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EMA/CVMP/751522/2013
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Closantel (bovine and ovine milk) – after the provisional maximum residue limit (MRL)

On 20 June 2014 the European Commission adopted a Regulation[1] establishing a MRL for closantel in bovine and ovine milk, valid throughout the European Union. This MRL was based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use (CVMP).

Closantel is used in bovine and ovine species for the treatment and control of adult and immature flukes, nematodes and larval stages of some arthropods and is administered subcutaneously or as a drench. In addition, bovine species may also be treated topically.

Closantel had MRLs already established[2] in muscle, fat, liver and kidney of bovine and ovine species. A provisional MRL in bovine and ovine milk had also been previously established[3] for closantel with an expiry date of 1 January 2014, further to a request from the Irish Medicines Board for the extrapolation of MRLs to milk.

The Irish Medicines Board submitted the responses to the list of questions further to the establishment of the provisional MRL to the European Medicines Agency on 6 September 2013.

Based on the data available, the CVMP recommended on 12 December 2013 the establishment of a MRL for closantel in bovine and ovine milk further to the extrapolation previously recommended and the establishment of the provisional MRL.

On 8 January 2014, the European Commission requested the review of the opinion in order to have further clarification with regard to the assessment of the outstanding issues identified at the time of the establishment of the provisional MRL in bovine and ovine milk.

On 15 January 2014 the CVMP adopted a revised opinion confirming the MRL extrapolated to bovine and ovine milk and recommending the removal of the provisional status of this MRL.

Subsequently the Commission recommended on 7 April 2014 that a MRL for closantel in bovine and ovine milk is established. This recommendation was confirmed on 28 April 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 20 June 2014.

[1] Commission Implementing Regulation (EU) No 682/2014, O.J. L 182, of 21.06.2014
[2] Commission Regulation (EEC) No 2901/93, O.J. L 264, of 23.10.93
[3] Commission Implementing Regulation (EU) No 221/2012, O.J. L 75, of 15.03.2012

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Summary of the scientific discussion for the extrapolation of MRLs

Substance name:	Closantel
Therapeutic class:	Antiparasitic agents/Agents against endoparasites
Procedure number:	EU/ART27/11/191/IMB
Requesting Member State:	Ireland
Target species:	Bovine and ovine milk
Intended therapeutic indication:	Treatment and control of adult and immature flukes, nematodes and larval stages of some arthropods
Route (s) of administration:	Subcutaneous injection, oral drench, topical

1. Introduction

Closantel is a salicylanilide which is used for the treatment and control of adult and immature flukes, nematodes and larval stages of some arthropods. Closantel is indicated for the treatment of cattle and sheep and is administered subcutaneously in the dose range of 2.5 to 5 mg/kg body weight. Closantel can also be administered as a drench at a dose of 10 mg/kg body weight. In addition, cattle may be treated topically at a dose rate of 20 mg/kg bodyweight. Closantel is often administered in combination with other anthelmintics.

Closantel was previously assessed by the CVMP resulting in the establishment of MRLs in bovine and ovine tissues.

On 19 August 2011, the Irish Medicines Board submitted a request for an opinion for the extrapolation of maximum residue limits for closantel to bovine and ovine milk. On 10 November 2011, the CVMP recommended a provisional MRL for closantel in milk.

The Commission subsequently amended the entry for closantel in table 1 of the Annex to Commission Regulation (EU) No 37/2010 of 22 December 2009, as follows:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Closantel	Closantel	Bovine	1000 µg/kg 3000 µg/kg 1000 µg/kg 3000 µg/kg	Muscle Fat Liver Kidney		Antiparasitic agents/Agents against endoparasites
		Ovine	1500 µg/kg 2000 µg/kg 1500 µg/kg 5000 µg/kg	Muscle Fat Liver Kidney		
		Bovine, ovine	45 µg/kg	Milk	Provisional MRLs expire on 1 January 2014	

Further to the establishment of a provisional maximum residue limit for closantel in bovine and ovine milk Ireland submitted to the European Medicines Agency on 6 September 2013, 30 October, 27 November and 2 December 2013 additional data concerning the validation of the analytical method in milk in order to allow for the establishment of a final MRL.

This EPMAR provides an overall assessment of the request for the extrapolation of the MRLs for closantel to milk and so covers the review performed for the establishment of the provisional MRLs in milk as well as the review of the additional data provided in response to the outstanding issues identified at the time of the establishment of the provisional MRLs in milk.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously performed a consumer safety evaluation for closantel and established a toxicological ADI of 0.03 mg/kg bw i.e. 1.8 mg/person based on the NOEL of 2.5 mg/kg bw/day in rats and applying a safety factor of 100. Therefore, no further assessment regarding the establishment of the ADI for the substance is required for the purpose of this request.

2.2. Residues assessment

For the assessment of the request for extrapolation the committee considered relevant residue data from the previous assessment and any new information made available as detailed below.

2.2.1. Pharmacokinetics in target species

Closantel is highly bound to plasma proteins and undergoes limited metabolism *in vivo*.

Experiments in sheep with radiolabelled-closantel have shown that plasma radioactivity is almost exclusively due to unmetabolised closantel. At least 80% of the radioactivity was excreted in faeces over 8 weeks, whilst less than 0.5% was recovered from urine. Ninety percent of excreted radioactivity was due to parent drug. Residual radioactivity in all tissues but liver was mainly due to the parent compound.

No radiolabelled pharmacokinetic data in milk are available and there is no information on metabolism in milk.

In a small, non-GLP study, a parallel decline of closantel concentration in plasma and milk in lactating cows has been shown with a plasma/milk concentration ratio in the order of 50/1. Three dairy cows were injected intramuscularly with a single dose of closantel at 5 mg/kg (5% injectable solution). Blood and milk samples were collected up to 35 days post-treatment. Closantel levels in plasma and milk were determined by HPLC-UV. Maximal closantel concentrations in plasma of 45 µg/ml were reached 2 to 4 days following administration. Maximal closantel concentrations in milk averaging 1 µg/ml were seen 4 days following administration. At day 35, the mean concentration of closantel in milk was 0.22 ± 0.08 µg/ml. The drug was eliminated from both plasma and milk with a half-life of approximately 12 days.

From the data available it can be concluded that residues of closantel persist in milk and that the parent compound will be the main residue in this food commodity.

2.2.2. Residue depletion studies

In a non-GLP study closantel was administered during the dry period in 4 pregnant dairy cows subcutaneously at a dose of 5 mg closantel/kg bw 30 or 60 days before expected calving. Residues of closantel were quantifiable in the milk until at least 29 days after calving. Residue values quantified by

LC-MS/MS ranged from 8 to 160 µg/kg. The half-lives of the residue concentrations in the milk ranged from 10 to 17 days.

In a recent GCP/GLP study 10 pregnant dairy cows were orally treated with closantel at the highest recommended dose of 10 mg closantel/kg bw at the beginning of the dry period (about 45 to 55 days before calving). Residues of closantel were quantifiable in the milk until at least 28 days after calving. After the colostrum period (i.e. from about 3 days after calving) the concentrations in milk decreased to 50 µg/kg or below. Milk residues values ranged from about 10 to 500 µg/kg. Overall, the median closantel concentrations at post colostrum time points were below 30 µg/kg. The 95th percentile values were below 50 µg/kg.

It can be concluded that following administration of closantel during the dry period residues of the parent compound are quantifiable in cow's milk and may persist for weeks.

No residue data are available for sheep milk.

Selection of marker residue and ratio of marker to total residues

No radiolabelled study is available to confirm the marker residue and on which to base a ratio of marker to total residues for milk. However, based on the fact that the parent compound closantel has been identified in bovine milk in a number of studies and considering that metabolism in plasma and tissues is known to be limited, closantel can be accepted as the marker residue in bovine and sheep milk.

Given the absence of total residue data in milk it is only possible to estimate the ratio of marker to total residues in milk; any such estimate must be sufficiently conservative to reflect the uncertainty inherent in such an estimate. Marker to total ratios established for cattle tissues were 1 in muscle, 0.7 in fat, 0.1 in liver, and 0.8 in kidney. Given the limited metabolic capacity of milk it is considered reasonable, in this case, to establish a ratio of marker to total residues identical to that established for fat, as fat also has limited metabolic capacity. It was therefore agreed to use the value of 0.7, established for fat as the ratio of marker to total residues in bovine milk.

It is noted that the ratios of marker to total residues established in sheep tissues are not the same as those established for bovine tissues: a ratio of 1 was established for sheep muscle, kidney and fat while a ratio of 0.6 was established for sheep liver. Nevertheless, for the purpose of establishing an MRL in milk and in view of the fact that ovine animals producing milk for human consumption are considered as a minor species, the marker to total residue of 0.7 agreed for bovine milk is also considered to be acceptable for ovine milk.

2.2.3. Monitoring or exposure data

Results of the Irish residue monitoring programme from 2008 to October 2010 were provided. Closantel was detected in four out of 284 samples in 2009 and in eight samples in 2010. Concentrations of closantel detected ranged from 1.01 µg/kg to 75.73 µg/kg.

2.2.4. Analytical method for monitoring of residues

A UPLC-MS/MS (ultra-performance liquid chromatography coupled to tandem mass spectrometry) method was developed by a national reference laboratory for the purpose of residue surveillance in cow's milk. The method is well described and considered in general suitable for monitoring of residues. A validation package was submitted based on the requirements of Volume 8 of The rules governing medicinal products in the European Union and was considered acceptable. The limit of quantification of the method is 22.5 µg/kg.

Although the analytical method proposed was considered generally suitable for monitoring of residues of closantel in milk, further stability and validation data were considered necessary concerning the specificity, accuracy, precision and linearity. In addition it was requested to demonstrate that the proposed analytical method is applicable to sheep milk. As a result of these deficiencies only provisional MRLs could be recommended.

The additional data provided in response to the outstanding issues identified in the EPMAR for the recommendation for a provisional MRL demonstrated that the method was specific for closantel and that the accuracy and precision were acceptable at the level of the proposed MRL. Repeatability and reproducibility were satisfactorily demonstrated. Linearity of the method was also demonstrated.

Additional data have also been provided to demonstrate the applicability of the method to sheep milk.

The proposed method has been reviewed by the relevant Union Reference Laboratory, which confirmed the overall suitability of the method.

The method can be considered validated for the purpose of monitoring residues in bovine and sheep milk.

2.2.5. Findings of EU or international scientific bodies

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the following MRLs: sheep: 1.5 mg/kg for muscle and liver, 5 mg/kg for kidney and 2 mg/kg for fat; cattle: 1 mg/kg for muscle and liver and 3 mg/kg for kidney and fat. No MRL was established for milk.

3. Risk management considerations

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

Microbiological effects are not expected for this substance therefore such data are not considered necessary.

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

The data provided for the scientific evaluation of closantel for the establishment of a maximum residue limit in milk were limited and do not comply with the requirements of Volume 8 of *The rules governing medicinal products in the European Union.* In particular the studies provided are not GLP compliant and no data on total residues in milk are available.

Recognising the deficiencies in the data presented the Committee took also into account the following:

- Although other flukicidal substances exist for which MRLs in ruminant milk have been established, these substances are not approved for the treatment of immature fluke, and consequently it is recognised that at present there is a lack of available products, authorised for the treatment of immature fluke in animals producing milk for human consumption;
- Liver fluke is a highly debilitating disease leading to loss of condition and ultimately cachexia and potentially death and therefore the availability of an adequate range of products for the treatment of immature fluke is essential in order to avoid unnecessary suffering of the animals;

- The establishment of a maximum residue limit is essential to provide the reference level for control purposes and to enable the use of the substance;
- The lack of available products coupled with welfare issues may lead to increased use of the products under non-authorised conditions.

3.3. Extrapolation of MRLs

Based on the existing MRL values, the daily intake of residues from bovine tissues equates to 1.7 mg (equivalent to 94.4% of the ADI), leaving 1 mg (equivalent to 5.6% of the ADI) for the establishment of a MRL for milk.

Given the available information relating to residues in other tissues closantel was retained as marker residue in milk and a ratio of marker to total residues of 0.7 was estimated.

Considering that the standard food basket indicates a consumption for milk of 1.5 kg per consumer per day, and in order to ensure that consumer exposure total residues of closantel remains below the ADI, the maximum allowable total residues in milk is 67 µg/kg.

In view of the information available and the risk management considerations the CVMP recommends the extrapolation of the existing MRLs for closantel in cattle to cattle milk. The proposed MRL is 45 µg/kg.

Although data were not provided to demonstrate the presence of the marker residue closantel in sheep milk, metabolism in plasma and tissues is known to be limited, and therefore closantel can be accepted as the marker residue in sheep milk. Although the ratios of marker to total residues established in sheep tissues are not the same as those established for bovine tissues, for the purpose of establishing an MRL in milk and in view of the fact that ovine animals producing milk for human consumption are considered as a minor species, the marker to total residue of 0.7 agreed for bovine milk is also considered to be acceptable for ovine milk.

Therefore the proposed MRL of 45 µg/kg for cattle can also be accepted for sheep milk.

Available data indicate that following the oral administration of 10 mg/kg bw to cows at the start of the dry period (i.e. 40 to 45 days before expected calving) 95th percentile residue values in milk were below 45 µg/l from 13 days after calving while median residue values were below 45 µg/l from two days after calving. It is also noted that closantel-containing products are authorised for administration by other routes (subcutaneous and topical) and this may impact on the time required for residues in milk to deplete to the level of the recommended MRL.

Calculation of theoretical daily intake of residues

Details used in the calculation of theoretical daily intake of residues from bovine tissues and milk (worst case scenario):

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	1000	1	300 µg
Fat	0.05	3000	0.7	214.3 µg
Liver	0.10	1000	0.1	1000 µg
Kidney	0.05	3000	0.8	187.5 µg

Milk	**1.50**	**45**	**0.7**	**96.4 µg**
Total				1798.2 µg (**100% of the ADI**)

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

Having considered that:

- a toxicological ADI of 0.03 mg/kg bw (i.e 1.8 mg/person) was previously established as the overall ADI for closantel;
- closantel has been accepted as the marker residue in cattle and sheep milk;
- the marker to total residues ratio of 0.7 established for fat was considered an suitable conservative value to be considered in relation to milk given that fat milk has limited metabolic capacity;
- there is a lack of available products for the treatment of immature fluke in animals producing milk for human consumption;
- there is a need for a reference level for control purposes and to enable the use of the substance; and that:
- following the responses to the outstanding issues identified in the EPMAR recommending a provisional MRL for closantel in bovine and ovine milk, the analytical method can be considered validated for the purpose of monitoring residues in bovine milk and applicable to ovine milk.

The CVMP confirms the maximum residue limit extrapolated to bovine and ovine milk and recommends by consensus the removal of the provisional status of the maximum residue limits in milk for closantel and the amendment of the entry in Table 1 (Allowed substances) 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
Closantel	Closantel	Bovine	1000 µg/kg 3000 µg/kg 1000 µg/kg 3000 µg/kg 45 µg/kg	Muscle Fat Liver Kidney Milk	NO ENTRY	Antiparasitic agents/Agents against endoparasites
		Ovine	1500 µg/kg 2000 µg/kg 1500 µg/kg 5000 µg/kg 45 µg/kg	Muscle Fat Liver Kidney Milk		

Based on these values, the theoretical maximum daily intake from bovine tissues and milk is 1798.2 µg, which corresponds to 100% of the ADI.

4. Background information on the procedure

Submission of the dossier	19 August 2011
Steps taken for assessment of the substance	
Clock started:	20 August 2011
CVMP opinion adopted (provisional MRLs):	10 November 2011
Submission of response to list of questions:	6 September 2013
Adoption of opinion:	12 December 2013
Request for the review by the Commission	8 January 2014
Revised opinion adopted	15 January 2014