EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Diclazuril (Rabbits)

On 2 December 2013 the European Commission adopted a Regulation[1] establishing maximum residue limits for diclazuril in rabbits, 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 diclazuril in all ruminants, pigs and poultry. Huvepharma NV submitted to the European Medicines Agency the application for the extension of maximum residue limits to poultry, on 29 August 2012.

Diclazuril is intended for use in rabbits as anticoccidial with a recommended dose of 0.05 mg/kg bw.

Based on the data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 7 February 2013 the extension of maximum residue limits for diclazuril to rabbits.

Subsequently the Commission recommended on 15 October 2013 that maximum residue limits in rabbits are established. This recommendation was confirmed on 5 November 2013 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 2 December 2013.

[1] Commission Implementing Regulation (EU) No 1235/2013, O.J.L322 , of 3 December 2013

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

Summary of the scientific discussion for the establishment of MRLs

Substance name:	Diclazuril
Therapeutic class:	Antiparasitic agents/ Agents acting against protozoa
Procedure number:	EU/12/201/HUV
Applicant:	Huvepharma NV
Target species:	Rabbits
Intended therapeutic indication:	Antococcidial
Route(s) of administration:	Oral

1. Introduction

Diclazuril is a benzeneacetonitrile derivative and its chemical name is (-2, 6-dichloro-α-4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)-benzeneacetonitrile. It is used as an anticoccidial in pigs, ruminants and poultry.

Diclazuril was previously assessed by the CVMP and a toxicological ADI of 30 µg/kg bw, *i.e* 1.8 mg/person was established.

Currently diclazuril 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 classification
Diclazuril	Not applicable	All ruminants porcine	No MRL required	Not applicable	For oral use only	No entry

In addition, the CVMP recommended on 16 April 2012 the establishment of MRLs for diclazuril in poultry in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Diclazuril	Diclazuril	Poultry	500 µg/kg 500 µg/kg 1500 µg/kg 1000 µg/kg	Muscle Fat and skin in natural proportions Liver Kidney	Not for use in animals from which eggs are produced for human consumption	Antiparasitic agents/ Agents acting against protozoa

An application has now been submitted by Huvepharma NV on 29 August 2012 for the establishment of MRLs for diclazuril in rabbits. The proposed indication for rabbits is anticoccidial with a recommended dose of 0.05 mg/kg bw.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously assessed the consumer safety of diclazuril and established an ADI of 30 µg/kg bw, *i.e* 1.8 mg/person based on the NOEL of 3 mg/kg bw from a 2-year chronic toxicity/carcinogenicity study in mice and applying an uncertainty factor of 100.

The same ADI value of 0.03 mg/kg bw was established by EFSA in 2007 and by the Joint WHO/FAO Expert Committee on Food Additives (JECFA) at its 50th meeting in 1998.

No further assessment regarding the consumer safety of the substance is required for the purpose of this application.

2.2. Residues assessment

In addition to the one residue depletion study in rabbits, published literature in the form of FAO Food and Nutrition Paper 41/8, 1996, and the EFSA Scientific Opinions were reviewed.

2.2.1. Pharmacokinetics in target species

Some published pharmacokinetic studies were described in the EFSA and FAO reports. From these studies a number of elements were demonstrated:

- biotransformation of diclazuril in rabbit is low;
- the major compound found in plasma, tissues, urine and faeces is the parent substance;
- although poorly absorbed following oral administration, diclazuril is detectable in plasma soon after administration. The terminal half-life is about 2 days. Following continuous administration of diclazuril medicated feed at 1 mg/kg in feed, the steady state was attained within 10 days of continuous dosing with plasma concentrations of about 900 µg/l.

2.2.2. Residue depletion studies

A non-radiometric depletion study was performed in rabbits following oral administration of diclazuril *via* feed at the dose rate of 0.05 mg/kg bw/day for 49 consecutivie days. The highest residues were found in liver and then in kidneys. The mean diclazuril concentrations were 1770 ± 251 µg/kg in liver, 817 ± 173 µg/kg in kidney, 173 ± 41 µg/kg in fat and 79 ± 19 µg/kg in muscle 3 hours following the end of treatment. Twenty-four hours following the end of treatment, the mean diclazuril concentrations were 1651 ± 514 µg/kg in liver, 610 ± 221 µg/kg in kidney, 143 ± 60 µg/kg in fat and 76 ± 18 µg/kg in muscle.

No radiolabelled studies were submitted but the EFSA Opinion included a radiolabelled study where two groups of three male rabbits (1.9 to 2.7 kg bw) received, for 14 consecutive days, a daily dose of ^{14}C-diclazuril (0.05 mg diclazuril/kg bw, equivalent to 1 mg diclazuril/kg feed), and were slaughtered after 1 and 3 days withdrawal periods. The mean total residue levels were 1636 ± 311 µg/kg in liver, 712 ± 143 µg/kg in kidney, 196 ± 44 µg/kg in fat and 71 ± 15 µg/kg in muscle 1 day following the end of treatment. Three days following the end of treatment, the mean diclazuril concentrations were 1331 ± 204 µg/kg in liver, 419 ± 132 µg/kg in kidney, 128 ± 46 µg/kg in fat and 38 ± 5 µg/kg in muscle.

In addition the following studies were reported in the original CVMP evaluation of diclazuril (EMEA/MRL/086/96-FINAL).

One radiometric depletion study was carried out in rabbits. Forty-eight hours after a single administration of 1 mg of ^{14}C-diclazuril per kgbw, radioactivity levels were measured in liver (2 mg equivalent diclazuril/kg), kidney (1.1 mg equivalent diclazuril/kg) and fat (0.03 mg equivalent diclazuril/kg). In muscle, the concentrations did not exceed 0.01 mg equivalent diclazuril/kg. The radioactivity elimination half-life was 2-2.5 days for all tissues, except for the liver (3 days).

In two non-radiometric depletion studies carried out in rabbits after administration of diclazuril in feed at doses equivalent to 0.067 mg/kgbw/day for 14 days, the concentrations of diclazuril in tissues at 24 h after administration, were 0.20 mg/kg in fat, 1.60 mg/kg in liver, 0.60 mg/kg in kidney. No residue could be detected in muscle tissue.

From all the data provided, the following conclusions can be drawn regarding the residue depletion of diclazuril in rabbits:

- residues deplete rapidly;
- the highest concentrations of diclazuril residues are found in liver and to a lesser extent in kidneys;
- the lowest residue levels are found in muscle.

From residue and metabolism studies, diclazuril seems slightly metabolised in rabbit. Based on the radiometric study reported in the EFSA Opinion, the CVMP agreed with its conclusions that diclazuril can be considered as the marker residue with ratios of marker to total residues of 0.8, 0.6, 0.7 and 0.5 for liver, kidney, muscle and fat respectively.

2.2.3. Monitoring or exposure data

No monitoring or exposure data were available.

2.2.4. Analytical method for monitoring of residues

An analytical method to assay diclazuril in edible rabbit tissues was well described. This LC-MS/MS method was fully validated in accordance with the requirements of 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. The limit of quantification was 50 µg/kg for all edible tissues.

2.2.5. Findings of EU or international scientific bodies

Both EFSA and Codex Alimentarius evaluated diclazuril and the following MRLs were established for rabbits:

Tissues	Rabbit MRLs (µg/kg)	
	EFSA	Codex
Muscle	150	500
Fat	300	1000
Liver	2500	3000
Kidney	1000	2000

3. Risk management considerations

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

During the original evaluation of diclazuril, it was found that diclazuril did not possess any antifungal activity and was devoid of activity at 100 µg/ml against *Bacillus subtilis* and *Sarcina lutea*. No further data were required for the purpose of this evaluation.

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

MRLs for diclazuril are established in the EU for its use as a feed additive in rabbits[2]. With a view to ensuring a consistent approach with regard to consumer safety and the feasibility of the monitoring of residues, the Committee took into account these MRLs when making its recommendation.

3.3. Elaboration of MRLs

Based on the information submitted, it can be seen that total residue levels were very low 1 day after treatment (12.8% of the ADI) and continued to deplete to 9.6% of the ADI after 3 days. As a result a "no MRL required" entry similar to the previous conclusion on ruminants and pigs could have been considered. However, considering that MRLs have already been established in the EU for diclazuril in rabbits for its use as a feed additive, the CVMP concluded that the EFSA evaluation of the substance can be supported and the same MRLs recommended as follows: muscle 150 µg/kg; fat 300 µg/kg; liver 2500 µg/kg and kidney 1000 µg/kg.

[2] Regulation (EC) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition

Calculation of theoretical daily intake of residues:

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.300	150	0.7	64.3
Fat	0.050	300	0.5	30
Liver	0.100	2500	0.8	312.5
Kidney	0.050	1000	0.6	83.3
Total				490.1 Representing 27.2% of the ADI

The CVMP notes that these MRLs are different to the ones established by Codex Alimentarius. However, in view of the fact that MRLs for diclazuril are established in the EU in rabbits for its use as a feed additive, further considerations on the possibility of harmonising the MRLs with Codex Alimentarius were not pursued.

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 recommended maximum residue limits established for diclazuril to other food producing species and food commodities. A "no MRL required" status has been established for oral use for all ruminants and porcine species and the establishment of MRLs in poultry have been recommended.

Taking into account the current scientific knowledge, the recommendations on extrapolation of the recommended MRLs for rabbits are justified as follows:

Animal species/ food commodities	Extrapola-tion possible (Yes/No)	Justification
Poultry eggs	No	No data are available that would allow conclusions to be drawn on the appropriate marker residue or marker to total residues ratio to use in eggs. No analytical method for monitoring of residues in eggs was available for evaluation.
Horses	No	Existing data indicate that the pattern of metabolites seen in rats, rabbits, chickens, turkeys, sheep, goats and cattle is similar and it can be expected that the parent compound would be a suitable marker residue in horses. However, no data are available to demonstrate that the analytical method used for monitoring of residues in rabbits is applicable for monitoring of residues in horse tissues.

Fin fish	No	Metabolism is generally less complicated in fish than in mammals or birds. Consequently, as the marker residue is the parent compound in rabbits it can be assumed that the parent compound would also be a suitable marker residue in fin fish meat. However, no analytical method for monitoring of residues in fin 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 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 rabbit 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 0.030 mg/kg bw (*i.e*. 1.80 mg/day per 60 kg bw person) was previously established as the overall ADI for diclazuril;
- the parent compound, *i.e*. diclazuril was retained as the marker residue;
- the ratios of marker to total residues calculated at 1 day were 0.7 in muscle, 0.5 in fat, 0.8 in liver and 0.6 in kidney;
- MRLs in rabbits have been established in the EU in relation to the use of diclazuril as a feed additive;
- a validated analytical method for the monitoring of residues of diclazuril in edible rabbit tissues is available;

the Committee recommends the establishment of maximum residue limits for diclazuril in rabbits and the modification of table 1 of the Annex to Regulation 37/2010 as follows:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Diclazuril	Diclazuril	Rabbit	150 µg/kg 300 µg/kg 2500 µg/kg 1000 µg/kg	Muscle Fat Liver Kidney		Antiparasitic agents/Agents acting against protozoa

Based on the recommended MRLs the theoretical maximum daily intake of residues from rabbit tissues represents 27.2% of the ADI.

4. Background information on the procedure

Submission of the dossier	29 August 2012
Steps taken for assessment of the substance	
Application validated:	12 September 2012
Clock started:	13 September 2012
CVMP opinion adopted:	7 February 2013