EMEA/MRL/037/95-FINAL

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CLORSULON

SUMMARY REPORT (1)

1. Clorsulon is a compound belonging to the benzenesulphonamide family which is recommended for the treatment and control of adult liver flukes (Fasciola hepatica and Fasciola gigantica) in cattle as suspensions for oral use or injectable formulations for subcutaneous administrations. The oral recommended level is 7 mg/kg bw and the subcutaneous one 2 mg per kg bw Frequently, Clorsulon is used in association with Ivermectin.

2. Clorsulon inhibits the enzymes implicated in the glycolytic pathway, the primary source of energy in flukes. Further investigations indicated 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.

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

4. In cattle, after intraruminal administrations of ^{14}C clorsulon at a level dose of 10 mg/kg bw, the maximum peak plasma levels (close to 3 mg/l) occurred at about 24 hours after dosing. The elimination of total radioactivity from plasma was biphasic and the concentrations of 0.014 ± 0.008 mg/l were still measured in plasma at 21 days after treatment. After subcutaneous administrations of 2 or 3 mg/kg bw, the maximum plasma concentrations (1.29 ± 0.32 and 2.50 ± 0.36 mg/l) were attained at 6 hours after the injection. At seven days, the plasma concentrations were close to the limit of 0.01 mg/l.

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

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

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

   After a daily oral administration of 20, 150 or 425 mg/kg bw of clorsulon during 13 weeks to rats, no NOEL could be established as significant increases in relative thyroid weights were observed in males with the lowest dosage.

7. Clorsulon alone or in combination with ivermectin were well tolerated by cattle a part for swellings at the subcutaneous injection sites.

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8. In the three-generation reproduction study carried out in rats (0, 3, 30, 300 mg/kg bw), the reproductive performance of the female rats, the viability and growth of the offspring in each generation were significantly affected at 300 mg/kg bw. There was no effect on the reproductive performance in the low and middle dosage.

9. Two teratogen studies (in mice 0, 25, 50, 100, 200 and 400 mg/kg b.w/day and in rabbits 0, 2, 10 and 50 mg/kg bw) did not reveal any potential teratogenic property of clorsulon up to 50 mg/kg bw.

   In mice, no maternotoxicity signs were reported up to 50 mg/kg bw. However, the high dosage of 50 mg/kg induce a decrease in the weight of foetuses. The NOEL for foetotoxicity was 10 mg/kg bw.

   In rabbits, maternotoxicity and foetoxicity signs (decrease in weights) appears at 10 mg/kg b.w and at 50 mg/kg bw respectively. NOELs for maternotoxicity and foetotoxicity were 2 and 10 mg/kg bw, respectively.

10. Mutagenic properties of Clorsulon were tested in three in vitro and two *in vivo* tests. The three *in vitro* tests, Ames test, Unscheduled DNA 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 : Bone marrow Micronucleus test (2000 mg/kg bw in mice) and the chromosomal aberration test (500 mg/kg bw in mice). The clastogenic properties of clorsulon might be related to bone marrow cytotoxicity.

11. 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 using in utero exposure of 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 carcinogen.

12. Based on the toxicological NOEL of 2 mg/kg bw/day of the three-month toxicity study performed in dogs, a provisional ADI of 0.002 mg/kg bw, i.e. 0.120 mg per person could be established by applying a safety factor of 1000. This safety factor incorporated an additional factor of 10 due to the inadequacies of the short-term studies.

13. 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 withdrawal periods were 7, 14 and 21 days post dose. 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 one 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.

14. In this same study (after a single intraruminal administration of 10 mg/kg bw of labelled ^{14}C clorsulon) the ratio of clorsulon vs. total residues could be established at the different withdrawal periods. At seven days post administration, these ratios were 75% in kidney, 55% liver, 41% in muscle. In fat, the ^{14}C clorsulon concentrations (0.011 to 0.020 mg equivalent/kg) were too low to establish this ratio.

15. Of the four depletion studies (one after oral administration and three after subcutaneous administrations), the most relevant data were those obtained after subcutaneous administrations.

    At one-day post-administration of 3 mg/kg bw of clorsulon to cattle by subcutaneous route, the highest concentrations were measured in kidney and liver (3.30 ± 0.37 & 2.20 ± 0.44 mg/kg). Seven days after treatment, clorsulon could only be detected in these two tissues (0.10 ± 0.07 and 0.04 ± 0.0005 mg/kg). The residues at the injection site declined rapidly and attained 0.02 ± 0.005 mg/kg at 7 days post-dose.

16. The analytical method used for assaying the concentrations of clorsulon in all edible tissues collected during the depletion studies indicate that 0.01 mg/kg could be considered as the limit of quantification. In the absence of clear information about the determination of the usual validation parameters (specially limits of quantification and of detection) it is not possible to retain this method as the routine analytical method. However, one analytical method for the determination and one GC/MS confirmatory method for the identification of clorsulon residues in kidney are available (limit of quantification 0.2 mg/kg).

17. The following points were considered in recommending provisional MRLs for clorsulon :

    - the provisional ADI for clorsulon is 0.002 mg/kg bw (i.e. 0.120 mg per person),
    - the marker residues is clorsulon,
    - at 7 days after intraruminal administration, clorsulon accounts approximately 75% of total residues in kidney, 55% in liver, 41% in muscle,
    - at three days after a subcutaneously with 3 mg/kg bw of clorsulon, the kidney and liver concentrations of clorsulon are about 7-fold and 3-fold higher than those of muscle,
    - although the limits of detection and quantification had not been properly determined in accordance with Volume VI of the "Rules Governing Medicinal Products in the European Community", there is an analytical method to quantify clorsulon residues at a level of 0.01 mg/kg in all edible tissues.

	MRLs Clorsulon marker residue (mg/kg)	Ratio of marker/total residues	Expressed in total residue (mg/kg)	x daily intake (kg)	Total residue intake (mg)
Muscle	0.050	40 %	0.125	0.300	0.0375
Liver	0.150	55 %	0.272	0.100	0.0272
Kidney	0.400	75 %	0.533	0.050	0.0266
Total (mg)					0.0913

In these conditions, the total amount of clorsulon and metabolites that could be daily ingested represents about 76% of the daily intake.

The following MRLs recommended are :

Pharmacological active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Clorsulon	Clorsulon	Bovine	50 µg/kg 150 µg/kg 400 µg/kg	Muscle Liver Kidney	Provisional MRLs expire on 01.01.2000

LIST OF QUESTIONS

The following information is required before final MRLs can be elaborated :

1. The applicant should precise the real coefficient of purity (98 or 99.9%) of clorsulon and indicate the percentage of every impurity.

2. The applicant should give information about the fraction of clorsulon excreted in faeces and urine in cattle.

3. In order to explain the toxicological significance of the bladder hyperplasia, the applicant referenced to two studies (TT #88-127-0 and TT #88-005-0) which are not provided. So, the applicant should provide these studies mentioned in Vol. 7, p. 4.

4. The applicant should provide a validated method in accordance with the Volume VI of the "Rules Governing Medicinal Products in the European Community" for the analysis of clorsulon in the cattle tissues for which provisional MRLs have been established. This method should be presented in the ISO 78/2 format.