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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

HALOFUGINONE

SUMMARY REPORT (1)

1. Halofuginone is a synthetic product derived from febrifugine. It belongs to the quinazolone group of chemicals. *Trans*-halofuginone is the active ingredient, the *cis*-isomer being present as an impurity. Halofuginone lactate, to which the current application is limited, is intended for the prevention of diarrhoea due to *Cryptosporidium parvum* in non ruminating calves of 4 days to 15 days of age at an oral therapeutic regimen equivalent to 0.10 mg/kg bw/day of halofuginone for 7 days.

   Halofuginone hydrobromide has been registered as an anticoccidial feed additive for broilers and for turkeys with a withdrawal period of 5 days in the Council Directive 91/248/EC of 12 April 1993 amending the Council Directive 70/524/EEC.

2. Most of the studies provided were carried out with the two salts of halofuginone: lactate and hydrobromide. In order to compare the results, the doses are expressed as halofuginone base equivalents.

3. In *in vitro* tests halofuginone inhibited the rate of the cell division of *Babesia equi*-infected lymphoblastoid cells. Halofuginone exerted its effect on *Theileria parva* transformed cells where the drug destroyed the whole cell-parasite complex. Electron microscopic studies showed that in lymph nodes from *Theileria parva*-infected calves, halofuginone only affected infected cells.

   Published data showed that halofuginone inhibits specifically and transiently collagen α1 (1) gene expression and collagen synthesis in skin fibroblasts from avian species and from humans (normal individual and scleroderma patients) at low concentrations (10^{-11}, 10^{-10} and 10^{-9} M, respectively).

4. After intravenous administration, halofuginone (salt not stated) caused effects on the cardiovascular systems in cats (at doses above or equal to 3 mg/kg bw) and in rats (at doses above 0.3 mg/kg bw). Effects on the central nervous system of the mouse (oral doses of halofuginone base ranging from 1 to 100 mg/kg bw) and transient effects on the central nervous system of the squirrel monkey (intravenous dosages of halofuginone base above 1 mg/kg bw) have been observed.

   After oral administration of 0.25 mg/kg bw ^{14}C-halofuginone hydrobromide to mice, 82.7% of the dose administered was excreted within 48 hours, predominantly via the faeces (65%). Approximately 90% of this radioactivity corresponded to unchanged halofuginone. The concentrations of radioactivity in liver declined from 220 µg equivalents halofuginone/kg at 24 hours to 130 µg/kg at 48 hours post-dose.

   In rats, about 78% of an oral dose administered (5 mg ^{14}C halofuginone hydrobromide/kg bw) was recovered within 24 hours, mainly in faeces (60%). No information on the radioactive components of excreta was provided.

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5. In a study carried out in three 1-week old male non-ruminating calves (cross-bred Hereford-Friesian), receiving 7 daily doses of ^{14}C-halofuginone lactate (0.10 mg/kg bw as halofuginone base), it was shown that the excretion of halofuginone lactate in calves was mainly via the urine. The urinary excretion of radioactivity after the last dose until sacrifice represented 10.0% (6 hours), 20% (24 hours) and 92% (48 hours) of radioactivity administered in the seventh dose. Due to the low number of animals (the urinary complete balance being obtained from results from one animal) and as the percent of recovery radioactivity was based on a comparison with the seventh dose administered, this metabolism study must be considered with caution. No balance elimination in calves can be made.

   In plasma, halofuginone represented only 6.5 to 10% of the total radioactivity. Halofuginone lactate was absorbed but this absorption was not quantifiable.

   In another study, 3-week old calves were treated via oral route, at the recommended therapeutic dose of halofuginone lactate (0.10 mg of base/kg bw/day), for seven days. The highest concentration of halofuginone in plasma (9 µg/l) occurred 6 hours after the seventh administration. The concentrations subsequently declined and halofuginone could no longer be detected by 7 days after the end of the medication (limit of quantification 1 µg/l).

   In a newly submitted pharmacokinetic study, eight 22 to 32 day-old calves of 52.6 kg bw were treated via oral route, at the recommended therapeutic dose of halofuginone lactate (0.10 mg of base/kg bw/day), for seven days. The highest concentration of halofuginone in plasma (6.66 µg/l) occurred 8 hours after the seventh administration. Then, the plasma concentrations decreased to reach 2.3 µg/l at 36 hours after the last administration and declined to be lower than the limit of quantification (1 µg/l) at later sampling time. The mean terminal half-life was 32.8 hours. Under these experimental conditions, no accumulation could be demonstrated. However, due to the large inter-individual variability and as halofuginone could not be detected in plasma of half of the animals, this result should be taken with caution.

   In a newly submitted GLP cross-over pharmacokinetic study, eight calves (10 to 15 day-old on the first day of the first administration and 17 to 22 day-old on the first day of the second administration) received halofuginone lactate by intravenous or oral routes at the recommended dose (0.10 mg of base /kg bw/day; 45 kg as mean bodyweight). After intravenous administration, the half-life of the elimination phase was 11.66 hours, the body clearance 0.6 l/kg.h and the mean residence time 16.7 hours. After a single oral administration, the highest concentration of halofuginone in plasma levels, 4.12 µg/l, was seen at 11 hours post dose. The oral half-life of the elimination phase, 30.84 hours, was three-fold higher than that calculated after intravenous administration. That means that a flip-flop phenomenon exists, the absorption phase being a restricting process for the pharmacokinetic behaviour for halofuginone. The oral bioavailability was 81.1%. Using these pharmacokinetic parameters, a simulation of repeated administrations showed that a possible accumulation of halofuginone in these young calves. The age and the weight of the animals may influence the accumulation of halofuginone.

6. The acute oral toxicity of halofuginone hydrobromide and lactate have been studied in mice, rats and rabbits. The LD_{50} were close to 30 mg/kg bw in rats and to 5 mg/kg bw in mice for both salts. In rats, after inhalation of halofuginone dust, an LC_{50} of 53 µg/l was determined. The dermal LD_{50} in rabbits was 16 mg/kg bw. The cis-isomer was 100-fold less toxic than the active ingredient (oral LD_{50} close to 430 mg/kg bw in mice).

7. Several oral repeated dose toxicity studies were conducted with the two halofuginone salts, hydrobromide and lactate in mice, rats and dogs.

In a bioequivalence study, mice received a single oral dose of halofuginone hydrobromide or lactate at a dose of 2 mg halofuginone base/kg bw. Due to the large inter-individual variability, it is not possible to conclude from a pharmacokinetic point of view on the bioequivalence between the two salts. The mean AUC_{0-8h} values determined for males and females were 103.37 and 82.65 µg.h/l for halofuginone lactate and hydrobromide, respectively. For males, the AUCs were in the same magnitude (83 µg.h/l for both salts) whereas for females the AUC measured for halofuginone lactate (157 µg.h/l) was higher than that of halofuginone hydrobromide (97.40 µg.h/l). However, from a toxicological point of view, as studies are conducted in both males and females, the results obtained for the hydrobromide salt can be taken into account to establish the safety profile of the lactate salt.

In two 4-week dietary toxicity studies, mice received halofuginone hydrobromide and lactate at doses of 0.070, 0.160 and 0.350 mg halofuginone base/kg bw/day. At the two highest doses, significant variations in haematology (cell volume, mean cell volume and in mean cell haemoglobin) were reported. At the highest dose, male mice also showed variations in blood chemistry (urea and cholesterol). The same toxicological profile was observed and the same NOEL (0.070 mg/kg bw/day) was retained for both salts.

In a 13-week toxicity study, rats received a diet containing halofuginone hydrobromide at doses of 0, 2, 5 and 10 mg/kg feed , equivalent to 0.13, 0.33 and 0.70 mg/kg bw/day for males and 0.16, 0.41 and 0.88 mg/kg bw/day in females. At the highest dose, 80% of the females showed fat deposition and vacuolation in the liver, associated with a minimal decrease in glycogen in the periportal hepatocytes. No adverse effects on haematology parameters and blood chemistry were reported. The NOEL was 0.13 to 0.16 mg/kg bw/day.

In a 13-week toxicity study, dogs received 0, 1.25, 2.5 and 5 mg/kg feed of halofuginone hydrobromide, approximately equivalent to 0, 0.034, 0.067 and 0.134 mg/kg bw/day expressed as base. A significant decrease in the mean cell volume was noted only in the highest dose group. As the haematological changes noticed for the intermediate dose group were within the biological variations, a NOEL of 2.5 mg/kg feed (0.067 mg/kg bw/day) was retained.

In a 26-week toxicity study, dogs received a diet containing hydrobromide at doses of 0, 1.25, 2.5 and 5 mg/kg feed in the diet, equal to 0.045, 0.086, 0.16 mg/kg bw/day in males and 0.039, 0.075, 0.17 mg/kg bw/day in females. Significant haematological changes (decrease in mean cell volume, in mean cell haemoglobin concentration, and/or in haemoglobin level) were noted at the highest dose. As the haematological changes noticed for the two other dose groups were within the biological variations, 2.5 mg/kg feed (0.075 to 0.086 mg/kg bw/day) was retained as NOEL.

8. Several tolerance studies were carried out in young calves (4 to 10 days of age at the beginning of the treatment). Calves received by gavage halofuginone at doses ranging from the recommended therapeutic regimen (0.1 mg/kg bw/day for 7 days) to doses corresponding to 25-fold this dosage.

   For doses corresponding to 15-fold and 25-fold the therapeutic dose, deaths occurred. When administered just after feeding, halofuginone lactate induced reversible gastro-intestinal inflammatory/necrotic lesions at dosages corresponding to 1, 2 and 3 times the intended dose. A possible effect of halofuginone cannot be ruled out. However, at the therapeutic dose, the product is clinically well tolerated.

   In another tolerance study, male non-suckling calves (24 to 66 hours of age) received by oral route the formulated product at doses of 0, 1 and 2 times the recommended dose (0.10 mg halofuginone base/kg bw/day for 7 days). In the highest dose group 25% of the animals died and lymphocytic depletion of the ileal Peyer's patches in 60% of the remaining animals on day 35 was observed. In the group treated at the recommended dosage no compound related histological findings were noted and a discreet depletion of ileal Peyer's patches was reported in one out of six animals. These results did not allow to conclude formally on the possible effects of this substance on the immunological status of the animals treated at the highest dose.

9. All studies on reproductive toxicity were conducted on mice, dogs and rats with halofuginone hydrobromide.

In mice, the administration of halofuginone at levels of 0, 0.25, 0.5, 1 mg/kg feed in the diet, approximately equivalent to 0, 0.034, 0.063 and 0.126 mg/kg bw/day, for 7 days prior to mating and during 2 weeks after mating did not induce adverse effects on fertility or on rearing performance up to 1 mg/kg feed (0.126 mg/kg bw/day).

In dogs, the administration of 2.5 and 5 mg/kg feed of halofuginone approximately equivalent to 0.067 and 0.134 mg/kg bw, for 68 weeks induced significant decreased of testicular length and width in all the animals treated. A decrease in fertility index was also reported. Although these differences were not statistically significant, they seemed to be compound- and dose-related and may be considered as having some biological significance. No NOEL was retained.

In a three-generation study, mice were dosed with halofuginone via the diet. The doses tested were 0.25, 0.5 and 1 mg/kg feed, approximately equivalent to 0, 0.034, 0.063 and 0.126 mg/kg bw/day. F3 pups issued from the highest dose group showed a significant lower mean weight and transient lower mean weight in the intermediate group. The body weight of male F0, F1 and F2 parents was lower than controls at the two highest doses for F0 and F1 and at the highest dose for F2. If it could be shown that the difference of body weight was without statistical differences when compared to the control groups for F0 parents for both groups, this difference was statistically significant for F1 male parents at the two highest dose groups and for F2 parents of the highest dose groups. Therefore, although the lower absolute male body weights for the F1 and F2 generations was principally due to lower weight gain prior to selection (i.e. during and immediately after lactating)**,** the NOEL retained was 0.25 mg/kg feed (0.034 mg/kg bw/day).

10. In a previously submitted study, halofuginone hydrobromide administered to pregnant rats either by gavage, at a single dose of 9.33 mg/kg bw on day 9, 10, 11, 12, 13 or 14 post-mating or administered in feed at 6 mg/kg feed (dose non stated in mg/kg bw) from the 10th to 20th day post-mating, did not induce adverse effects on pups. No NOEL could be retained from this study, because it was too poorly reported and not in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community.

    In a newly submitted teratogenicity study, halofuginone hydrobromide was administered to mated female rats by gavage at doses of 0, 0.17, 0.34 and 0.67 mg/kg bw/day from day 6 to day 17 of gestation. Maternotoxic signs (mortality, clinical signs, abortion) were noted in the highest dose group. The NOEL for maternotoxicity was 0.34 mg/kg bw/day. Halofuginone hydrobromide was not embryo/foetotoxic and not teratogene in rats up to an oral dose of 0.67 mg/kg bw/day.

    In a second teratogenicity study, halofuginone hydrobromide was administered to female rabbits by gavage at doses of 0, 0.0084, 0.025 and 0.076 mg/kg bw/day from day 6 to day 18 of gestation. Maternotoxic signs (mortality, lower body weight, lower rate of pregnancy) were noted at the highest dose. The NOEL for maternotoxicity was 0.025 mg/kg bw/day. Halofuginone hydrobromide was not embryo-/foetotoxic and not teratogene in rabbits up to an oral dose of 0.076 mg/kg bw/day.

11. The mutagenic potential of halofuginone and its two salts was investigated.

    Although most of the tests are poorly reported, it can be concluded that halofuginone (salt not stated) gave negative results in three *in vitro* tests: the mouse lymphoma assay, in an *in vitro* chromosomal aberration assay (on cultured human lymphocytes), in the DNA repair assay in human epithelioid cells and in three *in vivo* tests (*in vivo* the bone marrow micronucleus test in mice, *in vivo* metaphase analysis assay in rats, the host mediated assay in mice). Halofuginone gave positive results in the Ames test with *Salmonella typhimurium* strain TA1538 at dose levels of 1000 µg/plate with metabolic activation and halofuginone hydrobromide with strain TA98 for dose levels higher than 1000 µg/plate with and without metabolic activation .

    For halofuginone lactate, only two tests were reported: the Ames test and an *in vivo* bone marrow micronucleus test in mice. Halofuginone lactate only gave positive results in the *in vitro* test with *Salmonella typhimurium* strain TA98 at dose levels of 1000 µg/plate without and with metabolic activation.

    Considering that in the Ames tests, no dose-related in the number of revertants was noted and that the mouse lymphoma assay, which detects gene mutation, was negative, the Committee concluded that halofuginone is not likely to be genotoxic.

12. A carcinogenicity study was conducted in a derived strain of Swiss origin mice. Halofuginone hydrobromide was administered in the diet at doses equivalent to 0.03, 0.07 and 0.24 mg/kg bw/day. No carcinogenic potential could be seen.

    The oral administration of halofuginone (salt non stated) at doses equivalent to 0.29 to 0.36 mg/kg bw/day for 63 weeks induced no treatment-related histopathological changes and no increase in incidence of hepatic tumours, when administered to Sprague Dawley rats in their diet.

    In a 26-month long term toxicity/carcinogenicity study, Sprague Dawley rats received in their diet 0, 2.5, 5 and 10 mg/kg feed of halofuginone bromide equivalent to 0, 0.09, 0.18, 0.36 mg/kg bw/day for males and 0, 0.11, 0.23, 0.47 mg/kg bw/day for females. A toxicological NOEL was 2.5 mg/kg feed, i.e. 0.09 to 0.18 mg/kg bw/day based on haematological and histological results. No increase in the incidence in tumours and no treatment-specific tumours were noted when compared to controls. Halofuginone did not show any carcinogenic potential.

    Halofuginone did not show any carcinogenic potential in mice and rats.

13. Halofuginone was tested in vitro for its microbial activity against 135 aerobic and 75 anaerobic micro-organisms representative of the overall human and calf gut flora. No significant influence on the human and calf gut flora was demonstrated, the MIC values being higher than 128 µg/ml for the majority of the strain tested.

14. In the modified Buehler test on skin sensitisation performed on guinea-pigs, no cutaneous reaction attributable to the sensitisation potential of halofuginone was observed whereas in the maximisation method of Magnusson and Kligman, slight cutaneous reactions attributable to delayed contact hypersensitivity in 35% of the animals were seen.

15. When applied on shaved skin of rabbits and in eye mucosa, halofuginone and its two salts caused delayed systemic toxic effects and was irritant.

16. No data about observations in humans are available as this compound is not used in humans.

17. For six of eight toxicological studies carried out in rats, dogs and mice, the NOELs were in the same magnitude, 0.070 mg/kg bw/day or lower. However, in the 3-generation study carried out in mice and in the teratogenicity study carried out in rabbits, the NOELs were 0.0334 and 0.025 mg/kg bw/day respectively. Therefore, based on a NOEL of 0.03 mg/kg w/day and applying a safety factor of 100, a rounded toxicological ADI of 0.30 µg/kg bw/day, i.e. 18 µg per person can be established. Although the toxicological studies have been carried out with the hydrobromide salt, no additional factor was used as these studies were carried out in both sexes.

18. Two depletion tissue studies were provided in non-ruminating calves of 1-week and 3-week of age.

    In a radiometric study, halofuginone lactate was administered via the oral route at the recommended dose of ^{14}C-halofuginone lactate (0.10 mg of halofuginone base/kg bw/day for seven days) to three 1-week old calves, who were sacrificed at 6, 24 and 48 hours after the end of the treatment. Twenty-four and 48 hours after the end of oral administrations, very low amounts of total residues were measured in edible tissues : 40 µg equivalents halofuginone/kg in muscle and fat, 500 µg/kg in liver and 300 µg/kg in kidney. As these data were obtained in one animal per slaughtering time, no conclusion on the individual variation can be reached.

    Sixty eight to 95 % of the total radioactivity could be extracted from tissues by solvents.

    In all tissues, ^{14}C-halofuginone has been identified as being the major radioactive component and represented approximately 60 % of the total radioactivity in muscle, fat and kidney and 52.6 % in liver.

    The parent compound could be retained as marker residue.

In a non-radiometric depletion study, halofuginone lactate was administered in sixteen 3-week old calves via oral route at the recommended dose of 0.10 mg of halofuginone base/kg bw/day for seven days. Animals were slaughtered in groups of 4. Six hours after the last oral administration, 90 µg/kg of halofuginone in muscle, 220 µg/kg in fat, 500 µg/kg in liver and kidney were measured. At a 5-day withdrawal period, the residues of halofuginone were in the magnitude of 50 µg/kg for liver and kidney and below 25 µg/kg for muscle and fat. At a 7-day withdrawal period, the residues of halofuginone were in the magnitude of 50 µg/kg for all edible tissues except muscle (30 µg/kg).

19. An HPLC method with UV detection was proposed as the routine analytical method. All the parameters of validation were determined according to the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community, but some raw data are still missing and the analytical procedure is under validation for fat. It was concluded that the analytical method proposed has been properly validated for muscle, liver and kidney. The limits of quantification are 5 µg/kg for muscle and 10 µg/kg for liver, kidney and fat.

Conclusion and recommendation

Having considered that:

- a toxicological ADI of 0.30 µg/kg bw (i.e. 18 µg/person) was established for halofuginone,
- in the depletion studies, the concentrations of halofuginone in edible tissues being assayed with a less sensitive analytical method than that proposed as routine analytical method, it was not possible to take into account precisely the tissue distribution,
- the indication for use of halofuginone in non-ruminating calves of 4 to 15 days of age, who are unlikely to be sent for slaughter during or immediately after treatment,
- the parent compound can be retained as marker residue and represents 60% of the total residues in muscle, fat and kidney and 52.6% in liver up to 48 hours after treatment,
- that the analytical method proposed has been properly validated for muscle, liver and kidney and is under validation for fat. The limits of quantification of the analytical method for the determination of halofuginone in edible tissues are sufficient to set provisional MRLs according to the current CVMP approach (twice the LOQ),

the Committee for Veterinary Medicinal Products recommends the inclusion of Halofuginone. in Annex III 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
Halofuginone	Halofuginone	Bovine	10 µg/kg 25 µg/kg 30 µg/kg 30 µg/kg	Muscle Fat Liver Kidney	Provisional MRls expire on the 1.01.2001

Based on these MRLs values, the daily intake will represent about 85% of the ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of halofuginone in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should provide additional residue studies to determine the ratio of the marker residue versus total residues at longer withdrawal period and to determine the concentrations of the parent compound at longer withdrawal period in non ruminating calves of 4 days to 15 days of age at the therapeutic dosage and recommended treatment. Those studies should be done in a sufficient number of animals per slaughtering time.

2. The applicant should complete the validation of the routine analytical method for all bovine edible tissues in accordance with the Volume VI of the Rules Governing Medicinal Products in the European Community. All the raw data should be provided and the analytical method presented according to a recognised format ISO 78/2.