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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Nitroxinil (extrapolation of MRLs to bovine and ovine milk)

On 8 March 2012 the European Commission adopted a Regulation[1] establishing maximum residue limits for nitroxinil in bovine, ovine species, 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.

Nitroxinil is intended for use in cattle and sheep for the treatment of faciolosis and is administered by the subcutaneous route at a dose of 10 mg/kg bw.

Nitroxinil had maximum residue limits already established[2] for edible tissues of cattle and sheep.

The Irish Medicines Board submitted the application for the extrapolation of maximum residue limits to milk to the European Medicines Agency, on 30 August 2011.

Based on the available data, the Committee for Medicinal Products for Veterinary Use recommended on 10 November 2011 the extrapolation of maximum residue limits for nitroxinil to cattle and sheep milk.

Subsequently the Commission recommended on 26 January 2012 that maximum residue limits in bovine and ovine milk are established. This recommendation was confirmed on 16 February 2012 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 8 March 2012.

[1] Commission Implementing Regulation (EU) No ,O.J. L 71, of 09.03.2012
[2] Commission Regulation (EC) No 997/1999, O.J. L 122, of 12.05.1999

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Nitroxinil
Therapeutic class:	Antiparasitic agents/Agents against endoparasites
Procedure number:	EU/ART27/11/194/IMB
Requesting Member State:	Ireland
Target species:	Bovine and ovine milk
Intended therapeutic indication:	Fasciolicide effective against *Fasciola hepatica* with some activity against gastrointestinal nematodes (*Haemonchus contortus*)
Route (s) of administration:	Subcutaneous

1. Introduction

Nitroxinil is a halogenated phenol which is effective against *Fasciola hepatica* with some activity against gastrointestinal nematodes (*Haemonchus contortus*). Its mode of action is as an uncoupler of oxidative phosphorylation. Nitroxinil is indicated for the treatment of cattle and sheep and is administered by the subcutaneous route at a dose of 10 mg/kg bw.

Nitroxinil was previously assessed by the CVMP and a toxicological ADI of 5 µg/kg bw, i.e 300 µg/person was established.

Currently nitroxinil 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
Nitroxinil	Nitroxinil	Bovine, ovine	400 µg/kg 200 µg/kg 20 µg/kg 400 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Antiparasitic agents/Agents against endoparasites

On 30 August 2011 Ireland submitted to the European Medicines Agency a request for the extrapolation of the existing maximum residue limits for nitroxinil to bovine and ovine milk, pursuant to Article 27 of Regulation (EC) No 470/2009.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously performed a consumer safety evaluation for nitroxinil and established a toxicological ADI of 5 µg/kg bw, i.e 300 µg/person based on the NOEL of 500 µg/kg bw from a 90-day

repeat dose study in rats and applying an uncertainty factor of 100. Therefore, no further assessment regarding the establishment of the ADI for the substance is required for the purpose of this request.

2.2. Residues assessment

For the assessment of the request for extrapolation the committee considered relevant residue data from the previous assessment and any new information made available as detailed below.

2.2.1. Pharmacokinetics in target species

In cows, sheep and rabbits, nitroxinil is highly bound to plasma protein (97 to 98%). In all species, residues in plasma were higher than the residues in tissues and consisted almost entirely of nitroxinil. In all three species, nitroxinil is extensively metabolised.

In cattle, the percentage of marker residue to total residue was reported to be 2% for liver, 56% for kidney, 69% for muscle and 78% for fat at 5 days after treatment; 4% for liver, 34% for kidney, 100% for muscle and 100% for fat at 30 days after treatment; and 6% for liver, 19% for kidney, 100% for muscle and 90% for fat at 45 days after treatment.

In sheep, the tissue marker to total residue ratios were assessed in a single study by comparing the nitroxinil content of tissue when determined by HPLC with those determined by LSC. The percentage of marker residue to total residue for liver, kidney, muscle and fat were reported to have been 1%, 53%, 97% and 81% at 5 days after treatment.

Given the low percentage of residues present as parent compound in the liver, it can be concluded that the substance is extensively metabolised in the liver. It can also be concluded that the metabolites are very quickly eliminated by renal excretion, given the low percentage of parent substance found in the kidney while the percentage in plasma and muscle and fat remained particularly high.

No radiolabelled pharmacokinetic studies in milk were available and there was no information on metabolism in milk, however in view of the metabolism data available in plasma and tissues the percentage of the parent substance in milk is expected to be similarly high.

2.2.2. Residue depletion studies

Two residue studies were provided.

In a non-GLP study nitroxinil was administered subcutaneously to cattle at a dose of 10 mg/kg bw close to the time of parturition and resulted in detectable residues of nitroxinil in milk (100 to 300 µg/kg, expressed as µg/kg of the phenol in milk). By increasing the time between treatment and parturition, nitroxinil residues in milk were reduced. Residues of nitroxinil in samples of milk taken on the day of calving, 30-38 days after administration, and thereafter were found to be at or below the limit of detection (LOD) of the analytical method, which was 100 µg/kg. However, the analytical method had poor sensitivity with a LOD higher than the proposed MRL, making depletion difficult to assess. As the study was conducted in 1974 the data generated do not meet current standards and cannot be considered suitable to adequately characterise the depletion of residues in milk.

The second study, although non-GLP, appeared to have been well conducted and was well reported. The study included 35 test animals which were administered doses between 8.25 and 12.41 mg/kg bw as a single subcutaneous injection 43 to 80 days before expected calving. Residues of nitroxinil were quantifiable in milk for up to 38 days after calving. In general, the longer the time between treatment and calving the lower the concentrations of nitroxinil in milk. The highest concentrations of nitroxinil (Cmax) in each animal were found in the first milking and ranged from 1.6 to 476 µg/kg. Nitroxinil

residues were below the limit of detection of the method (0.24 µg/kg) between 67 and 106 days post-treatment (10 to 38 days post-calving).

In a recent published study, six dairy cows were treated during lactation with a 34% solution for injection containing nitroxinil. Milk samples were collected twice daily for 16 days and later at weekly intervals up to 58 days post-treatment. Nitroxinil was determined by ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) in negative ionization mode. Maximum concentrations of nitroxinil in samples were in the range of 688-1358 µg/kg, with residues persisting for 58 days in four of the six lactating cows.

A publicly available report written by the Australian Pesticides and Veterinary Medicines Authority indicates that residue levels in cow's milk ranged from 10 (limit of quantification) to less than 500 µg/kg following a single subcutaneous administration of a nitroxinil/clorsulon/ivermectin combination product 20 to 34 days prior to calving. The original data reviewed in the report are not available and consequently cannot be assessed.

No residue data were available on depletion of residues in ovine milk.

Selection of marker residue and target tissues

No radiolabelled data are available to confirm the identity of the marker residue. The parent compound nitroxinil has been detected in milk but there are no data on metabolites in milk.

However, given the low percentage of residues present as parent compound in the liver, it can be concluded that the substance is extensively metabolised in the liver. It can also be concluded that the metabolites are very quickly eliminated by renal excretion, given the low percentage of residues present as parent substance found in the kidney while the percentage in plasma and muscle and fat remained particularly high. This would lead to the fair assumption that the percentage of the parent substance in milk will also be high.

This, in combination with the fact that the parent substance was detected in milk following the use in dry cows and the fact that the parent substance was already retained as the marker residue for all other tissues, gives sufficient confidence to consider the marker residue as the parent compound, nitroxinil.

There is no information on metabolism in milk and no radiolabelled data on which to base a ratio of marker to total residues. However, pharmacokinetic data in cows and sheep available for the previous evaluation concerning the establishment of MRLs in tissues, indicate that nitroxinil is highly bound to plasma protein (97 to 98%) and that residues in plasma are higher than the residues in tissues and consisted almost entirely of nitroxinil. *In vivo* and *in vitro* studies indicated that the metabolism of nitroxinil was the same in cattle and sheep.

Nitroxinil was retained as the marker residue relevant to all tissues of bovine and ovine tissues. The percentage of marker residue to total residues was 4% for liver, 34% for kidney, 100% for muscle and 100% for fat at 30 days after treatment.

It is therefore expected that metabolism in the udder is limited and a conservative ratio of 0.5 can be estimated.

2.2.3. Monitoring or exposure data

Results of the Irish residue monitoring programme for 2009 and 2010 were provided. Nitroxinil was detected in seven out of 284 samples of tested bulk milk in 2009 and in five out of 179 samples of tested bulk milk in 2010 (data available in October 2010).

Levels detected ranged from 0.74 µg/kg to 7.84 µg/kg. In a number of cases the interval between treatment and detection could not be established. In those cases where the interval could be established it ranged from approximately 10 days (resulting in a residue level of 7.228 µg/kg) to 3 months (resulting in a maximum residue level of 3.3 µg/kg).

2.2.4. Analytical method for monitoring of residues

A UPLC-MS/MS (ultra-performance liquid chromatography coupled to tandem mass spectrometry) method was developed by an Irish national reference laboratory for the purposes of residue surveillance in cow's milk. The method is well described and validated in accordance with the requirements of Volume 8 of *The rules governing medicinal products in the European Union*. The calibration range for nitroxinil is 1 to 200 µg/kg and the limit of quantification is 1.0 µg/kg. The proposed method has been reviewed by the relevant European Union Reference Laboratory which confirmed the overall suitability of the method.

No data were provided to demonstrate the applicability of the method to sheep milk. However, in its assessment of the analytical method, the European Union Reference Laboratory considered that, although the higher fat content in sheep milk could create some difficulties as the sample preparation did not include a defattening process, the method seemed robust and should be applicable to sheep milk too.

2.2.5. Findings of EU or international scientific bodies

None available.

3. Risk management considerations

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

Microbiological effects are not expected for this substance therefore such data are not considered necessary.

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

The data provided for the scientific evaluation of nitroxinil were limited and do not comply with the requirements of Volume 8 of The rules governing medicinal products in the European Union. In particular, no radiolabelled data are available to enable determination of the appropriate marker residue in milk or the establishment of the ratio of marker to total residues. However pharmacokinetic data related to milk are available for cattle and indicate that nitroxinil can be accepted as the marker residue. Based on the metabolism pattern in plasma and tissues, a conservative ratio of marker to total residues of 0.5 can be estimated for milk.

Recognising the deficiencies in the data presented, the Committee took also into account the following:

- Although other flukicidal substances exist for which MRLs in ruminant milk have been established, these substances are not approved for the treatment of immature fluke, and consequently it is recognised that at present there is a lack of available products for the treatment of immature fluke in animals producing milk for human consumption;
- Liver fluke is a highly debilitating disease leading to loss of condition and ultimately cachexia and potentially death and therefore the availability of an adequate range of products for the treatment of fluke is essential in order to avoid unnecessary suffering of the animals;
- The establishment of a maximum residue limit is essential to provide the reference level for control purposes and to enable the use of the substance;
- The lack of available products coupled with welfare issues may lead to increased use of the products under non-authorised conditions.

3.3. Extrapolation of MRLs

Based on existing MRL values, the daily intake of residues from bovine and ovine tissues equates to 239 µg (equivalent to 79.6 % of the ADI), leaving 61 µg (equivalent to 20.4 % of the ADI) for establishment of a MRL for milk.

Given the available information relating to residues in other tissues, nitroxinil was retained as the marker residue in milk and a ratio of marker to total residue in milk of 0.5 was estimated.

Considering that the standard food basket indicates a consumption value for milk of 1.5 kg per consumer per day, and in order to ensure that consumer exposure to total residues of nitroxinil remains below the ADI, the maximum allowable total residue in milk would be 40 µg/kg. However, considering the percentage of marker residue to total residues in milk (0.5) a MRL of 20 µg/kg can be proposed.

In view of the information available and the risk management considerations the CVMP recommends the extrapolation of the existing MRLs for nitroxinil in cattle to cattle milk. The proposed MRL is 20 µg/kg.

Although data demonstrating the presence of the marker residue, nitroxnil, in sheep milk were not available i*n vivo* and *in vitro* studies indicate that the metabolism of nitroxinil was the same in cattle and sheep and therefore nitroxinil was accepted as marker residue in sheep. The ratio of marker to total residues estimated in cattle milk (0.5) was also considered acceptable for sheep milk. Therefore the proposed MRL of 20 µg/kg for cattle milk can also be accepted for sheep milk.

Calculation of theoretical daily intake of residues

Edible tissue or product	Daily consumption (kg)	MRL (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	400	1.00	120 µg
Fat	0.05	200	1.00	10 µg
Liver	0.10	20	0.04	50 µg
Kidney	0.05	400	0.34	59 µg
Milk	**1.5**	**20**	**0.50**	**60 µg**
Total				299 µg **(99.6 % of the ADI**)

Based on this MRL, milk would account for approximately 20 % of the ADI. Taking into account the residues in all tissues, the daily intake would represent less than 100% of the ADI.

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

Whereas:

- a toxicological ADI of 5 µg/kg bw (i.e 300 µg/person) was previously established as the overall ADI for nitroxinil;
- nitroxinil has been accepted as the marker residue in cattle and sheep milk;
- the ratio of marker to total residues was estimated to be 0.5;
- a validated analytical method for the monitoring of residues of nitroxinil in cattle milk is available, and is expected to be applicable to sheep milk as well;
- there is a lack of available products for the treatment of immature fluke in animals producing milk for human consumption;
- there is a need for a reference level for control purposes and to enable the use of the substance;

The CVMP recommends the extrapolation of maximum residue limits for nitroxinil to milk and the amendment of the entry for nitroxinil in 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
Nitroxinil	Nitroxinil	Bovine, ovine	400 µg/kg 200 µg/kg 20 µg/kg 400 µg/kg 20 µg/kg	Muscle Fat Liver Kidney Milk		Antiparasitic agents/Agents against endoparasites

Based on these MRL values, the theoretical maximum daily intake from tissues and milk is 299 µg which corresponds to approximately 100% of the ADI.

4. Background information on the procedure

Submission of the dossier	30 August 2011
Steps taken for assessment of the substance	
Clock started:	31 August 2011
CVMP Opinion adopted:	10 November 2011