SCIENTIFIC DISCUSSION

Name of the medicinal product:	HALOCUR
Marketing Authorisation Holder:	Intervet International B.V. Wim de Körverstraat 35 5831 AN Boxmeer The Netherlands
Active substance:	Halofuginone (as lactate salt)
Therapeutic indication(s):	In new born calves: ▪ Prevention of diarrhoea due to diagnosed *Cryptosporidium parvum*, in farms with history of cryptosporidiosis. Administration should start in the first 24 to 48 hours of age. ▪ Reduction of diarrhoea due to diagnosed *Cryptosporidium parvum*. Administration should start within 24 hours after the onset of diarrhoea. In both cases, the reduction of oocysts excretion has been demonstrated.
Target species:	New-born calves.

1. INTRODUCTION

Halocur is an oral solution containing halofuginone lactate as an active substance for administration by oral route to new-born calves. It is presented as a canary yellow homogeneous clear solution, miscible with water.

The active substance, halofuginone is an antiprotozoal agent of the quinazolinone derivatives group. The exact mechanism of action is unknown. The compound has a cryptosporidiostatic effect on *Cryptosporidium parvum.* It is mainly active on the free stages of the parasite (sporozoïte, merozoïte).

Halocur is indicated for new-born calves for prevention of diarrhoea due to *Cryptosporidium parvum*, on farms with a history of cryptosporidiosis and for reduction of diarrhoea due to *Cryptosporidium parvum.*

The oral solution is presented in two different forms, a portable bottle (HD-PE plastic) of 500 ml containing 490 ml of the oral solution and a portable bottle (HD-PE plastic) of 1000 ml containing 980 ml of the oral solution.

Halocur was eligible for the granting of a Community marketing authorisation via the centralised system since it was a product intended for food-producing animals and its active ingredient, halofuginone, had not been authorised for use in food-producing animals as a veterinary medicinal product on the date of entry into force of Council Regulation (EEC) No 2309/93 (i.e. on 1 January 1995), as provided for under the last indent of Part B of the Annex to that Regulation.

Summary on cryptosporidiosis

Cryptosporidiosis is a disease caused by the protozoan parasite, *Cryptosporidium parvum*. Clinical signs, present mainly as diarrhoea and can be encountered in new-born calves, aged from 5 to 15 days old. Oocyst excretion detected in older calves and adult cattle are generally asymptomatic.

The relation between oocyst excretion and diarrhoea in new-born calves has been clearly investigated in the literature and by field studies. In a prevalence study conducted in young calves of various origin, presence of Cryptosporidia was positively and significantly correlated to diarrhoea: oocyst excretion rate was 28.6 % in non diarrhoeic calves whereas it was 45.0 % in diarrhoeic calves.

The life cycle occurs within one host, and the pre-patent period ranges from 2 to 14 days in most domestic mammals. Most oocysts are excreted from the host in faecal material where they are directly infectious, but some oocysts can reinfect intestinal cells without external excretion.

Cryptosporidiosis is a zoonosis; human contamination can occur by direct contact between man and animal (ingestion or inhalation of oocysts), and by indirect means (e.g. through contamination of water).

Cryptosporidium parvum oocysts are very resistant to a range of environmental conditions, and no specific treatments are actually available.

2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The product contains per ml:

Active principle:

Ingredient	Amount	Quality standard
Halofuginone base (as lactate salt)	0.50 mg	Company monograph

Others ingredients:

Ingredient	Quality standard	Function
Benzoic acid	Ph. Eur. II 66	preservative
Lactic acid	Ph. Eur. II 458	solubilizing agent
Tartrazine (E 102)	French Ph. X Jan. 90	colouring agent
Water purified	Ph. Eur. II 8	solvent

Container:

- A 500 ml (containing 490 ml of solution) portable bottle made of translucent high density polyethylene. The bottle is closed with a polypropylene screw stopper fitted with a heat sealable aluminium polyethylene joint.
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- A 1000 ml (containing 980 ml of solution) portable bottle made of translucent high density polyethylene. The bottle is closed with a polypropylene screw stopper fitted with a heat sealable aluminium polyethylene joint.

CLINICAL TRIAL FORMULATIONS

The clinical trials were carried out directly with the formulation intended to be marketed.
Pre-clinical trials were carried out with an oral solution containing halofuginone used for theleriosis in Africa (TERIT).

The solution of 2.5 % of halofuginone was diluted with water to reach an halofuginone concentration of 0.05 %.
An in vivo bioequivalence study was performed between the TERIT solution and the Halocur solution. Results of the study show that both test products are equally effective in experimental *C. parvum* infection, in terms of oocyst excretion (no statistically significant difference between the two treated groups).

Development Pharmaceutics

1. Choice of the formulation:

As the packaging is intended to deliver multiple doses of product, a preservative was added to the solution. Two preservatives were tested: benzyl alcohol at 0.5 % and benzoic acid at 0.1 %. Benzoic acid was selected for its solubility (no crystallisation) and its good stability in the formula. The concentration of 0.1 % is introduced to ensure sufficient antimicrobial preservation.

Efficacy of antimicrobial preservation was checked on a batch packed in 100 ml vials stored at 40°C for 6 months and analysed after 0, 3 and 6 months. The test was conducted according to the Ph. Eur. monograph. Further results were provided on the efficacy of antimicrobial preservation analysed at 14 and 28 days in compliance with the Ph. Eur. monograph.

To check that the preparation was protected from the beginning to the end of the treatment (10 ml/day during 7 days), 10 ml was sampled each day from each of 14 vials tested during a week. The 10 ml sample collected the 7th day from all the vials were combined to give 140 ml of solution and tested. No physical and chemical alteration was observed after 7 days of treatment.

A solubilizing agent, lactic acid was selected by analogy with a marketed oral solution of halofuginone lactate, stable for 5 years. The same ratio lactic acid/halofuginone base contents was selected.

A colouring agent was added for safety reasons and to make the user aware of the administration route of the solution i.e. by oral route and not by injection. Four colouring agents were tested and tartrazine yellow was selected for its greater stability in acid medium. 0.003 g gives a coloration of sufficient intensity.

The pH was chosen as a function of the solubility of halofuginone lactate.

Stability studies showed that the enantiomeric purity of the finished product remains unchanged up to the end of shelf-life.

2. Choice of the primary packaging:

The packaging was selected on the basis of a daily dose volume to deliver at least the required number of doses for treatment: the 500 ml vial for 7 treatments and the 1000 ml vial for 14 treatments.

Studies were performed to investigate the compatibility of the solution with the stopper, and the solution was stored in amber glass vials of 100 ml and 500 ml. A selection of vials was stored in the upright position and the remainder inverted to establish the potential influence of the solution in contact with the stopper. The results were identical whatever the storage conditions.

For the 1000 ml bottle, 2 batches of solution were put at 40°C/75 % HR for 3 months (1 batch was in the upright position and the remainder inverted). The results showed that the contact of the solution with the joint did not generate any alteration of the solution quality.

The compatibility of the solution with the plastic packaging was investigated during the stability studies. Absorption of benzoic acid by the plastic container was observed (≈ 20 % after 6 months at 40°C).

Studies to demonstrate whether the packs were leakproof showed a loss of weight of approximately 0.95% in a 100 ml vial after 2 months at 50°C. Further results were provided on the 1000 ml bottle and showed that after 24 months at 25°C or 30°C the loss of weight is approximately 0.1 %.

B. DESCRIPTION OF METHOD OF PREPARATION

The manufacturing formula is given for a 110 litre batch.
The method of manufacture is divided into six steps:

- dissolution of benzoic acid with purified water.
- addition of lactic acid.
- dissolution of halofuginone.
- dissolution of tartrazine.
- clarifying filtration through a cartridge, 1.2 µm in porosity.
- distribution into vials.

A description of the equipment is provided by the applicant at each step of the process.
The manufacturing process is considered in compliance with the Guideline on 'Manufacture of the finished dosage form' (EMEA/CVMP/126/95).

In-process quality controls:

- dissolution of benzoic acid: check for total dissolution.
- dissolution of halofuginone lactate: check for total dissolution.
- dissolution of tartrazine: check for total dissolution.
- distribution into vials: every 30 min. on 5 vials (or bottles).
    * 500 ml vial (real filling volume 510 ml): 510 ± 10 ml.
    * 1000 ml vial (real filling volume 1020 ml): 1020 ± 10 ml.

VALIDATION OF THE MANUFACTURING PROCESS:

The content of the active ingredient was verified at the beginning, in the middle and at the end of the filling process. The manufacturing process produces a homogeneous solution (0.41 < CV < 1.51 %).

The solution was tested before and after the clarifying filtration for the compatibility of the filtering membrane with the solution. Results showed that physical and chemical degradation of the product due to the filtration are not observed.

The validation of the manufacturing process can be considered to be in compliance with the EEC guideline (Rules Governing Medicinal Products in the EC, vol. III) 'Development pharmaceutics and process validation'.

Results were provided for pilot batches and for an industrial scale batch.

C. CONTROL OF STARTING MATERIALS

The active ingredient halofuginone is not described in a pharmacopoeia but a monograph has been supplied.

The following tests are performed on the active ingredient:

Routine tests:

Characteristics:	Beige powder.
Identification tests:	Characteristic spectrum IR compared with a standard. Characteristic spectrum UV compared with a standard. Characteristic retention time by HPLC compared with a standard.
Purity and other tests:	Clarity and colour of the solution: clear and yellow. Water content (Karl Fisher): ≤ 0.5 % (m/m). Residual solvent (GC): ethyl acetate: ≤ 1.5 %. Sulphated ash: ≤ 0.1 %. Related substances (HPLC): * cis isomer : ≤ 0.5 % (m/m). * any other impurity : ≤ 0.5 % (m/m). * total content of other impurities : ≤ 1 % (m/m). Heavy metals : ≤ 20 ppm.

Assay(s):

- By a potentiometric method: 98 to 102 % (on the anhydrous and solvent free basis).
- Declared assay (calculated on the substance as is): state the result found.

Specific tests:

The following tests are performed on scale-up batches and on the first 5 industrial batches:

- Lactic acid content (potentiometric method): 17 to 21 % (m/m) (calculated on the anhydrous and solvent-free substance).
- Methanol (GC) : ≤ 500 ppm (tentative specification).
- Halofuginone enantiomer ratio (chiral HPLC): 45/55 to 55/45 % (tentative specification). If the results are proved to be consistent, this test will then be deleted.

In addition on the first 5 industrial batches:

- Bromide content: ≤ 0.1 % (tentative specification) is tested.

The quality control of the active ingredient is considered satisfactory.

Certificates for analysis were provided for the three starting materials of the active substance (ridane hydrobromide, cebrazolone and lactic acid). Specifications for routine control and the validated methods for control tests of the intermediate products: allylbromoridane and halofuginone hydrobromide have been provided as all specifications for the reagents and solvents used.

The potential impurities found in 4 batches were:

- cis isomer : ≤ 0.1 %.
- dichloro derivatives : ≤ 0.1 %
- dibromo derivatives : round 0.1 %.
- N-lactyl derivative : round 0.1 %.
- O-lactyl derivative : round 0.1 %.

The impurities are tested using an HPLC method with a limit of quantification equal to 0.05 %. The content of each of them is limited to 0.5 % and the sum of them should not exceed 1 %.

The residual solvents used in the last step of the synthesis of the active substance are methanol and ethyl acetate. Ethyl acetate is a non toxic solvent tested by a GC method allowing for a limit of quantification of 50 ppm. Its level is limited to 1.5 %. Methanol content was found to be less than 50 ppm on 4 pilot batches. It is not especially examined for in routine control procedure but it would be detected and quantified (limit of quantification of 50 ppm) with the GC method used for ethyl acetate in the case it would occur as residual solvent. However, it will be tested in-house on the first 5 industrial batches with a specification of 500 ppm.

The solubility of halofuginone lactate was determined in different aqueous solutions and organic solvents at 25°C. Halofuginone lactate is easily soluble in 0.1 N HCl, and in water and buffers whose pH is equal to or lower than 7, but practically insoluble in buffers whose pH is equal to or higher than 9 and in 0.1 N NaOH (solubility in aqueous medium increases when pH decreases).

All the excipients are described in a Pharmacopoeia and certificates of analysis are provided for each excipient.

The control procedure includes a description and a check of dimensional characteristics and an IR identification for each bottle and screw stopper. Drawing and certificates of analysis are provided.

D. CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

None.

E. CONTROL TESTS ON THE FINISHED PRODUCT

All the methods used for the control of the finished product are provided by the applicant.

1.1 General characteristics of the finished product

The following characteristics were determined:

- Appearance: canary yellow homogeneous clear solution, miscible with water.
- 500 ml vial:
  - extractable volume: ≥ 490 ml.
- 1000 ml bottle:
  - extractable volume: ≥ 980 ml.
- pH: 2 to 3.

- Related substances (HPLC):
  - cis isomer:
    - at release: ≤ 0.5 %.
    - at the end of shelf life: ≤ 0.7 %.
  - any other impurity:
    - at release: ≤ 0.5 %.
    - at the end of shelf life: ≤ 1.3 %.
  - total content of other impurities:
    - at release: ≤ 1 %.
    - at the end of shelf life: ≤ 2.3 %.

1.2 Identification and assay of active ingredient

- Identification test(s): by HPLC.
- Quantitative determination of active ingredients (HPLC):
  - at release : 47.5 to 52.5 mg / 100 ml (95-105 % of the label claim)
  - at the end of shelf life : 46.75 to 52.5 mg / 100 ml (93.5-105.0 % of the label claim).

The limits for the end of the shelf life are justified by the stability results.

1.3 Identification and assay of excipient constituents

- Identification test(s) for benzoic acid, lactic acid and tartrazine: by HPLC.

- Determination of benzoic acid content (HPLC):
  - at release : 90 to 110 mg / 100 ml (90-110 % of the label claim)
  - at the end of shelf life : 70 to 110 mg / 100 ml (70-110 % of the label claim).

The method validation for each of the assay methods is provided.
Results of the control of 3 batches gave proof of the homogeneity of the manufacturing process.

F. STABILITY

Stability studies on active constituents

One study was performed to determine the sensitivity of the active substance to light, heat, oxidation and humidity. The solid state was examined after exposure to dry heat and oxidation (in sealed ampoule, in a oven under air and under argon at 100°C during 7 days), to humid heat (in closed crystallising dish with a water tank, in a oven under air/humidity (100 % RH) at 80°C during 6 hours)

and in intense light (in sealed ampoule, in a suntest under air at ambient temperature during 24 hours). The appearance was tested, the impurity profile was determined by validated TLC and HPLC, and the assay value was obtained by a validated HPLC method.

The results indicated that halofuginone lactate:

- is sensitive to strong heat (in air or in argon) with a decrease of about 5 % of the assay value. The two main degradation products are the cis isomer and cebrazolone.
- is sensitive to humid heat with an increase of the impurities profile of 1.1 %.
- is stable to intense light.

Based on the previous elements, the active ingredient should be stored at a temperature not exceeding 30°C.

Semi-industrial batches were investigated in long term and accelerated conditions to determine a provisional shelf life:

- long term:
  - in brown glass vial, at - 20°C under air during 60 months.
  - in heat sealed low density double polyethylene bags introduced in a cardboard box, at + 2°C to + 8°C under air during 60 months (samples analysed only if a degradation of the product is observed at 25°C).
  - in heat sealed low density double polyethylene bags introduced in a cardboard box, at 25°C/60 % RH under air during 60 months.

- accelerated:
  - in heat sealed low density double polyethylene bags at 40°C/75 % RH under air during 12 months.
  - in heat sealed low density double polyethylene bags introduced in a cardboard box, at 60°C under air during 12 months.

The results show that there is no degradation at 25°C/60 % RH, and a slight degradation at 40°C/ 75 % RH (the colour become darker, the colour index increases by 90 to 100 units, the water content increase by 0.3 to 0.6 % and the cis isomer content increase by 0.2 to 0.4 %). There is a strong degradation at 60°C (the colour becomes darker from beige to dark brown, the colour index increases by 200 to 250 units and an increase of the impurities results in decreases of the assay values by 3 to 4 %).
The colour index is only a comparative test and it is not representative of the appearance of the sample.

Additional data provided after 36 months of storage under long term conditions confirmed good stability at 2°-8°C and 25°C/60 % RH. A shelf life of 36 months at 2°-8°C is granted.

Stability tests on the finished product

The applicant used the recommended conditions of the EEC guideline "Stability testing of new active substances and medicinal products" (vol. III, addendum 3).

In a preliminary study, the following conditions were applied:

- 4°C but analysed only if degradation at 25°C.
- 25°C/dark during 24 months.
- 25°C/light during 12 months.
- 40°C during 6 months.

The results showed no decrease of assay value, no modification of the appearance and of pH value of the solution, a slight increase of the cis isomer content at 40°C and of the total content of other

impurities at 25°C. A decrease of 21 % of the benzoic acid content also occurs at each temperature. The efficacy of antimicrobial preservation remains satisfactory after 6 months at 40°C.

In a formal study, the following conditions were applied:

Studies were performed on 100 ml and 1000 ml bottles during 36 months. Stability studies on final packaging (1000 ml) were performed during 36 months at 25°C/60%RH and 30°C/70%RH, and during 6 months at 40°C/75%RH and at room temperature under light.

After 6 months at 40°C no modification of the appearance of the solution or of the pH value were recorded. A slight decrease of the halofuginone content, a slight increase of the cis isomer content, an increase of the total content of other impurities, and a decrease of the preservative level however were observed.

After 36 months at 25°C and 30°C a slight increase of the cis isomer content and of the total content of other impurities, and a decrease of the preservative level were observed. All other parameters remain unchanged.

In-use shelf-life

Four studies were performed to simulate the different recommendations for use in practice (duration of 7 treatments for 500 ml vials, and duration of 14 treatments for 1000 ml vials). Two of the studies were performed over 30 days and the two others over 6 months. The results are satisfactory.
The proposed in-use shelf life is six months. The microbiological purity and the preservative efficacy have been investigated in the 6 months studies. The results are in compliance with the EP specifications. An in-use stability will be investigated on batches at the end of the shelf life.

In conclusion, the stability tests provided for the finished product are in compliance with the EEC guidelines and show that the product is stable under real conditions (25°C/60% RH and 30°C/70% RH during 36 months) and under accelerated conditions (40°C/75% RH during 6 months). The retained shelf life is 3 years and 6 months *in broached vials.*

3. OVERVIEW OF PART III OF THE DOSSIER: TOXICOLOGICAL AND PHARMACOLOGICAL ASPECTS

Halofuginone lactate, the active substance, has been assigned a maximum residue limit and was placed in Annex III of Council Regulation (EEC) No 2377/90 published in the Official Journal of the European Communities of 12 May 1995: Commission Regulation (EC) No 997/1999 of 11 May 1999. The other components of HALOCUR are preservatives and colouring compounds.

Benzoic acid, lactic acid and tartrazine yellow are E compounds (E numbers = E 213, E 270 and E 102 of EEC directive 95/2 of the 20/02/1995 respectively). These substances are listed in Annex II of Council Regulation EEC 2377/90, for all food producing species (Regulation n° 2034/96 of the 24 October 1996). These substances are considered as safe to the consumer and therefore no further studies about components of HALOCUR are required.

A. SAFETY TESTING

1. Single dose toxicity

The acute oral toxicity of halofuginone hydrobromide and lactate have been studied in mice, rats and rabbits. The LD_{50} was 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).

2. Repeated dose toxicity

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 $AUC_{0\text{-}8h}$ 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.

3. Tolerance in the target species

Tolerance is discussed under Part IV

4. Reproductive toxicity, including teratogenicity

a) Study of the effects on reproduction

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).

b) Embryotoxicity/fetotoxicity, including teratogenicity

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.

5. Mutagenicity

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.

6. Carcinogenicity

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.

Other requirements

1. Immunotoxicity

See 'Tolerance in the target species of animal' on pg. 23 Part IV.

2. Microbiological properties of residues

On the human gut flora

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.

ECOTOXICITY

Benzoic acid and lactic acid are highly degradable ubiquitous compounds. Tartrazine yellow can be considered as not of concern in this context because of very low concentration found in HALOCUR. The active ingredient is therefore the only substance to be considered for the ecotoxicity assessment.

Studies were performed between 1976 and 1984 for the registration of halofuginone hydrobromide as a food additive. These studies are performed with halofuginone or halofuginone hydrobromide. Because of similarities between halofuginone salts they are convenient for the ecotoxicological safety assessment of halofuginone lactate.

In Europe, halofuginone hydrobromide (stenorol) is used as an anticoccidial food additive in poultry (chicken and turkey). This salt was registered as a food additive under Council Directive 70/524 (EEC) N° E 764 (SCAN report 17/11/1982 and 8/02/1984). Maximum authorised level in chicken and turkey foodstuffs : 3 mg/kg.

One study investigated pharmacokinetics in veal calves using radiolabelled ^{14}C-lactalofuginone. Halofuginone lactate after 7 daily administration in veal calves is mainly excreted in urine. The main substance recovered in urine is halofuginone (from 17 to 36 %). Other metabolites were not identified. The other metabolites individually represent less than 20 % of the total radioactivity each, therefore for the ecotoxicity assessment, the residues are expressed as halofuginone.

One soil leaching study was performed using 500 µg ^{14}C halofuginone, 20 g of excreta from chickens treated with ^{14}C halofuginone or excreta from treated chickens pre-incubated in soil for 32 days added at the top of glass columns. Soil radioactivity was measured into 5 cm sections and elute in 10 ml fractions. The columns were eluted by 400 ml of water. There was no significant radioactivity below the first 5 cm after elution in the ^{14}C halofuginone columns. When un-incubated excreta were eluted, 80% of the applied radioactivity remained in the first 5 cm. 0.25% and 2.4% of the radioactivity was recovered in elutes for loamy sand and clay loam respectively. In 32 days pre-incubated excreta, 80 and 85 % of the applied radioactivity stayed bound to the first 5 cm and 1.1% and 0.3% respectively was found in the elutes from loamy sand and clay loam columns respectively. In conclusion, Halofuginone and related metabolites have a poor leaching potential (0.25 to 2.4% of the total amount of radioactivity found in the water elute). Halofuginone is unlikely to contaminate water courses.

Biodegradation of ^{14}C halofuginone and its metabolites in chicken excreta have been studied in laboratory and field conditions. A silty clay loam (Alcombury) and a sandy loam (Taunton) were studied in the laboratory. A field study was performed in Alcombury soil. Faeces analysis showed halofuginone and a conjugate of halofuginone.

The laboratory study showed that $^{14}CO_2$ was released regularly but in small amount (2 to 4% respectively after 16 weeks). Total quantity of radioactivity in soil samples related to the radioactivity applied varied from 80 to 120%. Solvent extracted radioactivity decreased from (54% and 62% to 24 and 17% respectively in 52 weeks) in relation to time while non extractable radioactivity increased. The nature of the radioactivity in soil extracts (by TLC) showed an increase of polar metabolites and a decrease of unchanged halofuginone. The apparent half-life in the decrease of halofuginone in the first 8 weeks were 15 and 20 days respectively. One of the most important metabolites accounted for up to 12% of the applied radioactivity. In the field study, the same pattern was observed over 32 weeks. The apparent half-life of halofuginone was 43 days. Radioactivity mainly stayed bound to the 0 - 5 cm soil layer. Halofuginone was shown to lead to more polar metabolites. Halofuginone and related compounds are closely bound to soil and do not leach below the 0 - 5 cm soil layer.

Excreta from chickens dosed with ^{14}C halofuginone at the intended dose was incorporated to soil. The soil/excreta was mixed and incorporated in pots at the dose rate of 50g/pot (corresponding to 10

tons/ha). Sugar beets, carrots and potatoes were sowed and grown to maturity. Concentration of radioactivity in plants (roots, leaves and tubers) were less than 4 ppb.

Soil treated by ^{14}C halofuginone (80 kg/ha) or treated by 80 tons/ha of excreta from chickens treated by ^{14}C halofuginone (equivalent to 55 kg halofuginone/ha) were used to grow plants. After 6, 10, 14 and 16 weeks, residue levels were measured. The following results were obtained:

Plant	^{14}C halofuginone treated soil		Excreta from ^{14}C halofuginone dosed chickens treated soil	
Lettuce	W6 W10	15.9 ppb 10.9 ppb	W6 W10	12.1 ppb 5.2 ppb
Tomato plant Tomato leaves and stalk Tomato repined fruit	W6 W16 W16	24.1 ppb 30 ppb <3 ppb	W6 W16 W16	10.2 ppb 20 ppb <3 ppb
Tobacco plants Tobacco leaves	W6 W14	12.6 ppb 24.4 ppb	W6 W14	8 ppb 13.6 ppb
Cucumber plant Cucumber fruit	W6 W10	28.6 ppb 1.6 ppb	W6 W10	16.9 ppb 1.9 ppb

The following results were obtained in five non GLP studies to investigate ecotoxicity to aquatic organisms; *Cyprinus carpio* LC50 : 0.3-0.7 mg/l (72h), *Salmo gairdneiri* LC50 : 1.8 mg/l (96 h), *Lepomis macrochirus* LC50 : 0.12 mg/l (96 h), *Daphnia magna* EC 50 : 0.02 mg/l (48 h) and *Chlorella pyrenoidosa* EC50 : 46 mg/l. Halofuginone is considered highly toxic to aquatic fauna.

Four more non-GLP studies were provided on the possible effects of phytotoxicity and toxicity to earthworms. The two studies on Earthworms (*Lumbricus terrestris)* showed no effect on mortality at dose up to 21 ppm and no effect on the body weight at dose up to 10 ppm. No phytotoxicity effects were found in tomatoes, lettuce, cucumber or tobacco on grounds treated with 0-480 g/ha. In one study some phytotoxicity signs in tobacco were allocated to manure or beddings from the chickens used to treat the soil.

Strains of amoeba of different origin are cultured in agar medium supplemented with 0, 2, 5 and 10 mg/l of halofuginone bromhydrate. Halofuginone hydrobromide at the 2 mg/l level inhibited most *Acanthamoeba* (10/16), *Naegleria* strains (5/8). Halofuginone is considered moderately toxic to algae.

The effects of faeces of chickens dosed with halofuginone on microbial nitrogen transformation in soil was investigated. Nitrification was unaffected in samples treated with faeces containing halofuginone and halofuginone metabolites.

Study of effects of halofuginone hydrobromide on the growth of pure culture of methanogenic bacteria showed that this substance inhibited the growth of *Methanobrevibacter arboriphilus* at 10 mg/l and *Methanococcus vannielli* at 0.1 mg/l. Batch fermentation studies showed that the concentration of 1 mg/l reduced methane production in the first days of incubation. After a few days, no difference in methane production was observed between control and the 100 mg/l batch. Higher concentrations showed partial or total inhibition of methane production. In semi-continuous fermentations, a significant decrease of methane production is observed when 30 mg/l concentrations are reached. Substance absorption to particles should explain higher sensitivity of pure culture compared to batch and semi-continuous fermentations.

Environmental exposure assessment

Environmental exposure occurs by spreading a mixture of manure and beddings collected from food additive use in poultry or from veterinary use in calves. In calves and poultry, halofuginone is excreted mostly in faeces. Faeces and bedding mixtures are spread over land according to EEC 91/676

directive. Halofuginone appears to be the main metabolite in urine. Such products are intended for use in intensive husbandries, therefore outdoor and non intensive husbandry situations are neglected.

Hypothetical calculations were provided to obtain a PEC of 13.8 µg/kg for halofuginone in soil due to its use as food additive in poultry (worst case scenario). A PEC of 0.7 µg/kg was calculated for halofuginone in soil due to its use as a veterinary medicinal product.

Since the trigger value of 10 µg/kg of soil is not exceeded even in a worst case scenario, a phase II assessment is not required according to the CVMP guideline 'Note for guidance on the environmental risk assessment of veterinary medicinal products other than GMO containing and immunological products' (EMEACVMP/055/96-FINAL).

In conclusion, halofuginone residues released from the veterinary use represents only 5% of amount related to the use as a food additive. The use as a food additive has been evaluated and authorised under EEC regulation (EEC Directive: 70/524 23/11/1970). The use of HALOCUR as a veterinary medicinal product cannot be a threat to terrestrial or aquatic ecosystems because of the low soil concentrations and the acceptable ecotoxicity profile. Therefore, the SPC now contains the following warning: 'Halocur should not come into watercourses, as this may be dangerous for fish and other aquatic organisms'.

User Safety

Three additional local toxicity studies were provided on the request of the CVMP. Two studies on skin sensitisation in guinea pig, one study on the acute dermal toxicity in rat.

During the induction period in the first sensitisation study in guinea pigs all treated animals showed a slight to moderate erythema and dryness of the skin on day 4. Crusts were observed in 7/20 animals and hypoactivity in 13/20 animals. No cutaneous reactions were observed after the challenge application.

In the second study, an erythema of grade 2 was observed in 7/20 animals and of grade 1 in 8/20 animals. 14/20 animals showed dryness of the skin. The test substance Halocur induced cutaneous reactions attributable to delayed contact hypersensitivity in 35% animals.

In the cat study, there was no mortality at doses up to 2000 mg/kg

It is known that halofuginone lactate and halofuginone hydrobromide show a significant acute toxicity by oral and dermal routes.

The exposure of the skin to the product when handling a contaminated vial, cap or syringe during repeated operations (up to 50 with a 500 ml vial for 50 kg calves) should be considered. Skin contact with the product may occur during the following operations:

- Skin contact with the cap and vial (screwing on, unscrewing the cap, squeezing the vial to fill the measuring compartment)
- Skin contact with the syringe (filling the syringe and adjusting the volume, emptying the syringe in the oral cavity of the calf).

It is accepted that the dermal acute toxicity of the product is limited with a LD_{50} > 2000 mg/kg (corresponding to 3.98-4.46 mg/kg halofuginone). However, the nature of the exposure of the skin (repetitive contact) is favourable to the development of skin allergies. Even if the erythema was moderate and rapidly resolved in the guinea pig study, the skin contact with the oral solution should be avoided.

Ocular exposure may result from accidental splashing. Halofuginone is an eye irritant and the formulation is acid pH =2-3. In case of accidental splashing in the eyes, the eye should therefore be rinsed with water. Medical advice is recommended in case of persistency of an irritation.

The following statements have now been included into the Product Literature:

- Repetitive contact with the product may lead to skin allergies
- Avoid skin and eye contact with the product. In case of skin and eye contact wash thoroughly the exposed area with clean water. If eye irritation persists, seek medical advice.
- Wear protective gloves while handling the product
- Wash hands after use

B. RESIDUE TESTING

Metabolism and residue kinetics

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).

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 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.

Depletion of residues

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).

Routine analytical method for the detection of residues

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. Further completeness of the validation of the routine analytical method is still required and is one of the two outstanding questions of the Summary Report.

Withdrawal period

On the request of the Committee a new tissue residue depletion study in young calves following a seven daily administration of Halocur had been provided in order to calculate the withdrawal period. Twenty-six male and female calves were used at an age of 4 - 16 days. The test drug was administered once daily during 7 consecutive days at a dosage of 0.1 mg halofuginone base/kg body weight. Slaughter times were 5, 10, 15 and 25 days post treatment. An HPLC method was used to analyse the residues (LOQ: 10 µg/kg for kidney, liver and fat, 5 µg/kg for muscle).

The mean concentration (µg/kg) of halofuginone in edible tissues, range of observed concentration and the number of animals showing tissue level above the MRL after oral administration of halofuginone at a dose rate of 0.1 mg/kg/day for 7 consecutive days are summarised in the following table:

Time post-treatment (days)		Tissue levels (µg/kg)			
		liver	**kidney**	**muscle**	**fat**
5	**mean ± sd** **range** **n>LOQ**	77.0 ± 21.01 57.17 - 99.75 5/5	76.23 ± 29.76 50.66 - 126.47 5/5	8.33 ± 2.74 <5 - 10.12 3/5	21.47 ± 4.49 < 10 - 24.64 2/5
10	**mean ± sd** **range** **n>LOQ**	24.40 ± 5.38 < 10 - 28.98 3/5	17.92 ± 9.05 10.78 - 29.14 5/5	<5 <5 0/5	< 10 < 10 0/5
15	**mean ± sd** **range** **n>LOQ**	< 10 < 10 0/5	10.62 < 10 - 10.62 1/5	<5 <5 0/5	< 10 < 10 0/5
25	**mean ± sd** **range** **n>LOQ** **LMR**	NA 30	NA 30	NA 10	NA 25

Mean ± sd : arithmetic mean and it standard deviation of tissue level above limit of quantification ; n>LOQ : number of animals which have tissue level above limit of quantification. NA : not assayed

As the results obtained at 15 days for all samples were negative the sample obtained at 25 days were not assayed.

The statistical approach for the calculation of the withdrawal period was only possible for residues depletion in kidney because for other tissues only one (for muscle and fat) or two (for liver) slaughter times showed quantified levels (i.e. > LOQ) Based on the pragmatic approach a withdrawal period of 10 days was determined. At this time all tissue levels are below the MRLs. As only one (for muscle and fat) or two (for liver) slaughter times showed quantified levels a safety span of 30 % is added to the withdrawal period. Consequently the withdrawal period is 13 days.

4. OVERVIEW OF PART IV OF THE DOSSIER: PRE-CLINICAL AND CLINICAL ASPECTS

I PRE-CLINICAL REQUIREMENTS

A. Pharmacology

A.1 Pharmacodynamics

Data provided on pharmacodynamics consist mainly of studies and publications on the efficacy and secondary pharmacological effects of halofuginone bromide. The mechanism of action is not described and no data are provided for the *in vitro* activity of halofuginone on *Cryptosporidium parvum*.

The secondary pharmacological effects for laboratory species have been provided. The main results (from non GLP studies) have shown that halofuginone bromide exerts effects on cardio vascular systems (bradycardia), in particular in the cat. In the mouse and in the squirrel monkey, effects on the central nervous system have been observed (a dose 1.0 mg/kg bw caused a definite activation of the electrocorticogram (EcoG) as indicated by a reduction in total activity and a reduced activity at all frequencies up to 30 Hz. *In vitro* data show halofuginone antagonises contractions of the guinea pig ileum provoked by acetylcholine, 5-hydroxytryptamine, barium chloride and histamine.
An *in vitro* study has been provided in order to establish IC_{50} and IC_{90} of halofuginone against *Cryptosporidium parvum*. Two human enterocytic cell lines (Caco2 and HCT-8) were used. Quantitation of *Cryptosporidium parvum* were performed by an immunofluorescent method by using human polyclonal anti *Cryptosporidium parvum* antibodies (1 :200) and revealed by using sheep anti human Ig GAM conjugated with FITC (1:100). Halofuginone lactate was used at concentrations ranged from 0.04 to 40 µg/ml. Results are expressed as the mean parasite counts per 30 microscopic field (x 1000). Three experiments were performed with six repetitions. The IC_{50} and the IC_{90} were calculated for both cell series. The determination of this parameter was obtained by graphical calculation for IC_{50} and by regression line for IC_{90}. The parameters are summarised in the following table.

	Cell series	
Parameters	**Caco2**	**HCT8**
IC_{50} (µg/ml)	0.073	4.59
IC_{90} (µg/ml)	0.078	4.38

The IC parameters are closed for the both cell series. The study provided allows establishing the IC[1] parameters for halofuginone against *Cryptosporidium parvum*. It is established a IC_{90} of 4.5 µg/ml.

No specific study on the physiological functions of the target species was provided.

A.2 Pharmacokinetics

The pharmacokinetic studies of halofuginone were conducted in lactating dairy cows at a dose rate of 1.2 mg lactalofuginone/kg bodyweight. The metabolism and the kinetics of halofuginone lactate in calves were documented in two study reports and one publication.

Different pharmacokinetic parameters were proposed in a study with four calves.

The Committee decided however that new results should be provided since:

- The dosage used in the original study was 10 fold higher than the therapeutic dose. No information was available on the pharmacokinetic linearity of halofuginone in plasma and consequently the results were not pertinent to the pharmacokinetic behaviour of

[1] IC = inhibiting concentration,

halofuginone after oral administration at a dose rate of 0.1 mg/kg in calves. Furthermore, accumulation was not adequately investigated because the number of administrations was insufficient.

- The drug was administered by an oral gavage in aqueous solution (rather than milk replacer solution).
- The study was performed in "calves" weighing 118-205 kg i.e. ruminating calves. The product is indicated for use in non-ruminant calves and this physiological discrepancy is expected to modify the kinetic profile;
- The formulation administered was not the same as the one under the assessment. The proof given on bioequivalence of the drug with Halocur was considered inadequate.

Two additional studies were then performed in accordance with the Volume VII Guideline on 'Conduct of Pharmacokinetic Studies in Animals' (The Rules Governing Medicinal Products in the European Union).

A study was performed using 8 pre-ruminating calves of 6 – 11 days old to establish pharmacokinetic parameters after intravenous or oral administration of 0.1 mg/kg of halofuginone base. Blood samples were taken over 36 hours after IV administration and over 96 hours after oral administration.

The mean (±SD) pharmacokinetic parameters of halofuginone after a single administration of halofuginone by intravenous or oral routes at a dose rate of 0.1 mg/kg are presented in the following table :

	Route of administration	
Pharmacokinetic parameters	**IV**	**Oral**
T1/2 ß (h) (*)	11.66 ± 4.43 (6.6 - 19.6)	30.84 ± 10.16(21.5 - 51.0)a
$AUC_{0\text{-}Inf}$ (ng h/ml)	171 ± 31	110.3 ± 42.2
MRT (h)	16.7 ± 5.1	22.0 ± 5.1
MAT (h)	-------	11.4 ± 4.1
$CLb_{0\text{-}Inf}$(L/h/kg)	0.604 ± 0.123	-------
Vc (L/kg)	0.94 ± 0.35	-------
$Vss_{0\text{-}Inf}$ (L/kg)	9.81 ± 2.621	-------
Tmax (h)	-------	11.0 ± 5.4
Cmax (ng/ml)	-------	4.12 ± 1.47
F (%)	------	81.1 ± 29.4

a : estimated on 5 calves, (*) harmonic mean with SD ; T1/2 ß :terminal elimination half-life, (in brackets the range are indicated); $AUC_{0\text{-}inf}$: Area under curve between 0 and infinity for IV and 0-Clast for oral; MAT : mean absorption time ; $CL_{0\text{-}inf}$: Whole body clearance, Vc : volume of central compartment ; $Vss_{0\text{-}inf}$: volume of distribution at steady state ; Cmax: maximal observed concentration, Tmax: time where the Cmax is observed; F: bioavailability, LOQ : 1 ng/ml

After intravenous administration the terminal half-life is 11.66 hours and 30.84 hours after single oral administration. The volume of distribution is high indicating a large distribution of halofuginone. After oral administration maximal concentration (4 ng/ml) is reached at 11 hours. The mean bioavalability is estimated at 81 %.

To determine the accumulation of halofuginone a study was performed with calves which received 0.1 mg halofuginone/kg bodyweight once a day for 7 days by oral administration. Ten male calves were used at the age of 22-32 days. The maximal concentration obtained after the first administration was 3.49 ng/ml and 4.25 - 6.66 ng/ml after the following administration. After the last administration the LOQ level was reached between 36 h and 120 h. The apparent elimination half life was estimated at 32.8 hours. A statistical analysis based on the linear regression model by least square shows that accumulation of halofuginone in the blood does not take place, but when the apparent terminal half life after oral administration of halofuginone is considered it is noted that a poor accumulation is possible.

Furthermore, halofuginone should bind to the solid contents of the intestinal lumen but as demonstrated in the pharmacokinetic study, the bioavailability was high, which suggested that if binding does exist it is reversible. Consequently it is clear that in the intestinal tract, halofuginone should reach target parasites.

B. Tolerance in the target species of animal

Different studies on the tolerance of the target species were provided:

In one study, twelve calves (6 new born pre-ruminating less than 1 week old weight ranging from 33-60 kg and 6 ruminating calves of unknown age and weight ranging from 75 to 160 kg) were allocated into 4 treated groups. The pre-ruminating calves (3 animals/group) were treated by 1 mg/kg (10x the therapeutic dose (TD)) and 1.5 mg/kg (15x the TD) for 3 consecutive days. Ruminating calves were treated by 1.5 and 2.5 mg/kg (15 and 25x the TD). The product, TERIT, halofuginone lactate 30 mg/ml corresponding to 25 mg/ml halofuginone base was administered by oral route after dilution into 30 ml milk or water. Animals were observed during 21 days. All animals treated by 15x and 25x the TD died (9/9). Pre ruminating calves treated by 15x the TD died before the third administration. Ruminating calves died a few days after the third treatment from D6 to D15. Death was preceded by anorexia, prostration and diarrhoea. One of the 3 pre-ruminating calves treated by 10x the TD died. The other animals of this group had profuse diarrhoea for a few days. Necropsy examination showed inflammation and congestion of the gastro-intestinal tract and adrenal hypertrophy. In some animals heart and kidney lesions were also recorded. Blood analysis showed hypoproteinemia, hyperuremia and high creatinin level suggesting protein digestive loss and renal failure. It is concluded that the product is lethal in pre-ruminating and ruminating calves at 10 times the TD and over.

Twenty-four calves (12M, 12 F) 7 days old and weighing 37-50 kg at the beginning of the treatment were allocated into 6 treated groups. Animals were dosed daily by 1x (groups 1, 2 and 3) or 3x (groups 4, 5 and 6) the TD for 7 consecutive days. The treatment was administered with a syringe by the oral route. In groups 1 and 4, animals were treated 2 hours before feeding, in groups 2 and 5 the animals were treated just after feeding, in groups 3 and 6 animals were treated 2 hours after feeding. Three animals in group 4, treated at 3x the TD two hours before feeding died during the period from D2 to D6. These animals became anorectic 24-36 hours before death. No other calves died during the study. Even at 3x the TD administered immediately or 2 hours after feeding, the product was clinically well tolerated. The incidence of diarrhoea increased by the end of the study and was possibly related to treatment, but this event cannot be interpreted fully since this study was not designed with a control group. Biochemistry findings showed a tendency for total protein to decrease in all groups but more particularly in groups 1, 4 and 6. Urea and creatinin was increased in groups treated by 3x the TD. Macroscopical and histopathological examination of animals treated at both doses showed significant gastro-intestinal congestion (particularly duodenum and jejunum). A slight acute epithelial nephritis in a calf treated with 1x the TD just after feeding was possibly related to treatment. Macroscopical and histopathological examination of the dead animals showed gastro-intestinal tract epithelium lysis as well as an epithelial nephritis (necrosis) in the 3 x the TD group dosed before feeding. The other animals treated by 1x and 3x the TD only showed blood stasis in the duodenum mucosae. The product is significantly better tolerated when administered after feeding.

Twenty four calves, aged 4-8 days at the beginning of trials were allocated into 2 groups (12 animals each). Animals were treated by 0x (control) or by 3x the TD (0.6 ml/kg/day of Halocur corresponding to 0.3 mg/kg/day halofuginone base) for 7 consecutive days. Animals were observed over 35 days in order to assess delayed toxicity. The treatment was administered by oral route with a syringe in the morning being given to calves just after food. The product used at 3x the TD induced moderate to severe signs of toxicity. During the treatment period 3/12 calves were found moribund on D7 or D8. They were prematurely sacrificed on D7 (1/12) or slaughtered on D8 as scheduled (2/12). During the 7 days treatment period, 7/12 of treated calves showed deshydratation (D7 and D8) and 5/12 showed apathy (D7). As these clinical findings were not observed in control calves, they were considered as related to treatment. Diarrhoea or liquid faeces were found on at least one occasion in 6/12 treated calves. Faecal index was higher in treated group (due to diarrhoea) on D5, D7 and D8. The calves

were given rehydrating solution, nutrient replacer of milk. In 3 calves due to apathy and milk refusal, specific treatments were administered. Compared to control, the number of treated calves given a treatment (rehydratation and other) was significantly higher in the treated group on D5 and D7. On D4 and D6, rectal temperature was higher in treated animals. Milk consumption and body weight were affected in treated animals. Milk consumption was lower in treated animals on D7 and correlatively body weight was lower in treated calves on D7. No significant difference in milk consumption or body weight was observed thereafter. Lower lymphocytes counts (-30% on D4 to D14), higher neutrophil counts (+76% to +106% on D4 and D7) and higher fibrinogen (+38%) on D14 were recorded. Significantly higher blood urea level was observed in treated calves on D4 (x2.2) and D7 (x3.3). Anti *Cryptosporidium parvum* IgM level was lower in treated animals on D14. No difference in anti *Cryptosporidium parvum* IgG level was recorded throughout the study. Local immunity assessed by anti *Cryptosporidium parvum* IgA in faeces showed that significantly lower levels were recorded on D11 but were significantly higher than control on D25. Necropsies performed on the 3 calves sacrificed on D7/D8 showed very extensive necrotic inflammatory lesions in the digestive tract. The animals slaughtered on D28 and D35 did not show any macroscopic lesions in the digestive tract, in liver or kidneys. Microscopic findings confirm necrotic lesions of the digestive tract in animals sacrificed on D7/8. A heavy lymphocytic depletion of all lymphoid organs predominate histopathologic changes on all treated animals slaughtered on D8. On D28, 2/4 animals in the treated group showed lymphocytic depletion in Peyer's plaques, this pattern appeared to be discrete on D35 (2/4 animals). The other organs did not show significant changes between groups. The changes observed in treated animals are likely to have resulted from gastro-intestinal necrosis/inflammatory lesions and renal failure.

Thirty six calves, aged 4-10 days at the beginning of trials were allocated into 3 groups. Animals were treated by 0x (control), 1x or 2x the TD (0.1 and 0.2 mg/kg/day halofuginone base / 0.2 and 0.4 ml/kg/day) for 7 consecutive days. Animals were observed during 35 days in order to assess delayed toxicity. The treatment was administered by the oral route with a syringe in the morning just after food was given to calves. No mortality occurred during the study. No significant clinical findings related to treatment such as dehydratation or prostration were recorded. Some transient clinical signs, unlikely to be related to treatment were observed in all groups (cough, apathy, respiratory infection). The only sign likely to be treatment related was diarrhoea occurring during treatment in 1/12 calves (8%) of the 1x the TD and in 1/12 calves in the 2x the TD dose. In both cases, faeces showed pathogens able to cause diarrhoea. Some other cases were recorded after the treatment period in all groups probably linked to rotavirus. Increased incidence of mucus in faeces was recorded on D6 in the treated calves. On D7, increased incidence of visible blood in faeces was recorded in the high dose group. Body weight gain was significantly increased in the group treated at the TD but not in the high dose group. The animals recovered with fasting and administration of concentrated nutrient. Haematological findings were slight lymphocytopenia from D4-D7 to D21 in both treated groups (20 and 35% respectively). From D4 to D7, a slight increase in urea was noticed in both treated groups (20-30% and 70-80% respectively). IgM level decreased as the dose administered increased on day 14. Cryptosporidia oocyst excretion was significantly lower in the treated calves on D7 and D11. On D18, oocyst excretion was increased as the dose administered increased. Macroscopic examination of gastro-intestinal tract on D8 in treated animals showed higher incidence of abomasum, colon and rectum lesions. No further visible lesions were recorded on D28 and D35. Histopathological examination performed on D8 showed gastro-intestinal lesions in all treated animals (4/4 in each group). These lesions (rectum and forestomachs) were of greater severity in the high dose group. Some congestion of villi was also recorded at the duodenum-jejunum junction. On D28, superficial micro abscesses were noted in 1/4 animal in each group. On D35, no treatment related lesions were recorded

Two more new studies performed in 1-3 day old calves were also provided. In the first study 24 male non-suckling calves (24-66 hours of age) were allocated into 3 groups of 8 animals. The recommended dose (100 µg/kg bw/d halofuginone base) and twice the recommended dose (200 µg/kg bw/d halofuginone base) were administered by the oral route (formulated halofuginone lactate) for 7 days, administered once daily after the morning feed. One group served as control. In the twice recommended dose group, two calves died on D 7 and D24 and in both cases, lymphocyte depletion was noticed. Their macroscopic examinations revealed a fibrinomatous pleuropericarditis or a

pulmonary oedema and a peritonitis secondary to an occlusion syndrome, and so these deaths were considered as not product related. Statistically significant higher relative proportions of neutrophils and lower proportions of lymphocytes were observed from D4 to D7. When compared to control values, the following differences were reported:

* lower mean leukocyte counts on days 21, 28 and 35 (-28%, -30 % and -24 % respectively) and statistically significant on day 21,
* higher mean values in neutrophil counts on days 4, 7, 14 and 21 (+50 %, +46 %, + 36 % and 22 % respectively) and statistically significant on day 4,
* statistically significant decrease in lymphocytes counts on days 21 and 35 (-40 % and -43 % respectively),
* moderate increase in urea levels on days 4 and 7: 1.8 and 2.1 fold higher than those of controls, respectively).

Histological findings were observed, which demonstrated moderate lymphocytic depletion of the ileal Peyer's plaques in 3/5 remaining animals on D35. In the recommended dose group, no adverse effects were observed and even a statistically significant better evolution of body weight was demonstrated compared to the control group. No compound related histological findings were noted. A discrete depletion of ileal Peyer's plaques was reported in 1/6 animals.
The death of 2 calves at twice the recommended dose was not considered as related to the treatment by the study director. The role of the test substance cannot be definitely ruled out since some lesions recognised as potentially due to Halocur (depletion of ileal Peyer's plaque) were recorded. The investigations performed on these 2 calves however allowed consideration of other causes. Clearly, the use of 1 to 3 days old calves in a preclinical safety study is very difficult to achieve due to the transportation. The calves treated at the therapeutic dose did not show any significant changes. This study also illustrates the very narrow safety index of this product.

One long term tolerance, multicentric, controlled, randomised study conducted in a blind manner used new-born calves under field conditions. Thirty six new born calves (18 males, 18 females), 61% of the Charolais breed, 33% Limousine breed and 6 % crossbreed from 3 farms (Moselle area, France), with a mean age of 31.7 hours old and a mean body weight of 46.9 kg were allocated in 3 groups of 12 animals each. Halocur (halofuginone lactate) was administered by the oral route for 7 days at the recommended dose (100 µg/kg bw/d halofuginone base) and twice the recommended dose (200 µg/kg bw/d halofuginone base). One group was used as a control. No mortality was observed in any group tested. The animals remained in healthy condition and no serious adverse reaction attributable to the treatment was observed within the study. On day 0, the 3 groups differed in terms of the following criteria: age on day 0 (the control group being older), presence of blood in faeces, white blood cell count and neutrophil differential count (these parameters being more elevated in the 200 µg/kg). These differences disappeared on D14. No statistically significant differences were recorded in lymphocytes counts despite a lower cell count in the group treated by 200 µg/kg ($p=0.13$, on days 7 and 14). Coronavirus and Cryptosporidia infections remained very discrete while E. coli and rotavirus were detected in about 50% of the animals. No significant differences between groups were demonstrated. The measurement of body weight suggests that the treatment might have a positive impact on the weight gain especially from D7 to D28.

An overall assessment of the recurrent toxicity symptoms seen in the above studies is summarised below.

Lymphocytopenia at the recommended dose

Published reports confirm that a considerable variation of the white blood cell counts exists among calves depending on age, muscular activity and emotional status and that WBC counts and neutrophiles/lymphocyte ratio show marked differences between calves in response to stress of birth. Moreover, WBC counts decreases as the calves get older and that lymphocytopenia and neutrophilia are regularly observed in new born calves.

In tolerance studies, WBC counts and lymphocyte counts showed some variations considered as within the normal range of calves. The decrease in WBC and lymphocytes counts was a trend mainly in the groups treated with x2 and x3 the therapeutic dose. At the therapeutic dose the lymphocyte count showed a 20% decrease on D7 only. The decrease observed never reached the statistical significance.

In the field tolerance study, at twice the therapeutic dose, the decrease observed was slight, transient and without any clinical consequence in the 56 days follow up.

Peyer's plaque lymphocytic depletion (PPLD)

In the tolerance studies, at 3 times the therapeutic dose in K95 TRI study, 4/4 calves sacrificed on day 8 and 2/4 calves sacrificed on day 28 showed a PPLD. This was not observed in calves sacrificed on D35. This depletion was therefore transient in nature. The other studies showed some PPLD in some animals at twice the recommended dose but it was difficult to draw clear conclusion about the involvement of the treatment. In the K96 TNB study, 1/8 calves showed a slight PPLD on day 8, this was no longer observed on D35.

Increased sensitivity to gastrointestinal pathogens

In the tolerance and clinical trials the occurrence and the involvement of pathogens has been the subject of follow up and review. Gastro-intestinal pathogens such as Campylobacter, Salmonella, Rotavirus and Coronavirus have been detected in different trials. No significant trend was seen in these studies, and it appears that the product does not increase the sensitivity of calves to gastro-intestinal pathogens during treatment and in subsequent weeks.

In conclusion, the lymphocytopenia and the WBC count decrease observed in the tolerance studies are of low magnitude and transient at the recommended dose and the reported values are in the normal range for animals of this age. The occurrence of PPLD in calves at the recommended dose and at twice the recommended dose could be due to the treatment but it is difficult to draw clear conclusions. This feature is transient in nature and the survey of the occurrence of other gastro-intestinal pathogens in field studies shows that the product does not increase the sensitivity of calves during the treatment and in subsequent weeks.

Kidney function

It was demonstrated that lesions similar to interstitial nephritis observed at necropsy were noticed both in control and treated groups and that no link with the blood urea nitrogen increases could be established. The non-specificity of blood urea nitrogen and the transient nature of the increase noticed in the tolerance studies should not be considered as an indication of a detrimental effect upon renal function.

Gastro-intestinal sensitivity

It is agreed that the different tolerance studies and clinical trials show that the product does not increase the incidence of the diarrhoea at the recommended dose. However, the SPC (5.8) refers to the nature of the symptoms which might occur at 2 times the recommended dose (diarrhoea, visible blood in faeces, decrease milk consumption, dehydration, apathy, prostration) and the necessity to apply strictly the recommended dosage.
Considering the tolerance studies and the inclusion criteria used in the clinical field trials, the product should not be administered in cases where diarrhoea has established for more than 24 hours, and in weak animals. Furthermore, the use in fasting animals should be contra-indicated for safety reasons.

In conclusion, it can be concluded that there is clearly a narrow safety index of this product and therefore the following warnings are required:

- Under SPC section 5.3. contra-indications:
  'Do not use in fasting animals'
  ' Do not use in case of diarrhoea established for more than 24 hours and in weak animals '

- Under SPC section 5.4. Special precautions for use:
  'Administer after colostrum feeding or after milk or milk replacer feeding only using either a syringe or any appropriate device for oral administration. Do not use on an empty stomach. For treatment of anorexic calves, the product should be administered in half a litre of an electrolyte solution. The animals should receive enough colostrum according to good breeding practice.'

- Under SPC section 5.8 Overdose:
  'As symptoms of toxicity may occur at 2 times the therapeutic dose, it is necessary to strictly apply the recommended dosage. Symptoms of toxicity include diarrhoea, visible blood in faeces, decline in milk consumption, dehydration, apathy and prostration. Should clinical signs of overdosing occur the treatment must be stopped immediately and the animal fed unmedicated milk. Rehydration may be necessary.'

C. Resistance

A study was performed on calves receiving oocyst excreted from low dose treated animals. These animals were treated by increased dosage. In parallel the efficacy of the drug was evaluated by inoculating the oocysts to other calves treated with the recommended dosage. The criteria observed were: mortality, weight and weight gain, oocyst shedding. No emergence of resistance is observed after 5 passages using 25 µg then 37.5, 50, 62.5 and 75 µg/kg/d for 7 days.

CHAPTER II CLINICAL REQUIREMENTS

The applicant submitted 5 dose-titration studies, 2 trials investigating local immunity, two prevalence studies, a therapeutic equivalence study, a trial on young goats on the mode of action of halofuginone, and three field trials.

Prevalence studies

An epidemiological survey, multicentric, prospective and descriptive trial was performed in 7 regions of France. One thousand six hundred and thirty calves were included throughout the 12 month survey period. Calves had a mean age of 8 ± 4 days and 94, 6 % were males. Diarrhoea was only noted in 5.2 % of the calves. About 17, 9 % of calves excreted oocysts of Cryptosporidium (95 % confidence interval is (16.1 % - 19.8 %). The minimum prevalence was recorded in July (7 %) and the maximum was observed in September (26 %). On a regional basis, the minimum was recorded in Brittany (13.3 %) and the maximum in Franche-Comté (25.3 %).

Another epidemiological survey, multicentric and descriptive study performed on 189 farms in France was also provided. A total of 440 calves, 4 to 21 days of age at the time of sample, developed diarrhoea for less than 72 hours. The mean age was 9.8 days, 46.3 % were males and 53.7 % were females. Diarrhoea was noted in 90.5 % of the calves: 41.8 % of the calves presented liquid diarrhoea and 43.9 % showed some degree of dehydratation. Prevalence of Cryptosporidium was confirmed in 43.4 % of calves. Minimum prevalence was recorded in January (32.9 %) and maximum was observed in December (53.0 %). Presence of Cryptosporidia was positively and significantly correlated to diarrhoea: oocysts excretion rate was 28.6 % in non-diarrhoea calves whereas it was 45.0 % in diarrhoeic calves. These results comfort the importance of Cryptosporidia in neonatal enteritis. Almost half of diarrhoeic calves excreted Cryptosporidia.

Dose titration studies

Except in one study where calves were inoculated with sporulated oocyst of *C.parvum*, calves involved in the dose titration studies were naturally infected by *C.parvum*.

Fifty, three to six day old calves, fed with replacement milk, having a mean body weight of 46.2 kg received 0.5 mg/kg of halofuginone lactate. Sixteen were untreated, 17 received treatment on days 1, 2, and 3 (group A) and 17 were treated on days 1, 4, and 7 (group B). During and after the period of medication, calves in both medicated groups showed a significant increase in diarrhoea and a significant weight loss. On Day 28, a significant lower weight gain was recorded in group A. Two calves also died in group A. All unmedicated calves excreted oocysts with a maximal excretion between Day 9 and Day 13 (mean log10 of 6.85 to 6.92). Excretion stopped in both medicated groups but reappeared 9 to 10 days after drug withdrawal. Birnavirus was isolated in the three groups.Natural immunity appears to have protected against *C.parvum* after two weeks of life ; on Day 20, only one calf in control group excreted oocysts.

Fifty three- to six-day old calves, fed with replacement milk, with a mean body weight of 47 kg received 0, 60 (during 14 days), 125 (day 1,4,7) or 250 µg/kg (day 1,4,7) of halofuginone lactate. There were no differences between numbers of animals showing diarrhoea in the different groups, nor on the weight gain. All unmedicated calves excreted oocysts (at different times) with a maximal excretion between day 7 and day 13. Excretion was completely stopped in medicated groups but reappeared 10 days after drug withdrawal in groups 3 and 4. In group 2, only 4 calves became positive again 6, 13 and 32 days after withdrawal. Rotavirus was isolated in 5 to 8 calves in each group. The drug was well tolerated. After continuous treatment (in group 2) a lower excretion of *C.parvum* has been observed than in calves treated alternatively (groups 3 and 4).

Fifty three- to six-day old calves, fed with replacement milk, with a mean body weight of 46.7 kg received 0, 30 (during 14 days), 60 (during 7 days) or 125 µg/kg (during 7 days) of halofuginone lactate. There were no differences between numbers of animals showing diarrhoea in the different groups, nor on the weight gain. Only discrete signs of diarrhoea were observed in different groups. All unmedicated calves excreted oocysts with a maximal excretion between Day 6 and Day 13. After Day 13, no excretion was observed. Excretion stopped in medicated groups but reappeared in some calves respectively 10 and 6 days after drug withdrawal in groups 3 and 4 (higher number of oocysts in group 4). In group 2, all calves became negative by the 6th day of medication, but low numbers of oocysts were detected again in 3/13 calves by the 8th day of medication. Rotavirus was isolated in 2 to 5 calves in each group. One can conclude that 60 or 125 µg/kg of halofuginone lactate was effective in stopping parasitologic excretion and consequently in reducing external contamination, however *C.parvum* didn't represent a real clinical problem in control group. In the unmedicated group IgA and IgM reached a maximum after 6 days (maximal oocyst excretion between Day 6 and Day 13) and declined when oocyst shedding stopped. Titers increased again by Day 27. In the medicated groups the rise of IgM and IgA was less pronounced during medication, but treatment did not inhibit immunological response. Decrease occurred more rapidly than in group 1 (and was the greatest in group 2). Titers increased again 7 days after drug withdrawal and that rise was greater in group 4 than in group 2. Group 3 was intermediate.

Twenty one-day-old calves were inoculated on day 0 orally with 10^6 sporulated oocysts of *C.parvum*. Four groups of 5 animals received respectively 0, 30, 60 and 120 µg/kg of halofuginone lactate from day 2 to day 8 post-infection. High mortality occurred in the unmedicated and 30 µg/kg groups in 3/5 calves in each group. No mortality was observed in other groups, only two cases of diarrhoea were noticed in the 60 µg/kg group. In unmedicated group, oocyst excretion started on Day 3, was maximal on Day 7 (score 4) and finished on Day 14. In the 30 µg/kg group, oocyst shedding wasn't totally reduced during treatment (score 1 - 1.5) but there was no re-excretion afterwards. In the 60 and 120 µg/kg groups there was total inhibition during treatment but excretion started again immediately after drug withdrawal in 60 µg/kg group and 10 days after withdrawal in 120 µg/kg group (score 1.5 - 2). In the unmedicated group, IgA and IgM increased during oocyst shedding and decreased after Day 14. IgG rose slightly until Day 14 and then decreased (dosage on 2 calves in each group, 3 were dead). In medicated groups, IgM activity was present in all groups during treatment whereas IgA activity was

different amongst the group. In 30 µg/kg group, IgA rose during treatment concurrent with oocyst shedding and in both 60 and 120 µg/kg IgA levels increased after drug withdrawal in association with a low oocyst output.

Fifty seven-day old calves, fed with milk replacer, with a mean body weight of 47.8 kg received 60 or 120 µg/kg of halofuginone lactate during 7 consecutive days. Sixteen calves were left untreated. At 112 days of age, five calves in each group were challenged orally with 10^7 oocysts of *C.parvum* and five calves served as control in each group. During 4 weeks, the following parameters were examined: clinical signs, oocyst excretion, local and serological antibodies, and incidence of Rotavirus and Salmonella were evaluated too. There were no differences between numbers of animals with diarrhoea in the different groups, nor in the weight gain. All unmedicated calves excreted oocysts (at different time) with a maximal excretion 7 days after arrival. Afterwards, only small peaks of excretion were seen on days 35, 49 and 98. Excretion was completely stopped in medicated groups on Day 7 and reappeared in some cases 7 days after drug withdrawal (low number of oocysts). Afterwards, small peaks of excretion were observed as in the unmedicated group. No Salmonella organisms were detected. Rotaviruses were eliminated in each group (8 to 14 calves) mainly 21 to 35 days after arrival, and that elimination was associated with liquid to mucus faeces. The kinetics of IgA, IgG, and IgM in medicated groups were similar to the kinetics detected in untreated group (somewhat lower than in unmedicated group but the difference was not significant). The animals remained completely refractory to the massive challenge as reflected in no clinical signs, or oocyst excretion being recorded. Only IgA in challenged calves rose slightly in comparison with control (but the difference was not significant). No differences could be established between both dosages of halofuginone.

In a multicentre, comparative study, the preventive and curative treatment on naturally infected calves was investigated. One hundred and sixty six neonatal Belgian Blue-White beef calves from three different farms received orally 0, 60 or 120 µg/kg of halofuginone lactate during 7 consecutive days. In each farm, calves were randomised to one of the three treatment groups. For some calves a preventive treatment was given from the 4th day of birth, and during 7 consecutive days. For others a curative treatment was given when first signs of diarrhoea occurred. All had received colostrum from their mother which were vaccinated against coronavirus, rotavirus and *E.coli* K88. After birth, calves were kept with their dam or were separated and fed with milk. Calves were not vaccinated, but were medicated parenterally with antibiotics (colistin or gentamicin) at 2 or 3 days of age because of endemic colibacillosis. The results were analysed per farm. In farm Durand: untreated calves showed diarrhoea between 4 and 21 days after birth with a maximum on Day 7. Diarrhoea was significantly reduced in medicated group, and almost completely prevented in the 120 µg/kg group during the first two weeks. A peak in incidence of diarrhoea was observed on day 14 in that 120 µg/kg group, a week after termination of treatment. All untreated calves excreted oocysts (maximum on Day 7, end by Day 18), whereas excretion was significantly lower in treated groups (and lower in 120 µg/kg than in 60 µg/kg group). No rise in excretion after termination of treatment was seen. Kinetics of antibodies were similar in the three groups. In farm Loyens: only slight diarrhoea was observed in the three groups, and oocysts excretion was very low in all calves (3/17 in untreated group). For the group that received curative treatment, in farm Durand, untreated calves showed diarrhoea between 11 and 21 days after birth (peak on Day 14), treatment began 8 ± 1 days after birth from the first signs of diarrhoea, but didn't reduce clinical signs in both treated groups (number of days of diarrhoea was the most important in 60 mg/kg group). Almost all calves excreted oocysts. Low diminution of oocyst excretion in treated groups was only observed between Day 11 and Day 14. Kinetics of antibodies didn't differ between the three groups, whether calves stayed with their dam or not. In farm Lummen: untreated calves showed diarrhoea between 4 and 21 days after birth (peak on Day 11), treatment began 8 ± 3 days after birth from the first signs of diarrhoea, but didn't reduce clinical signs in both treated groups. Excretion of oocysts was similar in the three groups (peak on Day 11), antibodies titres were similar too. It was concluded that neither clinical results nor parasitological results could support the curative efficacy of treatment. Concerning the preventive treatment: calves in farm Loyens weren't significantly infected by *C. parvum*, and a positive response could be demonstrated in that farm, whereas in farm Durand, prevention of diarrhoea was clearly demonstrated. Prevention of clinical signs and oocyst excretion was better with the upper dosage of 120 µg/kg of halofuginone lactate during 7 consecutive days.

A meta-analysis was conducted on studies which used the same dosage and the same protocol (natural condition of *C.parvum* infection). In total, 186 calves entered into the meta-analysis. The score counts and faecal indexes were compared at the different times for the groups taken two by two, and a dose effect relation was researched (Mantel-Haenszel Chi Squared linear correlation test). A significant difference in counts is noted between 60 µg/kg group and control group from days 4 to 11, and from days 4 to 14 between control and 120 µg/kg group. On days 7, 11 and 14, oocyst excretion is significantly less important in 120 µg/kg group than in 60 µg/kg group. A dose-effect relation is observed between Day 4 and Day 14. A significant difference in faecal index is noted between both treated groups and control group on Day 11. A dose-effect relation is observed between D4 and D11.

In conclusion the meta-analysis confirms the choice of a 120 µg/kg dosage on reduction of oocyst excretion. The role in prevention of diarrhoea was less demonstrable (difference only on day 11). The faecal indexes remained very low in control groups ; a mean score of 0.6 on Day 11 was the maximum registered in the meta-analysis.

Supplementary data

As previous studies were carried out using a 1:50 water dilution of halofuginone lactate concentrated formulation, a therapeutic equivalence study with Halocur was conducted. This controlled, randomised study was performed with 20 new-born (12 to 48 hours-old) calves, fed twice daily with reconstituted milk. Calves with oocysts in faecal samples at arrival were excluded. All calves were inoculated orally with 1 x 10^6 oocysts of *C.parvum* on Day 0. Treatment was given between day 2 and day 8. Four calves were untreated, 8 were treated at 100 µg/kg during 7 consecutive days with the 0.05% solution, 8 were treated at 100 µg/kg of hb during 7 consecutive days with Terit. The results were considered inconclusive for equivalence due to an inherent lack of statistical power.

Sixteen new-born male young goats, which received colostrum (heated one hour by 56°C) after birth and then reconstituted milk twice a day. The kids received 120 µg/kg of halofuginone lactate (2 ml of solution for 10 kg). Solution was administered in the morning, just before feeding, during 5 consecutive days. They were inoculated orally with 1 x 10^6 oocysts of *C.parvum* on Day 0.

N = 2 kids in each group

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Untreated	Treated 24 h before infection	Treated concurren-tly with infection	Treated 6 h after infection	Treated 12 h after infection	Treated 24 h after infection	Treated 48 h after infection	Treated 72 h after infection

Excretion was very considerable (mean 2.10^8) in untreated kids between D4 and Day 8.:
Compared to unmedicated groups, reduction of oocysts excretion in treated groups depended on time of treatment:

reduction of oocyst excretion	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
(in %)	96.13	92.72	86.79	78.25	41.03	93.12	67.25

It was noted that oocyst excretion could be different within one group (2 kids). From these results, it was supposed that halofuginone lactate acts on the free stages of *C.parvum* sporozoites (maximal efficacy when administered 24 hours before infection and concurrently with infection) and merozoites (efficacy 48 hours after inoculation). On the other hand, efficacy is low when parasites are in intestinal cells. Unfortunately, clinical signs of diarrhoea were not taken into account in that study. However again it was shown: the later the drug is administered, the lower is the reduction of oocyst excretion.

A multicentre, non-comparative study was performed on new-born calves with diarrhoea. Animals received 0.5 mg/kg of the Halofuginone lactate. Rehydratation therapy, antidiarrhoea agent and antibiotics completed the treatment. Faecal materials were sampled at first visit and examined for

presence of Cryptosporidium, Salmonella spp., enteropathogenic E.coli, and virus. Treatment began at first visit, but was continued only if presence of cryptosporidium was confirmed by microscopic examination. Five days later (and sometimes 15 days later), a new faecal sample was taken for Cryptosporidium research. Two hundred seventy four new-born calves were examined, oocysts were found in 105 of them (38.5 %). From these 105 cases (mainly < 16 day-old), cryptosporidium was considered to be responsible for diarrhoea in 67 cases (no other bacteria or virus isolated). Number of oocysts was high at first sampling (51.5 % score 4) but no relation could be established between quantity of oocyst and seriousness of clinical signs. In 84 cases, the second sample was performed (Day 5), 77 calves were negative for Cryptosporidium (91.7 %). 21.8 % of calves died. Diarrhoea ceased in 68.3 % of the cases. Due to the absence of a control group and the high dosage used, this study is of poor interest for the demonstration of efficacy of Halocur.

Field trials

A multicentre, comparative (versus placebo), randomised, double-blinded trial was performed using 40 livestock farms in 3 French departments (selection: history of cryptosporidiosis and a *C.parvum* positive sample). Calves received orally 2 ml/10 kg of a solution containing halofuginone or placebo, during 7 consecutive days when aged from 4 to 10 days, presenting a diarrhoea < 24 hours, as well as other calves aged from 4 to 10 days at time of intervention. The drug was administered with a syringe immediately after suckling, or in ½ litre of rehydrator. Use of sulfonamides, sulfones, nitrofuranes and all anticoccidial and antiprotozoal products was forbidden. Three hundred and eleven calves were included; most of them were suckling calves. 158 calves in the treated group, 153 in the control group. The mean age was 6 days, 53 % were males, mean weight = 45 kg. No differences were observed between the two groups in their general condition, faecal indexes and oocyst counts. Eighty two % of calves were presented in good general condition, 76.2 % having a normal appetite and 91.3 % absence of dehydration. 48.5 % of calves presented liquid or semi-liquid diarrhoea. 50.2 % of calves presented a positive oocyst count score. Relationship between diarrhoea and presence of *C.parvum* (in 108 cases both were present) was demonstrated in analysis ($p < 0.001$) as was presence of Coronavirus. No pathogenic agents were found in 31 % of cases. Rotavirus was isolated in 13.2 % of calves, and Coronavirus isolated in 6.4 %. Many calves received a concomitant treatment between D0 and D6: antibiotics were used in 65 % of cases, rehydrating agents and anti-diarrhoeals in 20 to 30 % of cases. The numbers of calves with Rotavirus and Coronavirus did not increase during the follow-up, and were not different between the two groups.

Evolution of oocysts count scores: number of calves presenting score 0 or superior or equal to 1;

	HALOCUR		PLACEBO	
Day of sample	Score = 0	Score ≥ 1	Score = 0	Score ≥ 1
D0	83	75	72	81
D3	33	125	24	129
D7	32	126	21	132
D14	118	40	110	43

Evolution of faecal indexes: number of calves presenting score 0 (absence of diarrhoea) or scores 1 and 2 (semi-liquid or liquid diarrhoea);

	HALOCUR		PLACEBO	
Day of sample	Score = 0	Scores 1 and 2	Score = 0	Scores 1 and 2
D0	81	77	79	74
D3	78	80	56	97
D7	89	69	59	94
D14	122	36	101	52

Failures (4 *vs* 9 cases) or relapses (4 *vs* 5 cases) as defined in the protocol did not differ between groups, but mortality was significantly higher in placebo group (12 cases *vs* 3). Study of adverse

events, including mortality, blood in faeces or the use of concomitant treatments did not result in differences between the two groups. The results of that trial did not support the efficacy of the drug in curative situation; oocyst counts presented the same evolution between Day 0 and Day 14 in both groups (approximately 80 % of calves excreted oocysts on Days 3 and 7, and 26 % on Day 14), and cessation of diarrhoea was recorded in only 11 to 17 % more calves in treated group than in control group.

Due to confusion between different clinical status on Day 0, a re-analysis of this study was performed considering:

- on the one hand, the calves which were diarrhoeic on D0 (48.5 % of the included calves had liquid or semi-liquid diarrhoea), and consequently were treated in a curative situation,
- on the other hand, the calves which were not diarrhoeic on Day 0, and consequently were treated in a preventive situation.

1 - Curative situation (n = 151):

70 % and 61 % of calves presented diarrhoea respectively on D0 and D7 in the placebo group versus 50 % and 41 % at the same dates in the treated group.
Concerning liquid diarrhoea, percentages were 31 % and 26 % in the placebo group *versus* 18 % and 12 % in the Halocur group at respectively D3 and D7.
A reduction of diarrhoea and essentially of liquid diarrhoea was observed in the treated group.

The statistical analysis was performed on the faecal index (0, 1 or 2).
Mean of 0.69 at D3 in treated group vs 1.01 in placebo group
Mean of 0.52 at D7 in treated group vs 0.86 in placebo group
Taking into account the effect of dehydration as a factor of confusion (more calves were dehydrated in the placebo group on Day 0), a significant improvement in favour of treated group is shown for faecal index ($p = 0.02$).
Furthermore, a significant effect of the treatment on the reduction of the Cryptosporidia count scores is noted ($p = 0.0005$)
Mortality : 10 deaths were recorded in the placebo group and 3 deaths in the treated group ($p = 0.12$)

2 - Preventive situation (n = 160):

No significant effects of treatment were shown on the oocyst count scores. On faecal indexes, difference at the limit of significance was shown ($p = 0.06$).
Mean of 0.78 at D3 in treated group vs 0.90 in placebo group
Mean of 0.67 at D7 in treated group vs 0.81 in placebo group
Mortality : 2 deaths were recorded in the placebo group and none in the treated group (NS).

This trial was the only field trial performed in curative situation. An effect of the treatment was shown on the reduction of diarrhoea. The effect of the treatment on mortality remains doubtful, and the effect on weigh gain was not investigated.
These results are not of sufficient clinical significance to support an indication of « treatment » of diarrhoea. The claim has to be limited to the results obtained i.e. the « reduction of diarrhoea due to *C.parvum* » with an important limitation to calves presenting a diarrhoea from less than 24 hours.
This trial was not demonstrative for a preventive effect but this was not the aim of the study/

A multicentre, comparative (versus placebo), randomised, double-blinded trial was performed using 6 industrial livestock farms. Calves aged from 8 to 15 days with or without diarrhoea were included however calves with a diarrhoea > 24 hours were not included. Calves received orally 2 ml/10 kg of a solution containing halofuginone or placebo, during 7 consecutive days. A total of 382 calves were included with 191 calves entered in each group. The mean age was 6 days, 91.6 % were males, mean weight = 52.4 kg.

Systematic concomitant treatments were given for all calves around D7 (colistin + oxytetracyclin in 3 farms, and colistin and ivomec in 3 other farms). In one trial all the calves were treated for pneumonia on day 15 and day 26. Posology was respected with mean daily volume of 10.5 ml and administered for a mean duration of 7 days and drug was administered directly with a syringe in 100 % of cases. Clinical and parasitological signs on day 0 showed no differences between the two groups in general condition; most of the calves presented in good general condition (99% of the calves), normal appetite (99.7%) and absence of dehydration (97.1 %). On oocysts counts, 83.3 % of calves were negative for Cryptosporidia. Coronavirus were found in 31 % of calves and Rotavirus in 9.7 %.

No pathogenic agents were found in 49 % of calves and 6.3 % of the calves presented a liquid or semi-liquid diarrhoea on Day 0. A statistical relationship was shown between presence of diarrhoea and presence of Cryptosporidia and Rotavirus. Evolution of other agents during study included numbers of calves with rotavirus (30 %) and coronavirus (35 %) which increased on Day 7. Rotavirus decreased (11.8 %) on Day 14, prevalence of coronavirus remained high (36.7 %). Analysis demonstrated a poorer result in the treated group. In fact, calves of each group were housed in separate rooms, and the problem was more probably linked to the housing than to the treatment.

Evolution of oocysts count scores: number of calves presenting score 0 or superior or equal to 1				
	HALOCUR		PLACEBO	
Day of sample	Score = 0	Score ≥ 1	Score = 0	Score ≥ 1
D0	158	33	160	31
D3	179	12	147	44
D7	187	4	92	99
D14	163	28	130	61

Reduction in oocyst count scores is statistically significant between D0 and D14 in favour of group HALOCUR (difference reached a maximum on Day 7). A significant farm site effect was seen.

Evolution of faecal indexes: number of calves presenting score 0 (absence of diarrhoea) or scores 1 and 2 (semi-liquid or liquid diarrhoea)				
	HALOCUR		PLACEBO	
Day of sample	Score = 0	Scores 1 and 2	Score = 0	Scores 1 and 2
D0	184	7	174	17
D3	145	46	152	39
D7	127	64	139	52
D14	152	39	160	31

Statistical analysis showed that there was no effect of treatment on the evolution of faecal indexes, from D0 to D14. A significant farm site effect was pointed out. No difference was found between the two groups for mortality (1 death in placebo group vs 3), blood in faeces and use of concomitant treatments (other than systematic). No adverse events required the drug withdrawal. In conclusion, considering that only 6.3 % of calves presented diarrhoea on Day 0, the clinical results did not support the prevention of diarrhoea as claimed by the applicant (no significant difference on faecal indexes). Possibly infection by *C.parvum* was insufficient to lead to clinical signs. The parasitologic efficacy of the drug is well demonstrated (on Day 7, 52 % in control group vs 2 % in treated group excreted oocysts).

The Committee requested new information in order to support the claim on 'prevention of diarrhoea'.

A new study was submitted:
A multicentred, comparative (*versus* placebo), randomised, double-blinded trial included 158 calves, aged from 24 to 48 hours, having yet received colostrum, not infected by a concomitant pathology or developing diarrhoea for more than 24 hours, not treated by anticoccidial or antiprotozoal product. The calves received orally 2 ml/10 kg of a solution containing halofuginone or placebo, during 7 consecutive days. Treated calves received 100 µg/kg of halofuginone base. The principal criterion for

efficacy were the oocyst count scores. Only 13.9 % of calves showed diarrhoea symptoms. Half of these calves were positive for E.coli K99.

Efficacy results:	HALOCUR					PLACEBO				
	V0	V4	V7	V14	V21	V0	V4	V7	V14	V21
mean oocyst scores	0.01	0.56	1.38	2.68	0.54	0.00	1.44	3.54	1.96	0.01
mean diarrhoea scores	0.24	0.35	0.53	0.34	0.18	0.15	0.65	1.06	0.25	0.15

On V7, 41/79 (51.9 %) calves had a score of 5 for the oocyst counts in the placebo group whereas only 9/78 (11.5 %) calves reached this score in the Halocur group. At this moment, 87.3 % of calves excreted oocysts in the placebo group instead of 48.7 % in the treated group. On V7, 73.8 % of untreated calves had signs of diarrhoea *versus* 41.0 % in the treated group. The statistical analysis shows a high significant effect on the evolution of Cryptosporidia counts in favour of the treated group ($p = 0.0001$). The same conclusion can be made for faecal indexes. The placebo group needed more concomitant treatments (rehydrators) than the treated group (48 % vs 32 %). Prevalence of rota-coronavirus was not different between groups (superior in treated group on V7, inferior on V14). Six deaths occurred in the placebo group and 5 deaths in the treated group. Calves were necropsied and no relation with treatment could be inferred.

In conclusion, the mean oocyst count scores showed that Halocur decreased the amount of excreted oocyst. On day 14 (8 days after the end of the treatment), there is an increase of the oocyst excretion in the treated group : in fact the peak of oocyst excretion is delayed, but at this time excretion is no longer responsible for clinical signs. On the overall trial period, the reduction of oocyst excretion in favour of the treated group is highly significant ($p = 0.0002$). The relation between the presence of *Crytosporidium* (83.7 % of calves excreted *C.parvum* in the placebo group) and diarrhoea is well demonstrated on V7 (73.8 % of calves of this group are diarrhoeic).

The inclusion of calves before 48 hours of age guarantees a true preventive situation: because of the prepatent period, diarrhoea cannot occur before 3 or 4 days of age. This trial is a good demonstration of the preventive effect of Halocur against diarrhoea due to *C.parvum*.

The effects of the product on mortality and on weight loss as suggested in the claim proposed by the applicant have not been demonstrated and can not be retained. The indications for use point out that the preventive use is limited to well identified at-risk calves, it means to farms with history of cryptosporidiosis.

In conclusion on part IV of the dossier, the Committee accepted to retain the following indications for Halocur:

In new born calves:

* Prevention of diarrhoea due to diagnosed *Cryptosporidium parvum*, in farms with history of cryptosporidiosis.
  Administration should start in the first 24 to 48 hours of age.

* Reduction of diarrhoea due to diagnosed *Cryptosporidium parvum*
  Administration should start within 24 hours after the onset of diarrhoea.

In both cases, the reduction of oocysts excretion has been demonstrated.

5. RISK-BENEFIT ASSESSMENT AND CONCLUSION

The data submitted in the dossier and in response to questions confirm the acceptability of the proposed formulation and the format in which it is presented, the suitability of the specification for the active ingredient, the method of manufacture of the product and the validity of the test methods applied to the product. The stability tests provided for the finished product are in compliance with the EEC guidelines and show that the product is stable under real conditions (25°C/60% RH and 30°C/70% RH during 36 months) and under accelerated conditions (40°C/75% RH during 6 months). The retained shelf life is 3 years and six months after opening.

The safety to the consumer of halofuginone lactate has been evaluated according to requirements laid down in European legislation for establishment of MRLs, and this active substance has been classified in annex III. Excipients are listed in Annex II of Council Regulation EEC 2377/90 for all food producing species. A withdrawal period of 13 days was determined in accordance with the CVMP note for guidance: 'Approach towards harmonisation of withdrawal period' (EMEA/CVMP/036/95).

The product is considered compliant with the European note for guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products.

For the ecotoxicity part, the applicant has provided an expertise according to the Note for guidance on the environmental risk assessment of veterinary medicinal products other than GMO containing and immunological products (EMEACVMP/055/96-FINAL). The use of halofuginone as a veterinary product leads to low concentrations in the environment, therefore no phase II assessment is necessary.

Concerning the operator's safety, safety precautionary measures are proposed in the SPC. Particularly the use of protective gloves is recommended in order to prevent for handling the product possible skin allergies as the product has been recognised to cause irritation in sensitive skins.

Halofuginone is an antiprotozoal agent of the quinazolinone derivatives group. The mechanism of action is unknown. The *in vitro* activity was established against *Crysptosporidium parvum.* The concentrations which inhibit 50 % or 90 % of parasites were established in *in vitro* system (Human enterocytic cell lines, Caco2 and HT-8). The values are respectively 0.075 µg/ml and 4.5 µg/ml.

The tolerance of the product has been investigated in six studies which lead to conclude that the safety margin of the product is narrow and that it is necessary to strictly apply the recommended dosage. Symptoms of toxicity may occur at 2 times the recommended dosage. Clinically, signs may include diarrhoea, blood in faeces, dehydration, apathy and prostation. Histopathologic examinations have revealed a transient lymphocytopenia and depletion of Peyer's patches at two or three times the recommended dosage. Halocur should not be used in the case of diarrhoea established for more than 24 hours nor in the case of weak or fasted animals.

In literature the data on resistance of parasites against halofuginone are limited. Data concerning the emergence of resistance mainly relate to *Emeira* species in chicken. To document the resistance in calves a study was performed to establish the emergence of resistance of *C. parvum* against halofuginone. After 5 passages using increasing doses from 25 µg/kg bw to 75 µg/kg bw for 7 consecutive days no emergence of resistance was observed.

The effect of Halocur oral solution for the prevention of diarrhoea due to *C.parvum* has been documented in field trials. Early treatment during the first 24 to 48 hours after birth is necessary to prevent diarrhoea. The curative situation was investigated in only one trial, and the results are not of obvious clinical significance to allow the term of « treatment of diarrhoea ». Considering that the claim has to be inferred from the results obtained in the trial, the claim of « reduction of diarrhoea due to *C.parvum* » has been considered more appropriate.

As potential immunotoxicity of the substance was recorded in the tolerance studies at two or three times the recommended dosage, it has been verified in the field trials that the treatment at the recommended dosage has no incidence on the occurrence of other gastro-intestinal pathogens in calves.

In conclusion, the following indications for use have been approved:

In new born calves:

* Prevention of diarrhoea due to diagnosed *Cryptosporidium parvum*, in farms with history of cryptosporidiosis.
  Administration should start in the first 24 to 48 hours of age.

* Reduction of diarrhoea due to diagnosed *Cryptosporidium parvum*
  Administration should start within 24 hours after the onset of diarrhoea.

In both cases, the reduction of oocysts excretion leading to a decrease of environmental contamination has been demonstrated.

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC (now Directive 2001/82/EC).

