9 January 2024
EMA/CVMP/511158/2023
Committee for Veterinary Medicinal Products (CVMP)

European public MRL assessment report (EPMAR)

Toltrazuril (Poultry eggs)

On 2 March 2023, the European Commission adopted a Regulation[1] establishing maximum residue limits for toltrazuril in poultry eggs, 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.

Toltrazuril is intended for use in chickens for the treatment of coccidiosis at an oral dose of 7 mg/kg bw/day for 2 days, administered in drinking water.

Toltrazuril had maximum residue limits already established[2] for tissues of all mammalian food producing species and poultry.

The Netherlands submitted to the European Medicines Agency a request for the extension of maximum residue limits on 29 June 2021.

Based on the original data in the dossier, the Committee for Veterinary Medicinal Products recommended on 9 December 2021 the extension of maximum residue limits for toltrazuril to poultry eggs.

Subsequently the Commission recommended on 17 December 2022 that maximum residue limits in poultry eggs are established. This recommendation was confirmed on 7 January 2023 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 2 March 2023.

[1] Commission Implementing Regulation (EU) No 37/2010 of 22 December 2009, O.J. L 67/38, of 03 March 2023
[2] Commission Implementing Regulation (EU) No 37/2010 of 22 December 2009, O.J. L 15/1, of 22 December 2009

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Toltrazuril
Therapeutic class:	Antiparasitic agents / agents acting against protozoa
Procedure number:	EMEA/V/MRL/003363/EXTN/0004
Applicant:	Medicines Evaluation Board Agency
Target species requested:	Chicken eggs
Intended therapeutic indication:	Treatment of coccidiosis
Route(s) of administration:	Oral

1. Introduction

Toltrazuril is a triazinetrione derivative used as anticoccidial agent. It is widely used in veterinary medicine in chickens, turkeys, pigs, and cattle for the treatment of coccidiosis. The doses in food producing species are: 7 mg/kg bw/day for two days administered in the drinking water for chickens and turkeys; 15 mg/kg bw in calves and 20 mg/kg bw in piglets and lambs given once as an oral suspension; 20 mg/kg bw or 45 mg/animal given intramuscularly in piglets.

Toltrazuril was previously assessed by the CVMP and a toxicological ADI of 2 µg/kg bw, i.e. 120/µg/person was established (EMEA/MRL/314/97-FINAL).

Currently, toltrazuril is included in Table 1 of the Annex to 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**
Toltrazuril	Toltrazuril sulfone	All mammalian food producing species	100 µg/kg 150 µg/kg 500 µg/kg 250 µg/kg	Muscle Fat Liver Kidney	For porcine species the fat MRL relates to 'skin and fat in natural proportions'. Not for use in animals from which milk is produced for human consumption. Not for use in animals from which eggs are produced for human consumption.	Antiparasitic agents/ Agents acting against protozoa
		Poultry	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg	Muscle Skin and fat Liver Kidney		

The Netherlands submitted a request for the extension of MRLs for toltrazuril to chicken eggs on 29 June 2021 pursuant to Article 9(1)(b) of Regulation (EC) No 470/2009. Toltrazuril is intended for use in chickens for the treatment of coccidiosis at an oral dose of 7 mg/kg bw/day for 2 days, administered in drinking water.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously assessed the consumer safety of toltrazuril and established an ADI of 2 µg/kg bw, i.e. 120 µg/person based on the threshold dose of 1 mg/kg bw/day for pre-neoplastic lesions retained from a carcinogenic study in rats and applying a safety factor of 500 (EMEA/MRL/314/97-FINAL). Therefore, no further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

Maximum residue limits for toltrazuril in chicken and poultry tissues were recommended in 1998 and the published Summary Report (EMEA/MRL/314/97-FINAL) describes pharmacokinetic and residues studies performed in these species. The current report relates specifically to a request for extension of the MRLs to eggs and only new data relevant to this specific request are described.

2.3. Pharmacokinetics in target species

No proprietary data relating to kinetics of toltrazuril in chicken following oral administration is available. Plasma kinetics of toltrazuril in chicken was previously assessed by the CVMP during the MRL application of toltrazuril in chicken (EMEA/MRL/314/97-FINAL).

A published paper (Kim et al., 2013[3]) examined plasma kinetics of toltrazuril and its two major metabolites (toltrazuril sulfoxide and toltrazuril sulfone) after oral administration in broilers. This published paper confirms previous conclusions reached by CVMP, i.e. that the metabolism is rapid and that the major metabolite is toltrazuril sulfone, the elimination of which is slow.

2.3.1. Residue depletion studies

Depletion in eggs

Four published papers and one technical report from the European Reference Laboratory for residues (EURL, Berlin) present useful information on the residue composition and the residue profile in eggs from laying hens after administration of the recommended dose of toltrazuril.

After administration in laying hens of 9.5 mg toltrazuril/kg bw/day for 2 days twice with a washout period of 5 days between the two treatments, it was noted that the main residue, toltrazuril sulfone, accumulated in yolk, in which levels were almost 20 times greater than in albumen. The decline of toltrazuril sulfone is slow and a significant concentration of residue (i.e. 1600 µg/kg) can still be found 18 days after the last dose (Mudler et al., 2005[4]).

[3] Kim, M. S., Park, B. K., Hwang, Y. H., Song, I. B., Kim, T. W., Cho, J. H., ... & Yun, H. I. (2013). Pharmacokinetics and metabolism of toltrazuril and its major metabolites after oral administration in broilers. The Journal of Poultry Science, 50, 257-261.

[4] Mulder, P. P. J., Balzer-Rutgers, P., Te Brinke, E. M., Bolck, Y. J. C., Berendsen, B. J. A., Gerçek, H., ... & Van Rhijn, J. A. (2005). Deposition and depletion of the coccidiostats toltrazuril and halofuginone in eggs. Analytica chimica acta, 529(1-2), 331-337.

Mudler and Van Rijn (2003[5]) found similar results after administration of 25 mg/L of toltrazuril via drinking water for 2 days twice with a washout period of 5 days between the two treatments.

Pietruk et al. (2021[6]) also showed that toltrazuril sulfone accumulates in yolk after administration via drinking water of 7 mg/kg bw/day of toltrazuril for 2 consecutive days. The depletion rate of toltrazuril sulfone was not constant during the study. During a first phase, the half-life value was lower, around 3.5 days, but with time the elimination slowed down and the half-life value increased to up to 5 days. The author concluded that the required time for the concentration of toltrazuril sulfone to reach zero (i.e. LOQ of 6.25 µg/kg) with 95% confidence is almost 70 days after the last administration.

Following an oral administration via drinking water of 7 mg toltrazuril/kg bw/day for 2 consecutive days in laying hens (N = 10, 15 weeks old), the individual residue concentrations of toltrazuril sulfone declined from 167.49 µg/kg at day 27 after treatment to below CCα (= 4.42 µg/kg) at day 55 after treatment. The residue concentrations of toltrazuril and toltrazuril sulfoxide were below their respective CCα at all-time points (Technical report of the EURL; EURL Berlin, 2020[7]). An LC-MS/MS validated method was used.

The following conclusions can be retained:

- Toltrazuril sulfone is the major residue;
- Residues are more concentrated in yolk versus egg white;
- The decrease of residues in whole egg is slow, corresponding to a half-life of about 4-5 days. However, it is notable that in the pharmacovigilance report from the Netherlands (see "Monitoring or exposure data", below), elimination appeared slower. One possibility is that the slower apparent elimination may have been associated with the conditions under which the birds were kept, which allowed coprophagic behaviour and consequent re-ingestion of residues.

Selection of marker residue and ratio of marker to total residues

In previous assessments of toltrazuril, the CVMP concluded that toltrazuril sulfone is the most appropriate marker residue as it represents close to 100% of the total residues in tissues of both chickens and turkeys (EMEA/MRL/314/97-FINAL). Data provided (Mudler et al., 2005; Pietruk et al., 2021; technical report of the EURL for residues; EURL Berlin, 2020) supports that toltrazuril sulfone can be considered as the marker residue for toltrazuril in eggs.

Mudler et al. (2005) showed that the ratio of marker to total residues was 0.95 and 0.99 at 7 and 11 days after the last treatment administration. It can be concluded that the ratio of marker to total residues is considered to be 1 for toltrazuril in eggs at 11 days after the treatment, as previously considered for edible tissues of poultry.

Monitoring or exposure data

In 2020, an incident was reported in Eudravigilance after treatment at the label dose to young laying hens (about 20 weeks of age) with a veterinary medicinal product containing toltrazuril. Following a withdrawal period of 7 days (in line with article 11(2) of Directive 2001/82/EC) the

5 Mulder, P.P.J., & Van Rijn, J.A. (2003). Multi-residue Screening for Coccidiostatic Compounds Used in Poultry Production. Institute for Food Safety (RIKILT), Report No. 2003.515.
6 Pietruk K, Olejnik M & Jedziniak P (2021) Risk of residues of toltrazuril sulfone in eggs after oral administration - could setting maximum residue limit be helpful? Food chemistry, 360, 130054.
7 Technical report 2019/2020 of the EURL for residues (EURL Berlin, 2020).

Dutch inspection unit required a check for residues before eggs could enter the food chain. The samples were analysed by an LC-MS/MS method with a decision limit (CCα) of 1.9 µg/kg (Van Ginkel, 2021). The marker residue was identified up to 96 days after treatment.

In the German control program, toltrazuril sulfone was found in eggs. Therefore a study was conducted by the EU Reference Laboratory for residues (EURL, Berlin), in which 15-week old pullets were treated with Toltra-K (25 mg/mL) via drinking water at 7 mg/kg bw/day for 2 consecutive days (see above, technical report of the EURL; EURL Berlin, 2020).

A report of the European Food Safety Authority (EFSA) on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products revealed findings of toltrazuril sulfone in egg: 5 non-compliant samples were found in a total of 163 egg samples taken in Latvia in 2018 (EFSA, 2020).

2.3.2. Analytical method for monitoring residues

The methods described in the published papers were not developed for the purposes of an MRL submission and so were not validated in line with VICH GL49. However, they are considered suitable for use in determining the marker residue and the ratio of marker to total residues.

The relevant European Reference Laboratory has developed an analytical method for monitoring toltrazuril residues in eggs. Toltrazuril and its two major metabolites, toltrazuril sulfone and sulfoxide were assessed by an LC/MS-MS method, with CCα of 6.47, 4.42 and 0.49 µg/kg, respectively.

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

The substance is not intended for use in dairy animals and therefore potential effects in dairy products were not investigated.

2.3.4. Findings of EU or international scientific bodies

Toltrazuril has not been evaluated for this purpose by EU or international scientific bodies.

3. Risk management recommendations

3.1. Availability of alternative medicines and other legitimate factors

Availability of alternative medicines

Coccidiosis in poultry can be treated with different pharmacologically active substances that have variable modes of action, including toltrazuril. Per substance, the spectrum of activity may differ and there is a limited number of substances authorised for laying hens. For example, amprolium is authorised for use in chickens and turkeys and has a withdrawal period of zero days for eggs; however, it is not active against all of the *Eimeria* species. Also, sulfadimidine is authorised but only for broiler chickens. Lasalocid has MRLs established for eggs but lasalocid-containing products are not authorised in all Member States. Diclazuril is not authorised for use in birds producing eggs for human consumption.

Some vaccines are available for the reduction of clinical symptoms, and/or for the reduction of oocyte shedding of some *Eimeria* species. However, these may only be suitable for use under particular conditions (e.g. environmental conditions that favour sporulation of the oocytes contained in the vaccine).

The availability of curative treatment options for such infections is important, also for vaccinated animals.

Technological aspects of food and feed production (potential effects on the microorganisms used for industrial food processing)

Chicken eggs are not further processed into food or feed products using microorganisms.

Other factors that should, if applicable, be taken into consideration in support of the MRL recommendation:

No other relevant factors were identified for consideration of the risk management recommendations.

3.2. Elaboration of MRLs

The following MRL is recommended based on the reasoning provided below:

Tissue	MRL (toltrazuril sulfone)
Eggs	140 µg/kg

Available residue depletion data in eggs does not allow direct derivation of an MRL. The recommended MRL is therefore based on what can be accepted taking the unused portion of the ADI into account.

The theoretical maximum daily intake (TMDI) of toltrazuril sulfone based on MRLs established for mammalian tissues is 112.2 µg while the TMDI based on MRLs established for poultry tissues is 112 µg.

In addition, a previous CVMP referral on toltrazuril in relation to environmental exposure (EMEA/402698/2008) concluded that up to 1.1 µg toltrazuril sulfone/L could be present in groundwater. Assuming all drinking water is derived from groundwater and assuming consumption of 2L drinking water per day, the intake of toltrazuril sulfone from this source amounts to 2.2 µg. Total consumer exposure to toltrazuril sulfone based on existing tissue MRLs and exposure from groundwater therefore amounts to 114.4 µg/person, which equates to 106.4 µg toltrazuril (based on a correction factor for the difference in molar weights of the metabolite and parent substance, i.e. 1.075) or 88.67% of the ADI. This leaves a maximum of 11.33% of the ADI (13.58 µg toltrazuril or 14.6 µg toltrazuril sulfone) for residues in eggs.

The standard food basket assumes 100 g consumption of eggs per day. Taking into account the ratio of marker to total residues in eggs of 1, this allows a maximum residue concentration of 146 µg/kg toltrazuril sulfone in eggs in order to be compatible with the ADI. As it is usual to set MRLs at rounded numbers an MRL of 140 µg/kg is recommended for the marker residue toltrazuril sulfone.

Calculation of theoretical daily intake of residues

Edible tissue or products	Daily consumption (kg)	MRL proposal µg/kg)	Ratio of the marker/total residue	Total residues (µg) (toltrazuril sulfone + toltrazuril)**	Total residues (µg) expressed as toltrazuril equivalents ***
Muscle*	0.30	100	0.85	35.3 (30 + 5.3)	33.20
Fat*	0.05	150	0.89	8.43 (7.5 + 0.93)	7.90
Liver*	0.10	500	0.92	54.3 (50 + 4.3)	50.86
Kidney*	0.05	250	0.94	13.3 (12.5 + 0.8)	12.43
Eggs	0.10	140	1	14 (14 + 0)	13.02
Total				125.3	117.41

* From mammalian species

** Total residues are considered to be made up primarily of toltrazuril sulfone, with the remaining residues assumed to consist of parent toltrazuril. The quantity of each is calculated using the ratio of marker to total residues

*** Toltrazuril sulfone residues are converted to toltrazuril equivalents using a conversion factor of 1.075 based on the molar weights of the two substances

Based on the above figures the maximum theoretical consumer intake of total residues is 125.3 µg, or 117.4 µg of toltrazuril equivalents, which corresponds to 97.8% of the ADI for toltrazuril (i.e. 120 µg/person). When a further 2.2 µg of toltrazuril sulfone residues in drinking water is added, which corresponds to 2.05 µg toltrazuril equivalents, a total of 99.5% of the ADI is used up.

The use of 99.5% of the ADI is considered justified in this case even though it will not leave enough space within the ADI to allow for possible future uses (i.e. in honey and milk production). Coccidiosis is not a disease with relevance for honey bees and use of toltrazuril in dairy animals is not expected as coccidiosis is primarily a disease of young animals.

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 toltrazuril 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
Poultry eggs	Yes	The similarity of metabolism in chickens and other poultry species has already been accepted as MRLs for tissues were previously extrapolated to all poultry species. Based on this accepted similarity of metabolism it is considered appropriate to extrapolate the recommended MRL for chicken eggs to eggs from other poultry species

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

Having considered that:

- the toxicological ADI of 2 µg/kg bw (i.e. 120 µg/person) was established as the overall ADI for toltrazuril,
- maximum residues limits have already been established in poultry tissues as well as in tissues of food producing mammals,
- detection of residues in eggs has been observed in the context of the administration of toltrazuril containing products which was in accordance with Article 11(2) of Directive 2001/82/EC,
- toltrazuril sulfone was retained as the marker residue in eggs,
- the ratio of marker to total residues is 1 in eggs 11 days following oral treatment,
- a validated analytical method is available indicating that residues in eggs can be adequately monitored,
- a maximum residue limit in eggs is proposed based on the unused portion of the ADI,
- extrapolation of the maximum residue limits recommended for chicken eggs to eggs of other poultry species is considered appropriate,

the Committee recommends the extension of maximum residue limits for toltrazuril to poultry eggs and the amendment of the entry for toltrazuril 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**
Toltrazuril	Toltrazuril sulfone	All mammalian food producing species	100 µg/kg 150 µg/kg 500 µg/kg 250 µg/kg	Muscle Fat Liver Kidney	For porcine species the fat MRL relates to 'skin and fat in natural proportions'. Not for use in animals from which milk is produced for human consumption.	Antiparasitic agents/ Agents acting against protozoa
		Poultry	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg 140 µg/kg	Muscle Skin and fat Liver Kidney Eggs	NO ENTRY	

6. Background information on the procedure

Submission of the dossier	29 June 2021
Steps taken for assessment of the substance	
Clock started:	9 July 2021
CVMP opinion adopted:	9 December 2021