15 January 2013
EMA/CVMP/914694/2011
Committee for Medicinal Products for Veterinary Use (CVMP)

European public MRL assessment report (EPMAR)

Fenbendazole (extension to chicken and extrapolation to all food producing species)

On 7 December 2012 the European Commission adopted a Regulation[1] establishing maximum residue limits for fenbendazole in all food producing species except fin fish, 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.

Maximum residue limits had previously been established for fenbendazole in all ruminants, pigs and horses. Intervet International BV submitted to the European Medicines Agency an application for the extension of maximum residue limits for fenbendazole to chicken, on 29 June 2011.

Fenbendazole is intended for use in poultry for treatment and control of pre-adult and adult stages of gastrointestinal nematodes in chickens infected with *Ascaridia galli* and *Heterakis gallinarum*. The proposed recommended dose is 1 mg/kg bw per day for 5 consecutive days. In ruminants, pigs and horses fenbendazole is used for the control of gastrointestinal roundworms, lung worms and tape worms.

Based on the data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 8 December 2011 the establishment of maximum residue limits for fenbendazole in chicken and the extrapolation of existing maximum residue limits to all food producing species except fish.

Subsequently the Commission recommended on 25 October 2012 that maximum residue limits in all food producing species except fin fish are established. This recommendation was confirmed on 15 November 2012 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 7 December 2012.

[1] Commission Implementing Regulation (EU) No 1161/2012, O.J. L 336/14, of 8.12.2012

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Fenbendazole
Therapeutic class:	Antiparasitic agents/Agents against endoparasites
Procedure number:	EU/11/189/INT
Applicant:	Intervet International BV
Target species:	Chicken
Intended therapeutic indication:	Treatment and control of pre-adult and adult stages of gastrointestinal nematodes in chickens infected with *Ascaridia galli* and *Heterakis gallinarum*
Route(s) of administration:	Oral

1. Introduction

Fenbendazole is a benzimidazole anthelmintic that is metabolised in mammals to a series of other benzimidazoles including oxfendazole. It is used for the control of gastrointestinal roundworms, lung worms and tape worms.

Fenbendazole was previously assessed by the CVMP, an ADI of 7 µg/kg bw i.e. 420 µg/person was established.

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

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classify-cation
Fenbendazole	Sum of extractable residues which may be oxidised to oxfenda-zole sulphone	All ruminants, porcine, *Equidae*	50 µg/kg 50 µg/kg 500 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	For porcine species the fat MRL relates to 'skin and fat in natural proportions'.	Antiparasitic agents/Agents against endoparasites
		All ruminants	10 µg/kg	Milk		

An application has now been submitted for the extension of fenbendazole to chicken. The proposed indication for chicken is treatment and control of pre-adult and adult stages of gastrointestinal nematodes in chickens infected with *Ascaridia galli* and *Heterakis gallinarum*. The proposed recommended dose is 1 mg/kg bw per day for 5 consecutive days.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP previously assessed the consumer safety of fenbendazole and agreed to adopt the ADI established for oxfendazole for fenbendazole and its produg febantel. The reason for having previously adopted such ADI is that all three compounds share a common metabolism and oxfendazole (the commom metabolite formed *in vivo*) was the most toxic. An ADI of 7 µg/kg bw, i.e. 420 µg/person was established by applying a safety factor of 100 to the NOEL of 0.65 mg/kg bw/day for hepatic vacuolation from a carcinogenicity study in rats treated with oxfendazole.

Therefore, no further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

A new formulation produced as sub-micron size particles of the active fenbendazole was developed for use in drinking water. This is a different formulation from the one used in the studies previously assessed by the CVMP for the establishment of MRLs in the other animal species. The new formulation was used in one pharmacokinetic study and in the residue studies in chicken.

2.2.1. Pharmacokinetics in target species

A pharmacokinetic study was conducted in two groups of hens where they were treated orally by gavage with two different fenbendazole oral formulations at a single dose rate of 5.0 mg fenbendazole/kg bw. Blood samples were collected at various intervals and the pharmacokinetic parameters compared. All the major metabolites of fenbendazole (oxfendazole and oxfendazole sulphone) were found in large quantities in plasma and the absorption of the new fenbendazole formulation was greater than the old one. C_{max} and AUC_{last} (area under the plasma concentration-time curve from time zero to time of last measurable concentration) for fenbendazole of the new formulation were 89.3 ng/ml and 637.4 (h.ng/ml) and that of the established formulation were 38.3 ng/ml and 357.0 h.ng/ml respectively. C_{max} and AUC_{last} for oxfendazole were 224.4 ng/ml and 2563.6 h.ng/ml for the new formulation and 140.6 ng/ml and 2022.9 h.ng/ml for the older formulation. C_{max} and AUC_{last} for oxfendazole sulfone were 311.8 ng/ml and 9572.5 h.ng/ml for the new formulation and 243.1 ng/ml and 7459.5 h.ng/ml for the older formulation.

Metabolism

A number of GLP radio-labelled studies were conducted in laying hens and broilers. In these metabolism studies the birds were administered [^{14}C]-fenbendazole orally in drinking water or by gavage. The birds were administered varying doses (1.5 or 5 mg fenbendazole/kg bw) for 5 or 12 days. Fenbendazole was found in eggs only whereas oxfendazole and oxfendazole sulphone and other metabolites were found in all tissues and eggs. The major metabolite was oxfendazole sulphone followed by oxfendazole. Other 4 metabolites were found in liver and kidney and eggs, these metabolites were more polar than fenbendazole.

In a pilot study, 3 laying hens (group A) were administered a 20% [^{14}C] fenbendazole suspension (diluted each morning in drinking water and divided in three doses administered approximately 3 hours apart) at a daily dose of 4.5 mg/kg bw daily for 12 days and eggs were collected from the last two treatment days until three days after the last treatment. Two broiler chicken and two laying hens (Group B) were administered daily for 7 days. One broiler chicken and one laying hen each were slaughtered 24 (group B I) and 48 hours (group B II) after the last administration and the 4 edible

tissues (muscle, liver, kidney and fat plus skin) were sampled. For broiler chickens, the highest radioactivity levels were found in liver (4300 and 2000 µg equivalent/kg) and in kidneys (2400 and 1200 µg equivalent/kg). Levels of radioactivity were lower in fat plus skin (500 and 300 µg equivalent/kg) and in muscle (400 and 200 µg equivalent/kg). The radioactivity levels were higher in laying hens when compared to broilers. Fenbendazole was only found in eggs whereas oxfendazole and oxfendazole sulphone were found in all tissues and eggs.

In a pivotal study, 3 broiler chickens and 3 laying hens per group were administered a daily dose of 1.5 mg [^{14}C]-fenbendazole/kg for 5 days. The five groups were slaughtered 24, 48, 72, 96 and 120 hours after the last administration. Residue levels in the four edible tissues (muscle, liver, kidney and skin plus fat) were sampled. Total radioactive residues were assayed in tissues by liquid scintillation counting. Metabolite profiling was performed in tissues after extraction and using an HPLC method with radioactive and MS/MS detection. The highest concentrations of total radioactive residue were observed 24 hours after the last treatment for all tissues. The maximum concentrations were 1733.71 µg equivalent/kg in liver, 1257.92 µg equivalent/kg in kidneys, 278.45 µg equivalent/kg in skin plus fat and 250.66 µg equivalent/kg in muscle. Total residue concentrations were still quantifiable 120 hours after the last treatment in all tissues. The highest concentrations of oxfendazole sulfone were observed 24 hours after the last treatment in all tissues. The maximum concentrations were 1106.33 µg/kg in liver, 769.83 µg/kg in kidneys, 285.47 µg/kg in skin plus fat and 180.57 µg/kg in muscle. Thereafter residue concentrations decline rapidly, with oxfendazole sulfone concentrations being below the lower limit of quantification 96 hours after the last treatment in skin plus fat and muscle and 120 hours after the last treatment in liver and kidneys. Total residue levels and oxfendazole sulphone levels were generally higher in laying hens.

In another pivotal study, 15 laying hens were administered a daily dose of 1.5 mg [^{14}C]-fenbendazole/kg for 5 days. Eggs were collected from day 2 to day 13. Total radioactive residues were assayed in eggs by liquid scintillation counting. The metabolite profiling was performed in eggs after extraction and using an HPLC method with radioactive and MS/MS detection. The highest total radioactive residue concentrations (328.45 µg/kg to 730.90 µg/kg) were observed, in most hens, in egg specimens collected on day 6 (1 day after last treatment). Thereafter, the total residue concentrations decline rapidly, but total radioactivity was still quantifiable in all samples (4.90 µg/kg to 52.01 µg/kg) on day 13 (8 days after last treatment). In most hens, the highest oxfendazole sulfone concentrations were observed in eggs collected on day 5 or day 6, and ranged from 508.76 µg/kg to 914.42 µg/kg. In all other egg samples, oxfendazole sulfone and oxfendazole were the only metabolites detected. Both were considered as major, with maximum 91.41% of the total radioactive residue for oxfendazole sulfone (on day 12) and maximum 36.32% (on day 2) of the total radioactive residue for oxfendazole. After 8 days (day 13) marker residue levels were not found in eggs.

2.2.2. Residue depletion studies

In a pilot study, 20 layer hens were treated with fenbendazole suspension with a total dose of at least 5 mg fenbendazole/kg bw (actual dose 4.99 – 8.33 mg fenbendazole/kg bw) subdivided over 3 consecutive days. The fenbendazole suspension was applied orally twice a day as medicated water. The applied volume was based on the highest body weight determined 1 day before the first treatment. Oxfendazole sulfone residues were detected in eggs from 1 day after the first treatment up to 8 days after the last treatment. The highest residues were determined 1 to 2 days after the last treatment in a concentration range between 559 and 850 µg/kg. No oxfendazole sulfone residues above the limit of quantification were detected 9 days after the last treatment and at later time points. The entire albumen and yolk from each egg was homogenized with a blender and levels of fenbendazole residues in eggs were determined (as the sum of extractable residues which may be

oxidized to oxfendazole sulfone) using a HPLC/fluorescence method (limit of quantification (LOQ) equal to 100 µg/kg).

In a pivotal study, 20 laying hens were administered 1 mg fenbendazole/kg body weight/day for 5 consecutive days diluted as medicated drinking water for voluntary uptake. Eggs from 12 hens (i.e. at least 10 eggs per sampling day) were analysed for fenbendazole residues determined as sum of all residues which can be oxidized to oxfendazole sulfone using a validated HPLC/fluorescence method (LOQ of 100 µg/kg). Eggs were analysed until residue concentrations were below the LOQ in all eggs on 3 consecutive days. Oxfendazole sulfone residues were detected in eggs from 1 day after the first treatment up to 8 days after the last treatment. The maximum concentrations of oxfendazole sulfone (170.86 to 572.32 µg/kg) were measured in the eggs laid one day after the last administration (study day 6). Residue concentrations then decreased rapidly, with levels below the lower limit of quantification (LOQ) in all eggs laid 7 days after the last administration (study day 12).

In a pivotal tissue residue depletion study, 91 healthy birds (23 laying hens, 23 roosters, 23 female broiler chickens and 23 male broiler chickens) were randomly allocated to 5 study groups of 12 birds each (3 roosters, 3 laying hens, 3 male broiler chickens and 3 female broiler chickens per study group) and 1 reserve group of 31 birds (7 roosters, 8 laying hens, 8 male broiler chickens and 8 female broiler chickens). The birds were administered 1 mg fenbendazole/kg bw/day for 5 consecutive days via drinking water (medicated water) for voluntary uptake for 5 days. Residue levels in liver ranged from 257 to 2102 µg/kg after 24 hours and from below LOQ to 63 µg/kg after 120 hours. In kidney, concentrations ranged from 189 to 1030 µg/kg after 24 hours and from below LOQ to 38 µg/kg after 120 hours. In muscle, concentrations ranged from below LOQ to 426 µg/kg after 24 hours and were below LOQ in all animals after 120 hours. In skin plus fat, concentrations ranged from 111 to 409 µg/kg after 24 hours and from below the limit of detection (LOD) to 31 µg/kg after 120 hours. Samples were analysed using a validated HPLC/fluorescence method with a lower LOQ of 25 µg/kg.

Selection of marker residue

The applicability of the selected marker residue was confirmed in radiolabeled studies. It is proposed that the same marker residues as currently established for other species be retained for chicken; sum of extractable residues which may be oxidised to oxfendazole sulphone.

Given the differences in the residue levels in laying hens and broilers, the ratio of marker to total residue was selected at the time point where residues fall below the proposed MRLs. It is proposed that currently established MRLs for other species (i.e. 50 µg/kg for muscle, kidneys and skin plus fat and 500 µg/kg for liver) are retained. Where different markers to total residue values are calculated for laying hens and broilers, the lowest one is selected. In cases where the marker to total residue is greater than one, it is proposed that 1 is used as the marker to total residue (worst case). For eggs, the marker to total residue was always above 1. Therefore, the following ratios of marker to total residues were derived; 0.29 for liver, 0.18 for kidneys and 1.0 for muscle, skin plus fat and eggs.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described in this report are available.

2.2.4. Analytical method for monitoring of residues

A validated HPLC/fluorescence method for the determination of the marker residue (sum of extractable residues which may be oxidised to oxfendazole sulphone) in chicken tissues (muscle, liver, kidney, skin plus fat) and eggs is available. The analytical method is based on the method already approved for the determination of the same marker residue in other species (ruminants, porcine, equidae). The limits

of detection for chicken tissues and eggs were 3.31, 4.95, 4.83, 3.43 and 22.20 µg/kg for muscle, liver, kidney, skin plus fat and eggs respectively, and the limit of quantification (LOQ) of the analytical method was 25 µg/kg for all tissues and 100 µg/kg for eggs. All other validation parameters were within the limits set out in Volume 8 of The Rules Governing Medicinal Products in the EU. Although the limits of quantification were lower than half of the proposed liver and egg MRLs, the validation range nonetheless covered concentrations ranging well below the proposed MRLs to twice the proposed MRLs. Therefore, the analytical method was considered to have been suitably validated.

2.2.5. Findings of EU or international scientific bodies

Codex Alimentarius has established MRLs for fenbendazole as follows: muscle, kidney and fat (cattle, horses, pigs, sheep and goats): 100 µg/kg; liver (cattle, horses, pigs, sheep and goats): 500 µg/kg: Milk (cattle and sheep): 100 µg/l. No MRLs have been established for fenbendazole in poultry.

3. Risk management considerations

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

Fenbendazole had no significant antibacterial activity and therefore potential effects in dairy products were not investigated.

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

None.

3.3. Elaboration of MRLs

The residue data submitted for chicken indicated that although a higher absorption is observed with the new formulation developed for administration in drinking water the residue distribution is similar. Therefore the same MRLs as currently established for all ruminants, pigs and *Equidae* can be recommeded for chicken tissues, i.e. 50 µg/kg for muscle, 50 µg/kg for fat, 500 µg/kg for liver and 50 µg/kg for kidney. For eggs a MRL of 1300 µg/kg can be recommended.

Calculation of theoretical daily intake of residues of chicken, eggs and milk:

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	50	1.0	15
Skin + Fat	0.09	50	1.0	5
Liver	0.10	500	0.29	172
Kidney	0.01	50	0.18	3
Eggs	0.10	1300	1.0	130
Milk	*1.5*	*10*	*0.20*	*75*
			Total	***400***
			ADI	***420***
			% of ADI used	***95.2***

Fenbendazole is not used as a pesticide and therefore the whole of the ADI is available for veterinary medicines.

The intake of residues from chicken tissues (without eggs) represents 46.5% of the ADI.

The intake from tissues (and milk) from the other species is 17% of the ADI

The theoretical daily residue intake from poultry tissues plus milk and eggs represents 95% of the ADI which represents worst case 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 recommended for fenbendazole in chicken (including eggs) and the existing MRLs for ruminants, porcine and *Equidae,* as well as the MRL for milk from ruminants, to other species/food commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

All food producing species, except fish	Yes	Currently the MRLs are established for all ruminants, porcine and *Equidae*. The same MRL values established for those species are now recommended for chicken tissues. Considering that specific residue data confirm a similar exposure of the consumer to residues from cattle, pig and chicken tissues, it can be assumed that the exposure assessment and ergo the risk characterisation on the basis of same MRLs for further species beyond these animal classes would be similar. Therefore extrapolation to all food producing species except fish can be recommended. An analytical method for the monitoring of fenbendazole residues in several animal species is available and is considered applicable to other species.
Fish	No	No data are available on extrapolation to fish. However, metabolism is generally less complicated in fish and given that the marker residue is the sum of extractable residues which may be oxidised to oxfendazole sulphone, in principle the same marker residue could be acceptable to fish. However, no information on the applicability of the analytical method to fish was available and therefore extrapolation of MRLs to fish are not recommended.
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 are required in order to allow adequate evaluation of the risk to consumer safety posed by residues in honey. No data are available to demonstrate that the analytical method used for monitoring of residues in other animals species tissues is applicable for monitoring of residues in honey.

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

Having considered that:

- the toxicological ADI of 7 µg/kg bw (i.e. 420 µg/person) was previously established as the overall ADI,
- the sum of extractable residues which may be oxidised to oxfendazole sulfone was retained as the marker residue,
- the ratios of marker to total residues were 1.0 in muscle, 1.0 in skin plus fat, 0.29 in liver, 0.18 in kidney and 1.0 in eggs,
- a validated analytical method for the monitoring of residues of fenbendazole in edible chicken tissues; muscle, liver, kidney and skin plus fat and eggs is available,
- MRLs are established on ruminants, pigs and *Equidae,*
- the same MRLs are also recommended to chickens,
- MRLs can be extrapolated to all food producing species except fish and honey,

the CVMP recommends the modification of maximum residue limits for fenbendazole and the amendment of table 1 of the Annex to 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
Fenbendazole	Sum of extractable residues which may be oxidised to oxfendazole sulfone	All food producing species except fish	50 µg/kg 50 µg/kg 500 µg/kg 50 µg/kg 10 µg/kg 1300 µg/kg	Muscle Fat Liver Kidney Milk Eggs	For porcine and poultry species the fat MRL relates to 'skin and fat in natural proportions'	Antiparasitic agents/Agents against endoparasites

The theoretical daily residue intake from poultry tissues plus milk and eggs (worst case scenario) represents 95% of the ADI.

4. Background information on the procedure

Submission of the dossier	29 June 2011
Steps taken for assessment of the substance	
Application validated:	13 July 2011
Clock started:	14 July 2011
CVMP opinion adopted:	8 December 2011