Annex I

List of the names, pharmaceutical form, strengths of the veterinary medicinal products, animal species, route of administration, applicants and marketing authorisation holders in the Member States

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
Austria	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Axentyl 200 mg/ml Injektionslösung für Rinder, Schafe, Ziegen und Schweine	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Austria	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 200 mg/ml Injektionslösung für Rinder, Schafe, Ziegen und Schweine	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Austria	Vana GmbH Wolfgang Schmälzl-Gasse 6 1020 Wien Austria	Vanatyl 200 mg/ml - Injektionslösung für Tiere	Tylosin base	200 mg/ml	Solution for injection	Cattle, dogs, goats, pigs, sheep	Sheep: Intramuscular route
Austria	CEVA Santé Animale 10 Avenue De La Ballastiere 33500 Libourne France	Tiljet	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Bulgaria	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 200 mg/ml solution for injection for cattle, sheep, goats and pigs	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Bulgaria	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Pharmasin 50 solution for injection	Tylosin base	50 mg/ml	Solution for injection	Cats, cattle, dogs, goats, pigs, sheep	Sheep: Intramuscular route
Bulgaria	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylovet B-200 solution for injection for cattle, sheep, goats, swine and dogs	Tylosin base	200 mg/ml	Solution for injection	Cattle, dogs, goats, pigs, sheep	Sheep: Intramuscular route
Bulgaria	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylovet B-50, solution for injection for cattle, sheep, goats, swine, dogs and cats	Tylosin base	50 mg/ml	Solution for injection	Cats, cattle, dogs, goats, pigs, sheep	Sheep: Intramuscular route
Bulgaria	CEVA Santé Animale	Tiljet 200 mg/ml	Tylosin	200	Solution for	Cattle,	Sheep:

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
	10 Avenue De La Ballastiere 33500 Libourne France	solution for injection for cattle, pigs, sheep and goat	base	mg/ml	injection	goats, pigs, sheep	Intramuscular route
Denmark	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Tylmasin	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Denmark	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylovet	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Denmark	Ceva Animal Health A/S Ladegårdsvej 2 DK-7100 Vejle Denmark	Tiljet	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Estonia	Interchemie Werken De Adelaar Eesti AS Vanapere tee 14 Püünsi, Viimsi vald 74013 Harju maakond Estonia	Macrolan-200	Tylosin base	200 mg/ml	Solution for injection	Cattle, pigs, sheep	Sheep: Intramuscular route
Estonia	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
France	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	AXENTYL 200 MG/ML SOLUTION INJECTABLE POUR BOVINS, OVINS, CAPRINS ET PORCINS	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
France	Bimeda Animal Health Unit 2/3/4 Airton Close Tallaght Dublin 24 Ireland	BILOVET 200 MG/ML SOLUTION INJECTABLE	Tylosin base	200 mg/ml	Solution for injection	Sheep	Sheep: Intramuscular route
France	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen	PHARMASIN 200 MG/ML SOLUTION INJECTABLE POUR	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
	Belgium	BOVINS OVINS CAPRINS ET PORCINS					
France	LILLY France 24 Boulevard Vital Bouhot 92200 Neuilly Sur Seine France	TYLAN 200	Tylosin base	200 mg/ml	Solution for injection	Sheep	Sheep: Intramuscular route
France	CEVA Santé Animale 10 Avenue De La Ballastiere 33500 Libourne France	TILJET 200 MG/ML SOLUTION INJECTABLE POUR BOVINS OVINS CAPRINS ET PORCINS	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Germany	CEVA Santé Animale 10 Avenue De La Ballastiere 33500 Libourne France	Tiljet 200mg/ml solution for injection for cattle, pigs, sheep and goats	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Germany	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylmasin 200 mg/ml Injektionslösung für Rinder, Schafe, Ziegen und Schweine	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Germany	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 200 mg/ml Injektionslösung für Rinder, Schafe, Ziegen und Schweine	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Greece	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Greece	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylmasin	Tylosin tartrate	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Greece	Ceva Hellas LLC 15 Agiou Nikolaou Street 17455, Alimos Greece	Tiljet	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
Greece	A. Nikolakopoulos S.A. 115 Galatsi Avenue 11146, Galatsi Greece	Tilocen	Tylosin tartrate	200 mg/ml	Solution for injection	Cattle, sheep, pigs, dogs, cats	Sheep: Intramuscular route
Greece	Anafasis LTD Santorinis 3 Pallouriotissa 1048 Nicosia Cyprus	Tylosin/Anafasis	Tylosin tartrate	200 mg/ml	Solution for injection	Cattle, sheep, pigs, dogs, cats	Sheep: Intramuscular route
Hungary	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 200 mg/ml oldatos injekció szarvasmarhák, juhok, kecskék és sertések részére	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Hungary	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	TYLMASIN 200 mg/ml oldatos injekció szarvasmarhák, juhok, kecskék és sertések részére	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Ireland	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 200 mg/ml solution for injection for cattle, sheep, goats and pigs	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Ireland	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylosin Biovet JSC 200 mg/ml solution for injection for cattle, sheep, goats and pigs	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Ireland	CEVA Santé Animale 10 Avenue De La Ballastiere 33500 Libourne France	Tiljet Solution for Injection	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Italy	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	AXENTYL 200 mg/ml soluzione iniettabile per bovini, ovini, caprini e suini	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Italy	Huvepharma NV Uitbreidingstraat 80	PHARMASIN 200 mg/ml soluzione iniettabile per	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs,	Sheep: Intramuscular

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
	2600 Antwerpen Belgium	bovini, ovini, caprini e suini				sheep	route
Latvia	Interchemie Werken De Adelaar Eesti AS Vanapere tee 14 Püünsi, Viimsi vald 74013 Harju maakond Estonia	LV Macrolan	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route.
Latvia	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Lithuania	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 50 mg/ml injekcinis tirpalas kiaulėms, galvijams, avims, ožkoms, šunims ir katėms	Tylosin base	50 mg/ml	Solution for injection	Cats, cattle, dogs, goats, pigs, sheep	Sheep: Intramuscular route
Lithuania	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 200 mg/ml injekcinis tirpalas kiaulėms, galvijams, avims ir ožkoms	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Poland	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylozyna Biovet JSC	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Poland	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Poland	Ceva Animal Health Polska Sp. z o.o. Ul. Okrzei 1a Warszawa 03-715 Poland	Tiljet 200 mg/ml solution for injection for cattle, pigs, sheep and goats	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Portugal	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera	AXENTYL 200 mg/ml soluzione iniettabile per bovini, ovini, caprini e	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route

Member State EU/EEA	Applicant / Marketing authorisation holder	Name	INN	Strength	Pharmaceutical form	Animal species	Route of administration
	Bulgaria	suini					
Portugal	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 200 mg/ml soluzione iniettabile per bovini, ovini, caprini e suini	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Romania	S.C. Pasteur - Filiala Filipesti Str. Principala, nr. 944 Filipestii de Padure Jud. Prahova Romania	Tilozina FP	Tylosin tartrate	200 mg/ml	Solution for injection	Sheep	Sheep: Intramuscular route
Romania	Romvac Company SA Sos. Centurii nr.7 07718, Voluntari Jud. Ilfov Romania	Tylavet	Tylosin tartrate	200 mg/ml	Solution for injection	Sheep	Sheep: Intramuscular route
Romania	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 200 mg/ml	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Romania	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	Tylmasin 200 mg/ml	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Spain	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	PHARMASIN 200 mg/ml SOLUCION INYECTABLE PARA BOVINO OVINO CAPRINO Y PORCINO	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Spain	Biovet JSC 39 Petar Rakov Str. 4550 Peshtera Bulgaria	TILOSINA BIOVET JSC 200 mg/ml SOLUCION INYECTABLE PARA BOVINO OVINO CAPRINO Y PORCINO	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route
Spain	CEVA SALUD ANIMAL, S.A. Avda. Diagonal, 609-615 08028 Barcelona Spain	TILJET 200 mg/ml SOLUCION INYECTABLE PARA BOVINO, OVINO, CAPRINO Y PORCINO	Tylosin base	200 mg/ml	Solution for injection	Cattle, goats, pigs, sheep	Sheep: Intramuscular route

Annex II

Scientific conclusions and the grounds for amendment of the summaries of product characteristics, labelling and package leaflets

Overall summary of the scientific evaluation of veterinary medicinal products containing tylosin presented as solution for injection to be administered to sheep (see Annex I)

1. Introduction

Tylosin is a macrolide antibiotic which is produced by *Streptomyces fradiae*. It is active mostly against Gram-positive bacteria and mycoplasmas. It is ineffective against Enterobacteriaceae. Tylosin and its phosphate and tartrate salts are used in veterinary medicines for the treatment of conditions caused by sensitive organisms. It may be administered by oral or parenteral routes. Macrolides are categorised as critically important antimicrobials both in human and veterinary medicine, however tylosin is not used in human medicine.

An application was submitted under Article 13(1) of Directive 2001/82/EC, i.e., a generic application for a marketing authorisation under the decentralised procedure for the veterinary medicinal product Tylovectin, containing 200 mg of tylosin per ml, with France as Reference Member State (FR/V/0323/001/DC). The reference product is Tylan 200 solution for injection, which has been authorised in various Member States.

The application of the generic product (Tylovectin) concerned cattle, pigs, sheep and goats as the target species, which is in line with the reference product as authorised in France (Tylan 200). However, in some Member States, including the Netherlands, the reference product has not been authorised for use in sheep.

The proposed sheep meat withdrawal period for the generic product Tylovectin was 42 days, in line with the French reference product, Tylan 200.

From the information on the reference product (Tylan 200) provided by the Reference Member State France, it became clear that the withdrawal period for sheep was based on an extrapolation from the withdrawal period for cattle of 28 days. No specific residue data were available for the target species sheep.

In accordance with the current legislation and relevant guidelines, maximum residue limits withdrawal periods have to be established on the basis of species-specific residue depletion data for all major food producing species. Sheep (meat) is considered a major food producing species. The CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1)[1] allows extrapolation of the withdrawal period between animal species, but only from a major species to a minor species.

In absence of residue depletion data in the major species sheep, the Netherlands considered that the withdrawal period for sheep of the reference product (Tylan 200) has not been adequately justified. Therefore, the appropriateness of the withdrawal period for sheep of the reference product is uncertain.

It has been noted that there are different approved withdrawal periods for sheep (milk, meat and offal) for veterinary medicinal products containing tylosin presented as solution for injection to be administered to sheep across the EU, e.g. sheep meat and offal from 8 days to 42 days; sheep milk from 4 days to 7 days. Therefore, the Netherlands considered that it is necessary to refer the matter to

[1] CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1) - https://www.ema.europa.eu/documents/scientific-guideline/guideline-safety-residue-data-requirements-pharmaceutical-veterinary-medicinal-products-intended/limited-market_en.pdf

the CVMP in the interests of protecting consumer safety in the Union, and requested the Committee to review all available residue depletion data and recommend withdrawal periods for sheep (meat and offal, and milk).

2. Discussion of data available

Qualitative and quantitative composition

Information was received regarding the composition of the affected products (n=50). For three of the products the concentration of tylosin is 50 mg/ml, whereas for the other 47 products it is 200 mg/ml.

Regardless of the tylosin strength, the formulation of 49 of the concerned products is very similar. For these 49 products, excipients listed are benzyl alcohol and propylene glycol (in concentrations that differ slightly between products), as well as small concentrations of different pH adjustors. Within this group of 49 products, most products contain tylosin base as active substance, whereas only five products contain tylosin tartrate. The chemical forms of the active substance, being tylosin base or tartrate, have been considered to have similar behaviours (FAO, 2006[2]), and tylosin being a weak base with a pK_a of 7.73, can be expected to ionize and form salts under physiological conditions (i.e. at pH 7.4). Hence, the chemical form is not considered to effect residue absorption from tissues (including the injection site). The differences in concentrations of benzyl alcohol and propylene glycol, and in pH adjustors, have also been considered not to have an impact on residue absorption from tissues and the injection site.

For one product (Tilocen), the solvent polyethylene glycol 400 was used instead of propylene glycol. While minor differences in concentrations of the same excipients have been considered not to have an impact on residue depletion, different solvents can have an effect on the viscosity of the formulations which can affect absorption. Indeed, published data has shown that the rate of absorption from the injection site is inversely proportional to the viscosity of the product, i.e. the higher the viscosity, the slower the rate of absorption (Kakemi *et al.*,1972[3]). Given that the viscosities of propylene glycol and polyethylene glycol 400 are different, a significant impact on the absorption and residue depletion, and consequently on the withdrawal periods needed for tylosin solutions for injections with these different excipients cannot be excluded.

Residue depletion in sheep meat and offal

Two residue depletion studies were submitted by two of the concerned marketing authorisation holders. Results from one of the studies could not be used, since data on residue concentrations at the injection site was missing. The other submitted study was a GLP-compliant residue study with tylosin solution for injection, conducted in sheep via intramuscular administration. This study was done with the product Tylosin/Anafasis, containing benzyl alcohol and propylene glycol as excipients. From this study, the injection site was confirmed as the withdrawal period determining tissue. Data from this study could not be used as a basis for extrapolating the withdrawal period for the only product containing the excipient polyethylene glycol 400 (Tilocen, marketing authorisation holder: A. Nikolakopoulos S.A), as differences between the absorption of residues from the injection site for these two products (i.e., given the differences on viscosities from products with different excipients) cannot be excluded, as mentioned in the section above.

[2] Joint FAO/WHO Expert Committee on Food Additives. Meeting. (2006). *Residue Evaluation of Certain Veterinary Drugs: Joint FAO/WHO Expert Committee on Food Additives, 66th Meeting 2006* (Vol. 2). Food & Agriculture Org.
[3] Kakemi, K., Sezaki, H., Okumura, K., Kobayashi, H., & Furusawa, S. (1972). Absorption of Drugs from the Skeletal Muscle of the Rats.(3). Effect of Watersoluble Adjuvants and Vehicles on the Intramuscular Absorption. *Chemical and Pharmaceutical Bulletin*, *20*(3), 443-451.

The results from this residue study have been used for establishing the withdrawal periods for the remaining 49 out of the 50 products concerned, i.e. for all those products considered to be comparable qualitatively regarding excipients.

The residue study was performed in sheep at a dose of 10 mg/kg bodyweight (bw), administered every 12 hours for 3 consecutive days. Tylosin concentrations in tissues (muscle, fat, liver, kidney, and injection site) were measured at 3, 7, 10 and 21 days post-treatment using a validated high performance liquid chromatography method. The recommended treatment and dose of Tylosin/Anafasis is 10 mg/kg bw for 3-5 days, although most products recommend only 3 days of treatment. As the animals in the study were treated twice daily, as opposed to once daily as usually recommended, the study design can be considered to represent a worst-case scenario. In this study, however, additional shortcomings were identified, these being that the number of treated animals (n=3) was below the number required in VICH GL 48[4], animals were very young (12 kg mean bw), and also the sampling of the injection site did not include an outer ring sample.

After analysis of the residue data for the injection site using the recommended EMA statistical software (WT 1.4), it was concluded that not all the statistical requirements (i.e., F-test) were met. Therefore the Committee used the 'alternative' method for determining the withdrawal period, in line with CVMP note for guidance on the approach towards harmonisation of withdrawal periods (EMEA/CVMP/036/95)[5]. At day 21 after the last dose was administered, the residue concentrations in all samples of the injection site were below the established maximum residue limit (MRL) for tylosin in sheep meat of 100 µg/kg. An additional safety factor of 30% was considered necessary in view of the limited number of animals treated (n=3), their weight (12 kg mean bw) and consequently the small injection volume required to treat these three lambs (0.6 ml), together with the lack of analysis of injection site ring samples, resulting in a withdrawal period of 28 days.

It is noted that this product can be used in adult sheep and that adult sheep would require treatment with higher injection volumes. As the withdrawal period is established taking into account persistence of residues in the injection sites, and a higher injection volume may cause higher local residues in injection sites, a longer withdrawal period would be required for adult sheep treated with tylosin intramuscularly. Whereas, according to current standards, residue depletion studies should be conducted in animals that are representative of the target population, it has not been possible to obtain such data within the context of this referral procedure. Hence, an option to overcome the lack of data in adult sheep would be to limit the injection volume, to the volume used in the residue study available with Tylosin/Anafasis (of 0.6 ml). Nevertheless, as limiting the injection volume would require adult animals having to be injected 8 to 10 times per treatment dose, this is not considered appropriate for both practical and animal welfare considerations.

From the residue data available, the treatment of a 50 kg sheep would require an injection volume of 2.5 ml. This represents a deposit at the injection site of approximately four times the amount of tylosin used in the residue study available, of 0.6 ml. A half-life ($T_{1/2}$) of approximately two days for the absorption from the injection site was determined from the available residue study. If, theoretically, a larger injection would have the same absorption rate as established for a volume of 0.6 ml, then the withdrawal period would need to be extended by approximately 5 days, i.e., a total of 33 days (resulting from 21 days, plus a 30% safety span, and the additional 5 days). Nevertheless, it has been reported that the rate of absorption from the injection site depends on a number of factors (e.g. injection volume, excipients, lipophilicity of the active substance), and without specific residue data,

[4] VICH topic GL48: Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker-residue-depletion studies to establish product withdrawal periods (EMA/CVMP/VICH/463199/2009) https://www.ema.europa.eu/documents/scientific-guideline/vich-gl48-studies-evaluate-metabolism-residue-kinetics-veterinary-drugs-food-producing-animals_en.pdf

[5] CVMP note for guidance on the approach towards harmonisation of withdrawal periods (EMEA/CVMP/036/95) - link

the influence of increasing the injection volume/site of injection on the absorption rate is difficult to quantify.

It is worth noting that the withdrawal period for sheep meat for many of the products that fall within the scope of this procedure, including the generic product Tylovectin and the reference product Tylan 200 as authorised in France, is 42 days. Theoretically, a withdrawal period of 42 days would accommodate for a decrease in release rate of more than 250%. Whereas these theoretical calculations are not scientifically substantiated by data, it is reasonable to assume that a withdrawal period of 42 days would ensure that consumers would not be exposed to residues of concern if the injection volume was to be restricted to 2.5 ml per injection site.

Hence, and in view of the limited data and considerations mentioned above, the Committee considered that harmonising the withdrawal periods of 49 out of 50 of the products concerned within the scope of this referral procedure, to a withdrawal period of 42 days for meat and offal from sheep, would be a conservative approach to ensure consumer safety (i.e., to ensure that residues at the injection site would be below the established MRL for tylosin meat (100 µg/kg)).

For sheep over 50 kg, the CVMP recommends that the dose is divided over two injection sites.

Residue depletion in sheep milk

No proprietary residue depletion studies in ewes were submitted. However, two publically available studies were made available to CVMP. The first study was conducted with 50 mg/ml tylosin (Ivatyl 50 inj. ad us. vet. Bioveta, Czech Republic)[6]. In this study, seven ewes (Slovak merino) were treated intramuscularly with tylosin base 50 mg per ml at a dosage of 100 mg per 10 kg bw, for five consecutive days. Milk samples were analysed using a high-performance liquid chromatography method with solid phase extraction. Tylosin residues were detected in milk during the treatment, and were below the established MRL for tylosin in cattle milk (50 µg/kg) 36 hours after the last administration, and were not detected 48 hours after the last treatment dose.

A second study was conducted with Najdi ewes with 200 mg/ml tylosin (Tylan 200)[7]. The objective of this study was to evaluate kinetics, and the residual decline of tylosin in milk and plasma of lactating ewes, following a single intramuscular injection of tylosin at the dose of 10 mg/kg bw. However, the actual residue concentrations were not given, and the study was done using a single injection rather than repeated injections. Hence, the Committee concluded that the data is considered to have a limited value for the scope of this referral procedure.

While the residue studies mentioned above were made available, milk from sheep is considered a minor food commodity, and withdrawal periods for sheep milk can be extrapolated from those for cows' milk in accordance with the CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1). This approach was proposed by some of the concerned marketing authorisation holders, and it is supported by the CVMP.

In addition, JECFA at their 66th meeting reviewed tylosin and compared the residue depletion of tylosin in milk from cows and sheep (JECFA, 2006[8]).The results show that tylosin residues deplete faster in milk from ewes, than in milk from cows. Two days after the last dose, tylosin could no longer be detected in sheep milk. Thus, these data indicate that a 1:1 extrapolation of the milk withdrawal period from cows to ewes is justified.

[6] Nagy, J., Popelka, P., Sokol, J., Turek, P., & Neuschl, J. (2001). The excretion of tylosin residues in ewes milk after its experimental administration. *Folia Veterinaria (Slovak Republic)*.
[7] Al-Wabel, N. A. (2008). The pharmacokinetics and milk residual behaviour of tylosin in lactating Najdi ewes. *Iranian Journal of Veterinary Research*, *9*(2), 138-143
[8] http://www.fao.org/tempref/AG/agn/food/tylosin_2006.pdf

Discussion

There are 50 products within the scope of this referral procedure. The formulation of 49 of the concerned products is very similar. For these 49 products, excipients listed are benzyl alcohol and propylene glycol (in concentrations that differ slightly between products), as well as small concentrations of different pH adjustors. The remaining product contains polyethylene glycol 400 instead of propylene glycol. While minor differences on concentrations of the same excipients have been considered not to have an impact on residue depletion from the injection site, different solvents can have an effect on the viscosity of the formulations, and consequently affect absorption of residues from the injection site. Given that the injection site is the withdrawal period determining tissue, extrapolation of residue data from products containing propylene glycol to that containing polyethylene glycol 400 cannot be justified.

A GLP-compliant residue study with tylosin solution for injection, conducted in sheep via intramuscular administration was made available to CVMP. This study was conducted with the product Tylosin/Anafasis, containing benzyl alcohol and propylene glycol as excipients. Hence, data from this study could not be extrapolated to the only product containing the excipient polyethylene glycol 400 (Tilocen, marketing authorisation holder: A. Nikolakopoulos S.A), for the reason mentioned above. Although a number of shortcoming were identified from this study (more specifically that the number of treated animals (n=3) was below the number required in VICH GL 48, animals were very young (12 kg mean bw), and also the sampling of the injection site did not include an outer ring sample), the results showed that the injection site is the withdrawal period determining tissue and results from this residue study have been used for establishing the withdrawal periods for 49 out of the 50 products concerned.

At day 21 after the last dose was administered, the residue concentrations in the injection site were all below the established MRL for tylosin in sheep meat, of 100 µg/kg. However, to address certain deficiencies in the data set, an additional safety factor of 30% was considered necessary resulting in a withdrawal period of 28 days. Also, as the product can be used in adult sheep, and higher injection volumes would then be required, it has been theoretically calculated that a longer withdrawal period of five days (i.e., a total of 33 days) could account for the increased volume needed to treat sheep up to 50 kg of weight, with a maximum volume of 2.5 ml. However, the rate of absorption from the injection site depends on a complexity of factors, and without specific residue data, the influence of increasing the injection volume/ site of injection on the absorption rate is difficult to quantify.

It is noted that a withdrawal period of 42 days for meat and offal is currently established for several of the products within this referral procedure. The Committee considered that harmonising the withdrawal periods of 49 of the products concerned within the scope of this referral procedure to a withdrawal period of 42 days for meat and offal from sheep, would be a conservative approach to ensure consumer safety (i.e., that residues from the injection site would be below the established MRL for tylosin meat (100 µg/kg)), as a withdrawal period of 42 days would accommodate for a decrease in release rate of more than 250% and ensure consumer safety if the injection volume was to be restricted to 2.5 ml per injection site. For sheep over 50 kg, however, the CVMP recommends that the dose is divided over two injection sites (maximum injection volume per site of 2.5 ml).

Regarding the establishment of withdrawal periods for sheep milk, no proprietary residue depletion studies in ewes were submitted. However, two publically[9,10] available studies were made available to CVMP. From these two published studies, one did not provide data that could be used within the scope of this referral procedure. The other publically available study showed that residues in sheep milk were

[9] Nagy, J., Popelka, P., Sokol, J., Turek, P., & Neuschl, J. (2001). The excretion of tylosin residues in ewes milk after its experimental administration. *Folia Veterinaria (Slovak Republic)*.
[10] Al-Wabel, N. A. (2008). The pharmacokinetics and milk residual behaviour of tylosin in lactating Najdi ewes. *Iranian Journal of Veterinary Research*, *9*(2), 138-143

below the MRLs for tylosin in cattle milk (50 µg/kg), 36 hours after the last administration, and were not detected 48 hours after the last treatment dose.

Milk from sheep is, however, considered a minor food commodity, and withdrawal periods for sheep milk can be extrapolated from those for cows' milk, in accordance with the CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1). The reference product Tylan 200 has a sheep milk withdrawal period of 108 hours, and this is the same withdrawal period for the milk of cattle in most Member States for the same product. Consequently, the CVMP is proposing to harmonise the milk withdrawal period to products concerned to 108 hours following the approached outlined in the guideline mentioned above.

3. Benefit-risk assessment

Introduction

The CVMP was requested to review all available residue depletion data for the veterinary medicinal products containing tylosin presented as a solution for injection for intramuscular use in sheep and recommend withdrawal periods for meat, offal and milk derived from treated sheep.

Benefit assessment

While the efficacy of the concerned products in sheep has not been specifically assessed as part of this referral, the products under assessment are considered to be effective in the treatment and prevention of infestations, which include respiratory infections caused by Gram-positive microorganisms, and mastitis caused by Gram-positive microorganisms.

Risk assessment

Quality, target animal safety, user safety, and the environmental risk for the concerned veterinary medicinal products have not been assessed in this referral procedure.

A risk has been identified regarding the length of the authorised withdrawal periods for sheep (meat and offal, and milk), which, for some products, may be insufficient to allow residues of tylosin to fall below the authorised MRLs in all edible tissues by the end of the withdrawal period, thereby posing a risk to consumers of meat, offal and milk from sheep treated with these products.

On the basis of a proprietary residue depletion study in sheep tissues, a withdrawal period for sheep meat and offal of 42 days could be derived for 49 concerned products. Data extrapolation was done based on the excipients composition of the products concerned.

For sheep milk, literature data showed that the tylosin residue depletion from sheep milk is faster than depletion from cow's milk. In accordance with CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1), the milk withdrawal period for cattle milk of 108 hours has been also established as the withdrawal period for sheep milk.

For the product containing the excipient polyethylene glycol 400 (Tilocen, marketing authorisation holder: A. Nikolakopoulos S.A), no suitable residue depletion data are available for establishing a withdrawal period for sheep meat, offal, and milk.

Risk management or mitigation measures

To ensure the safety of consumers of food and food products derived from animals treated with products containing tylosin, the European Commission has set MRLs for tylosin in the edible tissues of

sheep. In order for tylosin-derived residues to deplete below the MRLs, a sufficient time between treatment and slaughter must be allowed.

The Committee considered that for the 49 products containing benzyl alcohol and propylene glycol, the differences in concentrations will not lead to different rates of absorption from the injection site. However, as the injection site is the withdrawal period determining tissue, for the product containing polyethylene glycol 400 (Tilocen, marketing authorisation holder: A. Nikolakopoulos S.A), and in view of the effect of this excipient on product viscosity, a different rate of absorption of residues from the injection site cannot be ruled out. Hence, extrapolation is not warranted.

A GLP compliant residue depletion study was provided by one of the marketing authorisation holders involved in the procedure, and while a number of shortcoming on the study design were identified, the data allowed the recommendation to have a 42 day withdrawal period for meat and offal for 49 out of the 50 concerned products, i.e., for products containing 50 mg/ml and 200 mg/ml tylosin as a solution for injection for intramuscular use in sheep, containing benzyl alcohol and propylene glycol as excipients.

Data from the literature indicated that the residue depletion from milk from sheep is faster than from milk from cows. Milk from sheep is considered a minor food commodity, and in line with the CVMP guideline on safety and residue data requirements for pharmaceutical veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/SWP/66781/2005–Rev.1) extrapolation can be done from cow's milk to sheep milk. Following this approach a withdrawal period for sheep milk can be established at 108 hours.

An additional risk mitigation measure of restricting the maximum injection volumes to 2.5 ml for sheep of 50 or less kg of bodyweight was considered by the CVMP. For sheep over 50 kg of bodyweight, the CVMP recommends that the dose is divided over two injection sites.

Evaluation and conclusions on the benefit-risk balance

Having considered the grounds for referral and the data available, the CVMP concluded that for 49 out of 50 products, these being all those containing benzyl alcohol and propylene glycol as excipients, the withdrawal periods for meat and offal derived from treated sheep should be amended to 42 days, and the withdrawal periods for milk derived from treated sheep should be amended to 108 hours to provide assurance for consumer safety.

For these 49 concerned veterinary medicinal products, the overall benefit-risk balance remains positive subject to the recommended changes in the product information (see Annex III).

For the product Tilocen (marketing authorisation holder: A. Nikolakopoulos S.A) containing tylosin presented as solution for injection for use in target species sheep, and containing polyethylene glycol 400, there was no data available that could be used to determine adequate withdrawal periods that could assure that after 42 days after treatment, the residues are below the established MRLs for tylosin in sheep.

Therefore, the Committee considers that the benefit-risk balance for the veterinary medicinal product Tilocen (marketing authorisation holder: A. Nikolakopoulos S.A) containing tylosin presented as solution for injection for use in target species sheep, and containing polyethylene glycol 400 is not favourable in the absence of adequate residue depletion data in sheep to justify a withdrawal period of 42 days for sheep meat and offal, and 108 hours for sheep milk. Consequently, for this product the Committee recommends the variation of the existing marketing authorisations in order to remove any reference to the target species sheep.

Grounds for amendment of the summaries of product characteristics, labelling and package leaflets

Whereas,

- from the data provided the formulation of 49 of the concerned products is very similar. For these 49 products, excipients listed are benzyl alcohol and propylene glycol, as well as small concentrations of different pH adjustors. The differences on concentrations of benzyl alcohol and propylene glycol, and on pH adjustors have been considered not to have an impact on residue absorption from the injection site. For only one concerned product (Tilocen, marketing authorisation holder: A. Nikolakopoulos S.A), the solvent polyethylene glycol 400 is used instead of propylene glycol. Given that the viscosities of propylene glycol and polyethylene glycol 400 are different, a significant impact on the absorption and residue depletion from the injections site, and consequently on the withdrawal periods cannot be excluded.
- a GLP compliant residue study conducted with tylosin solution for injection, used intramuscularly in sheep was provided, and from which withdrawal periods could be established for meat and offal of treated sheep for 49 concerned products, i.e., those with excipients benzyl alcohol and propylene glycol. This withdrawal period was considered adequate and safe for the consumer as far as the injection volume was restricted.
- no suitable residue depletion study was made available the results from which could be extrapolated to the concerned product Tilocen (marketing authorisation holder: A. Nikolakopoulos S.A) containing tylosin presented as solution for injection for use in the target species sheep, and containing polyethylene glycol 400 as excipient.
- milk from sheep is a minor food commodity and the and withdrawal periods for sheep milk can be extrapolated from those for cows' milk. In addition, publically available data showed that residues in sheep milk deplete faster than in cow's milk, and could not be detected 48 hours after the last treatment dose.
- on the basis of the available data, the CVMP considered the withdrawal periods for meat and offal, and milk derived from treated sheep should be amended to provide assurance for consumer safety for all concerned veterinary medicinal products, as referred in Annex I, except for Tilocen (marketing authorisation holder: A. Nikolakopoulos S.A);
- on the basis of the available data, the CVMP considered the withdrawal periods for meat and offal, and milk derived from treated sheep cannot be established for Tilocen (marketing authorisation holder: A. Nikolakopoulos S.A), as referred in Annex I.

the CVMP has recommended variations of the marketing authorisations for veterinary medicinal products containing tylosin presented as a solution for injection for intramuscular use in sheep in order to amend the summaries of product characteristics, labelling and package leaflets in line with recommended changes in the product information as set out in Annex III.

Annex III

Amendments in the relevant sections of the summary of product characteristics, labelling and package leaflet

A. For Tilocen listed in Annex I (Marketing Authorisation Holder: Nikolakopoulos S.A)

All references to target species sheep should be deleted from the summary of product characteristics, labelling and package leaflet.

B. For all other products listed in Annex I

Summary of product characteristics

4.9 Amounts to be administered and administration route

For sheep over 50 kg of bodyweight, the injection should be divided over two injection sites (maximum 2.5 ml injection volume per injection site).

4.11 Withdrawal period(s)

Sheep:

Meat and offal: 42 days.

Milk: 108 hours

Labelling

8. WITHDRAWAL PERIOD

Sheep:

Meat and offal: 42 days.

Milk: 108 hours

Package leaflet

8. DOSAGE FOR EACH SPECIES, ROUTE AND METHOD OF ADMINISTRATION

For sheep over 50 kg of bodyweight, the injection should be divided over two injection sites (maximum 2.5 ml injection volume per injection site).

10. WITHDRAWAL PERIOD

Sheep:

Meat and offal: 42 days.

Milk: 108 hours