens for fattening).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 animal(s), consumer, user and the environment and the efficacy of the product Maxiban® G160 (narasin and nicarbazin), when used under the conditions described in Table 1.

[4] OJ L 268, 18.10.2003, p.29
[5] Priestley Road Basingstoke, Hampshire RG24 9NL United Kingdom
[6] EFSA Dossier reference: FAD-2008-0037

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Maxiban G160
Registration number/EC No/No (if appropriate)	E772
Category of additive	Coccidiostats and histomonostats
Functional group of additive	n/a

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Narasin 80g activity /kg Nicarbazin: 80g/kg Soyabean oil or mineral oil: 10-30 g/kg Vermiculite: 0-20g/kg Microtracer red: 11g/kg Corn cob grits or rice hulls qs 1 kg	**(a) Narasin** $C_{43}H_{72}O_{11}$ CAS number: 55134-13-9 Polyether monocarboxylic acid produced by Streptomyces aureofaciens (NRRL 8092) in granular form **(b) Nicarbazin** $C_{19}H_{18}N6O6$ CAS number: 330-95-0 Equimolecular complex of 1,3-bis(4-nitrophenyl)urea and 4,6-dimethylpyrimidin-2-ol, in granular form	**(a) Narasin** narasin A activity: ≥ 85% (b) **Nicarbazin** Related impurities: ≤ 1%	**Narasin** is quantified using a HPLC equipped with a post-column derivatization. Narasin is reacted with vanillin, and the resulting products are measured by a variable wavelength detector operating at 520nm. **Nicarbazin** is a 1:1 molar mixture of 4,4[1]-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). It is assayed using a reverse-phase isocratic method, which measures the DNC moiety at a wavelength of 340nm

Trade name (if appropriate)	Maxiban G160
Name of the holder of authorisation (if appropriate)	Eli Lilly and Company Ltd.

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		mg/kg of complete feedingstuffs		
Chickens for fattening	-	80	100	5 days

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Indicate in the instructions for use: 'Dangerous for equines'. 'This feedingstuff contains an ionophore: simultaneous use with certain medicinal substances (e.g. tiamulin) can be contra-indicated.'

Specific conditions or restrictions for handling (if appropriate)	-
Post market monitoring (if appropriate)	Post marketing monitoring will be conducted using an already established pharmacovigilance system.
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
4,4' dinitrocarbanilide (DNC)	Chickens for fattening	Liver	750 μg/kg

ASSESSMENT

1. Introduction

The additive Maxiban® G160 is composed of two active substances, narasin and nicarbazin, and is intended to be used as coccidiostat for chickens for fattening at a dose range of 40–50 mg narasin/kg plus 40–50 mg nicarbazin/kg complete feed. It is currently authorised for chickens for fattening under Regulation (EC) No 2430/1999[7] until 30 September 2009.

The active substance narasin, as the only active substance of Monteban® G100, has already been assessed by the FEEDAP Panel in 2004 (EFSA, 2004). Monteban® G100 (containing 10 % narasin activity) is authorised by Commission Regulation (EC) 545/2006[8] until August 2014 at a dose of 60–70 mg narasin/kg feed. However, the FEEDAP Panel mentioned in its opinion in 2004 (EFSA, 2004) that it cannot be excluded that the use of Monteban® G100 at the recommended dose range poses a risk for soil organisms and that insufficient data was provided to assess the risk for the aquatic environment and secondary poisoning.

The active substance nicarbazin, as the only active substance of Koffogran, has already been assessed by the FEEDAP Panel in 2003 (EFSA, 2003). Koffogran (containing 25 % nicarbazin) is proposed for use at a dose of 100–125 mg nicarbazin/kg feed for chickens for fattening. The FEEDAP Panel identified some deficiencies in the toxicological and environmental data.

The FEEDAP Panel has recently assessed nicarbazin on the basis of a supplementary dossier (EFSA, 2010a) and adopted an opinion in favour of a zero-day withdrawal period for narasin from Monteban® G100 (maximum dose 70 mg/kg feed) (EFSA, 2010b).

Maxiban® G160 and Monteban® G100 are represented by the same applicant and nicarbazin in Koffogran by another applicant. EFSA received a cross-reference letter from the applicant of Koffogran allowing the sharing of the safety and technical data in the Koffogran dossier in the context of this opinion.

2. Characterisation of the additive

2.1. Identity of the additive

Maxiban® G160 contains two active ingredients, narasin (a fermentation product) and nicarbazin, at a level of 80 g/kg each. It is intended to control coccidiosis in chickens for fattening. The composition of Maxiban® G160 is summarised in Table 2.

[7] OJ C 50, 25.2.2004, p.1
[8] OJ L 94, 1.4.2006, p.26

Table 2: Composition of Maxiban® G160

Ingredients	g/kg Maxiban® G160
Active ingredients	
Narasin activity (not less than 85 % as narasin A)[a]	80
Nicarbazin[b]	80
Other ingredients	
Anti-dusting oil (soybean oil or mineral oil)	10
Microtracer-F-Red	11
Anticaking agent (Vermiculite)	0–20 g
Rice Hulls	q.s. 1000

[a] From a granulated narasin product (Narasin Granulated) containing about 13 % narasin activity[9]
[b] From a granulated nicarbazin product (Nicarbazin Granulated) containing about 30 % nicarbazin[10]

Granulated narasin consists of fermentation solids and manufacturing aids such as clay. Granulated nicarbazin contains also corn cobs and polyethylene glycol (PEG).

Microtracer-F-Red consists of coloured uniformly sized iron particles. It has an average arsenic content of about 50 mg/kg (20–66 mg/kg, 17 samples) estimated by atomic absorption.[11] Dioxins/ furans were below the detection limit.[12]

Vermiculite is an approved feed additive as anticaking agent.

Maxiban® G160 is a free flowing mixture of tan-to-yellow particles and grey brown particles. Content uniformity has been examined in five batches which contained 80.5 ± 0.3 mg narasin/kg and 80.9 ± 0.9 mg nicarbazin /kg.

Three batches of Maxiban® G160 have been tested for dioxins[13] and heavy metals.[14] The results are summarised in Table 3.

Table 3: Dioxins and heavy metal content measured in three batches of Maxiban® G160

Maxiban® G160 batch	TEQ (WHO) ng/kg	Arsenic mg/kg	Cadmium mg/kg	Lead mg/kg	Mercury mg/kg
931DW3	0.25	0.84	0.16	2.8	<0.04
933DW3	0.36	0.78	0.17	2.5	<0.04
935DW3	0.29	1.36	0.14	2.3	<0.04

The FEEDAP Panel has no safety concerns on the measured values.

Because granulated narasin is a fermentation product, data on microbial contamination and data certifying the absence of the production strain (*Streptomyces aureofaciens* strain NRRL 8092) are required. The applicant submitted instead data on Monteban® G100 (three to five batches),[15] which is based on the same fermentation product. The FEEDAP Panel considers these data representative for Maxiban® G160.

[9] Supplementary information July 2009
[10] Supplementary information July 2009
[11] Supplementary information/July 2009/Appendix 1
[12] Supplementary information/July 2009/Appendix 2
[13] Supplementary information/July 2009/Appendix 3
[14] Supplementary information/July 2009/Appendix 4
[15] Supplementary information/July 2009/Appendix 5

The microbiological parameters assessed included total viable aerobic count (TVAC), *Enterobacteriaceae*, *Streptomyces*, moulds, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. The analysis of three batches revealed the absence of *Salmonella* and *E. coli* in 25 g and 1 g, respectively, and counts of *S. aureus* lower than 3 CFU/g. High counts of moulds (up to 2.6 x 10^4 cfu/g) and *Enterobacteriaceae* (up to >1000 cfu/g) were reported by the applicant in the three analysed Monteban® G100 batches. The analysis of the rice hulls showed high levels of microbial counts (1.7 x 10^5 cfu/g) suggesting that this excipient contributes to the high microbial load of the product.

Three lots of Maxiban® G160 were analysed for heavy metals and arsenic (As < 1.4 , Cd < 0.17, Cu < 33, Pb < 2.8 and Hg < 0.04 mg/kg).

The particle sizes of both narasin and nicarbazin granules are controlled prior to manufacture of Maxiban® G160. Specifications for narasin granulated are 99 % of the particles < 600 µm, not more than 15 % < 100 µm, and for nicarbazin granulated not more than 2 % < 180 µm. Sieve analysis[16] of three lots of Maxiban® G160 showed between 1.6 and 2.7 % for particle < 100 µm and 0.8 to 1.5 for particles < 50 µm. The dusting potential of the Maxiban® G160 measured in three batches by Stauber-Heubach test was 0.2–0.585 g/m^3.

2.2. Characterisation of the active substances

Narasin is a polyether ionophore that exhibits both antimicrobial and anticoccidial activities. According to the applicant at least 85 % of the narasin activity should be due to narasin A ([α-ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3.]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]tetrahydro-3,5-dimethyl-2H-pyran-2-acetic acid], see Figure 1).

The molecular formula is $C_{43}H_{72}O_{11}$; CAS-No: 55134-13-9.

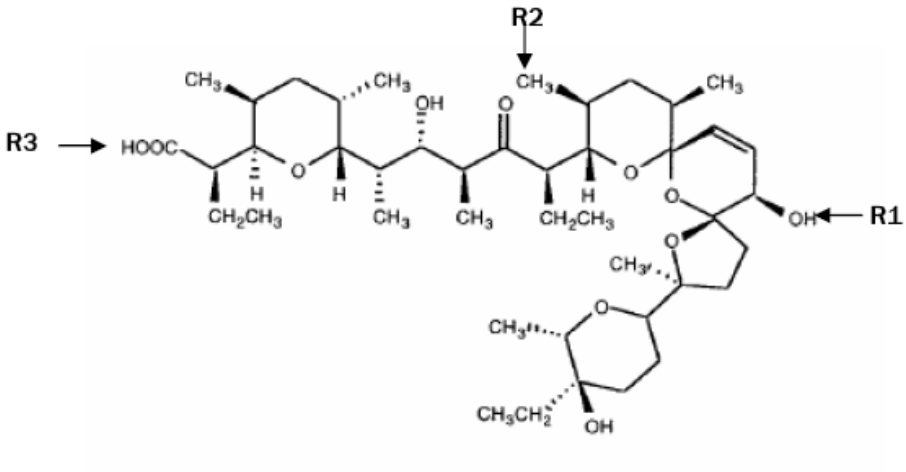

Figure 1. Structural formula of narasin A

The structural variants of narasin are given in Table 4.

[16] Supplementary information/July 2009/Appendix 7

Table 4: Structural variants of narasin

Structural variants of Narasin	R1	R2	R3
A	OH	CH_3	COOH
B	=O	CH_3	COOH
D	OH	C_2H_5	COOH
I	OH	CH_3	$COOCH_3$

Nicarbazin (see Figure 2) is an equimolar complex of 1,3-bis(4-nitrophenyl)urea , also known as N,N bis (4 nitrophenyl) urea or 4,4-dinitrocarbanilide (DNC), and 4,6-dimethylpyrimidin-2-ol, also known as 2-hydroxy-4,6-dimethylpyrimidine (HDP), CAS-No: 330-95-0. Nicarbazin purity is higher than 95.1 %.[17] Potential impurities are free HDP (max. 3.0 %), paranitroaniline (max. 0.5 %), ammonia (max. 0.5 %), chloride (max 0.3 %), sulphate (max. 1.5 %), methanol (max. 0.02 %), diethylbenzene (max. 0.02 %) and other unspecified impurities 0.2 %.

PNA was analysed in six batches between 0.1 and 0.2 %.[18] It did also not increase during a storage period of 36 months.[19] The specifications for residual solvents are below the VICH[20] thresholds, they were in six batches below 0.01 %.[21] Control methods are in place.

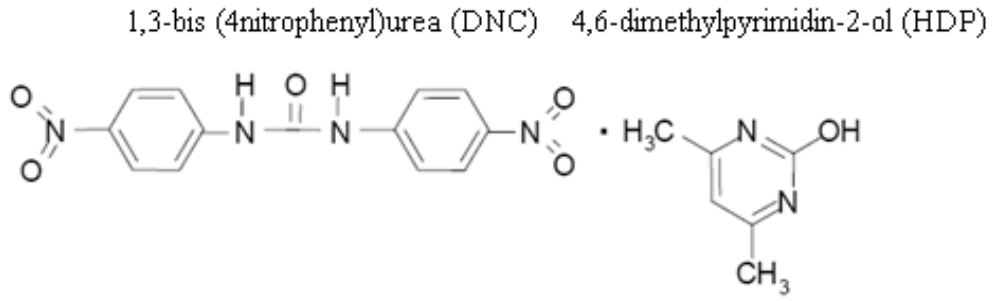

Figure 2. Structural formula of nicarbazin

The relevant properties of narasin and nicarbazin are given in Table 5.

Table 5: Relevant properties of narasin and nicarbazin

	Narasin	Nicarbazin
Dissociation constant	1.3×10^{-8}	-
pKa value	7.9 (80 % DMF)	-
Log K_{ow}	4.85 (pH 8)	DNC: 3.6 (pH 5-9) HDP: -0.94 (pH 5-9)
Water Solubility[22]	102mg/L at pH 7 681mg/L at pH 9	DNC: <0.02 mg/L (pH range 4-9)[a] HDP: appr. 70 g/L (pH range 4-9)

[a] In the aquatic acute toxicity study DNC water solubility was 0.07 mg/L.

[17] Cross-reference/Koffogran technical dossier/Monograph section
[18] Cross-reference/Koffogran technical dossier/Quality folder/Appendix 21
[19] Cross-reference/Koffogran technical dossier/Quality folder/Appendix 22
[20] http://www.vichsec.org/en/guidelines.htm
[21] Cross-reference/Koffogran technical dossier/Quality folder/Appendix 21
[22] Supplementary information/July 2009/Appendix 10

2.3. Manufacturing processes

2.3.1. Active substance(s)/agent(s)

The production processes of narasin and nicarbazin are fully described in the dossier.

Narasin is produced by fermentation from a culture of *Streptomyces aureofaciens* strain NRRL 8092 or of strains derived from NRRL 8092 and selected for high productivity. The strain NRRL 8092 has been deposited in the stock culture collection of the Northern Marketing and Nutrition Research Division (Illinois, USA). In the final step of the production, the fermentation product containing narasin is granulated together with processing aids through pelleting.

Nicarbazin is synthesised from 4,4'-dinitrocarbanilide (DNC) and 2- hydroxy-4-6-dimethylpyrimidine (HDP) using a chemical process.

2.3.2. Additive

Maxiban® G160 is manufactured by mixing the two raw products containing the active substances (granulated narasin and granulated nicarbazin) with other ingredients (see Table 2).

2.4. Physico-chemical and technological properties of the additive

2.4.1. Stability

2.4.1.1. Shelf life of the additive

The applicant reports the stability of a total of 31 batches of Maxiban® G160. Twenty-three batches were stored at 25 °C up to 24 months (eight batches only up to 12 months), six batches at 37 °C up to 12 months. The initial values of the 23 batches were 80.6 $\pm$ 1.3 g narasin/kg and 79.8 $\pm$ 1.9 g nicarbazin/kg Maxiban® G160; after 24 months 81.1 $\pm$ 1.5 g narasin and 80.3 $\pm$ 2.2 g nicarbazin were measured. Accelerated stability test gave similar pictures (initial 80.4 $\pm$ 1.4 g, after 12 months 81.5 $\pm$ 1.7 g narasin; initial 79.2 $\pm$ 1.2, after 12 months 80.5 $\pm$ 1.9 g nicarbazin/kg). The data provided support the shelf life of 24 months proposed by the applicant.

2.4.1.2. Stability of the additive used in premixtures and feedingstuffs

The stability of the product when mixed in premixtures was studied in two tests. In a first test,[23] the three batches of the additive were added to a complete vitamin-mineral premixture (including trace elements) containing choline chloride and the level of supplementation was of 5 g/kg of each of the active substances. Samples were kept at 25 °C/60 % relative humidity (RH) for six months. The recovery for narasin and nicarbazin was ~100 % at the end point. In the second test,[24] a vitamin-mineral premixture (composition no specified) was supplemented with Maxiban® G160 (one batch) to obtain a level of 10 g/kg of each of the active substances. The premixture was kept at 25 °C/ambient RH for 13 months, or at 37 °C/75 % RH for six months. The sample kept at 25 °C showed no reduction on the active substance content while the sample kept at 37 °C showed a recovery of 88 % of the initial activity after six months.

The stability of the product during the preparation of feed and during its storage was determined in different tests.

[23] Technical Dossier/Section II/Appendix 2
[24] Technical Dossier/ Section II /Appendix 5

In a first test,[25] the stability of the additive to pelleting was tested: two batches of broiler feed were supplemented at 40+40 mg narasin+nicarbazin/kg or at 50+50 mg narasin+nicarbazin/kg. The feed was pelleted at 70–80°C, pelleting did not modify the content of the active substances.

In a second test,[26] a standard broiler mash feed was supplemented at 40+40 mg narasin+nicarbazin/kg (two batches) or at 50+50 mg narasin+nicarbazin/kg (one batch). The mash feeds were kept at 25 °C/60 % RH or at 40 °C/75 % RH for four and three months, respectively. The recovery values showed no modification when the samples were kept at 25 °C, no reduction of the narasin content when kept at 37 °C and 80 % recovery of nicarbazin after three months when kept at 37 °C. The mash feeds were pelleted at 70 °C and no reduction of the content of the active substances were found. Those pelleted feeds were kept under the same conditions as the mash feed, and the results found were in the same line as in the mash feed.

In the third test,[27] four lots of poultry feed were manufactured: two lots to contain 30+30 mg/kg and two lots to contain 50+50 mg narasin+nicarbazin/kg. A portion of each mash feed was pelleted at 79–83 °C. The samples were stored at 25 °C/ambient RH or 37 °C/35 % or 75 % RH. They were assayed for narasin and nicarbazin, initially and at monthly intervals, during three months. A statistical analysis of the results showed that 90 % of the claimed potency was maintained for at least three months under all the three conditions.

Finally, in another test[28] one lot of starter feed was manufactured in a horizontal ribbon blender to contain 50+50 mg narasin+nicarbazin/kg from Maxiban® G160. The mash feed was pelleted (pellet temperature of 75 °C). Samples were packaged and stored under the conditions described above. The pelleted feed samples were assayed for narasin and nicarbazin, initially, at monthly intervals during three months and at six months. After storage at 25 °C for six months, the pelleted feed retained about 96 % of the initial content of narasin and nicarbazin. After storage at 37 °C for six months, the pelleted feed showed about 91 % and 87 % of the initial values for narasin and nicarbazin.

2.4.2. Homogeneity

Ten samples of each of the two premixtures (5+5 g narasin+nicarbazin/kg) were analysed in one study.[29] The coefficients of variation were 2.2 and 3.3% for nicarbazin, and 4.3 and 5.0 % for narasin,.

The homogeneity of the distribution of the active substances was studied in ten samples each of two batches of both a mash and pelleted broiler feed[30] (supplementation at 40+40 and 50+50 mg narasin+nicarbazin/kg, respectively). The coefficients of variation in the mash feed were 5.3 and 7.3 % for nicarbazin, and 2.9 and 5.5 % for narasin, respectively. In the pelleted feed, the coefficients of variation were 2.0 and 2.2 % for the nicarbazin and 2.8 and 4.6 % for the narasin, respectively.

2.5. Conditions of use

The recommended inclusion level of Maxiban® G160 in complete feed for chickens for fattening is 0.5 to 0.625 g/kg equivalent to 40+40 to 50+50 mg narasin+nicarbazin/kg. The applicant proposes a withdrawal period of five days.

[25] Technical Dossier/ Section II /Appendix 6
[26] Technical Dossier/ Section II /Appendix 2
[27] Technical Dossier/ Section II /Appendix 24
[28] Technical Dossier/ Section II /Appendix 25
[29] Technical Dossier/Section II/Appendix 2
[30] Technical Dossier/Section II/Appendix 2

2.6. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active substances in animal feed and the marker substances in tissues. The Executive Summary of the CRL report can be found in Appendix A.

3. Safety

3.1. Safety for the target species

In a toxicity test on target animals,[31] four-week-old broiler chickens were given single oral doses of 40+40, 63+63, 100+100 or 160+160 mg narasin+nicarbazin/kg body weight. Three consecutive trials were conducted with ten birds per sex and dose each, resulting in a total of 240 birds (without control group). The birds were observed for 14 days. The average LD_{50} was 67.8+67.8 mg narasin+nicarbazin/kg bw for male and 68.4 + 68.4 mg narasin + nicarbazin/kg bw for female chickens. Gross lesions of ventral soiling, petechial hemorrhages of the heart, pallor in the heart and skeletal muscles and histological lesions of skeletal muscle (degeneration and necrosis) were attributed to the treatment.

3.1.1. Tolerance studies in the target species

A total of 2120 newly-hatched broiler chickens (Hubbard X White Mountain) were fed 0+0, 50+50, 62.5 + 62.5, 150+150 and 250+250 mg narasin+nicarbazin/kg diet, respectively, for 45 days, followed by a five-day withdrawal period.[32] Twenty birds of each sex, selected at random from each treatment group, were bled for clinical chemistry, after which they were killed for necropsy and histopathological examination. Tissues from birds that died during the study were also examined grossly and microscopically.

There were no treatment-related changes in clinical pathology, growth performance or mortality at 50+50 and 62.5+62.5 mg narasin+nicarbazin/kg feed. At the higher doses, however, there were dose-related adverse changes in some of those values. Congestive heart failure was observed in 12 birds (8 males, 4 females) at 150+150 mg narasin+nicarbazin/kg and in 69 birds (47 males, 22 females) at 250 +250 mg narasin+nicarbazin/kg, respectively. The low incidence of heart failure in the two low dose groups was not associated with increased mortality and did not appear to be treatment-related. Unusually, there was no finding of congestive heart failure in the control birds. The applicant conducted therefore an additional tolerance study to confirm the absence of adverse effects at 50+50 mg narasin + nicarbazin/kg. Due to the higher sensitivity of males, the additional study was conducted with male birds only.

The additional study consisted of two replicates, in each of which 2100 one-day-old male chickens (Hubbard X White Mountain) were fed 0+0, 50+50, 62.5+62.5, 150+150, 250+250, 80+0 and 0+125 mg narasin+nicarbazin/kg feed, respectively, for 49 days, followed by a five-day withdrawal period. The birds were observed for physical signs of toxicity. Growth performance data were determined at four weeks, seven weeks and at the end of the withdrawal period. Thirty birds selected at random were taken from each group and killed for necropsy. Tissues from these birds and all the birds that died during the study were examined grossly and microscopically.

Mortality was significantly increased in birds given 250+250 mg narasin+nicarbazin/kg feed and in those given 125 mg nicarbazin alone. In the latter group, the increase in mortality was probably related to high ambient temperature during the study. Mortality due to heat stress is a known phenomenon of nicarbazin treatment. Mortality in the groups given 50+50, 62.5+62.5, 150+150 and 80+0 mg narasin+nicarbazin/kg feed was similar to that in the controls. There were no other observed signs of

[31] Supplementary information/July 2009/Appendix 13
[32] Technical dossier/Section III/ Appendix 2

toxicity in any treatment group; however, changes in the growth performance parameters were observed due to a treatment-related reduction in feed consumption. Growth depression was found in the 150+150, 250+250 and 0+125 mg narasin + nicarbazin groups; increased feed conversion ratios in the first two groups.

A treatment-related increase in the incidence and severity of congestive heart failure, myocardial degeneration and skeletal muscle degeneration and regeneration was observed in birds given 150+150 and 250+250 mg narasin+nicarbazin/kg feed. There were no such changes in the other groups. In contrast to the previous study, congestive heart failure occurred at the expected incidence in control birds in the two replicates. The incidence in each control group was 2 of 300 birds (0.67 %), similar to that found in the two lowest groups (50+50 and 62.5+62.5 mg narasin+nicarbazin/kg feed), in all three studies. This indicates that the few random cases of congestive heart failure in the low dose groups of both studies occurred spontaneously and were not related to treatment.

In summary, there were no adverse findings in birds given 80 mg narasin alone or those given 50+50 or 62.5+62.5 mg narasin+nicarbazin/kg feed. The margin of safety (the margin between the maximum proposed dose level and the level resulting in adverse effects) is not less than 1.25 (62.5+62.5/50+50).

3.1.2. Tolerance for non-target animals

In its former opinion on Monteban® G100 (EFSA, 2004), the FEEDAP Panel mentioned that horses are particularly susceptible to narasin as well as to other polyether ionophores. Reports on poisoning incidents suggest that narasin is toxic to turkeys and rabbits at levels below those used in the prevention of coccidiosis in chickens for fattening.

3.1.3. Microbiological safety of the additive

The MIC of nicarbazin against two strains of *Staphylococcus aureus*, two strains of *Salmonella* Typhimurium, three strains of *Klebsiella pneumoniae*, three strains of *Escherichia coli* and two strains of *Clostridium perfringens* have been determined using the plate dilution method and found in all cases to be > 128 µg/mL.[33]

While nicarbazin does not have antibacterial activity, the typical MIC range of narasin for *Bacteroides* was found to be 0.25–2 µg/mL, when tested with 60 *Bacteroides* isolates of poultry origin using the plate dilution method. For two of the isolates, the MIC was exceptionally high (64 µg/mL).[34] The birds, from which the bacterial isolates had been obtained, had received either untreated feed or narasin-nicarbazin mixture (50 mg/kg feed) for 30 days. All the isolates were tested against narasin, while the 20 isolates from untreated control birds were also tested against narasin-nicarbazin mixture (1:1). The presence of nicarbazin did not affect the MICs against narasin, indicating no antibacterial interaction between the two coccidiostats. In the same study, 20 isolates of both *E. coli* and *Staphylococcus* were also tested and found resistant. While resistance is typical to Gram-negatives, *Staphylococcus* are supposed to show some degree of sensitivity.

The sensitivity of *Staphylococcus* to narasin was confirmed in a study[35] with poultry isolates of *Staphylococcus* and streptococci (13 each), in which the MIC ranges in the plate dilution assays were ≤ 2 µg/mL and 0.125–8 µg/mL, respectively.

Further studies[36] were performed on *Enterococcus faecium* and *E. faecalis*, isolated from Maxiban® G160 treated chickens. The total number of isolates from Maxiban® G160 treated birds were 60 (*E. faecium*) and 80 (*E. faecalis*).The isolation period spanned the whole treatment (39 days), and the withdrawal period (10–15 days). The MIC determinations with narasin, nicarbazin or narasin+

[33] Technical dossier/Section III/Appendix 3
[34] Technical dossier/Section III/Appendix 4
[35] Technical dossier/Section III/Appendix 5
[36] Technical dossier/Section III/Appendix 6

nicarbazin (1:1 mixture) indicated no antimicrobial activity by nicarbazin, while the enterococcal MICs to narasin ranged between 0.25–4 µg/mL (*E. faecium*) and 0.125–4 µg/mL (*E. faecalis*). The MICs for the nicarbazin-narasin mixture indicated no interference by the two coccidiostats.

Occurrence of enterococcal resistance to narasin has been observed in monitoring programs (Norm-VET, 2006; SVARM 2007, FINRES-Vet 2005 - 2006), and complete cross-resistance between narasin and salinomycin has also been reported (Butaye et al., 2000).

The studies reviewed in the previous FEEDAP opinion on Monteban® G100 (EFSA, 2004) do not indicate any effects on *Salmonella* shedding as a result of narasin administration.

3.1.4. Interactions/incompatibilities

In its former opinion on Monteban® G100 (EFSA, 2004), the FEEDAP Panel concluded that 'the known history of use of narasin has shown that incompatibilities or interactions with feedingstuffs, carriers, or other approved additives are not to be expected. On the other hand it is well known from the literature that severe interactions between the polyether ionophore coccidiostats and the diterpene-antibiotic tiamulin as well as other antimicrobials (mainly macrolides) may occur. Therefore the simultaneous use of Monteban® G100 and certain antibiotic drugs (i.e. tiamulin) is contra-indicated.' The FEEDAP panel concludes that the same contra-indications would apply to Maxiban® G160 due to its narasin content.

3.1.5. Conclusions on the safety for the target species

Severe signs of intolerance including growth depression, reduced feed conversion ratio, increased incidence of congestive heart failure and other cardiac and skeletal muscle alterations and increased mortality, were observed in chickens for fattening given 150+150 and 250+250 mg narasin+nicarbazin/kg feed. No adverse effects were found in birds given 50+50 and 62.5+62.5 mg narasin+nicarbazin/kg feed, respectively. The margin of safety for Maxiban® G160 in chickens for fattening is small but at least equal to 1.25 (62.5+62.5/50+50).

The FEEDAP Panel therefore concludes that Maxiban® G160 up to the highest use level (0.625 g Maxiban® G160/kg feed corresponding to 50+50 mg narasin+nicarbazin) is safe for chickens for fattening.

Nicarbazin does not display any antimicrobial properties and consequently no microbiological safety concern is associated with this compound. However, the other active substance of Maxiban® G160 (narasin) has MICs against several intestinal bacteria species clearly lower than four times 40–50 mg/kg (the recommended Maxiban® G160 concentrations in feed). Therefore, the selection of antimicrobial resistances should be considered.

Narasin from Maxiban® G160 may be dangerous to equines. Attention should be taken to avoid cross-contamination of feedingstuffs destined for turkeys and rabbits. Interactions between the polyether ionophore coccidiostats and the diterpene-antibiotic tiamulin as well as other antimicrobials (mainly macrolides) are demonstrated in the literature. Therefore the simultaneous use of Maxiban® G160 and certain antibiotic drugs (i.e. tiamulin) is contra-indicated.

3.2. Safety for the consumer

3.2.1. Metabolism and residue studies

3.2.1.1. Metabolism

Narasin

The data submitted concerning the metabolic fate of narasin in the chicken and rat include the studies already assessed by the FEEDAP Panel and presented in a previous opinion on Monteban® G100 (EFSA, 2004). Therefore, the same conclusions can be retained:

i) The main metabolic pathway of narasin in the chicken and rat involves oxidative processes leading to the formation of di-, tri- and tetra-hydroxynarasins as well as keto-narasins.

ii) Unchanged narasin is a minor component (up to 5 %) of chicken excreta in the feed dose range proposed, whereas a great number of metabolites have been identified. Two major di-hydroxy and two major tri-hydroxy narasin metabolites represented together about 30 % of the whole narasin related excreted compounds.

iii) Narasin metabolites in tissues and excreta are qualitatively similar. The liver is the target tissue. A great number of narasin metabolites represent each less than 10 % of the whole tissue residues. However, for control purposes skin/fat and narasin should be retained as practical target tissue and marker residue.

Nicarbazin

The data submitted concerning the metabolic fate of nicarbazin in the chicken and rat include the studies already assessed by the FEEDAP Panel and presented in a previous opinion (EFSA, 2003), a study[37] not submitted and therefore not assessed by the FEEDAP Panel in 2003, as well as a recent study performed using [^{14}C]-HDP-nicarbazin (EFSA, 2010a). All these studies are consistent and the main conclusions are the following:

i) The metabolic steady state is reached after six days.
ii) The metabolic fate of nicarbazin shows that the DNC and HDP components of the complex are split and behave independently, the DNC moiety (and metabolites) being mainly excreted in the faeces (46 % unchanged DNC) while the HDP moiety mainly appears in the urine (90 % unchanged HDP).
iii) DNC metabolic fate has been established and its main metabolites identified in the excreta, bile and tissues: monoacetylamino-DNC resulting from the reduction and acetylation of one nitro group, diacetylamino-DNC corresponding to the reduction and acetylation of both nitro groups, and N,N'-1,4-phenylenebis(acetamide) resulting from the split, reduction and acetylation of the molecule.
iv) Unchanged HDP represents about 85 % of total HDP related residues in liver and kidney, the other metabolites representing less than 10 %.
v) Liver is the target tissue and DNC can be considered as the marker residue.
vi) Nicarbazin metabolic fate is qualitatively similar in the chicken and the rat.

Interaction of nicarbazin constituents

As reported in the recent EFSA opinion on Koffogran (EFSA, 2010a), the bioavailability of DNC administered as nicarbazin to the rat is considerably higher than that of DNC given alone or mixed with HDP in a comparable 1:1 molecular ratio.

[37] Technical dossier/Section III/Appendix 13

Interaction narasin/nicarbazin

A study[38] concerning the metabolic fate of narasin with and without simultaneous administration of nicarbazin has been submitted. Two groups of five chickens (three weeks of age) received for five consecutive days either a diet supplemented with 50 mg [^{14}C]-narasin/kg or the same diet added with 50 mg nicarbazin/kg. The excreta were collected and analysed by LC/MS/LSC. The metabolic profiles of narasin were qualitatively and quantitatively similar whatever the treatment.

Three studies, which were not submitted in the framework of the former assessment of nicarbazin, highlighted the question of the eventual interference of narasin on the metabolism and residues of nicarbazin.

In the first study,[39] four groups of chickens (three males and three females per group, seven weeks old) received for five consecutive days either 50 mg [^{14}C]-narasin/kg feed alone, 50 mg [^{14}C]-DNC-nicarbazin/kg feed alone, 50 mg [^{14}C]-narasin plus 50 mg unlabelled nicarbazin/kg feed or 50 mg [^{14}C]-DNC-nicarbazin plus 50 mg unlabelled narasin/kg feed. Animals were killed at zero-day withdrawal time.

The results indicated that the combination of narasin and nicarbazin administered at the same dosage increased considerably DNC-derived total residues in the liver, kidney, muscle, fat and skin/fat by 20 %, 35 %, 42 %, 21 % and 4 % (not significant), respectively. The ratio DNC vs. total radioactivity was not modified.

A second study[40] was carried out with chickens (five males and five females per group, six weeks old) that received 50 mg [^{14}C]-DNC-nicarbazin/kg feed with or without 50 mg unlabelled narasin/kg feed for five consecutive days. Animals were killed at zero-day withdrawal time. Excreta, bile and tissues were sampled.

The main results were the following: i) the patterns of nicarbazin metabolite distribution in the excreta, bile, liver and kidneys were not modified by the simultaneous administration of narasin (only verifiable for excreta), and ii) DNC-derived total residues in tissues were considerably higher with combined narasin administration, i.e. 25 %, 37 %, 51 %, 40 % and 38 % for the liver, kidneys, muscle, fat and skin/fat, respectively.

The third study[41] was carried out following the same protocol as for the second, but with [^{14}C]-HDP-nicarbazin.

No difference in [^{14}C]-HDP-nicarbazin derived total residues in tissues was found when narasin was co-administered. HDP represented 71 %, 67 % and 84 % of total residues in the liver, kidney and muscle, respectively. The metabolism of the HDP component of nicarbazin was affected neither qualitatively nor quantitatively by narasin.

3.2.1.2. Residues

Narasin

A study[42] of the residue depletion of narasin in tissues of chickens grown in field conditions has been performed. Groups of six birds (one day old, three of each sex) were administered feed supplemented with 50 mg narasin and 50 mg nicarbazin from Maxiban® G160/kg (analytically controlled) for 35 days. Groups of animals were slaughtered after zero, three, five and seven days of withdrawal and

[38] Technical dossier/Section III/Appendix 14
[39] Technical dossier/Section III/Appendix 11
[40] Technical dossier/Section III/ Appendix 10
[41] Technical dossier/Section III/ Appendix 13
[42] Technical dossier/Section III/ Appendix 17

tissues sampled. Narasin residues were measured using an analytical method with a LOQ of 0.025 mg/kg.

After a zero-day withdrawal time, all the results were below or close to (skin/fat) the LOQ, whereas they were all below after three days.

Nicarbazin

Total residues

Two studies concerning the kinetics of tissue residues in chickens fed feedingstuffs supplemented with either 125 mg [^{14}C]-HDP-nicarbazin or 125 mg [^{14}C]-DNC-nicarbazin /kg have been submitted[43, 44] and analysed in the opinion on Koffogran (EFSA, 2010a). Although the total residue figures cannot be retained for nicarbazin-derived residues due to the higher dosage used and the absence of a potential interaction with narasin, the following conclusions must be considered:

iv) Total residues resulting from either the HDP or the DNC moieties of nicarbazin (administered at the highest recommended dose) indicate that (i) HDP-related residues are much lower than those derived from DNC, (ii) after five days all tissues are essentially devoid of HDP-derived residues, and (iii) DNC appears to be the marker residue. Following nicarbazin withdrawal, DNC residues decline rapidly from tissues, liver being the target tissue.

v) The ratios DNC vs. total DNC-related residues calculated for the different tissues at one-day withdrawal time were 0.4, 0.3, 0.2 and 0.4 for the liver, kidney, muscle and skin/fat, respectively.

Marker residue

The residue depletion of nicarbazin, measured as DNC, has been followed at the same time as that of narasin, in the study already described (see narasin above).

DNC residues were quantified using an analytical method with LOQs of 0.050, 0.100, 0.025 and 0.025 mg/kg for the liver, kidney, muscle and skin/fat, respectively. The results are presented in Table 6.

Table 6: DNC residue depletion in tissues of chickens fed a diet supplemented with 50+50 mg narasin+nicarbazin from Maxiban® G160/kg for 35 days followed by a withdrawal period (mg/kg tissue)

Withdrawal period (day)	Liver	Kidney	Muscle	Skin/fat
0	9.190 ± 0.956	4.290 ± 1.034	1.610 ± 0.149	2.040 ± 0.479
3	2.450 ± 0.284	0.295 ± 0.111	0.187 ± 0.028	0.313 ± 0.042
5	0.355 ± 0.071	<LOQ[a]	<0.028	0.060 ± 0.016
7	< 0.088	<LOQ	< LOQ	<0.026

[a] LOQ = 0.05, 0.1, 0.025 and 0.025 mg kg^{-1} for the liver, kidney, muscle and skin/fat

3.2.1.3. Conclusion

The metabolism and residue data for narasin and nicarbazin, administered separately, have already been described and assessed (EFSA, 2003, 2004, 2010a, 2010b).

Additional information concerning Maxiban® G160 indicates that the simultaneous administration of nicarbazin and narasin increases DNC-labelled nicarbazin derived total residues by 20 % (liver) to 50 % (muscle), whereas no modification is observed for HDP-labelled nicarbazin derived residues.

[43] Technical dossier/Section III/Appendix 15
[44] Technical dossier/Section III/Appendix 16

3.2.2. Toxicological studies

3.2.2.1. Acute toxicity

The acute toxicity of narasin was summarised by the FEEDAP Panel in its opinion on Monteban® G 100 (EFSA, 2004). The oral LD_{50} values for mycelial narasin were about 16 mg/kg bw in mice and 19 mg/kg bw in rats.

Two acute studies in mice and rats on nicarbazin assessed in the previous opinion (EFSA, 2003) were both pre-GLP (observation time after dosing was shorter than required in OECD guideline). The results showed that nicarbazin was of low acute toxicity.

An additional study[45] examined the influence of nicarbazin (1000 mg/kg bw) on the acute toxicity of narasin in rats. The single oral doses of narasin were 0, 11, 18, 27.5 or 40 mg/kg bw, administered simultaneously with nicarbazin. The nicarbazin did not increase the acute toxicity of narasin.

3.2.2.2. Genotoxicity (mutagenicity and clastogenicity)

Narasin

In its opinion on Monteban® G100 (EFSA, 2004), the FEEDAP Panel summarised the studies. Narasin was tested in reverse mutation assays in bacteria (standard test strains of *Salmonella* Typhimurium and *Escherichia coli*), a mouse lymphoma assay, a cytogenetics assay in CHL cells, an *in vitro* UDS assay in rat hepatocytes and an *in vivo* test for sister chromatid exchanges in the bone marrow of orally-dosed Chinese hamsters. All tests were negative.

Nicarbazin

In its opinion on Koffogran (EFSA, 2010a), the FEEDAP Panel concluded that:

> 'Nicarbazin gave positive results for mutagenicity in two bacterial studies in some *Salmonella* strains. Since genotoxicity of nicarbazin was absent in mammalian systems (micronucleus and unscheduled DNA synthesis and mouse lymphoma assay, including *in vivo* studies of effects in two somatic tissues), the FEEDAP Panel concludes that nicarbazin is not genotoxic.'

Narasin + nicarbazin

The following tests with the combination narasin+nicarbazin have been provided: (i) a gradient plate assay[46] for bacterial mutation using eight strains of *Salmonella* Typhimurium (G46, TA1535, TA100, C3076, TA1537, D3052, TA1538 and TA98) and two strains of *E. coli* (WP2 and WP2uvrA$^-$), with and without metabolic activation by S9 mix in rats. N-Methyl-N`-nitro-N-nitrosoguanidine (MNNG) and 2-aminoanthracene (9AmAc) served as positive controls. Concentrations between 0.1 and 1000 µg/mL were used; (ii) an Ames assay[47] for bacterial mutation using five strains of *Salmonella* Typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) with and without metabolic activation by S9 mix prepared from rat livers induced by Aroclor 1254. MNNG, 2-nitrofluorene (2NF) and 9AmAc served as positive controls in the non-activated test, while 9AmAc served as positive controls in the activated test. The concentrations per plate were 50–1000µg); iii) an unscheduled DNA synthesis assay in primary cultures of adult rat hepatocytes incubated for 20 hours with concentrations between 0.005 and 10µg/ml. MNNG and 2-acetylaminoflourene served as positive controls with and without metabolic activation; (iv) an *in vitro* assay for forward mutation[48] using L5178Y TK +/- mouse lymphoma cells. Ethylmethanesulfonate and methylcholanthrene served as positive controls.

[45] Technical dossier/Section III/Appendix 36
[46] Technical dossier/Section III/Appendix 39
[47] Technical dossier/Section III/Appendix 40
[48] Technical dossier/Section III/Appendix 42

The concentrations for treatment were between 0.1 and 20 µg/ml; (v) an *in vitro* Chromosome Aberration study[49] in Chinese Hamster Ovary Cells in the presence and absence of Arochlor-induced rat liver S9 activation systems. Mitomycin C served as positive control in the non-activated cells, while cyclophosphamide were used in the activated system; (vi) an *in vivo* test for chromosomal damage[50] in bone marrow cells of Chinese hamsters (treated orally), with cyclophosphamide as positive control, and; (vii) an *in vivo* test[51] for the induction of micronuclei in polychromatic erythrocytes in bone marrow of mice, at dose levels of 4–16 mg/kg bw (oral administration) and with cyclophosphamide as positive control.

All of the systems described above exerted mutagenic or clastogenic reactions by the administration of substances used as positive controls, whereas the combined product containing narasin and nicarbazin showed negative results. This confirms the results obtained with the individual substances.

3.2.2.3. Sub-chronic oral toxicity

Narasin

Sub-chronic studies with narasin (two non-GLP studies in mice, one non-GLP in rat, two (of which one non-GLP) in dogs), identified 1 mg/kg bw as the lowest NOAEL in the dog studies (EFSA, 2004).

Nicarbazin

For nicarbazin, two 90-day studies in rats were provided. One study was conducted with nicarbazin,[52] the other with DNC.[53] These studies were assessed by the FEEDAP Panel in 2010 (EFSA, 2010a) with the following conclusion:

> 'In a sub-chronic rat study, nicarbazin exerted adverse effects at all the dose levels tested (lowest dose 181 mg/kg bw/day). A NOAEL could not be established. A sub-chronic rat study using DNC alone was performed. No DNC related effects were observed at the highest dose tested and a NOAEL of 709 mg DNC/kg bw was derived. These apparently conflicting results may be related to the higher systemic exposure of rats to DNC when administered as nicarbazin instead of DNC alone (or as a simple mixture with HDP).'

Narasin + nicarbazin

A study[54] was conducted in rats (20/sex) fed 0+0, 7.5+7.5 , 20+20 or 60+60 mg narasin+nicarbazin/kg feed which correspond to approximately 0.55, 1.45 and 4.3 mg of each coccidiostat/kg bw, for three months, with the intention to evaluate the potential synergistic toxic effects between narasin and nicarbazin.

There were no deaths or physical signs of toxicity attributable to treatment, nor any toxicologically significant changes in haematology, clinical chemistry, urine analysis or pathology. Ophthalmological examinations showed no differences between controls and dosed groups.

Treatment-related effects were restricted to animals receiving 60+60 mg narasin+nicarbazin/kg and consisted of a slight, but significant, reduction of group mean body weights and/or body weight gains. A NOAEL of 20+20 mg narasin+nicarbazin was identified in this study (around 1.45 mg/kg bw for each compound). This NOAEL is in the range already described after administration of narasin alone to rats. Therefore, synergistic effects are unlikely to occur.

[49] Technical dossier/Section III/Appendix 45
[50] Technical dossier/Section III/Appendix 43
[51] Technical dossier/Section III/Appendix 44
[52] Supplemetary information/September 2009/Appendix 1
[53] Supplementary information/October 2009
[54] Technical Dossier/Section III/Appendix 37

3.2.2.4. One- and two-year toxicity/carcinogenicity studies

In its former assessment of Monteban® G100 (EFSA, 2004), the FEEDAP derived from a one-year dog study the lowest NOAEL of 0.5 mg narasin/kg bw, based on neurological observations and histopathological effects. This NOAEL is retained for further safety considerations.

In its recent assessment of nicarbazin (EFSA, 2010a), the FEEDAP Panel assessed two two-year toxicity studies one performed in the rat and another in the dog and identified as the lowest NOAEL 154 mg DNC (+ 51 mg HDP)/kg bw derived from the dog study.

Chronic toxicological studies with the combination narasin+nicarbazin were not provided.

3.2.2.5. Reproduction toxicity including developmental toxicity

In its former assessment of Monteban® G100 (EFSA, 2004), the FEEDAP Panel identified (i) from a three-generation reproduction study on rats, NOAELs of 0.7–1.5 mg narasin/kg bw and 1.0–1.8 mg/kg bw in male and female parental animals, respectively; (ii) from a developmental study in rats, a NOAEL of 0.7–1.3 mg narasin/kg bw based on maternal toxicity, and; (iii) from a developmental study in rabbits, a NOAEL for maternal toxicity of 0.6 mg narasin/kg bw. At the top dose there was no evidence of developmental toxicity in the rat (3.5 mg/kg bw) and of teratogenicity in the rabbit (1.2 mg/kg bw).

Concerning reproduction toxicity of nicarbazin, the FEEDAP Panel summarised the studies submitted (EFSA, 2010a) as follows:

> 'Despite of the shortcomings of the rat multigeneration studies no major concern appeared at the highest doses tested (300 mg DNC + 100 mg HDP/kg bw/day). Despite some deficiencies in the reporting of data, the FEEDAP Panel concludes there are no concerns on developmental toxicity in rats up to 70 mg nicarbazin/kg bw, based on fetotoxic effects at 200 mg/kg bw. The NOAEL for maternal toxicity in the rabbit study can be conservatively established as 60 mg nicarbazin/kg bw, whereas the NOAEL for developmental toxicity was 120 mg nicarbazin/kg bw (the highest dose given).'

The potential for reproductive toxicity of the combined administration of narasin+nicarbazin was evaluated in a one-generation study[55] in rats (25 females/dose). Doses of 0+0, 0.25+0.25, 0.75+0.75 or 2.25+2.25 mg narasin+nicarbazin/kg bw per day were administered orally to mated female rats on gestation day-6 to day-15. On gestation day-20, the females were killed and evaluated for reproductive performance and the fetuses were examined for external, visceral and skeletal anomalies. Maternal toxicity, consisting of one death, diarrhoea and depressed weight gain and feed consumption, was confined to the high dose group. Prenatal toxicity also occurred in the high dose group. The mean percentage of live fetuses was slightly depressed, due primarily to three females completely resorbing their litters. Fetal body weights were also depressed at this dose level. No structural malformations were seen in the fetuses. The percentages of normal, variant and abnormal fetuses did not differ significantly between the test groups. The NOAEL for maternal and prenatal toxicity was 0.75+0.75 mg narasin+nicarbazin/kg bw. There was no teratogenicity at 2.25+2.25 mg narasin+nicarbazin/kg bw, the highest dose tested.

The results of this study were similar to those of a study conducted previously as a developmental study on rats with narasin alone, with a NOAEL for maternal toxicity of 0.7–1.3 mg/kg bw (EFSA, 2004). However, in the study with narasin alone no resorption of the litters took place and no depression of the fetus weights was found. There is some uncertainty to conclude that the presence of nicarbazin does not modify the maternal or fetal toxicity of narasin. Concerning the consequences, it should be noted that (i) the consumer will not be exposed to nicarbazin but to DNC and to a much lesser extent to HDP, and (ii) the additive is not foreseen for use in laying/breeding poultry.

[55] Technical dossier/Section III/Appendix 38

3.2.2.6. Pharmacological studies

The FEEDAP Panel reported in its opinion on Monteban® G100 (EFSA, 2004) a NOAEL of 1.53 mg narasin/kg bw for effects on the heart following a single oral dose to dogs.

3.2.2.7. Conclusions on toxicological studies

There was no evidence resulting from the toxicological studies for any significant interaction between narasin and nicarbazin.

3.2.3. Assessment of the consumer safety

3.2.3.1. Proposal for the acceptable daily intake (ADI)

In its former assessment of narasin (EFSA, 2004), the FEEDAP Panel established an ADI of 0.005 mg/kg bw (equal to 300 µg/person/day for a 60 kg person), applying an uncertainty factor of 100, based on the NOAEL of a one-year dog toxicity study (0.5 mg/kg bw/day). The same ADI has been retained by JECFA (WHO, 2009).

In its recent assessment of nicarbazin (EFSA, 2010a), the FEEDAP Panel concluded that DNC ADI would protect the consumer as it is only exposed to DNC. An ADI of 0.77 mg/kg bw for DNC was set, derived from the NOAEL of the two-year dog study (154 mg DNC/kg bw) applying a safety factor of 200.

The ADIs established for the single compounds in Maxiban® G160 can be used in assessing the safety of consumers exposed to foodstuffs from chickens for fattening treated with Maxiban® G160.

3.2.3.2. Consumer exposure and proposal for maximum residue limits (MRLs)

Narasin

The highest dose of narasin from Maxiban® G160 (50 mg/kg feed) is lower than the maximum dose (70 mg/kg feed) authorised for use of Monteban® G100. As nicarbazin does not interfere with the metabolic fate and residue status of narasin in chickens, it can be anticipated that total narasin-related residues after administration of Maxiban® G160 would not be higher than those from Monteban® G100 after a similar zero-day withdrawal period. Consequently, consumer exposure to total narasin-derived residues would also comply with the ADI.

After a zero-day withdrawal period, narasin concentrations measured in the different edible tissues (see Section 3.2.1.2) are in the same range as those measured with narasin from Monteban® G100. Therefore, the enforced uniform MRLs of 50 µg narasin/kg edible tissue also apply to narasin from Maxiban® G160.

At present, a validated and sensitive analytical method for the detection of narasin in tissues at EU level is only available for liver and muscle tissues (EFSA, 2010b). Considering the low consumer exposure to total narasin residues by kidney and skin/fat, setting MRLs could be temporarily restricted to MRLs for liver and muscle. This would adequately protect the consumer.

Nicarbazin

Despite the observed interaction resulting in an increase of DNC residues when nicarbazin is administered simultaneously with narasin, the DNC residues measured at a zero-day withdrawal time after administration of the highest recommended dose of Maxiban® G160 (50 mg nicarbazin/kg feed) (Table 6) are almost identical to those measured at a one-day withdrawal time after administration of

the highest recommended dose of Koffogran (125 mg nicarbazin/kg feed, see Table 6 in EFSA, 2010a).

Consequently, (i) consumer exposure to total DNC-derived residues back calculated using the same ratios marker/total residues established after a one-day withdrawal (worst case scenario) would also comply with the ADI, and (ii) the MRLs for DNC in liver (15 mg/kg), kidney (6 mg/kg), muscle (4 mg/kg) and skin/fat (4 mg/kg), proposed in the Koffogran opinion (EFSA, 2010a), would also apply for nicarbazin from Maxiban® G160.

3.2.3.3. Withdrawal period

The DNC residues after administering Maxiban® G160 at a zero-day withdrawal comply with the DNC ADI (4%). Therefore a zero-day withdrawal period for Maxiban® G160 is considered appropriate.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

No studies were provided.

3.3.2. Dusting potential of Maxiban® G160

A Stauber-Heubach test was performed with Maxiban® G160. The dust was analysed for narasin and nicarbazin. The results are summarised in Table 8.

Table 8: Narasin and nicarbazin in dust from Maxiban® G160

Maxiban® G160	Dust (g/m^3)	Narasin in dust (%)	Narasin from dust in air (mg/m^3)	Nicarbazin in dust (%)	Nicarbazin from dust in air (mg/m^3)
Lot A 187781	0.200	0.97	1.94	0.18	0.36
Lot A 206899	0.500	1.50	7.50	0.18	0.90
Lot A 226086	0.585	1.45	8.48	0.17	0.99

The particle size distribution in the dust from Maxiban® G160 (two samples) was determined by Laser diffraction. The results are given in Table 9. About 50 % of the dust particles are of respirable size (≤ 10.5-11.5 µm). Both samples contain particulate matter with particle sizes < 10 µm, but limit values concerning alveolar dust (100 % < 7.1 µm 50 % < 5.0 µm), which are assumed to be health hazardous,56 are not obtained. About 2.5 % of particles in both samples are ≤ 1 µm.

Table 9: Particle size (µm) of dust from Maxiban® G160

Maxiban dust	10 percentile	50 percentile	99 percentile
Lot A 206899	3.90	11.49	31.58
Lot A 226086	5.32	10.48	36.13

3.3.3. Worker/user inhalatory exposure estimate

There are different operations in a premixture factory during which the worker could be exposed to dust from Maxiban® G160. The scenario and the resulting calculations are described in Appendix B-1.

[56] TA Luft 2002 in: Feldhaus/Hansel Bundesimmissionsschutzgesetz, 15. Auflage 2002, C.F. Müller Verlag, Heidelberg

The narasin and nicarbazin uptake of persons handling Maxiban® G160 in a premixture factory via the respiratory route is estimated to 113 and 14 µg, respectively, during an eight-hour working day. This total inhalable amount does not quantitatively reach the lower respiratory tract (trachea, bronchi, bronchioles, alveoli). Particles in the nasopharynx, trachea, bronchi and broncheoles may adhere to the mucus and be absorbed only at a very low level. It can be assumed that the mucus is expectorated, swallowed and absorbed in the gastrointestinal tract in a similar way to dietary exposure, with involvement of the gut flora and first-pass metabolism by the liver.

The Maxiban® G160 dust consists of about 80 % of particles which are assumed to reach the absorptive surface of the alveoli (≤ 16 µm, DIN EN 481, see Appendix B-2). The amount of narasin from fine dust particles reaching the alveoli is calculated on the basis of Appendix B-2 and the laser diffraction analysis of sample 206899.

Whereas the sum of particles ≤ 16 µm in sample 206899 is 79.7 % of total dust, its amount reaching the alveoli is only 11.0 %. During an eight-hour working day (six minutes direct exposure) only 12.4 µg narasin (0.21 µg/kg bw) and 1.54 µg nicarbazin (0.026 µg/kg bw) would reach the alveoli. The NOAEL in two studies with inhalatory exposure of dogs (higher respiration frequency) to narasin (from Monteban® G100) for four hours daily (longer exposure) was ten times higher. Data on inhalatory toxicity of nicarbazin on laboratory animals are not available. The FEEDAP Panel concluded in its recent opinion on Koffogran (EFSA. 2010), where the worst case calculation of inhalatory exposure resulted in a ten times higher value, that 'In view of this assessment and the extensive experience of handling Koffogran/nicarbazin in the workplace and the lack of evidence of any adverse consequence in users the FEEDAP Panel concludes that no special safety measures are required for users above those normally employed for handling dust-generating products.'

3.3.4. Effects on eyes and skin

Studies with Monteban® G100 (EFSA, 2004) showed in both rabbits and dogs eye irritating properties of narasin.

A skin sensitisation study in guinea pigs (Buehler - topical patch method, 1965) was performed with Maxiban® G160, described as a granular formulation containing narasin and nicarbazin probably corresponding to Maxiban® G160.[57] For each treatment, 12 animals were topically treated, under occlusion, with 50 mg Maxiban® G160 for a minimum of six hours, three times weekly for two weeks. Six animals received the test article only during a challenge phase ten days after the induction phase. During the induction phase, dermal irritation in the form of slight erythema and edema was observed after 72 hours. The challenge phase performed with the same Maxiban® G160 dose resulted in comparable irritations. The study did not meet the requirements of OECD guideline 406 because a non-irritant dose required for a sensitisation test was not identified. The results were not clearly indicative for a sensitisation potential. However, in a local lymph node assay with Monteban® G100 (EFSA, 2004) narasin has been identified as a potential skin sensitiser.

3.3.5. Systemic toxicity

In inhalation studies in dogs with Monteban® G100 (EFSA, 2004), a concentration of 1.1 mg narasin/m^3 exerted neurological symptoms like ataxia, limb paresis, tremors and ocular irritation. Different serum enzymes were elevated (creatinine phosphokinase, ALT, AST and LDH). A NOAEL was defined to be 0.11 mg narasin/m^3.

3.3.6. Conclusion on the safety for the user

Maxiban® G160 is considered as a slight skin irritant. Since Monteban® G100 containing narasin, one of the two active substances in Maxiban® G160, is an eye irritant and a skin sensitiser, and because no

[57] Technical dossier/Section III/Appendix 35

(conclusive) studies with Maxiban® G160 were provided, the FEEDAP Panel considers Maxiban® G160 as an eye irritant and a skin sensitiser.

In the light of the assessment of the potential inhalatory exposure and the extensive experience of handling the product in the workplace and the lack of evidence of any adverse consequence in users, the FEEDAP Panel concludes that no special safety measures are required for users above those normally employed for handling dust-generating products.

3.4. Safety for the environment

The active ingredients are not physiological/natural substances of established safety for the environment. The additive is not intended either for companion animals. Consequently, the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC).

In Phase I and II initially a total residues approach will be taken to estimate a worst case $PEC_{initial}$. It will be assumed that both the additives narasin and nicarbazin are excreted 100 % as parent drug. Nicarbazin is an equimolar complex of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6 dimethylpyrimidine (HDP) in a 70:30 w/w ratio, which splits during the intestinal passage. Consequently, the environmental risk assessment should not consider nicarbazin but both components separately. Distribution in the environment is based on the properties of the individual components of Maxiban® G160 as long as no data on relevant metabolites and on potential interaction are submitted.

3.4.1. Exposure assessment

3.4.1.1. Fate and behaviour

Fate in manure

No data on the fate of narasin and nicarbazin in manure of the target animals have been submitted.

Fate in soil

Adsorption

Narasin

The adsorption and desorption behaviour of narasin was determined in five soils using five test concentrations following to OECD guideline 106.[58] The pH of the soils ranged from 5.2 to 7.7, and the soil organic carbon content ranged from 0.7 % to 5.0 %. Correlation coefficients indicated that the isotherms followed the Freundlich equation well. The K_{oc} values ranged from 873 to 2576, with a mean value of 1357.

DNC

The adsorption/desorption behaviour of [^{14}C]-DNC was investigated in three soil types: sandy loam, clay loam and silty clay loam.[59] The pH of the soils ranged from 6.1 to 7.5, and the soil organic carbon content ranged from 1.3 % to 3.1 %.

As adsorption was rapid and complete, the procedures outlined in OECD guideline 106 could not be followed completely. An important deviation is that the test was performed with two instead of five concentrations, which hampers the interpretation of the test results and no accurate K_d/K_{oc} can be obtained. At the lowest concentration (0.02 µg/L), the K_{oc} values were much lower, ranging from 16137 – 21962, than at the highest concentration tested (0.15 µg/L), ranging from 6 2591 to 12 3923.

[58] Technical dossier/Section III/Appendix 46
[59] Technical dossier/Section III/Appendix 48

As the highest test concentration was close to the water solubility, the high K_d/K_{oc} is considered to be an over prediction of the adsorption to soil. Therefore, the lowest values are considered to be more reliable. As only three soils are tested, the lowest K_{oc} value is used for the risk assessment.

HDP

The adsorption/desorption behaviour of [^{14}C]-HDP was investigated in three soil types: sandy loam, clay loam and silty clay loam.[60] The pH of the soils ranged from 6.1 to 7.5, and the soil organic carbon content ranged from 1.3 % to 3.1 %. As no adsorption equilibrium could be established, the procedures outlined in OECD guideline 106 could not be followed completely. Another important deviation is that the test was performed with two instead of five concentrations. Both test concentrations did however result in comparable K_{oc} values ranging from 33 to 114. As only three soils are tested the lowest K_{oc} value is used for the risk assessment.

Degradation

Narasin

A GLP-compliant study, following the SETAC (1995) guideline recommended for the aerobic degradation of pesticides, was performed on the aerobic degradation of [^{14}C]-narasin in three soils (sandy loam, a silt loan and a clay loam).[61] HPLC and TLC were applied for the identification of potential metabolites and a CO_2 trap for quantifying mineralisation. The duration of the study was 84 days with samples analysed on days 0, 7, 14, 21, 28, 42, 56, 70 and 84. Several metabolites where found but not identified. Only one metabolite was found at concentrations higher than 10 % of the total radioactivity with a maximum of 16 % in silt loam at 70 days after application, decreasing to 14 % at 84 days. Mineralisation to CO_2 was the main degradation process, accounting for 64 % in sandy loam, 19 % in silt loam and 54 % in clay loam. Non-extractable residues accounted for 18 %, 20 % and 25 %, respectively. The DT_{50} values for sandy loam, silt loam and clay loam were 21, 49, and 29 days; the DT_{90} values were 69, 162 and 96 days, respectively.

DNC

The aerobic degradation of DNC in accordance with OECD 307 was evaluated in sandy loam, sandy clay loam and silt loam soils using [^{14}C]-DNC and included the estimation of the fate and behaviour in soil.[62] Evolved [^{14}C]-O_2 was low throughout the study period, for all soils, accounting for 1 to 2 % of applied radioactivity after 120 days. Chromatographic analyses indicated that DNC was the only significant component present in all soil types. At 64 and 120 days, up to four minor components ($\leq$ 3 % each) were detected but not identified. The dissipation was mainly attributed to the formation of bound residues accounting for 27 % in the sandy loam. In the other soil types, the non-extractable residues were not determined. The DT_{50}, based on first order kinetics, was 239, 193 and 257 days in sandy loam, sandy clay loam and silt loam soil types, respectively.

HDP

The aerobic degradation of [^{14}C]-HDP was studied as described above for DNC, using the same soil types.[63] The formation of [^{14}C]-O_2 was relatively high throughout the study, accounting for 22 to 31% of applied radioactivity after 120 days. HDP was the only significant component present in all samples from all soil types; no metabolites representing more than 10 % of the total radioactivity applied were found. Dissipation was strongly attributed to the fast formation of non-extractable residues as demonstrated in sandy loam soil (not determined in the other soil types). Based on linear regression and first order kinetics, the DT_{50} was calculated as six, seven and three days, the DT_{90} as 20, 23 and 11 days, in sandy loam, sandy clay loam and silt loam, respectively.

[60] Technical dossier/Section III/Appendix 49
[61] Technical dossier/Section III/Appendix 50
[62] Technical dossier/Section III/Appendix 51
[63] Technical dossier/Section III/Appendix 52

Fate in water

No data have been submitted on (photo-)biodegradation of narasin, DNC and HDP in water.

3.4.1.2. Predicted environmental concentrations (PECs)

The methods to estimate PEC in manure, soil, groundwater and surface water are described in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008). The calculated values are shown in Table 10.

Table 10: Initial predicted environmental concentrations (PECs) of DNC, HDP and narasin in soil (µg/kg), groundwater and surface water (µg/L)

Compartment	Arable Land		
	DNC[(a)]	HDP	narasin
Soil	276	76	260
Groundwater	0.96	110	10.8
Surface water	0.32	37	3.6

(a) The DT_{50} of DNC is > 60 days, therefore a PEC plateau is calculated to take the potential of accumulation in soil into account.

All Phase I PEC trigger values for soil and groundwater are exceeded. Therefore, a Phase II assessment is considered necessary.

3.4.2. Effect assessment

3.4.2.1. Toxicity to soil organisms

Effects on soil micro-organisms

Narasin

The effects of narasin on soil carbon respiration and soil nitrification were determined following OECD guidelines 217 and 216, respectively.[64] No effects above the 25 % trigger selected for these studies were observed for concentrations equal to or lower than 17.43 mg/kg soil. Therefore, the NOEC for effects on soil respiration and soil nitrification is established at 17.43 mg/kg soil.

DNC and HDP

The effects of DNC and HDP on soil nitrification were determined in two separate tests using sandy loam soil, following OECD guideline 216.[65,66] No effects above the 25 % trigger selected for these studies were observed for concentrations equal to or lower than 8 mg DNC and 3.5 mg/kg soil. Therefore, the NOEC for effects on soil respiration and soil nitrification is established at 8 mg DNC and 3.5 mg HDP/kg soil.

Hansen et al. (2009) investigated the antibacterial potency of narasin and nicarbazin separately and in combination, using a soil bacteria bioassay based on a pour (agar) plate method according to ISO guideline 15522. This technique is used for counting viable bacteria in order to obtain dose-response curves. Bacteria were extracted from common soil type in Denmark, which has been used for organic farming with application of swine manure since 1990. Narasin exhibited an EC_{50} value of 19.6 µM, while nicarbazin exhibited an EC_{50} value above the maximum tested concentration of 21 µM.

[64] Technical dossier/Section III/Appendix 59
[65] Technical dossier/Section III/Appendix 60
[66] Technical dossier/Section III/Appendix 61

Assuming that the adsorption of narasin, DNC and HDP is related to the organic carbon content in soil, the EC_{50} values could be translated towards a concentration in soil using the same calculation method to determine the concentration in pore water/groundwater. For narasin, the EC_{50} value of 19.6 µM would be equal to 270 mg/kg based on the soil characteristics used in the nitrification study. For DNC and HDP, the observed EC_{50} value would be equal to > 1400 and > 1.4 mg/kg, respectively. This indicates that the results of Hansen et al. are not contradictory to the results of the soil nitrification and respiration studies.

Remarkably, when both substances were tested in combination (1:1 w/w), the toxicity increased by more than one order of magnitude: 0.4 µM (i.e. 0.5 mg narasin+nicarbazin/mL). No explanation could be given for this synergistic effect; however, a possible combined effect between the tested compound and the solvent DMSO could no be excluded. By contrast, in *Bacteroides* isolates of poultry origin, the presence of nicarbazin did not affect the MICs against narasin (see Section 3.1.2).

It is not easy to translate the combined effect to the soil matrix as narasin, DNC and HDP have different adsorption properties and will therefore not be present in the same ratio in pore water as it has been tested in the bioassay. The combined effect will therefore not be used in the PNEC derivation, but its relevance will be discussed in the risk characterisation.

Effects on plants

Narasin

A study was conducted on the toxicity of narasin to winter oat, radish and mung bean following OECD guideline 208.[67] The study indicates significant differences among tested species, with radish (*Raphanus sativus*) being the most sensitive. The LC_{50} for emergence is reported as 5.07 mg/kg soil, and an EC_{50} and NOEC for growth higher than 3.38 and 0.375 mg/kg soil, respectively.

In an older greenhouse study,[68] the phytotoxicity was tested in soil incorporated with floor pen litter from chickens fed with 40 and 80 mg narasin/kg feed. The litter contained 5.2 and 8.4 mg narasin/kg. From each litter, three exposure levels were obtained by mixing 15, 37.5 and 75 g litter with 5 kg soil. Based on the highest litter concentration this would have resulted in a maximum exposure level of 0.13 mg narasin/kg soil. Fourteen plants species (including both mono- and dicotyledonous plants) were tested and none of these species showed a sign of injury after a growing period of 24 days.

The test results are difficult to validate as no information on the test conditions, soil characteristics, analytical method and data, and on the method to measure injury of the plants was provided.

HDP

The toxicity of HDP[69] on the following terrestrial plants has been tested in loamy sand soils: Oats (*Avena sativa*), lettuce (*Lactuca sativa*), turnip (*Brassica rapa*), perennial ryegrass (*Lolium perenne*), mung bean (*Phaseolus aureus*) and radish (*R. sativus*). OECD guideline 208 was not completely followed as instead of using a concentration range, effects were measured at one, five and ten times the estimated PEC of 0.35 mg/kg. No effect of HDP on the emergence and growth of lettuce, oats, ryegrass and turnip seedlings. HDP did have a phytotoxic effect on the emergence of both radish and mung bean, resulting in LC_{50} values of 2.8 and 2.9 mg/kg, respectively. The mean radish shoot fresh weight was higher in the x10 rate group compared with the controls although this was not confirmed with the dry weight analysis. For mung bean, the total dry weight at 3.5 mg/kg (x10) was significantly lower than the controls. However, fewer plants emerged in the 3.5 mg/kg group than in the controls. The NOEC for both plants was 1.75 mg/kg.

[67] Technical dossier/Section III/Appendix 56
[68] Supplemetary information/July 2009/ERA/Appendix 5
[69] Technical dossier/Section III/Appendix 57

DNC

The toxicity of DNC[70] to terrestrial plants has been tested using the same species and test design as for HDP (see above). Effects were measured at one, five and ten times the estimated PEC of 0.8 mg/kg. No effects were observed on the emergence of any of the crop species tested. DNC did not have any effect on the growth of lettuce, mung bean, radish, ryegrass and turnip seedlings. The mean fresh weight of oats decreased slightly, but not significantly, as the concentration of DNC increased. The NOEC was 8 mg/kg.

Effect on earthworms

Narasin

The effect of narasin on *E. foetida* was tested[71] in an artificial soil (70 % sand, 20 % clay and 10 % organic matter) under laboratory conditions using six test concentrations up to 270 mg narasin/kg soil (each treatment in four fold), an exposure period of 14 days at 20–22 °C, according OECD guideline 207. The reported 14 days LC_{50} was 46.4 mg/kg.

DNC and HDP

The effect of DNC[72] and HDP[73] on *E. foetida* was tested in two separate studies in an artificial soil (70 % sand, 20 % clay and 10 % organic matter) under laboratory conditions using six test concentrations of 0, 95, 171, 309, 556 and 1 000 mg/kg soil (each treatment in four fold), an exposure period of 14 days at 21–22 °C, according to OECD guideline 207. No mortality occurred at any test concentration. The LC_{50} for both DNC and HDP is > 1 000 mg/kg soil.

3.4.2.2. Toxicity to aquatic organisms

Narasin

The toxicity of narasin to the unicellular algae *Selenastrum capricornutum* was determined following OECD guideline 201.[74] The reported 72 hours EC_{50} based on growth rate was 2.92 mg/L.

The acute toxicity of narasin to *Daphnia magna* was determined following the US EPA and ASTM guidelines, which are similar to OECD guideline 202.[75] Toxicity is reported as measured concentrations and therefore the study is considered valid. The reported 48 hours EC_{50} was 20.56 mg/L.

The acute toxicity of narasin to rainbow trout (*Oncorhynchus mykiss*) juveniles was determined following the US EPA and ASTM guidelines, which are similar to OECD guideline 203.[76,77] The 96 hours LC_{50} based on measured concentration was 2.23 mg/L.

No data on the toxicity of narasin to sediment dwelling organisms has been submitted.

Nicarbazin

The acute toxicity of nicarbazin to *D. magna*[78] and rainbow trout (*O. mykiss)* was determined following the US EPA and ASTM guidelines, which are similar to OECD guideline 202. Toxicity was

[70] Technical dossier/Section III/Appendix 58
[71] Technical dossier/Section III/Appendix 61
[72] Technical dossier/Section III/Appendix 54
[73] Technical dossier/Section III/Appendix 55
[74] Technical dossier/Section III/Appendix 67
[75] Technical dossier/Section III/Appendix 63
[76] Technical dossier/Section III/Appendix 65
[77] Technical dossier/Section III/Appendix 66
[78] Technical dossier/Section III/Appendix 64

performed as a limit test at a concentration of 100 mg nicarbazin/L. Because DNC is considered by the applicant insoluble in water, only HDP was measured. The measured concentration in the Daphnia study was 24.2 mg HDP/L, in the trout study 26.7 mg HDP/L, both concentrations remaining stable up to the end of the test. No acute toxicity was observed in Daphnia indicating that the LC_{50} for HDP is > 24.2 mg/L. No acute toxicity in trout was observed indicating that the LC_{50} for HDP is > 26.7 mg/L.

As DNC was not analysed in neither of the studies, it is unclear to which DNC concentration the animals were exposed.

In a more recent study, the toxicity of DNC to *D. magna*,[79] rainbow trout (*O. mykiss)*[80] and Blue Gill *(Lepomis macrochirus)*[81] were determined following OECD guidelines 202 and 203. All test conditions were within the acceptable limits. In these studies also the exposure concentrations were measured. The toxicity to *D. magna* was tested in a dose range, whereas the toxicity to the two fish species was tested in a limit test at a nominal concentration of 100 µg/L, resulting in measured concentrations around the reported maximum water solubility (i.e. 69 and 72 µg/L, respectively). The 48 hours EC_{50} for *D. magna* and the 96 hours LC_{50} for *O. mykiss* and *L. macrochirus* could not be determined because $E(L)C_{50}$ was not reached up to the maximum water solubility.

In an older study, not reported in detail, both DNC and HDP were tested for acute toxicity to the algae *Chlorella pyrenoidosa*, *D. magna* and the fishes *Poecilia reticulata* (guppy) and *O. mykiss* (rainbow trout).[82] The study has already been assessed by the FEEDAP Panel in its former opinion on Koffogran (EFSA, 2003). Because of the deficiencies in analysing essential parameters and in reporting data, the FEEDAP Panel does not consider this study valid, as it did not in 2003.

The applicant was informed that a full assessment of the algae study[83] would not be possible because information on the chemical analysis and detailed experimental results are lacking. The applicant informed EFSA that he was unable to obtain more details about this study. The applicant submitted instead for consideration that there should be no reason for doubts about the reliability of the study performed by a well-reputed research institute. The FEEDAP Panel could not follow those arguments and reiterates its position that in the absence of raw data, the toxicity of DNC and HDP to algae cannot be evaluated.

No data on the toxicity of DNC and HDP to sediment dwelling organisms has been submitted.

3.4.2.3. Conclusion

PNEC for the terrestrial compartment

The most sensitive endpoint observed for narasin is the EC_{50} for growth for plants of 3.38 mg/kg. Using an assessment factor of 100, the resulting PNEC for the soil compartment is 34 µg/kg. For HDP, the most sensitive endpoint is seed emergence with a NOEC of 1.75 mg/kg. This results in a PNEC of 0.18 mg/kg using an assessment factor of 10. For DNC, the lowest endpoint is a NOEC of 8 mg/kg for plants and micro-organisms, resulting in a PNEC of 0.8 mg/kg using an assessment factor of 10.

PNEC for the aquatic compartment

The lowest toxicity value for narasin was found for fish with a $L(E)C_{50}$ value of 2.2 mg narasin/L. By applying a safety factor of 1 000, the PNEC for aquatic organisms is 2.2 µg narasin/L.

[79] Supplemetary information/July 2009/ERA/Appendix 7
[80] Supplemetary information/July 2009/ERA/Appendix 8
[81] Supplemetary information/July 2009/ERA/Appendix 9
[82] Technichal dossier/Section III/Appendix 69
[83] Technical dossier/Section III/Appendix 69

Although the toxicity for *D. magna* and fish has sufficiently been investigated, for both DNC and HDP the toxicity data for algae could not be evaluated due to insufficient data. Consequently, a PNEC for the aquatic environment for both DNC and HDP can still not be determined.

3.4.2.4. Bioaccumulation

No experimentally determined bioconcentration factors (BCF) for earthworm and fish have been submitted. Since the log K_{ow} for narasin and DNC is 4.85 and 3.6, respectively, both components have a potential for bioaccumulation.

3.4.3. Risk Characterisation

3.4.3.1. Risk for soil

Based on the assumption that 100 % of the highest use dose is excreted as parent compound, a risk based on the sensitivity of plants is identified for narasin. For HDP and DNC no risks are identified.

The risk for narasin can be refined based on the metabolism data showing that 5 % of the dose is excreted as parent compound and 30 % is excreted as metabolites having not more than 20 % of the ionophoric activity (as a worst case assumption). Hence it is justified to consider only 11 % of the total narasin-related excreted products as environmentally relevant. The resulting PEC:PNEC ratio is < 1 (Table 11), indicating that narasin would not pose a risk for the terrestrial environment.

As explained in Section 3.4.2, it is difficult to assess the relevance of the observed synergistic effect of narasin and nicarbazin on micro-organisms. However, since the estimated concentrations in pore water for narasin and DNC are far below the EC_{50} at which this combined effect was observed, it is considered unlikely that a combined effect of narasin and nicarbazin on micro-organisms would occur under the condition of use of Maxiban® G160.

It should be noted that the PNEC for DNC is based on an unbounded NOEC. Furthermore, in a plant study with nicarbazin no effects were observed up to 1 000 mg/kg (EFSA, 2003). Considering that HDP is excreted by approximately 65 % of the dose, an unacceptable risk as a result of a combined effect of narasin, DNC and HDP is therefore not expected.

Table 11: PEC/PNEC comparisons based on 100 % of the proposed recommended dose and refined based on metabolism data

Compound	PEC_{soil} (mg/kg)	PNEC (mg/kg)	PEC/PNEC
Narasin	0.26	0.034	7.6
	0.029 (refined)	0.034	0.84
DNC	0.27	0.8	0.3
HDP	0.078	0.18	0.4

3.4.3.2. Risk for surface water

Based on the assumption that 100 % of the proposed recommended dose is excreted as parent compound, the PEC/PNEC ratio for narasin is 1.7 (3.6/2.2). After refinement of the PEC based on metabolism data, the PEC/PNEC is < 1, indicating that narasin would not pose a risk for the aquatic environment, including sediment dwelling organisms.

Since the toxicity data on algae submitted for DNC and HDP could not be evaluated, the risk for the aquatic environment for these compounds can not fully be assessed.

It should be noted that for crustaceans and fish no acute toxicity was observed up to water solubility. An absence of short-term toxicity does not, however, necessarily mean that a substance has no long-term toxicity. As a general rule used for industrial chemicals and biocides, long-term toxicity tests are required for substances with log K_{ow} > 3 (or BCF > 100) and a PEC > 1/100th of the water solubility. The initial PEC for both HDP and DNC is < 1/100th of the water solubility. In addition, QSAR calculations for amides and substituted urea, using EcoSAR v.1.0, show that the chronic toxicity value of DNC is > 10 higher than the initial PEC. The FEEDAP Panel therefore believes there is a sufficient margin of safety and does not consider chronic toxicity studies for crustaceans and fish necessary. For the same reason it is not expected that the initial PEC for sediment based on equilibrium partitioning will cause an unacceptable risk for sediment dwelling organisms. No further tests are considered necessary.

3.4.3.3. Risk for groundwater

Based on the screening model described in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008), the PEC ground water for narasin, DNC and DHP is > 0.1 μg/L. However, taking the reported degradation rates in soil into account and using the FOCUS PEARL model, the calculated concentrations did not exceed the groundwater trigger of 0.1 μg/L.

3.4.3.4. Risk for secondary poisoning

Narasin and DNC have a log K_{ow} of 4.85 and 3.6, respectively, and therefore both substances have a potential for bioaccumulation. No bioconcentration factor (BCF) values for earthworms and fish have been provided. Based on target species data, the lowest NOAEL for narasin and nicarbazin in birds/mammals is 20 and 25 mg/kg feed, respectively. Using an assessment factor of 30 in accordance to the REACH guidance for existing chemicals, the $PNEC_{oral}$ is 0.67 and 0.83 mg/kg feed, respectively. The calculated PEC_{oral} for narasin and DNC for fish eating birds/mammals and worms eating birds/mammals are presented in Table 12 (assuming that 50 % of the diet is taken from exposed soil/water) (Table 12). The PEC/PNEC ratios both for surface water and soil are below 1. The FEEDAP Panel therefore concludes that there are no safety concerns with regard to secondary poisoning.

Table 12: Risk assessment for secondary poisoning for narasin and DNC based on 100 % of the proposed recommended dose

	$PEC_{oral, sw}$ (mg/kg)	**$PEC_{oral, soil}$ (mg/kg)**	**$PNEC_{oral}$ (mg/kg)**	**PEC/$PNEC_{sw}$**	**PEC/$PNEC_{soil}$**
Narasin	0.034	0.37	0.67	0.051	0.56
DNC	0.026	0.093	0.83	0.031	0.11

3.4.3. Conclusion

Considering the condition of use of Maxiban® G160 and based on the data provided, a safety concern for the soil compartment, groundwater or by secondary poisoning could not be identified. In the absence of raw toxicity data for algae the risk for surface water can, however, not be assessed.

4. Efficacy

No recent dose titration studies under experimental conditions are required for the re-evaluation of a coccidiostat. The applicant consequently did not submit new data for this purpose. To assess the efficacy of a coccidiostat already in use for several years, three recent floor pen studies and three recent field trials are considered necessary, conducted under current use conditions (and using recently isolated *Eimeria* strains in floor pen studies).

4.1. Controlled battery cage experiments (artificial single and mixed infections)

A total of 13 battery trials with a total of 9 100 birds were performed to establish the optimum ratio and dose of narasin and nicarbazin.[84] The chickens were infected with different strains of *Eimeria* (*E. tenella*, *E. acervulina* and *E. maxima*) that varied in the resistance to narasin and nicarbazin. Doses of 0, 20, 40 60, 80, 100 and 120 mg/kg feed of each coccidiostat alone or in combination, but not exceeding 120 mg/kg feed for the total anticoccidial concentration. Parameters measured were body weight, oocyst excretion and lesion score.

The results indicated that the optimum narasin:nicarbazin ratio would be 1:1 at doses between 40 and 60 mg/kg of each substance (the maximum dose tested).

A total of 95 trials on 12 390 birds were conducted in order to establish the most efficacious dose of a 1:1 narasin to nicarbazin mixture. The experimental plan followed an incomplete two-factorial design (uninfected-untreated (UU), infected-untreated (IU), infected-treated (IT)). The 1:1 ratio of narasin and nicarbazin was tested at 20, 30, 40 or 50 mg/kg feed of each of the coccidiostats. Birds were inoculated with mixed species of *Eimeria* (*E. tenella*, *E. acervulina*, *E. maxima*, *E. mivati*, *E. brunetti and E. necatrix*) (32 trials, containing at least two strains). Some of the *Eimeria* strains tested were resistant to nicarbazin, to narasin and to both products. Parameters measured were performance parameters, mortality, oocyst excretion and lesion score.

The overall best results were obtained with doses from 40:40 to 50:50 mg/kg of each coccidiostat. The optimum dose depended on the *Eimeria* susceptibility. Strains resistent to nicarbazin only required doses of at least 40:40 mg/kg, to narasin (only in 87 % of the cases) doses equal to or higher than 40:40 mg/kg and to both components doses of 50:50 mg/kg of each coccidiostat.

4.2. Controlled floor pen studies

Four series of floor pen trials were conducted: 13 studies in the US, ten studies in Europe in 1983-1984, a further 14 floor pen studies in Europe in 1986-87 and three recent studies in Europe in 2006-2007. Only the latter studies were considered.

The recent studies[85,86,87] showed a similar experimental design consisting of an uninfected and untreated group (UU), an infected and untreated group (IU), and an infected and treated (IT) group receiving 40 mg narasin and 40 mg nicarbazin/kg feed (80 mg Maxiban® G160/kg feed). One-day-old chickens (separated by sex) were distributed in pens and fed the experimental diet for at least 35 (trials 1 and 2) and up to 42 days in trial 3. Animals were inoculated via feed on day 14 of experiment with the prepared inoculums (for details see Table 13).

Body weight, feed conversion ratio and mortality were measured for the treatment periods. Intestinal lesions were scored on day 20. Faecal samples were obtained on day 13, 21, 28, 35/37 for determination of oocysts per gram faeces.

Performance data are summarised in Table 13. No significant differences were observed concerning mortality in trial 2 and 3, but in trial 1 the infected-untreated group showed a significant higher ($P < 0.05$) mortality compared to UU group and the IT group (Maxiban® G160). Body weight gain and feed conversion were significantly improved in the IT groups compared to the IU group but did not reach the level of the UU groups.

[84] Technical dossier/Section 4/Appendix 1-9
[85] Technical Dossier/Section IV/Appendix 19
[86] Technical Dossier/Section IV/Appendix 20
[87] Technical Dossier/Section IV/Appendix 21

Table 13: Experimental design of three floor pen studies and performance data[1]

Trial	birds per pen (replicates per treatment)	Inoculum characteristics (intended dose per bird)	Treatments	Mortality (%)	Weight gain (g)	FCR (g g^{-1})
1[88]	50 (10)	1.5 x 10^5 *E. acervulina* 7.5 x 10^4 *E. maxima* 7.5 x 10^4 *E. brunetti*	Uninfected-untreated Infected-untreated Infected-treated	9.0^b 19.2^a 7.6^b	2101^a 1739^c 1996^b	1.62^c 1.83^a 1.69^b
2[89]	50 (10)	2.5 x 10^5 *E. acervulina* 7.5 x 10^4 *E. necatrix*	Uninfected-untreated Infected-untreated Infected-treated	6.4 10.4 7.2	1979^a 1629^c 1886^b	1.73^b 1.99^a 1.67^b
3[90]	45 (10)	6.6 x 10^4 *E. acervulina* 7.5 x 10^3 *E. maxima* 7.5 x 10^3 *E. tenella*	Uninfected-untreated Infected-untreated Infected-treated	2.2 1.3 2.2	2385^a 2230^c 2295^b	2.13^c 2.29^a 2.23^b

[1] The parameters refer to the 35 d period under experiment for trial 1 and 2, and to the 42 d period for trial 3.
[a,b,c] Means within the same column and within the same trial with different superscript are different ($P < 0.05$).

The Maxiban® G160 treatment decreased significantly the oocysts excretion (Table 14) in two of the three trials when compared to the UU group. The effect was found after seven days post-inoculation and disappeared after 14 days.

Table 14: Faecal oocysts counts/g faeces before and at different days post-infection

Trial[1]	Treatments	Day 0[2]	Day 7	Day 14	Day 21[3]
1	Uninfected-untreated	0	0^c	0^b	0.5^b
	Infected-untreated	0	243000^a	53055^a	11475^a
	Infected-treated	0	81449^b	62175^a	12150^a
2	Uninfected-untreated	0	0^c	0^b	817^b
	Infected-untreated	0	208590^a	27850^a	25055^a
	Infected-treated	0	41397^b	49385^a	25900^a
3	Uninfected-untreated	0	0^b	0^b	2^c
	Infected-untreated	0	2425^a	1853^a	508^b
	Infected-treated	0	2391^a	1157^a	751^a

[1] For references see Table 13.
[2] The inoculation was carried out in all the trials on day 14. The sampling was carried out one day before the infection in trial 1 and 2 and on the same day on trial 3.
[3] The values for the third trial refer to day 23 post-infection.
[a,b,c,] Means within the same column and within the same trial with different superscript are different ($P < 0.05$).

The lesion scores (Table 15) were the lowest in the UU group, the addition of Maxiban® G160 to the diets reduced the scores in 50 % of the observations when compared to the IU group.

[88] Technical Dossier/Section IV/Appendix 19
[89] Technical Dossier/Section IV/Appendix 20
[90] Technical Dossier/Section IV/Appendix 21

Table 15: Lesion score in different regions of the intestine six days after inoculation

Trial[1]	Treatments	Small intestine			Caeca
		Upper	Middle	Lower	
1	Uninfected-untreated	0.00[b]	0.00[c]	0.00[a]	0.00[b]
	Infected-untreated	0.50[a]	0.60[a]	0.84[a]	0.22[a]
	Infected-treated	0.90[a]	0.32[b]	0.16[b]	0.00[b]
2	Uninfected-untreated	0.00[c]	0.00[b]	0.00	0.00
	Infected-untreated	0.92[a]	0.82[a]	0.10	0.02
	Infected-treated	0.46[b]	0.32[b]	0.04	0.00
3	Uninfected-untreated	0.00[c]	0.08[b]	0.00	0.00[b]
	Infected-untreated	1.46[a]	0.97[a]	0.20	2.63[a]
	Infected-treated	1.13[b]	0.82[a]	0.02	2.24[a]

[1] For references see Table 13.
[a,b,c] Means within the same column and within the same trial with different superscript are different ($P < 0.05$).

4.3. Controlled field trials

A total of four field trials were carried out in two different countries (Germany and France). The trials had a similar design allowing to carry out a meta-analysis of the results.

In all the trials, two treatments were considered; a group of chickens fed Maxiban® G160 at 80 mg/kg and a group of chickens fed a polyether coccidiostat at 60 mg/kg (confirmed by analyses) from day 1 of age to day 27 of age in Trial 1 and 2 (Germany)[91,92] and to day 30 of age in Trial 3 and 4 (France).[93,94] After withdrawal periods the broilers were slaughtered (day 35 in Trial 1, day 36 in Trial 2, day 39 in Trial 3 and day 40 in Trial 4). The birds were not infected, the exposure depended on the natural occurrence of field *Eimeria*.

A total of 22 000 (Trial 1), 9 150 (Trial 2), 6 000 (Trial 3) and 5 500 (Trial 4) chickens for fattening (commercial strains, male and female) were included in the respective trials. There were one replicate per treatment in Trial 2 and two replicates per treatment in the other trials; consequently, seven replicates per treatment were available for statistical evaluation.

Birds were monitored daily for general condition and mortality. Faecal samples were collected on days 7, 14, 21, 28 and 35. During general health examinations, two pooled samples from ten individual droppings were collected per group and oocyst counts were performed. Birds were selected for lesion scoring in the intestine on day 7, 14, 21, 28, and 35 (10 in trial 1 and 2; 20 in trial 3 and 4). Final body weight was taken as the main parameter in the statistical evaluation. In Trial 1 and 2, the birds were individually weighted on day 1 and day 36, in Trial 3 and 4, group weight was determined. The total number of birds weighted was 200/22 000 in Trial 1, 2 000/9 150 in Trial 2, 400/6 000 in Trial 3 (group size 100 birds) and 400/55 000 in Trial 4 (group size 100 birds).

The results of the meta-analysis of all four trials are summarised in Table 16. In all four trials the mortality (2.1–7.5 %) was lower in Maxiban® G160 groups than in the polyether coccidiostat groups. The body weight of chickens for fattening of the Maxiban® G160 groups was higher than of the polyether coccidiostat groups in the meta-analyis (and in all trials). The improvement of feed to gain ratio in the Maxiban® G160 groups followed the same pattern.

In Trial 1, no coccidial lesions were seen, but the presence of oocysts showed that there was a natural exposure to field *Eimeria*. The total oocyst counts for all samples and times were lower for Maxiban® G160 than for the coccidiostat used for comparison.

[91] Technical dossier/Section IV/Appendix 22
[92] Technical dossier/Section IV/Appendix 23
[93] Technical dossier/Section IV/Appendix 19
[94] Technical dossier/Section IV/Appendix 20

In Trial 2 and 3, the total scores of coccidial lesions in the Maxiban® G160 group were lower than in the polyether coccidiostat group, but the total oocyst count for all samples and times were higher for the Maxiban® G160 group.

In Trial 4, the total scores of coccidial intestinal lesions in the Maxiban® G160 group were lower than in the coccidiostat used for comparison, and so were the total oocyst counts for all samples and times for the Maxiban® G160 group.

Table 16: Overall results of the meta-analyses carried out for the results obtained in the four field trials

Parameter	Maxiban® G160 (80 mg/kg)	Polyether coccidiostat	*P* - value
Final body weight (kg)	1.85	1.77	0.01
Feed intake (kg)	3.08	3.07	0.91
Feed : gain ratio (kg/kg)	1.63	1.71	0.08
Mortality (%)	3.47	3.83	0.09

4.4. Studies on the quality of the animal product

The potential influence of Maxiban® G160 at the highest recommended dose on the flavour of cooked chicken meat was examined.[95]

A total of 408 one-day-old chickens (Hubbard x Hubbard, both sexes) was distributed to a total of 16 pens (four replicates per treatment and sex). Two dietary treatments were considered, a control diet and a diet containing 100 mg Maxiban® G160/kg (confirmed by analysis). The starter diet was fed from day 1 to 21, followed by the grower diet until day 45, and the unsupplemented diet for a four-day withdrawal period. On day 49, the birds were slaughtered, the carcass weight was recorded, the carcasses were frozen until analysis for flavour. The zootechnical performance of the two groups did not differ.

A triangular test performed by 12 trained individuals did not show detectable differences in flavour for either dark or white meat, whether fried or baked, and whether reheated or not between the two groups.

4.5. Conclusion on efficacy

The optimum dose range was 80–100 mg active substances from Maxiban® G160/kg with a narasin to nicarbazin ratio of 1:1. In three floor pen studies with artificial mixed infection, Maxiban® G160 at the lowest proposed dose was effective against *Eimeria* infection as shown by a significant improvement of weight gain and feed to gain ratio, and by a reduction of the intestinal lesion score. The meta-analysis of the results of four field trials, also performed with the lowest proposed dose, showed a significant increase in body weight compared to a treated control group and a tendency for a better feed to gain ratio. It should be noted that performance data included an eight- to ten-day withdrawal period and were based on reduced animal samples, which would not have been representative due to the small size and limited number of replicates in some trials. Another positive finding based on all birds was a tendency to reduced mortality. Considering that the control group received feed supplemented with another coccidiostat authorised, Maxiban® G160 is considered to be at least as effective as that coccidiostat under field conditions.

Maxiban® G160 at the highest proposed dose does not influence the quality of animal product as shown by a panel triangular test with processed chicken meat compared to an untreated control.

[95] Technical dossier/Section IV/Appendix 27

5. Post-market monitoring

No specific risks associated with the use of Maxiban® G160 could be identified. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[96] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The FEEDAP Panel concludes that Maxiban® G160 up to the highest use level (0.625 g Maxiban® G160/kg feed corresponding to 50+50 mg narasin+nicarbazin) is safe for chickens for fattening. The margin of safety for Maxiban® G160 in chickens for fattening is small but at least equal to 1.25 (62.5+62.5/50+50).

Narasin is toxic to equines, turkeys and rabbits at levels below those used in the prevention of coccidiosis in chickens. Interactions between the polyether ionophore coccidiostats, including narasin, and tiamulin as well as other antimicrobials (mainly macrolides) are demonstrated in the literature.

Nicarbazin does not display any antimicrobial properties and consequently no microbiological safety concern is associated with this compound. However, narasin has an antimicrobial activity against several Gram-positive intestinal bacterial species (0.25–4.0 mg/L).

The metabolism and residue data for narasin and nicarbazin administered separately have already been described and assessed. Additional information concerning Maxiban® G160 indicates that the simultaneous administration of nicarbazin and narasin increased DNC-labelled nicarbazin derived total residues by 20 % (liver) to 50 % (muscle), whereas no modification was observed for HDP-labelled nicarbazin derived residues.

There was no evidence resulting from the toxicological studies for any significant interaction between narasin and nicarbazin.

An ADI for narasin of 0.005 mg/kg bw (equal to 300 µg/person/day for a 60 kg person) was established in 2004 by the FEEDAP Panel, based on the NOAEL of a one-year dog toxicity study (0.5 mg/kg bw/day) and applying an uncertainty factor of 100. The same ADI has been retained by JECFA. In its assessment of nicarbazin in 2010, the FEEDAP Panel concluded that an ADI for DNC would protect the consumers as they are only exposed to DNC. An ADI of 0.77 mg/kg bw for DNC was set, derived from the NOAEL of the two-year dog study (154 mg DNC/kg bw) and applying a safety factor of 200. The ADIs established for narasin and nicarbazin when used alone can be applied to the same compounds in Maxiban® G160 in assessing the safety of consumers exposed to foodstuffs from chickens for fattening treated with Maxiban® G160.

The FEEDAP Panel expects that narasin-related residues following the administration of Maxiban® G160 are not higher than those from Monteban® G100 after a zero-day withdrawal period. The MRLs set for narasin from Monteban® G100 (50 µg narasin/kg liver, kidney, muscle and skin/fat) could consequently be applied to narasin from Maxiban® G160.

Consumer exposure to the whole DNC-derived residues present in chicken tissues (fed with 125 mg nicarbazin /kg) at a one-day withdrawal period complies with the ADI. The MRLs for DNC in liver (15 mg/kg), kidney (6 mg/kg), muscle (4 mg/kg) and skin/fat (4 mg/kg) proposed in the Koffogran opinion would also apply for nicarbazin from Maxiban® G160.

[96] OJ L 35, 8.2.2005, p.1

The DNC residues following the administration of Maxiban® G160 at a zero-day withdrawal time comply with the DNC ADI (4 %). Therefore, a zero-day withdrawal period for Maxiban® G160 is considered appropriate.

Maxiban® G160 is considered as a slight skin irritant. Since narasin from Monteban® G100 is an eye irritant and a skin sensitiser, and no (conclusive) studies with Maxiban® G160 were provided, the FEEDAP Panel considers Maxiban® G160 as an eye irritant and a skin sensitiser. The inhalatory risk from Maxiban® G160 dust for users is considered negligibly small for nicarbazin, but deserves minimisation for narasin by safety measures normally employed for handling dust-generating products (i.e. masks).

Considering the condition of use of Maxiban® G160 and based on the data provided, a safety concern for the soil compartment, groundwater or by secondary poisoning could not be identified. In the absence of raw data for the algae toxicity study with nicarbazin, the risk of Maxiban® G160 for surface water compartment could not be assessed.

The optimum dose range was established to be 80–100 mg active substances from Maxiban® G160/kg with a narasin to nicarbazin ratio of 1:1. The FEEDAP Panel concluded, based on the results of three floor pen studies and four field trials, that Maxiban® G160 at the lowest proposed dose (40+40 mg nicarbazin+narasin/kg complete feed) is effective in controlling coccidiosis in chickens for fattening.

No effect of Maxiban® G160 used at the highest proposed dose is expected on the quality of the animal product.

RECOMMENDATIONS

In the conditions of use, the minimum and maximum contents should not address the sum of the two substances as proposed by the applicant. The FEEDAP Panel recommends expressing the minimum content as 40+40 mg nicarbazin+narasin and the maximum content as 50+50 mg nicarbazin+narasin.

Narasin is toxic to horses, turkeys and rabbits at levels below those used in the prevention of coccidiosis in chickens. Consequently, the instructions for use should indicate: Dangerous for equines, turkeys and rabbits. These instructions should also contain: 'The simultaneous use of Maxiban® G160 and certain antibiotic drugs (i.e. tiamulin) is contra-indicated.'

The FEEDAP Panel reiterates it recommendation already made in the opinion on Koffogran that p-nitroaniline in nicarbazin should be minimised to the lowest concentration possible (from 0.5 % to 0.1 %).

Since the MICs of narasin against intestinal Gram-positive bacteria are clearly lower than four times 40–50 mg/kg feed (corresponding to Maxiban® G160 concentrations), the possible development of cross-resistances to clinically relevant antibiotics should be followed either in connection with standard monitoring programs or as a part of post-market monitoring as stated in the FEEDAP Technical Guidance on Microbial Studies.[97]

Field monitoring of *Eimeria* spp. resistance to narasin and nicarbazin should be undertaken, preferably during the latter part of the period of authorisation.

Resistance development in Enterococci against narasin, including cross-resistance to clinical relevant antibiotics, should be monitored.

Considering the low consumer exposure to total narasin residues through the consumption of kidney and skin/fat, the FEEDAP Panel is of the opinion that setting MRLs could be temporarily restricted to MRLs for liver and muscle. This would adequately protect the consumer.

[97] The EFSA Journal (2008) 836, 1 -3.

DOCUMENTATION PROVIDED TO EFSA

1. Maxiban® G160 (narasin+nicarbazin). July 2008. Submitted by Eli Lilly and Company Limited.
2. Cross-reference letter (90-day study in rat with nicarbazin) from Phibro Animal Health. March 2009.
3. Cross-reference letter (manufacturing process of nicarbazin) from Phibro Animal Health. May 2009.
4. Cross-reference letter (safety and technical data) from Phibro Animal Health. June 2009.
5. Access letter for DNC aquatic toxicity studies and water solubility data from Innolytics, LLC. August 2009.
6. Maxiban® G160 (narasin+nicarbazin). Supplementary information. July 2009. Eli Lilly and Company Limited.
7. Maxiban® G160 (narasin+nicarbazin). Supplementary information. September 2009. Eli Lilly and Company Limited.
8. Maxiban® G160 (narasin+nicarbazin). Supplementary information. November 2009. Eli Lilly and Company Limited.
9. Comments from Member States received through the ScienceNet.

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APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Maxiban® G160

Maxiban G160 is a product already authorised as feed additive by Regulation (EC) No 2430/1999, under the category 'coccidiostats and histomonostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agents of Maxiban G160 are narasin and nicarbazin. The authorised inclusion level in complete feed is 40 to 50 mg of narasin + 40 to 50 mg of nicarbazin per kilogram.

In the current application the re-authorisation is sought for Maxiban G160 according to Article 10 (2) of Regulation (EC) No 1831/2003. Specifically, re-authorisation is sought to use Maxiban G160 for the control of coccidiosis in chickens for fattening.

The Maxiban G160 is a free-flowing mixture of tan-to-yellow particles and grey-brown particles, which contains granular narasin, granular nicarbazin, vegetable diluent, anti-dusting oil and microtracer.

The narasin concentration in the feed additive is expressed in terms of narasin 'g activity' which is calculated from the measured concentration of narasin components A, D and I.

The CRL recommends the standardized method EN 14183 for the determination of narasin in the feed additive (Maxiban G160), premixtures and feedingstuffs for official control purposes in the frame of the Maxiban G160 authorisation. The method is based on high performance liquid chromatography (HPLC) with post-column derivatisation (PCD) and ultraviolet (UV) detection and its performance characteristics are: - a limit of quantification (LOQ) of 2 mg/kg; - a relative standard deviations for repeatability (RSDr) ranging from 1.3 to 5.0 % and a relative standard deviations for reproducibility (RSDR) ranging from 4.6 to 12.6 % depending on matrix and concentration level.

The CRL recommends the standardized method prEN 15782 suitable for the determination of nicarbazin in the feed additive (Maxiban G160), premixture and feedingstuffs for official control purposes in the frame of the Maxiban G160 authorisation. The method is based on high performance liquid chromatography (HPLC) equipped with ultraviolet/visible (UV/VIS) detection and its performance characteristics are: - LOQ = 20 mg/kg; - RSDr ranging from 2.6 to 10.2 % and - RSDR ranging from 4.8 to 12.3 % depending on matrix and concentration levels.

Regarding residues in tissue the applicant proposed for the marker residue 4,4’ dinitrocarbanilide (DNC) a maximum residues limit (MRL) of 750 µg/kg in liver of chicken for fattening. For official control of this level the CRL recommends a method of the Community Reference Laboratory for Residues of Veterinary Drugs (Berlin) based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The method has been validated in accordance with the requirements of Commission Decision (EC) No 657/200298.

Further testing or validation is not considered necessary.

[98] Nicarbazin belongs to group B of Annex I of Council Directive 96/23/EC[98]. Analytical methods for the determination of this substance in the target matrices for official control purposes have to comply with the criteria specified in Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)

APPENDIX B-1

There are different operations in a premixture factory during which the worker could be exposed to dust from Maxiban® G160:

- Taking Maxiban® G160 from its bag for weighing in the dispensary,
- Emptying bags of previously weighed material in the hopper or mixers,
- Packing the final premixture.

Factors to be considered in a worst case scenario:

- A factory with a large throughput can prepare 40 premixture batches per day (8 hours per shift),
- The maximum time for weighing/emptying is 20 seconds,
- In the same factory about 20 % of premixtures[99] may contain Maxiban® G160,
- All dust comes from Maxiban® G160,
- Total air volume available for inspiration is saturated with Maxiban® G160 dust
- The maximum dust concentration is 0.6 g m^{-3} (see Table 8),
- The maximum narasin concentration in dust is 1.5 % (see Table 8),
- The total breathed air per worker is 10 m^3 per 8 hours = 1.25 m^3 per hour,
- The use of personal protection equipment (coverall, goggles, gloves and mask of the type P2, that reduces the inhalation exposure to 10 %).

The scenario:

Batches with potential exposure	40 (total) x 0.2 (percentage of Maxiban® G160 containing premixtures) = 8 batches
Time of exposure	8 (batches) x 20 sec = 160 seconds ~ 3 minutes, for safety reasons 6 minutes of contact with Maxiban® G160 will be considered.
Inhaled air during exposure	1.25 m^3 per hour x 0.1 hours (6 minutes) = 0.125 m^3
Narasin in air	0.6 g/m^3 (Maxiban® G160 dust) x 1.5 (% narasin in dust) = 0.009 g/m^3
Nicarbazin in air	0.6 g/m^3 (Maxiban® G160 dust) x 0.18 (% nicarbacin in dust) = 0.00108 g/m^3
Narasin in inhaled air	0.009 g/m^3 x 0.125 m^3 = 0.001125 g (1.125 mg)
Reduction by filter mask	1.125 mg x 0.1 (reduction to 10 %) = 0.113 mg
Nicarbazin in inhaled air	0.00108 g/m^3 x 0.125 m^3 = 0.000135 g (0.135 mg)
Reduction by filter mask	0.135 mg x 0.1 (reduction to 10 %) = 0.014 mg

[99] In 2008, the total feed production volume in the EU is estimated to be 150,570,000 t, 32.5 % of the total is poultry feed. From the data of the member states it can be extracted that not more than 50 % would be broiler feed, the animal category, for which Maxiban is applied for authorisation. 16.25 % is rounded to 20 %.

APPENDIX B-2

Numerical values of the separation curve following DIN EN 481 to compile the aerosol fraction relevant for occupational health, related to the whole air transported Aerosol.

Aerodynamic Diameter (µm)	Breathable fraction (%)	Thoracic fraction (%)	Alveolar fraction (%)
0	100	100	100
1	97.1	97.1	97.1
2	94.3	94.3	91.4
3	91.7	91.7	73.9
4	89.3	89	50
5	87	85.4	30
6	84.9	80.5	16.8
7	82.9	74.2	9
8	80.9	66.6	4.8
9	79.1	58.3	2.5
10	77.4	50	1.3
11	75.8	42.1	0.7
12	74.3	34.9	0.4
13	72.9	28.6	0.2
14	71.6	23.2	0.2
15	70.3	18.7	0.1
16	69.1	15	0
18	67	9.5	
20	65.1	5.9	
25	61.2	1.8	
30	58.3	0.6	
35	56.1	0.2	
40	54.5	0.1	
50	52.5	0	
60	51.4		
80	50.4		
100	50.1		

1. Inhalable fraction: the separation curve corresponds to the average probability of inhalation

2. Thoracic fraction: the separation curve corresponds to the average probability for particles entering the tracheo-bronchial-tree and the alveolar area

3. Alveolar (respirable) fraction: That fraction is part of a thoracic fraction. The separation curve corresponds to the average probability for particles entering the alveolar area

4. Extrathoracic fraction: This fraction results from the difference between the inhalable fraction and the thoracic fraction

5. Trachebronchial fraction: This fraction results from the difference between the thoracic fraction and the aveolar fraction. The separation curve is not numerically defined.