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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Rafoxanide (bovine and ovine milk) – extension of provisional MRLs

On 14 April 2016 the European Commission adopted a Regulation¹ establishing a provisional maximum residue limit for rafoxanide 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).

Rafoxanide is used in bovine and ovine species for the treatment of fasciolosis and active against gastrointestinal nematodes and sheep nasal bot fly.

Maximum residue limits for rafoxanide were previously established² in muscle, fat, liver and kidney of bovine and ovine species.

The Irish Medicines Board submitted a request for the extrapolation of the maximum residue limits to milk to the European Medicines Agency, on 19 August 2011.

Based on the available data the Committee for Medicinal Products for Veterinary Use (CVMP) recommended, on 12 December 2013, the establishment of a provisional maximum residue limit for rafoxanide in bovine and ovine milk.

The provisional maximum residue limit was adopted by the Commission on 20 June 2014, with an expiry date of 31 December 2015³.

On 27 October 2015 the Irish Healthcare Products Regulatory Authority (previously the Irish Medicines Board) requested an extension to the period of time applying to the provisional maximum residue limit, in order to allow completion of ongoing work on the validation of the analytical method.

Based on the request from the Irish Healthcare Products Regulatory Authority, the Committee for Medicinal Products for Veterinary Use (CVMP) recommended, on 6 November 2015, a 2-year extension of the provisional maximum residue limit for rafoxanide in bovine and ovine milk.

Subsequently, on 23 February 2016, the Commission recommended the amendment of the Annex to Regulation (EU) No 37/2010 and the establishment of an expiry date of 31 December 2017 for the provisional maximum residue limit in bovine and ovine milk. This recommendation was confirmed on 15 March 2016 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 14 April 2016.

¹ Commission Implementing Regulation (EU) No 2016/576, O.J. L 99, of 15.04.2016

² Commission Regulation (EU) No 1478/2001, O.J. L 195, of 19.07.2001

³ Commission Implementing Regulation (EU) No 681/2014, O.J. L 182, of 21.06.2014



Summary of the scientific discussion for the extrapolation of MRLs

Substance name: Rafoxanide
 Therapeutic class: Antiparasitic agents/Agents against endoparasites
 Procedure number: EU/ART27/11/192/IMB
 Requesting Member State: Ireland
 Target species: Bovine and ovine milk
 Intended therapeutic indication: Fasciolicide and active against gastrointestinal nematodes and sheep nasal bot fly
 Route of administration: Oral

1. Introduction

Rafoxanide is a halogenated salicylanilide, [3'-chloro-4'-(p-chlorophenoxy)-3,5-diiodosalicyl-anilide] used as a fasciolicide in cattle and sheep. Rafoxanide is also active against gastrointestinal nematodes (*Haemonchus*, *Bunostomum*, *Oesophagostomum*, and *Gaigeria* species) and against the sheep nasal bot fly (*Oestrus ovis*). Rafoxanide products are currently marketed in the EU for the treatment of cattle and sheep. It is generally administered by the oral route at a dose of 11.25 mg/kg body weight.

Rafoxanide was previously assessed by the CVMP and a toxicological ADI of 2 µg/kg, i.e. 120 µg per person was established.

Currently, rafoxanide is included in 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
Rafoxanide	Rafoxanide	Bovine	30 µg/kg 30 µg/kg 10 µg/kg 40 µg/kg	Muscle Fat Liver Kidney	NO ENTRY	Antiparasitic agents/Agents against endoparasites
		Ovine	100 µg/kg 250 µg/kg 150 µg/kg 150 µg/kg	Muscle Fat Liver Kidney		
		Bovine, ovine	10 µg/kg	Milk	Provisional MRL shall expire on 31 December 2015	

A provisional maximum residue limit was recommended for rafoxanide in milk as issues relating to the analytical method proposed for monitoring of residues remained to be resolved. Further to the establishment of the provisional MRL, the applicant has initiated further work in order to validate the analytical method. The studies are currently still ongoing.

The scientific assessment previously carried out by the Committee leading to the recommendation for the establishment of a provisional MRL in bovine and ovine milk is reported in the paragraphs below. Section 2.2.4 on the analytical method for monitoring of residues has been updated to reflect that scientific

studies to further validate the analytical method in milk are still ongoing and the conclusions and recommendations presented in section 3 have been modified accordingly.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously performed a consumer safety evaluation for rafxanide and established a toxicological ADI of 2 µg/kg, i.e. 120 µg per person based on a NOEL of 400 µg/kg bw/day from a thirteen week study in dogs and applying a safety factor of 200. Therefore, no further assessment regarding the establishment of the ADI of the substance is required for the purpose of this request.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

No pharmacokinetic data for milk were made available in sheep and cattle.

The pharmacokinetic data previously assessed by the CVMP is reported in the paragraphs below.

In sheep, after an oral administration of ¹⁴C-rafoxanide at a dose of 11.25 mg/kg bw, about 0.12% of the administered dose was recovered in urine within 3 days. The major metabolite was 3,5-diiodosalicylic acid, representing 91% of the excreted radioactivity; rafxanide accounted for only 9%. No information on the percentage of radioactivity excreted via faeces was given. The maximum plasma concentration of radioactivity (19 750 µg equivalents rafxanide/kg) occurred between 1.4 to 1.8 days after administration. An apparent radioactive elimination half-life of 8.9 ± 1.2 days was calculated. This persistence of rafxanide residues was due to the fact that the compound was strongly bound to plasma proteins (greater than 99%). No information on the ratio of the parent compound to total radioactivity in plasma was available.

In cattle, after an oral administration of ¹⁴C-rafoxanide at a recommended therapeutic dose of 11.25 mg/kg bw, less than 0.6% of the administered dose was recovered in the urine within 6 days. Unchanged rafxanide and 3,5-diiodosalicylic acid were the 2 major substances detected. However, due to low concentrations of these compounds and as the different system of chromatographic analysis led to different separation, no figures can be given. No information on the percentage of radioactivity excreted via faeces was given. The maximum plasma levels (close to 20 000 µg equivalent rafxanide/kg) occurred at 1.8 days post dose. The apparent radioactive elimination half-life was 3.87 ± 0.59 days. No information on the ratio of the parent compound to total radioactivity in plasma was available.

2.2.2. Residue depletion studies

In a non-GLP study nine pregnant dairy cows were treated with a single dose (11.21 to 11.27 mg/kg bw) of a 3% oral suspension at the start of the dry period. Calving occurred between 26 and 62 days after treatment. Milk samples were analysed using UPLC-MS/MS.

Highest concentrations of rafxanide occur in cows that calve less than 35 days after treatment and in the milk produced immediately after calving. The concentrations ranged from lower than 1.0 µg/kg to 50 µg/kg. After the colostrum period, this is from about 3 days after calving, concentrations of rafxanide in milk are typically low (<10 µg/kg) but persist at concentrations above the limit of quantification of the analytical method (1.0 µg/kg) for between 10 and 38 days post-calving. Mean concentrations are lower than 10 µg/kg at 2 days post-calving.

Administration to lactating cows by oral drench of 11.75 mg/kg resulted in high concentrations in milk with maximum 3 to 4 days after treatment ($516 \pm 138 \mu\text{g}/\text{kg}$).

Selection of marker residue and ratio of marker to total residues

No radiolabelled data are available to confirm the identity of the marker residue and on which to base a ratio of marker to total residues. The parent compound, rafoxanide, has been detected in milk but there are no data on metabolites in milk.

Standard procedure is to determine the ratio of marker to total residues from radiolabelled depletion studies and the value is chosen at a time point at which residue intake will be below the ADI.

Since the metabolism of rafoxanide in milk has not been characterised and the ratio has not been determined, any approach to setting a ratio of marker to total residues should be suitably conservative. In bovines, rafoxanide represented 50%, 50% and 30% of the total residues in muscle, fat and kidney, respectively, 14 days after treatment. In ovines rafoxanide represented 100%, 88%, 50% and 50% of the total residues in muscle, fat, liver and kidney, respectively, 30 days after treatment.

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. Therefore the value of 0.5, established for bovine fat, is retained as the ratio of marker to total residues in bovine milk.

It is proposed to use the worst case value of 0.5, established for fat, as the ratio of marker to total residues in bovine milk.

2.2.3. Monitoring or exposure data

Results of the Irish national residue monitoring programme for 2008 to October 2010 were provided. Rafoxanide was detected in one of 284 samples of tested bulk milk in 2009. The level of rafoxanide in the positive sample was $9.83 \mu\text{g}/\text{kg}$ but no information is available on when the animal(s) was treated with rafoxanide. Rafoxanide was not detected in any samples in either 2008 or 2010.

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 an Irish national reference laboratory for the purposes of residue surveillance in cow's milk. However, the method is not fully validated in line with the requirements of Volume 8 of *The rules governing medicinal products in the European Union*. In particular, additional information is needed on preparation samples, accuracy, precision, stability. Also the concentration range over which the method was validated did not include twice the MRL proposed. The limit of quantification is $6.0 \mu\text{g}/\text{kg}$.

Concerning the applicability of the analytical method in sheep milk, a similarity in the validation of the parameters is noticed when bovine, caprine and ovine milks are compared. However, further clarification on the validation for bovine milk is required before a final conclusion on the applicability of the proposed analytical method in sheep milk is drawn.

The proposed method has been reviewed by the relevant European Union Reference Laboratory, which confirmed the overall suitability of the method but highlighted the fact that the validation range did not cover the relevant limits.

Work has been initiated to further validate the method but the studies have not been completed yet.

2.2.5. Findings of EU or international scientific bodies

No information was available on evaluation from other scientific bodies.

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 rafoxanide 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 ovine tissues, which represents a worst case calculation, equates to 89.3 µg (equivalent to 74.3% of the ADI), leaving 30.8 µg (equivalent to 25.7% of the ADI) for the establishment of a MRL for milk.

Given the available information relating to residues in other tissues, rafoxanide was retained as marker residue in bovine milk and a ratio of marker to total residues of 0.5 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 rafoxanide remains below the ADI, the maximum allowable total residues in milk is 20 µg/kg.

In view of the information available and the risk management considerations the CVMP recommends the extrapolation of the existing MRLs for rafoxanide in cattle to cattle milk. The proposed MRL is 10 µg/kg.

Although data were not provided to demonstrate the presence of the marker residue rafoxanide in sheep milk, metabolism in plasma and tissues is known to be limited, and therefore rafoxanide 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 0.5 agreed for bovine milk is also considered to be acceptable for ovine milk.

Therefore the proposed MRL of 10 µg/kg for cattle can also be accepted for sheep milk.

In view of the deficiencies already highlighted in this report concerning the analytical method only a provisional MRL can be recommended at this stage.

Available data indicate that following the oral administration of 11.2 mg/kg bw to cows with a dry period of at least 54 days residue values in milk were below 10 µg/l from 3 days after calving while median residue values were below 10 µg/l from two days after calving. It is also noted that rafoxanide-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 ovine tissues and milk (worse 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	100	1.00	30 µg
Fat	0.05	250	0.88	14.2 µg
Liver	0.10	150	0.50	30 µg
Kidney	0.05	150	0.50	15 µg
Milk	1.50	10	0.05	30 µg
Total				119.2 µg (99.33% of the ADI)

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

Whereas:

- the toxicological ADI of 2 µg/kg bw (i.e 120 µg/person) was established as the overall ADI for rafoxanide;
- rafoxanide has been accepted as the marker residue in cattle and sheep milk;
- the marker to total residues ratio of 0.5 established for fat was considered a suitably 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;
- no residue depletion studies are available to demonstrate the depletion of residues in milk;

and having considered that:

- an analytical method for the monitoring of residues of rafoxanide in milk is available but not adequately validated;
- there are no grounds for supposing that residues of that substance at the level proposed constitute a hazard to human health;
- data needed to further validate the analytical method in milk are being generated and an extension of the time period applying to the provisional maximum residue limits would allow completion of the studies in progress;

the Committee, having considered the request from Ireland and the information provided on the ongoing studies for the validation of the analytical method in milk, recommends in accordance with Article 14(4) of Regulation (EC) 470/2009, the extension of the time period applying to the provisional maximum residue limit for rafoxanide in bovine and ovine milk and the amendment of the entry for rafoxanide 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
Rafoxanide	Rafoxanide	Bovine	30 µg/kg 30 µg/kg 10 µg/kg 40 µg/kg	Muscle Fat Liver Kidney	NO ENTRY	Antiparasitic agents/Agents against endoparasites
		Ovine	100 µg/kg 250 µg/kg 150 µg/kg 150 µg/kg	Muscle Fat Liver Kidney		
		Bovine, ovine	10 µg/kg	Milk	Provisional MRL expires on 31 December 2017	

Based on these values, the theoretical maximum daily intake from ovine tissues and milk is 119.2 µg, which corresponds to 99.3% of the ADI.

4. List of questions

1. The analytical method for rafoxanide residues in milk should be further validated, in line with the requirements specified in Volume 8 of *The rules governing medicinal products in the European Union*. In particular the concentration range over which the method was validated did not include the relevant limits (MRL value and twice the MRL proposed) and the documentation in relation to accuracy, precision and stability data for the proposed analytical method was not provided.

5. Background information on the procedure

Submission of the dossier	19 August 2011
Steps taken for assessment of the substance	
Clock started:	20 August 2011
List of questions adopted:	10 November 2011
Submission of response to list of questions:	6 September 2013
CVMP opinion on provisional MRLs adopted:	12 December 2013
CVMP opinion on extension of provisional MRLs adopted:	6 November 2015