The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CLORSULON

SUMMARY REPORT (2)

1. 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 provisional toxicological ADI of 0.002 mg/kg bw had previously been established by the Committee for Veterinary Medicinal Products, based on the toxicological NOEL of 2 mg/kg bw/day observed in the 3-month toxicity study in dogs, by applying a safety factor of 1000, incorporating an additional factor of 10 due to the inadequacies of the short-term studies.

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

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

2. Additional data on toxicity and on the routine analytical method to establish final MRLs were now provided.

3. 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.

4. 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.

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5. 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.

6. 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.

7. Short-term toxicity studies of 1 month duration were carried out in dogs and in rats with high dose 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.

8. 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 dose group, a significant increase by 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 only significant increase by approximately 35% in the relative thyroid weight was found for males without histological findings. No NOEL could be established due to the significant increases in relative thyroid weights at the lowest dose tested.

   In a 54-week oral toxicity study in rats with a 27-week interim necropsy, groups of 60 albino rats (30 animals per sex and dose) received clorsulon by gavage at doses of 0 (0.5% aqueous methylcellulose), 0.2, 2 and 20 mg/kg bw/day. At interim sacrifice (10 animals per sex and dose), hyperplasia of the urinary bladder was reported in 4 and 7 males treated at 2 and 20 mg/kg bw, respectively. In females, this effect was only reported in 2 animals treated at the highest dose. At terminal sacrifice this finding was not so clear with 0 male and 1 female of the 2 mg/kg bw group, and 8 males and 2 females of the highest dose group showing urinary bladder hyperplasia. An increase in incidence and concentration of triplate phosphate crystals primarily in males, which became more prominent in week 51 was also described in the two highest dose groups. At the lowest dose, 0.2 mg/kg bw/day, only a significant increase of pH in urine of males was reported. In absence of hyperplasia of the urinary bladder, of histopathological effects in kidney and of triplate phosphate crystals, this dose of 0.2 mg/kg bw/day was retained as a LOEL.

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

10. In a 3-generation study carried out in rats (0, 3, 30, 300 mg/kg bw orally), the reproductive performance of females 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.

11. 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 up to 50 mg/kg bw/day, the highest dose tested.

    In mice, no signs of maternotoxicity were reported 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.

12. Mutagenic properties of clorsulon were tested in three *in vitro* and two *in vivo* tests. The three *in vitro* tests, *Salmonella*-microsomal assay, unscheduled synthesis 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, a bone marrow micronucleus test (oral doses up to 2000 mg/kg bw in mice) and the chromosomal aberration test (oral doses up to 500 mg/kg bw in mice).

13. 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 carcinogenic.

14. Based on the toxicological LOEL of 0.2 mg/kg bw/day of the 54-week toxicity study performed in rats, an ADI of 0.001 mg/kg bw, i.e. 0.060 mg/person was established by applying a safety factor of 200.

15. 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 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.

16. 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 post administration, these percentage values were 75% in kidney, 55% in liver, 41% in muscle. In fat, the ^{14}C-clorsulon concentrations (11 to 20 µg equivalents clorsulon/kg) were too low to establish this 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.

17. 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 post dosing, significant amounts of residues were measured in liver (187 µg equivalents clorsulon/kg) and in kidney (373 µg equivalents clorsulon/kg). They declined to reach 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. They declined to reach 50, 140 and 330 µg/kg in muscle, liver and kidney 3 days post dosing, whereas clorsulon could not be detected in fat. At 7 days post dosing, 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 at day 1 post injection to 390 and to 20 µg/kg at 3 and 7 days post injection.

18. 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.

Conclusions and recommendation

Having considered that:

- an ADI of 0.001 mg/kg bw (i.e. 0.060 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 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 Veterinary Medicinal Products recommends the inclusion of clorsulon for bovine 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	

Based on these MRLs, the daily intake of total residues will represent about 95% of the acceptable daily intake.