ANNEX I

LIST OF THE NAMES, PHARMACEUTICAL FORM, STRENGTH OF THE VETERINARY MEDICINAL PRODUCT, ANIMAL SPECIES, ROUTE OF ADMINISTRATION, APPLICANT IN THE MEMBER STATES

Member State	Applicant	Invented name	Pharmaceutical form	Strength	Animal species	Recommended dose
Belgium	Laboratorios Karizoo S.A. Mas Pujades 11-12 Pol. Ind. La Borda 08140 Caldes de Montbui SPAIN	Enro-K 10% Oral Solution	Oral solution	100mg/ml	Chickens and turkeys	50 ml of product per 100 litres water or 10 mg of the active ingredient per kg bodyweight per day. The treatment should continue for a minimum of 3 days. For the treatment of salmonellosis treatment should be extended to 5 days.
Czech Republic	Laboratorios Karizoo S.A. Mas Pujades 11-12 Pol. Ind. La Borda 08140 Caldes de Montbui SPAIN	Enro-K 10% Oral Solution	Oral solution	100mg/ml	Chickens and turkeys	50 ml of product per 100 litres water or 10 mg of the active ingredient per kg bodyweight per day. The treatment should continue for a minimum of 3 days. For the treatment of salmonellosis treatment should be extended to 5 days.
Germany	Laboratorios Karizoo S.A. Mas Pujades 11-12 Pol. Ind. La Borda 08140 Caldes de Montbui SPAIN	Enro-K 10% Oral Solution	Oral solution	100mg/ml	Chickens and turkeys	50 ml of product per 100 litres water or 10 mg of the active ingredient per kg bodyweight per day. The treatment should continue for a minimum of 3 days. For the treatment of salmonellosis treatment should be extended to 5 days.
Ireland	Laboratorios Karizoo S.A. Mas Pujades 11-12 Pol. Ind. La Borda 08140 Caldes de Montbui SPAIN	Enro-K 10% Oral Solution	Oral solution	100mg/ml	Chickens and turkeys	50 ml of product per 100 litres water or 10 mg of the active ingredient per kg bodyweight per day. The treatment should continue for a minimum of 3 days. For the treatment of salmonellosis treatment should be extended to 5 days.
Poland	Laboratorios Karizoo S.A. Mas Pujades 11-12 Pol. Ind. La Borda 08140 Caldes de Montbui SPAIN	Enro-K 10% Oral Solution	Oral solution	100mg/ml	Chickens and turkeys	50 ml of product per 100 litres water or 10 mg of the active ingredient per kg bodyweight per day. The treatment should continue for a minimum of 3 days. For the treatment of salmonellosis treatment should be extended to 5 days.

ANNEX II

SCIENTIFIC CONCLUSIONS

OVERALL SUMMARY OF THE SCIENTIFIC CONCLUSIONS

1. Introduction

Enro-K 10% Oral Solution contains 100 mg enrofloxacin as active ingredient per 1 ml product. Enrofloxacin is a synthetic, broad spectrum antimicrobial substance, belonging to the fluoroquinoline group of antibiotics. The product is intended to be used for the treatment of diseases of the respiratory and alimentary tracts of bacterial or mycoplasmal origin in chickens and turkeys.

During the decentralised procedure concerns were raised that Enro-K 10% Oral Solution may present a potential serious risk to the environment regarding risk to blue-green algae and to terrestrial plants. In particular the adequacy of the data provided in respect to the environmental risk assessment regarding these two points was questioned.

2. Assessment of the environmental risk

The applicant provided an environmental risk assessment for Enro-K 10% Oral Solution that followed in general the established guidelines and recommendations. The submitted environmental risk assessment report is based on new studies initiated by the applicant and on bibliographical data extracted from peer reviewed journals.

The Phase I environmental risk assessment following the VICH guideline results in Predicted Environmental Concentrations (PECs) in soil for Enro-K 10% Oral Solution for broiler chickens (10 mg/kg bw/day, for 5 days) above 100 µg/kg, thus requiring a Phase II assessment. The calculations for the minor species turkeys were not considered further in this referral as the risk in any case will be lower.

2.1. Environmental fate studies

2.1.1 Metabolism and excretion

Ciprofloxacin is the main metabolite of enrofloxacin. As ciprofloxacin represents only 2% of the total outcome of the enrofloxacin metabolism in chickens it is assumed that any potential risk of metabolites is encompassed by using the total residue approach assuming that the total dose is excreted as parent compound or as metabolites with a toxicity that is similar to the parent compound.

2.1.2 Soil adsorption and desorption

A study, published in the peer-reviewed and open literature (Nowara et al 1997)[1] indicated that enrofloxacin and other fluoroquinolones are strongly adsorbed to soils with high content in clay minerals of montmorillonite and kaolinite, basically through electrostatic interactions between ionized carboxylated groups of fluoroquinolones and exchangeable cations within clay layers.

The K_{oc} was calculated as 110,885 l kg/[1](geometric mean).

2.1.3 Soil degradation

The disappearance of non-labelled enrofloxacin was investigated according to OECD guideline 307 in four soils with different content of organic carbon and clay and pH values.

The soil characteristics and resulting DT50 and DT90 values were: DT50: 141, 103, 99 and 149 days; DT90: 469, 342, 330 and 495 days.

[1] Nowara A, Burhenne J, Spiteller M. 1997. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J Agric Food Chem: 45, 1459-63.

The test follows the recommended test guideline with no major deviation and was conducted in compliance with GLP. The number and variation in the soil types are considered acceptable. The difference in DT50 and DT90 values are relative small. If one assumes a worst case situation, then the highest DT50 should be used for further assessment (e.g. $PEC_{Soil\text{-}plateau}$ calculations). If a less conservative approach is taken, the geometric mean of the four DT50 values could be used, i.e. 121 days.

The Committee recognised that the design of the study had deficiencies but agreed that the results can be used for the PEC calculations.

2.2 Calculation of PEC values

2.2.1 $PEC_{soil\text{-}plateau}$

Using the current guidelines the calculated $PEC_{soil\text{-}plateau}$ is 542.9 µg/kg. If the geometric mean of DT50 values from the four soils is used, the $PEC_{soil\text{-}plateau}$ is 506 µg/kg.

2.2.2 $PEC_{groundwater}$ (PEC_{gw})

The resulting PEC_{gw} value is 0.057 µg/l, which is below the critical trigger value of 0.1 µg/l.

Overall, the CVMP agreed that enrofloxacin is unlikely to pose any significant risk to ground water.

2.2.3 $PEC_{surfacewater}$ (PEC_{sw})

Application of the CVMP guideline Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38 results in an estimate of $PEC_{surfacewater}$ of 0.019 µg/l.

2.3.Effect studies and calculation of PNEC values

2.3.1 Algae

A study taken from the open literature, Robinson et al. (2005)[2], studied the toxicity of enrofloxacin to algae *Microcystis aeruginosa* (cyanobacteria or blue-green algae) and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*, green alga). The EC50 values for *M. aeruginosa* and *P. subcapitata* were 49 and 3,100 µg/l, respectively. The application of an assessment factor of 100 leads to a PNEC value of 0.49 µg/l for algae.

Antibiotics are likely to be toxic to algae and cyanobacteria. This is a major reason why the EU (EMEA/CVMP) has made the testing of cyanobacteria mandatory for all antibiotics. No further consideration is made to the data of the green algae as this was more than 50 times less sensitive to enrofloxacin.

The study by Robinson et al 2005 is, overall, a well reported scientific paper, which has been published in a well established international peer reviewed Journal. However, the data for the test on cyanobacteria *Microcystis aeruginosa* does not meet validity criteria required in OECD guideline 201. The coefficient of variation (CoV) was not provided, however the relatively narrow span of the 95% confidence interval around the estimated EC50 values qualitatively indicates an acceptable variation in data. The growth rates are unlikely to have been exponential, and therefore the tests are likely to have been conducted under sub-optimal sensitivity. The correlation between measured parameters and dry weight of biomass should have been reported.

[2] Robinson AA, Belden JB, Lydy MJ. 2005. Toxicity of Fluoroquinolone antibiotics to aquatic organisms. Env. Toxicol Chem 24: 423-430.

Given the uncertainty of the fate and disappearance of enrofloxacin during the course of the test, the CVMP cannot support using the nominal concentrations for the establishment of EC50 values. It is not clear to what extent a population recovery may have occurred at the end of the test period. With the existing disappearance data it would be necessary to use a time-weighted-average approach.

The geometric mean concentration during exposure was used to estimate a more realistic effect-measure compared to the nominal concentrations as also suggested in OECD 201. Only two data points are available, i.e. the start and end of the study, where unfortunately no precise level of concentration recovery was reported (less than 20%).

The EC50 based on the initial exposure concentration can be estimated to be 51.45 µg/l when considering the EC50 of 49 µg/l based on the nominal start concentration (equal to 100% recovery) and an initial average recovery of 105% of the nominal concentration.

The EC50 based on the final exposure concentration can be estimated to be 4.9 µg/l when considering the EC50 of 49 µg/l based on the nominal start concentration (equal to 100% recovery) and an average recovery of 10% of the nominal concentration (mean of the range 0 to 20%). The geometric mean of these two EC50 values is 15.9 µg/l.

The CVMP concludes that it is not feasible to estimate an exact PNEC value for cyanobacteria based on the study by Robinson et al 2005. Consequently no risk quotient (PEC/PNEC) is derived for cyanobacteria.

2.3.2 Aquatic invertebrates (*Daphnia magna*)

Robinson et al. (2005)[2] studied the toxicity of enrofloxacin to *Daphnia magna* (waterflea).

Although not following the recommended OECD guideline, the results are sufficient to demonstrate that enrofloxacin is not likely to pose any risk to aquatic invertebrates. A conservative PNEC value could hence be established at 10 µg/l. Using this PNEC value and the PEC_{sw} established above (0.019 µg/l) a risk quotient of less than 0.01 can be established for aquatic invertebrates.

2.3.3 Fish

Robinson et al. (2005)[2] studied the toxicity of enrofloxacin to *Pimephales promelas* (fathead minnow).

Although not following the recommended OECD guideline, the results are sufficient to demonstrate that enrofloxacin is not likely to pose any risk to fish. A conservative PNEC value could hence be established at 10 µg/l. Using this PNEC value and the PEC_{sw} established above (0.019 µg/l) a risk quotient of less than 0.01 can be established for fish.

2.3.4 Microorganisms

The effect of enrofloxacin on nitrogen transformation by soil organisms was investigated according to OECD guidelines 216 and EU Test Method C.21.

The NOEC for the tested loamy sand field soil was found to be 2.9 mg enrofloxacin per kg soil (dry mass). The EC50 for nitrogen transformation was estimated to be above 29 mg/kg soil. From this data it can be concluded that there would be no risk at environmental concentrations 10 times the PEC_{soil} value established above (542.9 µg/kg).

2.3.5 Plants

A terrestrial plant growth test according to OECD guideline 208 was provided.

The effect of enrofloxacin on the emergence and growth of terrestrial plant seedlings was investigated. The following test species were used: *Cucumis sativus* (cucumber), *Lactuca sativa* (lettuce), *Phaseolus aureus* (mung bean), *Avena sativa* (oat), *Triticum aestivum* (wheat) and *Secale cereale* (rye).

The seeds were placed in a natural sandy soil containing the test item at nominal concentrations of 10, 31.6, 100, 316 and 1000 mg enrofloxacin/kg dry soil (cucumber, lettuce, mung bean, oat and wheat) and 3, 12, 48, 192 and 768 mg enrofloxacin/kg dry soil (rye), respectively.

There was no concentration dependent effect on seedling emergence due to enrofloxacin up to the highest concentration tested. However, growth (on a fresh weight per plant basis) of all species tested was significantly inhibited by enrofloxacin. For all species tested a clear concentration dependent effect occurred and EC50 and NOEC values were found in the range of 124 to 435 and less than 10 to 100 mg/kg, respectively. Rye was the most sensitive species, with an EC50 of 124 mg/kg.

The plant test follows the recommended test guideline with no major deviation and was conducted in compliance with GLP. All validity criteria of the guideline were fulfilled. The proposed EC50 values are acceptable and can be used to derive a PNEC value of 1.24 mg/kg (1240 µg/kg). Using this PNEC value and the PEC_{soil} established above (542.9 µg/kg) a risk quotient of 0.44 can be established for plants.

A study, taken from the open literature, Boxall et al (2006)[3], was also provided. The study was not considered further as it contained insufficient detail. The newly performed study was preferred to assess the effect of enrofloxacin on the emergence and growth of terrestrial plants.

2.3.6 Earthworms

An earthworm reproduction test according to OECD guideline 222 was performed.

The test follows the recommended test guideline with no major deviation and was conducted in compliance with GLP. All validity criteria of the guideline were fulfilled. The proposed NOEC values are acceptable and can be used to derive a PNEC value of 100 mg/kg (100,000 µg/kg). Using this PNEC value and the PEC_{soil} established above (542.9 µg/kg) the risk quotient can be concluded to be far below 1 for earthworms.

2.4 Risk characterisation

Risk characterisation is calculated from the risk quotient (RQ), which should be less than 1 in all taxonomic levels to consider that enrofloxacin does not represent a risk to the environment.

It can be concluded that enrofloxacin does not pose a risk to fresh water invertebrates, fish, soil micro-organisms and soil invertebrates. Higher tier modelling has also revealed a negligible risk for ground water contamination.

The CVMP agrees that the applicant has met all the requirements for the plant toxicity evaluation, i.e. new toxicity data and the use of the $PEC_{soil\text{-}plateau}$ based on new degradation studies.

[3] Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levy LS. Uptake of Veterinary Medicines from Soils into Plants. J Agric Food Chem. 2006;54, 2288-97

The CVMP concluded that the provided information on toxicity to cyanobacteria is insufficient for a full risk characterisation, as the information found in the peer reviewed paper by Robinson et al 2005 does not allow for a full assessment of e.g. the validity of data and the exposure concentration. However, it is the view of the CVMP, that a margin of more than 800 between the estimated PEC_{sw} (0.019 µg/l) and the EC50 calculated on the basis of the data in the Robinson et al paper (15.9 µg/l) indicates a very low exposure to cyanobacteria as a result of the use of this product, and gives reassurance that the actual risk to cyanobacteria is acceptable. A study taken from the open literature, Knapp et al (2005)[4], indicated that exposure is very low.

GROUNDS FOR RECOMMENDATION OF THE GRANTING OF A MARKETING AUTHORISATION

A comprehensive set of data needed to conduct an environmental risk assessment according to the VICH and CVMP guidelines was provided. Some of the data are new studies conducted according to the recommended OECD guidelines and in compliance with GLP, those studies were performed following questions raised during the Decentralised Procedure. Other data are taken from peer reviewed articles published in the open literature. From this data set, it is concluded that enrofloxacin, used according to the applicant's indications, does not pose a risk to fresh water invertebrates, fish, soil micro-organism, plants and soil invertebrates. Higher tier modelling has furthermore revealed a negligible risk for ground water contamination.

The CVMP concludes from the new studies provided that the applicant has met the requirements for the plant toxicity evaluation.

The CVMP is of the opinion that the provided information on toxicity to cyanobacteria is insufficient for a full risk characterisation, as the information found in the peer reviewed paper by Robinson et al 2005, does not allow for a full assessment of e.g. the validity of data and the exposure concentration. However, a margin of more than 800 between the estimated PEC_{sw} and the EC50 calculated on the basis of the data in the Robinson et al paper indicates a very low exposure to cyanobacteria as a result of the use of this product, and gives reassurance that the actual risk to cyanobacteria is acceptable.

The CVMP therefore concludes that the objections raised by Germany should not prevent the granting of a Marketing Authorisation and that the dossier as submitted in the referral procedure fulfils the necessary requirements with regards to the risk assessment for cyanobacteria (blue-green algae) and terrestrial plants.

[4] Knapp CW, Cardoza LA, Hawes JN, Wellington EMH, Larive CK, Graham DW. Fate and Effects of Enrofloxacin in Aquatic Systems under Different Light Conditions. Environmental Science & Technology. 2005;39:9140-6.

ANNEX III

SUMMARY OF PRODUCT CHARACTERISTICS, LABELLING AND PACKAGE LEAFLET

The valid Summary of Product Characteristics, labelling and package leaflet are the final versions achieved during the Coordination group procedure.