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Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

EUROPEAN PUBLIC MRL ASSESSMENT REPORT (EPMAR)

CLORSULON
Cattle

INTRODUCTION

Maximum residue limits were first established in the European Union for clorsulon in bovine in June 1996[1] with a provisional status. Final maximum residue limits were established on 10 September 1999[2] following the favourable opinion adopted by the Committee for Veterinary Medicinal Products.

Clorsulon is intended for use in cattle for control of adult liver flukes (*Fasciola hepatica* and *Fasciola gigantica*) by subcutaneous injection.

In March 2008 it was drawn to the Committee's attention that the study on which the ADI was based used a related substance rather than clorsulon itself. In response, the CVMP reviewed this aspect of the previous assessment of the substance and, in July 2008, agreed to revise the ADI previously established for clorsulon.

EXPLANATORY NOTE

The European Public MRL Assessment Report is a public document giving the overview of the assessment carried out by the Committee for Medicinal Products for Veterinary Use (CVMP) of an application submitted for the establishment of maximum residue limits (MRLs). The document is based on the CVMP assessment report of the application from which confidential information has been deleted.

KEY WORDS: clorsulon, bovine, cattle

[1] Commission Regulation (EC) No 1147/1996, OJ L151, of 26.06.96
[2] Commission Regulation (EC) No 1942/1999, O.J. L241, of 11.09.99

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SUMMARY OF THE SCIENTIFIC DISCUSSION FOR THE ESTABLISHMENT OF MRLs

Substance name: Clorsulon

Procedure number: 94/7/114/MSD

Applicant: Mérial

Target species: Cattle

Intended therapeutic indication: Control of adult liver flukes (*Fasciola hepatica* and *Fasciola gigantica*)

Route (s) of administration: Subcutaneous

1 Introduction

Clorsulon is a compound belonging to the benzenesulphonamide family which is recommended for the control of adult liver flukes (Fasciola hepatica and Fasciola gigantica) in cattle as suspensions for oral use or injectable formulations for subcutaneous administration. The recommended dose is 7 mg/kg bw by the oral route and 2 mg/kg bw by the subcutaneous route. Frequently, clorsulon is used in association with ivermectin.

A toxicological ADI of 0.06 mg/kg bw was previously established by the Committee for Veterinary Medicinal Products, based on the toxicological LOEL of 0.2 mg/kg bw/day observed in the 54-week toxicity study performed in rats, and applying a safety factor of 200.

Currently, clorsulon is included in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Clorsulon	Clorsulon	Bovine	35 µg/kg 100 µg/kg 200 µg/kg	Muscle Liver Kidney	

In March 2008 it was drawn to the Committee's attention that the study on which the ADI was based used a related substance rather than clorsulon itself. In response, the CVMP reviewed this aspect of the previous assessment of the substance.

2. Safety assessment

Clorsulon was first assessed by the CVMP in November 1995. At that time the CVMP established a provisional toxicological ADI of 0.002 mg/kg bw, i.e. 0.120 mg/ person based on the toxicological NOEL of 2 mg/kg bw/day retained from the 3-month toxicity study performed in dogs, applying a safety factor of 1000 in order to account for the inadequacies of the short-term studies.

In April 1999, further to the assessment of the responses to the list of questions, the Committee established a toxicological ADI of 0.06 mg/kg based on the toxicological LOEL of 0.2 mg/kg bw/day observed in the 54-week toxicity study performed in rats, applying a safety factor of 200.

The evaluation that follows has been updated, compared to the April 1999 version (EMEA/CVMP/590/99-FINAL), to reflect the fact that the 54-week oral toxicity study in rats did not use clorsulon. As the ADI established in April 1999 was based on this study, the ADI has been amended to reflect this altered assessment.

2.1 Overview of pharmacological properties

Clorsulon inhibits the enzymes involved in the glycolytic pathway, the primary source of energy in flukes. Further investigations indicate that clorsulon is a competitive inhibitor of 8-phosphoglycerate kinase and phospho-glyceromutase and blocks the oxidation of glucose to acetate and propionate. Clorsulon also depresses ATP levels in the fluke.

Clorsulon appears to have pharmacological activity as a carbonic anhydrase inhibitor, as evidenced by significant increases in urinary pH, urinary volume and urinary sodium concentrations observed at all dose levels (0.2, 2 and 20 mg/kg bw/day) in a 54-week repeated dose rat toxicity study. The benzenesulphonamide family of compounds has the potential to decrease renal tubular resorption of sodium in order to decrease the excretion of hydrogen ions. Consequently, excretion of sodium, potassium and carbonate ions as well as water are increased. These effects are reported to be short-lived. No NOEL for these effects has been identified.

Pharmacokinetic properties (mainly in laboratory animals)

After single oral administrations of clorsulon at doses ranging from 0.25 to 15.8 mg/kg bw to rats experimentally infested with flukes, it was shown that clorsulon is absorbed by flukes.

2.2 Calculation of pharmacological ADI

At the time of the original assessment, it was considered that that it was not necessary to establish a pharmacological ADI.

2.3 Overview of toxicology

Acute toxicity and tolerance in target species

The acute toxicity of clorsulon was tested after oral and intraperitoneal administration in mice and rats. The oral LD_{50} values were higher than 10000 mg/kg bw in both species while those obtained after intraperitoneal administration ranged from 678 to 938 mg/kg bw.

Repeated dose toxicity

Short-term toxicity studies of 1 month duration were carried out in dogs and in rats with high doses of clorsulon. In dogs, in all treated groups (10 to 900 mg/kg bw/day), the post-mortem examination revealed haemosiderosis in liver and in spleen, bone marrow hyperplasia, extramedullary haematopoiesis, and inflammatory cellular infiltration in the choroid plexus and salivary gland. In female rats, reductions of thyroid weights were recorded for the range of doses tested (10 to 640 mg/kg bw/day). Hyperplasia of the bladder epithelium was observed at 160 and 640 mg/kg bw/day in both sexes. No NOEL could be retained.

In a 14-week study in dogs (0, 2, 8, 32 mg/kg bw/day, orally), a NOEL of 2 mg/kg bw/day could be retained based on the absence of effect on the thyroid weights.

In a 13-week oral toxicity study in rats, groups of 10 animals per sex received clorsulon in their diet at daily doses of 20, 150 or 425 mg/kg bw. In the highest dose group increases in relative organ weights (thyroid, adrenal, brain, kidney, spleen, lung) were reported. Urinary bladder hyperplasia occurred in 7 males and in 1 female, and kidney pelvic epithelial hyperplasia in 1 male and in 5 females. Thyroid follicular cell hyperplasia was only seen in 4 males. In the 150 mg/kg bw/day dose group, a significant increase (35%) in relative thyroid weight was found for males associated with 3 cases of thyroid follicular cell hyperplasia. Urinary bladder hyperplasia was reported in 6 males. In the lowest dose group a significant increase (approximately 35%) in relative thyroid weight was found for males, with no histological correlates. No NOEL could be established due to the significant increases in relative thyroid weights at the lowest dose tested.

A 54-week oral toxicity study conducted in rats using the carbonic anhydrase inhibitor acetazolamide led to the suggestion that the bladder hyperplasia seen in clorsulon-treated dogs and rats could be considered as secondary to changes in urine composition arising as a result of the inhibition of carbonic anhydrase, and that clorsulon does not directly act on the bladder to cause hyperplasia.

Tolerance in target species

Clorsulon alone or in combination with ivermectin was well tolerated by cattle apart from for swellings at the subcutaneous injection sites.

Effects on reproduction including developmental effects

In a 3-generation study carried out in rats (0, 3, 30, 300 mg/kg bw orally), the reproductive performance of female rats, viability, and growth of offspring in each generation were significantly affected at 300 mg/kg bw. There was no effect on the reproductive performance at the low and middle dose. A NOEL of 30 mg/kg bw/day was retained from this study.

Two teratogenicity studies in mice and rabbits at doses of 0, 2, 10 and 50 mg/kg bw/day, orally did not reveal any teratogenic potential of clorsulon at doses of up to 50 mg/kg bw/day.

In mice, no signs of maternotoxicity were reported at doses of up to 50 mg/kg bw, orally. However, the high dose of 50 mg/kg bw induced a significant decrease in the weight of foetuses. The NOEL for foetotoxicity was 10 mg/kg bw/day.

In rabbits signs of materno- and foetotoxicity (decrease in weights) appeared at 10 mg/kg bw/day and at 50 mg/kg bw/day, orally respectively. The NOELs for maternotoxicity and foetotoxicity were 2 and 10 mg/kg bw/day, respectively.

Mutagenicity

Mutagenic properties of clorsulon were tested in three *in vitro* and two *in vivo* tests. The three *in vitro* tests, *Salmonella*-microsomal assay, unscheduled DNA synthesis in human MRL-90 fibroblasts and measurement of DNA single strand breaks by alkaline elution in human MRL-90 fibroblasts gave negative results. However, positive results were obtained in the two *in vivo* tests, a bone marrow micronucleus test (oral doses of up to 2000 mg/kg bw in mice) and the chromosomal aberration test (oral doses of up to 500 mg/kg bw in mice).

Carcinogenicity

Two carcinogenicity studies were carried out in mice (44, 120 and 306 mg/kg bw/day for 2 years). These studies were inadequate due to low survival (20%) of animals. A study was conducted in rats that included in utero exposure to clorsulon (3.8, 12.6 and 48.8 mg/kg bw/day for 126 weeks - approximately 50% survival). Despite the inadequacies identified there was no evidence of carcinogenicity and it was concluded that clorsulon was not carcinogenic.

2.4 Calculation of the toxicological ADI

Although urinary bladder hyperplasia was observed in the 13-week oral toxicity study in rats, data generated with another carbonic anhydrase inhibitor, acetazolamide, suggested that the bladder hyperplasia could be considered as consequential to changes in urine composition. While two in-vivo mutagenicity studies (bone marrow micronucleous test and chromosomal aberration tests) were positive, carcinogenicity data led to the conclusion that clorsulon was not carcinogenic.

Therefore, based on the toxicological NOEL of 2 mg/kg bw/day from the three-month toxicity study performed in dogs, an ADI of 0.002 mg/kg bw, i.e. 0.120 mg per person was established by applying a safety factor of 1000. This safety factor combines both the “standard” safety factor of 100 and an additional safety factor of 10 as a result of the positive clastogenicity results and inadequacies in the carcinogenicity studies.

2.5 Overview of microbiological effects

Potential effects on human gut flora

In view of the nature of the substance, consideration of the microbiological effects was not considered relevant.

2.6 Calculation of microbiological ADI

Not relevant.

2.7 Observations in humans

Clorsulon is not used in human medicine; no information from use in humans was available.

2.8 Overview of evaluation by other international committees

No information on evaluation carried out by other international committees was available.

2.9 Overall conclusions on the ADI

The toxicological endpoints were considered the most relevant for the safety assessment of clorsulon. Therefore the toxicological ADI of 0.002 mg/kg bw (i.e. 120 mg/ person) was retained as the overall ADI for clorsulon.

3. Residues assessment

3.1 Pharmacokinetics in target species

In cattle, after intraruminal administration of ^{14}C-clorsulon at a dose of 10 mg/kg bw, maximum plasma levels (close to 3000 µg/l) were observed about 24 hours after dosing. The elimination of total radioactivity from plasma was biphasic. The mean concentration in plasma was 14 µg/l at 21 days after treatment. After subcutaneous administration of 2 or 3 mg/kg bw, maximum plasma concentrations (1290 and 2500 µg/l) were attained 6 hours after the injection. At seven days, the plasma concentrations were close to the limit of detection (10 µg/l). After a single intraruminal administration of 6.6 mg ^{35}S-clorsulon/kg bw or of 15 mg ^{14}C-clorsulon/kg bw, about 90% of the administered dose was excreted within 7 days, the major fraction being excreted in the faeces (approximately 70%) and a minor fraction (about 30%) in urine.

3.2 Residue depletion studies

Metabolism studies were carried out after administration of a single intraruminal dose of 10 mg/kg bw of labelled ^{14}C-clorsulon to steers. The animals were slaughtered 7, 14 and 21 days after administration. About 80% of the radioactive residues in the kidney and liver tissues were extractable by organic solvents. After acid hydrolysis of liver extracts, metabolism studies revealed 2 major metabolites which were confirmed by mass spectrometry and nuclear magnetic resonance: acetaldehyde derivative (2.9%) and butyric acid derivative (6.2%). Several other compounds were isolated. Ten were less polar and three more polar entities. No single compound accounted for more than 5% of the total residue. In kidney, the major component recovered was the unchanged drug. Other residues recovered consisted of less polar (at least 5) and more polar (at least 3) entities. None of these components accounted for more than 5% of the total radioactivity.

In this same study (after a single intraruminal administration of 10 mg/kg bw of ^{14}C-labelled clorsulon) the percentage of clorsulon to total residues could be established at the different time points (7, 14 and 21 days). At 7 days after administration, these percentage values were 75% in kidney, 55% in liver, and 41% in muscle. In fat, the ^{14}C-clorsulon concentrations (11 to 20 µg equivalents clorsulon/kg) were too low to establish a percentage. At 14 and 21 days post administration, the respective values were 67 and 74% in kidney, and 47 and 61% in liver whereas the percentages could not be established in muscle as the concentrations were too low.

Of the 4 residue depletion studies (1 after oral administration and 3 after subcutaneous administration), the most relevant data were those obtained after subcutaneous administration.

In one radiometric study, groups of 3 cattle received 2 mg/kg bw of ^{14}C-clorsulon by the subcutaneous route. At 3 days after administration, significant amounts of residues were measured in liver (187 µg equivalents clorsulon/kg) and in kidney (373 µg equivalents clorsulon/kg). Residue levels declined to 75 and 154 µg equivalents clorsulon/kg in liver and kidney at 5 days post injection. No data for the other edible tissues were provided.

In a non-radiometric study, 1 day after administration of 3 mg/kg bw of clorsulon to cattle by the subcutaneous route, residues peaked at mean values of 610 µg/kg, 130 µg/kg, 2200 µg/kg and 3330 µg/kg in muscle, fat, liver and kidney, respectively. Residue levels declined to 50, 140 and 330 µg/kg in muscle, liver and kidney 3 days after administration, whereas clorsulon could not be detected in fat. At 7 days after administration, only low concentrations of clorsulon could be measured in liver (10 µg/kg) and in kidney (20 µg/kg). The residues at the injection site depleted from 5800 µg/kg 1 day after administration to 390 and to 20 µg/kg at 3 and 7 days after administration.

3.3 Selection of marker residue and target tissues

At 7 days after intraruminal administration the parent compound represented approximately 75, 55 and 41% of total residues in kidney, liver and muscle, respectively. Residues in fat were low and the percentage of residues made up by the parent substance could not be established. Clorsulon was therefore retained as marker residue.

Residue concentrations were low and undetectable 3 days after sub-cutaneous administration, therefore fat was not considered a relevant target tissue.

Elaboration of MRLs

Maximum residue limits for liver and kidney were set as 100 µg/kg and 200 µg/kg, respectively. Residues in muscle were below the limit of detection of the analytical method (25 µg/kg). A MRL for muscle was set at 35 µg/kg.

Reference to MRLs established by other international committees

No information was available on the establishment of maximum residue limits for clorsulon by other international committees.

3.4 Calculation of theoretical daily intake of residues

Detailed calculation of theoretical daily intake of residues

Edible tissue or products	Daily consumption (kg)	MRL (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	35	0.41	25.6
Fat	0.05	---	---	---
Liver	0.10	100	0.55	18
Kidney	0.05	200	0.75	13.3

Based on these MRLs, the daily intake of total residues (0.057 mg) represents about 48% of the acceptable daily intake (0.120 mg/person).

3.5 Analytical method for monitoring of residues

The proposed routine analytical method for the determination of residues of clorsulon in edible tissues of bovine was based on HPLC with UV detection. The method was described according to the ISO 78/2 format. The limits of quantification were 25 µg/kg for muscle and fat, 50 µg/kg for liver and 100 µg/kg for kidney.

4. Conclusions and recommendation

Having considered that:

- a revised ADI of 0.002 mg/kg bw (i.e. 0.120 mg/person) was established for clorsulon,
- clorsulon was identified as the marker residue in edible tissues of bovine and that at 7 days after intraruminal administration it accounts for approximately 75% of total residues in kidney, 55% in liver, and 41% in muscle,
- the tissue distribution at 7 days after administration of 3 mg/kg bw clorsulon by the subcutaneous route showed that the kidney concentrations of clorsulon are about twice the concentrations in liver,
- the concentrations of clorsulon in muscle are below the limit of quantification, the MRL value allocated for muscle is slightly higher than the limit of quantification,
- clorsulon concentrations in fat are too low to set an MRL for this tissue,
- a fully validated analytical method for the determination of residues of clorsulon in edible tissues is available;

the Committee for Medicinal Products for Veterinary Use concluded that no amendment to the current MRLs established for clorsulon in bovine species should be recommended.

Clorsulon is currently included in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Clorsulon	Clorsulon	Bovine	35 µg/kg 100 µg/kg 200 µg/kg	Muscle Liver Kidney	