4 February 2013
EMA/CVMP/78198/2012
Committee for Medicinal Products for Veterinary Use (CVMP)

European public MRL assessment report (EPMAR)

Monensin (modification of MRLs)

On 23 January 2013 the European Commission adopted a Regulation[1] modifying the maximum residue limits for monensin in bovine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Monensin is used in lactating dairy cattle for control of ketosis as an oral device (controlled release capsule) releasing monensin in the rumen.

Maximum residue limits were initially established in 2007[2] for monensin in bovine species.

Eli Lilly and Company Limited submitted the application for the modification of maximum residue limits to the European Medicines Agency, on 1 June 2011.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 10 November 2011 the establishment of maximum residue limits for monensin in bovine species.

Subsequently the Commission recommended on 6 December 2012 that maximum residue limits in bovine species are established. This recommendation was confirmed on 27 December 2012 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 23 January 2013.

[1] Commission Implementing Regulation (EU) No 59/2013,O.J. L 21/21, of 24.01.2013
[2] Commission Regulation (EC) No 1353/2007, O.J. L 303, of 20.11.2007

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Monensin
Therapeutic class:	Anti-infectious agents/Antibiotics
Procedure number:	EU/11/187/ELY
Applicant:	Eli Lilly and Company Limited
Target species:	Bovine
Intended therapeutic indication:	Ketosis
Route(s) of administration:	Oral

1. Introduction

Monensin (CAS 17090-79-8, CAS 22373-78-0 for sodium salt) is a polyether antibiotic from the group of carboxylic ionophores produced by *Streptomyces cinnamonensis*. Monensin is used as the sodium salt. It is composed of the analogues A, B, C and D with monensin A being the major component (equivalent to 98%).

In veterinary medicine monensin is used in lactating dairy cattle for control of ketosis as an oral device (controlled release capsule) releasing monensin in the rumen at an average dose rate of 335 mg/day over 95 days.

Monensin was previously assessed by the CVMP and a pharmacological ADI of 3 µg/kg bw i.e. 180 µg/person was established.

Currently monensin is included in table 1 of the Annex to Commission Regulation (EU) No 37/2010 of 22 December 2009 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Monensin	Monensin A	Bovine	2 µg/kg 10 µg/kg 30 µg/kg 2 µg/kg 2 µg/kg	Muscle Fat Liver Kidney Milk	NO ENTRY	Anti-infectious agents/ Antibiotics

Eli Lilly and Company Limited submitted an application for the modification of the maximum residue limits for monensin in bovine species (kidney and liver) to the European Medicines Agency, on 1 June 2011. The request for modification was aimed at increasing the MRLs in liver and kidney in order to reflect the residue depletion following administration of the final formulation of the commercial product and to allow for practicable withdrawal periods.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP previously assessed the consumer safety of monensin and initially calculated a pharmacological ADI of 3.45 µg/kg bw based on the NOEL of 345 µg/kg bw for cardiovascular effects retained from a dog study and applying an uncertainty factor of 100

Monensin was also assessed by the European Food Safety Authority (EFSA). The FEEDAP Panel of the EFSA also considered the pharmacological end-points in the same dog pivotal study as the most relevant to derive the ADI for monensin. When calculating the ADI, the FEEDAP Panel of the EFSA rounded the NOEL to 0.3 mg/kg bw/day and obtained an ADI of 3 µg/kg bw i.e. 180 µg/person.

For the benefit of harmonisation, the CVMP agreed to retain the same ADI for monensin as calculated by the FEEDAP Panel of the EFSA of 3 µg/kg bw (i.e. 180 µg/person). No further assessment regarding the consumer safety of the substance is required for the purpose of this modification application.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

Data on the absorption, distribution, metabolism and excretion of monensin have previously been evaluated and the conclusions are summarised below.

Monensin and its metabolites were shown to be predominantly excreted in the bile. Biliary excretion in cattle was reported to correspond to about 35% of the oral dose. Orally dosed radioactivity was nearly completely recovered in faeces, whereas urinary excretion was negligible. Parent monensin and five metabolites resulting from O-demethylation and oxidation reactions were identified in liver, bile and/or faeces from monensin treated cattle. The compounds represented about 30% of total radioactivity in liver and 67% in faeces. Unchanged monensin represented only a limited fraction of the monensin-related compounds in liver and faeces (less than 10%) and 2% in milk. The pattern of metabolites seen in cattle was qualitatively comparable to that seen in rats.

Steady-state in milk is reached within 5 days after the beginning of treatment.

It was conservatively estimated that major monensin metabolites retain no more than 50% of the pharmacological and microbiological activity of the parent compound.

2.2.2. Residue depletion studies

The CVMP previoulsy assessed several residue studies (labelled and non-labelled) in cattle. None of the studies used the intended preparation (intra-ruminal controlled release capsule, providing continuous release of the substance) over the full treatment period, but used gelatine capsules administered twice a day.

In a new residue study a total of 22 lactating cows (564 to 799 kg bw) were orally treated with the new formulated intraruminal monensin controlled release capsule, delivering approximately 335 mg monensin/day for 95 days. The therapeutic dose rates were in the range of 0.6 to 0.4 mg/kg bw/day depending on the bodyweight of the animals. Tissue samples of liver, kidney, muscle and fat were collected from 10 animals 14 days after administration of the Controlled release capsule. Milk samples were collected from 22 cows twice daily at 12 hour intervals for 28 consecutive milkings up to 336 hours after application. Monensin A was assayed in bovine tissues and milk using a validated HPLC/MS/MS method with a limit of quantification (LOQ) of 0.75 µg/kg for all tissues except for fat, for which the LOQ was 1.0 µg/kg, and for milk, for which the LOQ was 0.45 µg/kg.

Monensin A was detected in tissues and milk at very low concentrations only. Highest concentrations were observed in liver with a mean value of 14.95 µg/kg (range: 2.54 to 26.3 µg/kg), followed by fat with 1.92 µg/kg (range: less than 1.0 to 5.32 µg/kg), kidney with 0.67 µg/kg (range: less than 0.75 to 1.45 µg/kg) and muscle with 0.46 µg/kg (range : below the limit of detection to 0.84 µg/kg). In kidney and muscle monensin A concentrations were below the limit of quantification in more than half

of the samples. In milk mean parent monensin concentrations were below the limit of quantification of 0.45 µg/kg in all samples except one milking (0.48 µg/kg). Results indicate that the MRL of 2 µg/kg in milk will not be exceeded.

This new study is considered to better represent the use of the product in dairy cattle (intra-ruminal administration with continuous release of the substance).

Selection of marker residue and target tissues

The CVMP previously retained monensin A as the marker residue for monesin and established marker to total residues ratios of 0.05 for muscle, liver, kidney and fat tissues and 0.027 for milk.

2.2.3. Monitoring or exposure data

Not provided.

2.2.4. Analytical method for monitoring of residues

An LC-MS/MS method to determine monensin A in tissues and milk of cattle was developed as the regulatory method based on the new MRL values.

The method was described in an internationally recognised format based on ISO 78/2. The limit of quantification was 0.75 µg/kg in muscle, 1.0 µg/kg in fat, 15 µg/kg in liver and 4 µg/kg in kidney. In milk the limit of quantification was 0.45 µg/kg. The limit of detection was 0.0409, 0.0823, 0.0379, 0.269 and 0.0852 µg/kg in milk, liver, kidney, muscle and fat, respectively. The method is validated according to Volume 8 of the Rules Governing Medicinal Products in the European Union. The analytical method was reviewed by the relevant European Reference Laboratory, which confirmed the suitability of the method for monitoring of residues.

2.2.5. Findings of EU or international scientific bodies

The FEEDAP Panel of EFSA assessed monensin with regard to its use as a feed additive. MRLs of 0.008 mg/kg were established for fattening chicken and turkey wet liver, kidney, muscle and 0.025 mg/kg for wet skin plus fat of fattening chicken and turkey.

Monensin was also evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2008. The JECFA recommended maximum residue limits for cattle, sheep and goats as follows: 100 µg/kg for fat, 10 µg/kg for kidney, 20 µg/kg for liver, 10 µg/kg for muscle and 2 µg/l for milk. For chicken, turkey and quail maximum residue limits were recommended as follows: 100 µg/kg for fat, 10 µg/kg for kidney, 10 µg/kg for liver and 10 µg/kg for muscle. The marker residue established by Codex Alimentarius in 2009 was monensin, which is different to the one established by CVMP (monensin A). In 2011 JECFA recommended the modification of the MRL previously established for cattle liver to 100 µg/kg, and recommended a marker residue, in this tissue, of monensin A.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

The CVMP has previously assessed the potential effects of monensin on the microorganisms used for food industrial processing. Monensin was tested against a panel of dairy starter cultures. Cultures found to be most susceptible to monensin were *Lactobacillus acidophilus* La-5 (MIC equal to 1 µg/ml)

and *Streptococcus thermophilus* TH-4 (MIC equal to 2 µg/ml). Monensin MICs against all other cultures were 4 µg/ml or above. *Lactobacillus lactis* was not included in the testing programme. The NOEL of monensin with regard to milk acidification rate of commercial dairy starter cultures was established as 0.1 µg/ml.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

The request for modification of the MRLs was made in order to reflect the residue depletion following administration of a controlled release formulation for intra-ruminal administration. The CVMP recognised that this type of formulation provides a valuable addition to available therapies for the control of ketosis. Once the intra-ruminal formulation has been administered monensin will be released continously over a period of approximatly 95 days. If residues are not below the MRLs during the treatment period, the withdrawal period for the product would be longer than 95 days, which would not be practicable. In practice, the only practicable withdrawal period for a controlled release formulation would be zero days, as this would allow use of milk and meat derived from animals actively receiving treatment. The Committee therefore considered whether MRLs could be set at a level that would allow a zero day withdrawal period while ensuring consumer safety.

3.3. Elaboration of MRLs

Based on the new data provided using the final formulation of the commercial product (intra-ruminal controlled release capsule), the MRLs for liver and kidney can be increased by a factor of 1.7 and 5, respectively i.e. 50 µg/kg for liver and 10 µg/kg for kidney. No changes are required for fat (10 µg/kg), muscle and milk (2 µg/kg). The Committee noted that these MRLs would allow a zero day withdrawal period for the proposed controlled release formulation.

The CVMP notes that the modification of the existing EU MRLs for liver and kidney in cattle will lead to greater disharmonisation with the Codex MRLs. However, the new residue data reflect the residues depletion following the intended administration of the final product, allowing for the establishment of practical withdrawal periods.

Calculation of theoretical daily intake of residues

As detailed in section 2.2.1 pharmacokinetics, the CVMP conservatively estimated that the metabolites of monensin have no more than 50% of the pharmacological and microbiological activity of the parent compound. This means that, in terms of consumer safety, the effect of exposure to a given amount of monensin metabolites would be equivalent or less than the effect of exposure to half that amount of parent compound. This was taken into account in calculating the theoretical maximum daily intake (TMDI) by allocating the portion of residues present as monensin parent compound an activity of 100% while the remainder of the residues were allocated an activity of 50%.

Calculation of theoretical maximum daily intake for monensin derived residues in bovine tissues and milk, with the proposed increased levels for liver and kidney:

Edible tissue or products	*MRL proposal (µg/kg)*	*Ratio of the marker to total residue*	*Concen-tration of parent com-pound in tissue (µg/kg)*	*Concen-tration of metabolite in tissue (µg/kg)*	*Concen-ration of metabolite in tissue, expressed as parent compound equivalents[1] (µg/kg)*	*Total concen-tration of residues (parent compound and metabolites) in tissues expressed as parent compound equivalents (µg/kg)*	*Daily consum-ption (kg)*	*Amount of residues ingested per edible tissue or product (µg)*
Muscle	*2*	*0.05*	*2*	*38*	*19*	*21*	*0.30*	*6.3*
Fat	*10*	*0.05*	*10*	*190*	*95*	*105*	*0.05*	*5.25*
Liver	*50*	*0.05*	*50*	*950*	*475*	*525*	*0.10*	*52.5*
Kidney	*10*	*0.05*	*10*	*190*	*95*	*105*	*0.05*	*5.25*
Milk	*2*	*0.027*	*2*	*72.1*	*36*	*38*	*1.50*	*57*
TMDI (µg)								*126.3*
% of ADI								*70.2*

[1]Metabolites comprises 50% of ionophoretic activity of parent monensin

Taking into account the MRLs established by the FEEDAP Panel of the EFSA for chicken tissues represent 51% of the ADI, while the MRLs recommended by the CVMP for cattle tissues represent 38.6% of the ADI. Therefore, the worst case theoretical maximum daily intake arises from the ingestion of chicken tissues (51%) and milk (31.7%). This represents 82.7% of the ADI.

In conclusion, the ADI will not be exceeded irrespective of whether cattle or chicken meat is considered in the theoretical maximum daily intake calculation.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/2009 the CVMP considered the possibility of extrapolating the maximum residue limits established for monensin based on data in cattle to other food producing species and food commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
Sheep	No	No specific pharmacokinetic or residue data were available for sheep and consequently the marker residue and the ratio of

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
		marker to total residues could not be derived. As sheep meat is consumed on a regular basis and in large quantities, species specific data are considered necessary in order to quantify the possible impact on consumer safety of exposure to residues. No analytical method suitable for the monitoring of monensin residues in sheep tissues was available for evaluation.
Goats	No	No specific pharmacokinetic or residue data were available for goats and consequently the marker residue could not be confirmed. No data was available to demonstrate that the analytical method for monitoring of residues in cattle tissues and milk is applicable for monitoring of residues in goat tissues and milk.
Pigs	No	Metabolism can be significantly different in pigs compared to cattle. Consequently species specific metabolism and residue data are needed in order to draw conclusions on safe MRL levels. No analytical method for monitoring of residues in pig tissues was available for evaluation.
Poultry (including eggs)	No	Metabolism in poultry can be significantly different compared to cattle. Consequently species specific metabolism and residue data are needed in order to draw conclusions on safe MRL levels. No analytical method for monitoring of residues in poultry tissues (or eggs) was available for evaluation.
Horses	No	Monensin is known to be highly toxic for horses and therefore considerations of extrapolation of MRLs for horses are not considered appropriate.
Rabbits	No	No specific pharmacokinetic or residue data were available for rabbits and therefore the marker residue could not be confirmed. No data are available to demonstrate that the analytical method used for monitoring of residues in cattle is applicable for monitoring of residues in rabbit tissues.
Fin fish	No	Metabolism is generally less complicated in fish than in cattle. But, as the parent compound is not the marker residue in cattle it cannot be assumed that the marker residue for cattle would also be a suitable marker residue in fish tissues. In addition, as fish meat is consumed on a regular basis and in large quantities, species specific data are considered

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
		necessary in order to allow adequate evaluation of the risk to the consumer posed by residues in fish meat. No analytical method for monitoring of residues in fish meat was available for evaluation.
Honey	No	Residue depletion in honey does not occur through metabolism and consequently conclusions drawn from data in other food products cannot be extrapolated to honey. Honey specific data is required in order to quantify the possible impact on consumer safety of exposure to residues. No data are available to demonstrate that the analytical method used for monitoring of residues in cattle tissues and milk is applicable for monitoring of residues in honey.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the pharmacological ADI of 3 µg/kg bw (i.e. 180 µg/person) was established as the overall ADI for monensin,
- monensin is extensively metabolised,
- monensin A was retained as the marker residue,
- marker residue depletion data from a new study using the intended veterinary medicinal product were available,
- the ratios of marker to total residues were calculated to be approximately 0.05 in tissues and 0.027 in milk,
- ionophoric and microbiological activity of monensin derived metabolites was largely reduced, and consequently a conservative estimate of 50% of activity was considered appropriate for intake calculations,
- a validated analytical method for the monitoring of residues of monensin A in edible bovine tissues (liver, kidney, muscle, fat) and milk is available,

the Committee recommends the modification of maximum residue limits for monensin in liver and kidney and the amendment the entry for monensin in table 1 of the Annex to Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Monensin	Monensin A	Bovine	2 µg/kg 10 µg/kg 50 µg/kg 10 µg/kg 2 µg/kg	Muscle Fat Liver Kidney Milk		Anti-infectious agents/Antibiotics

Taking into account the MRLs established by the FEEDAP Panel of the EFSA for chicken tissues and the CVMP MRLs for milk and the consideration of 50% activity of monensin metabolites, the theoretical maximum daily intake (from chicken tissue and milk) represents 82.7% of the ADI.

4. Background information on the procedure

Submission of the dossier	1 June 2011
Steps taken for assessment of the substance	
Application validated:	15 June 2011
Clock started:	16 June 2011
List of questions adopted:	10 November 2011
Consolidated response to list of questions submitted:	11 January 2012
Clock restarted:	12 January 2012
CVMP opinion adopted:	8 March 2012
Request for review by the Commission	23 April 2012
Revised CVMP opinion adopted	16 May 2012