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Committee for Veterinary Medicinal Products (CVMP)

European public MRL assessment report (EPMAR)

Rafoxanide (bovine and ovine milk)

On 20 October 2023, the European Commission adopted a Regulation[1] establishing maximum residue limits for rafoxanide in milk, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Veterinary Medicinal Products.

Rafoxanide is intended for use in ruminants as a fasciolicide, and active against gastrointestinal nematodes and sheep nasal bot fly, at a dose of 11.25 mg/kg body weight generally administered by the oral route.

Following a request from Ireland for the extrapolation of existing MRLs, provisional maximum residue limits in milk were established for bovine and ovine milk pending submission of validation data on the analytical method[2].

Ireland submitted to the European Medicines Agency a dossier aimed at the establishment of final MRLs in bovine and ovine milk on 21 February 2023.

Based on the original data in the dossier, the Committee for Veterinary Medicinal Products recommended on 20 April 2023 the extrapolation of maximum residue limits for rafoxanide in all ruminants and ovine milk.

Subsequently the Commission recommended on 4 September 2023 that maximum residue limits in all ruminants and ovine milk are established. This recommendation was confirmed on 19 September 2023 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 20 October 2023.

[1] Commission Implementing Regulation (EU) No 2023/2203, O.J. L, of 23 October 2023
[2] Commission Implementing Regulation (EU) No 2016/576, O.J. L 99, of 15.04.2016

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Rafoxanide (bovine and ovine milk)

Summary of the scientific discussion for the extrapolation of MRLs

Substance name:	Rafoxanide
Therapeutic class:	Antiparasitic agents/Agents against endoparasites
Procedure number:	EMEA/V/MRL/003450/EXPL/0005
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:

Pharmaco-logically 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 2017	

The provisional MRL for bovine and ovine milk expired on 13 December 2017. Subsequent to that, on the 21 February 2023, Ireland submitted to the European Medicines Agency, a request for an opinion

on the extrapolation of maximum residue limits for rafoxanide to bovine and ovine milk, pursuant to the second paragraph of Article 27 of Regulation (EC) No 470/2009.

The scientific assessment carried out by the Committee leading to the recommendation for the establishment of MRLs in bovine and ovine milk is reported in the paragraphs below. The considerations are largely identical to those that led to the establishment of a provisional MRL in bovine and ovine milk in 2014.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously performed a consumer safety evaluation for rafoxanide 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 ^{14}C-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; rafoxanide 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 rafoxanide/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 rafoxanide 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 ^{14}C-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 rafoxanide 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 rafoxanide/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 rafoxanide 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 rafoxanide 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 ± 138 µg/kg).

There are no data available for the depletion of residues in ovine milk.

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.

Article 7(e) of Commission Regulation 2017/880 of 23 May 2017 indicates that, when extrapolating from tissues to milk, consideration shall be given to the physicochemical characteristics of the substance and whether these suggest accumulation in milk. From published information, it is noted that log Kow is about 7-8, indicating that rafoxanide would accumulate in fat. 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 worst-case value of 0.5, established for bovine fat, is retained 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 µg/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 VICH GL 49. In particular, some information is missing on preparation of samples, accuracy, precision, stability. Also, the concentration range over which the method was validated did not include the MRL proposed. The limit of quantification is 6.0 µg/kg.

Concerning the applicability of the analytical method in sheep and goat milk, a similarity in the validation of the parameters of accuracy and precision is noticed when bovine, caprine and ovine milks are compared.

The proposed method has been reviewed by the relevant European Union Reference Laboratory, which confirmed the suitability of the method to control the proposed MRL with comparable performance for milk from the different animal species.

Notwithstanding the deficiencies identified, the data provided indicate that the analytical method has the potential to comply with the requirements of Regulation (EU) 2018/782. That is, the marker residue rafoxanide can be measured in bovine milk, in accordance with the guidance on validation of the analytical method as detailed in VICH GL49. Moreover, it is considered that the requirements for extrapolation of the Commission Regulation (EU) 2017/880 on extrapolation of MRLs are fulfilled, specifically Article 4, that "a suitably validated method is available for the reference species". This is the case as a validated analytical method for bovine and ovine tissue is available.

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 fully meet the criteria described in Commission Regulation (EU) 2018/782 of 29 May 2018. In particular, the studies provided are not GLP compliant and no data on total residues in milk are available. However, the current evaluation relates to an extrapolation request in line with Regulation 2017/880 and is considered to meet the relevant requirements.

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 products under non-authorised conditions.

3.3. Extrapolation of MRLs

The extrapolation to milk has been assessed in line with the requirements laid down in Commission Regulation 2017/880 of 23 May 2017, particularly articles 4 and 7 of that regulation.

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 from bovine and ovine meat depletion profile.

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 would be 10 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.

The analytical method for milk was not fully validated. However, requirements of Commission Regulation (EU) 2017/880 on extrapolation of MRLs have been fulfilled, notably a validated analytical method for monitoring residues in bovine and ovine tissues is available. Moreover, in any future application for marketing authorisation for rafoxanide in milk, a fully validated analytical method in support of residue depletion studies will be required.

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**	**0.5**	**30 µg**
Total				119.2 µg (**99.33% of the ADI**)

4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits recommended for rafoxanide to other food producing species and commodities. Taking into account the provisions laid down in Commission Regulation (EU) 2017/880, the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	**Extrapolation possible (Yes/No)**	**Justification**
All ruminants, including milk	Yes	Pharmacokinetics and residue data in ruminants other than cattle and sheep are not available. However, as other ruminants are considered minor species, in line with Commission Regulation (EU) 2017/880, extrapolation of the MRLs recommended for bovine (lower than for sheep meat) can be accepted.
Other species than ruminants	No	Pharmacokinetic and residue data were only available in bovine and ovine. In the absence of supporting data demonstrating similarity of metabolism, and in line with Commission Regulation (EU) 2017/880, extrapolation to unrelated species is not recommended.

5. 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 bovine fat was considered a suitably conservative value to be considered in relation to milk given that 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 having considered that:

- an analytical method for the monitoring of residues of rafoxanide in milk is available and considered fit for purpose while not fully validated;
- a validated analytical method for monitoring residues in bovine and ovine tissues is available;

the Committee, having considered the request from Ireland, recommends the extrapolation of maximum residue limits for rafoxanide to milk in bovine and ovine and the amendment of the entry in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Rafoxanide	Rafoxanide	All ruminants except ovine	30 µg/kg 30 µg/kg 10 µg/kg 40 µg/kg 10 µg/kg	Muscle Fat Liver Kidney Milk	NO ENTRY	Antiparasitic agents/Agents (acting) against endoparasites
		Ovine	100 µg/kg 250 µg/kg 150 µg/kg 150 µg/kg 10 µg/kg	Muscle Fat Liver Kidney Milk		

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.

6. Background information on the procedure

Submission of the dossier	20 February 2023
Steps taken for assessment of the substance	
Clock started:	21 February 2023
CVMP opinion on MRLs adopted:	20 April 2023