ANNEX I

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

Member State	Applicant / Marketing Authorisation Holder	Invented name	Pharmaceutical form	Strength	Animal species	Frequency and route of administration
The Netherlands[1]	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Austria[1]	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 1g/g Granulat zur Herstellung	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Belgium	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Bulgaria	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Czech Republic	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Denmark	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Germany	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer

[1] Marketing Authorisation granted

Member State	Applicant / Marketing Authorisation Holder	Invented name	Pharmaceutical form	Strength	Animal species	Frequency and route of administration
Hungary	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Ireland[1]	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Italy	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Tylmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Poland	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Portugal	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Romania	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
Spain	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 1g/g granulado para solución oral	Granules for oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer
United Kingdom[1]	Huvepharma NV Uitbreidingstraat 80 2600 Antwerpen Belgium	Pharmasin 100% W/W Water Soluble Granules	Oral solution	100% w/w	Pigs, broilers, pullets, turkeys and calves	Oral administration through the drinking water, milk or milk-replacer

ANNEX II

SCIENTIFIC CONCLUSIONS AND GROUNDS FOR THE REFUSAL TO GRANT NEW MARKETING AUTHORISATIONS AND FOR THE REVOCATION OF EXISTING MARKETING AUTHORISATIONS

OVERALL SUMMARY OF THE SCIENTIFIC EVALUATION

1. Introduction

Pharmasin 100% W/W Water Soluble Granules and its associated names is presented as water soluble granules containing tylosin tartrate, which is a macrolide antibiotic. The product is a generic of the reference product Tylan W.O and intended to be used as follows:

- In pigs: for treatment and prevention of porcine intestinal adenomatosis (ileitis) associated with *Lawsonia intracellularis* and for the treatment and prevention of enzootic pneumonia caused by *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis;*
- In calves: for the treatment and prevention of pneumonia caused by *Mycoplasma spp;*
- In chickens (broilers, pullets): for the treatment and prevention of chronic respiratory diseases (CRD) caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and for the treatment and prevention of necrotic enteritis caused by *Clostridium perfringens;*
- In turkeys for the treatment and prevention of infectious sinusitis caused by *Mycoplasma gallisepticum.*

During the decentralised procedure concerns were raised that Pharmasin 100% W/W Water Soluble Granules may present a potential serious risk to public health regarding risk to algae and to terrestrial plants. In particular the adequacy of the data provided in respect to the effects on aquatic and terrestrial organisms (Tier A) was questioned as these data do not enable a conclusion to be drawn on the environmental risk assessment.

In its opinion of 10 December 2008 the CVMP considered that the nitrogen transformation study provided was inadequate and that no firm conclusions could be drawn from the study and therefore the test results provided did not exclude a risk for soil microorganisms. Similarly the study with terrestrial plants was considered inadequate and due to the uncertainty regarding the NOECs derived in the study a risk for plants could not be excluded.

In the detailed grounds for the re-examination of the opinion Huvepharma NV argued that the data from the soil nitrification study and the plant growth study are in line with the current guidance and should be considered as conclusive. The decision on the acceptability of the data from these studies was of key importance to the re-examination and so both studies have been re-evaluated critically.

2. Assessment of the environmental risk

Huvepharma NV provided an environmental risk assessment for Pharmasin 100% W/W Water Soluble Granules that followed in general the established guidelines and recommendations. However, the CVMP does not agree with the approach and conclusions in respect to specific parts of the assessment and therefore the overall conclusions on the environmental risk assessment.

The Phase I environmental risk assessment following the VICH guideline results in Predicted Environmental Concentrations (PECs) in soil for Pharmasin 100% W/W Water Soluble Granules for all target species above 100 µg/kg, thus requiring a Phase II assessment. The $PECs_{soil}$ are: calves: 3199 µg/kg; pigs: 1738 µg/kg; broilers: 4435 µg/kg and turkeys 2210 µg/kg.

The aspects of the Phase II assessment considered in this referral are the effects on the terrestrial compartment and the effects on the aquatic compartment.

2.1 Risk to terrestrial compartment

2.1.1 Effects on soil invertebrates

The assessment of the effects of Pharmasin 100% W/W Water Soluble Granules on soil invertebrates was based on a published study. The experiments cover the effects of tylosin at

concentrations between 0 and 5000 mg/kg soil (dry weight) on collembolans, earthworms and enchytraeids but did not use standardised methods.

Based on the lowest reported EC10 of 149 mg/kg (dry weight) and applying an assessment factor (AF) of 10 a PNEC of 14.9 mg/kg for soil invertebrates can be derived.

The resulting PEC/PNEC ratios are below 1 for all four target animal species, therefore it can be concluded that the risk for soil invertebrates is low.

2.1.2 Effect on nitrate production by soil microorganisms

A soil nitrogen transformation study was provided; the study was conducted based on OECD Guideline 216, however not according to GLP specifications and with some modifications to the test protocol. The reporting and analysis of the results did not follow the OECD guideline. The study was performed with concentrations of tylosin in soil of 7264 μg/kg, which was derived from the maximum PEC of tylosin in soil arising from the use of other Pharmasin products, and at 5 times, 10 times, 25 times and 125 times this maximum PEC value.

The study is considered to suffer from a number of deviations described below.

For the soil nitrification study to be valid the difference between control replicates should be less than ±15%. The study does not fully comply with this criterion as the differences between 2 replicates of control soil at day 14 were above 15%.

There were some deviations from the OECD guideline: the biomass in the test soil was lower than required at day 0; it was not clear if the soil had been stored for more than 3 months before the study was carried out; the maximum water holding capacity was not provided so it was not possible to conclude if the soil complied with the requirements for moisture content during the study. These deviations from the OECD guideline may have contributed to the high standard deviations seen in nitrate production at each of the time points and concentrations tested.

The rate of nitrate production was not provided as required by the OECD guideline; instead the concentration of nitrate at each time point was given. At a later date data were presented in a form that enabled the comparison of nitrate production between control and treatment soils.

There was very low nitrate production in the control soil between days 0 and 7, but this phenomenon was not observed at later time points. The choice of 7264 μg/kg as the lowest PEC can be criticised as it is 1.6 times greater than the maximum PEC_{soil} produced by use of this product, but the choice was justified in the report as a conservative approach.

The difference in nitrate production between control and the lowest (1X) PEC at 28 days is 23% which is only just below the 25% maximum allowed by the OECD guideline. Given all the problems with the study recorded above, the fact that the value approaches the maximum difference allowed is a cause for concern.

After careful evaluation of all the information it is considered that due to the inadequacy of the data provided no firm conclusions can be drawn from this study. The results of the study cannot be used in the environmental risk assessment.

2.1.3 Effect on emergence and growth of plants

A plant toxicity study based on OECD Guideline 208 but not conducted under GLP was provided. The study in five plant species, one monocotyledon (wheat) and four dicotyledons (courgette, French bean, radish and sunflower), was performed to assess the effects of tylosin tartrate, at concentrations of multiples of the maximum PEC for other Pharmasin products calculated at 7264.2 μg/kg, on seedling emergence and early growth of higher plants following exposure to the test substance in a sandy loam soil. Only for courgette was one concentration below this PEC (0.25 PEC).

Some serious deficiencies with the plant study were identified. As germination (emergence) was expressed as percentage of the control in the report it is not possible to conclude that the criteria that seedling emergence is at least 70% was met. The seedlings should not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants should exhibit only normal variation in growth and morphology for that particular species. Partial chlorosis, extensive chlorosis and extensive chlorosis with leaf wilt was seen with all plant species in at least one test concentration, however information on phytotoxicity was not presented for the controls, so it was concluded that this criterion was not met. As the results of the control seedlings were not reported it is not possible to conclude if the mean survival of emerged control seedlings is at least 90% for the duration of the study as required by the OECD guidance. In the main study the test on wheat, radish and French bean was carried out 3 weeks before the test on courgette and sunflower. No detailed records of greenhouse conditions except temperature are provided in the report so it is impossible to conclude if environmental conditions were identical. As the tests were carried out 3 weeks apart it is unlikely that conditions were the same.

Many of the failings described above arise from the poor reporting of the study which does not follow the requirements of the OECD guideline and may be a consequence of using a non-GLP compliant laboratory. The key omission in the report is the absence of tables for all endpoints determined in the study, for example the numbers of seeds germinating and the biomass (growth) is not reported for the control groups of any of the plants tested.

Based on the information presented it is not possible to tell if two of the criteria, seedling emergence of at least 70% and mean survival of emerged control seedlings of at least 90% for the duration of the study, were met. The other two acceptability criteria, seedlings not exhibiting visible phytotoxicity and identical environmental conditions were not met. From the information provided it cannot be shown that any of the required acceptance criteria for this type of study have been met.

The plant study did not meet the requirements of the VICH Phase II guideline in respect of the assessment of the risk for plants. The first main experiment was carried out on wheat, radish and French bean. This part of the experiment can be considered the Tier A study on three species. In order to follow VICH guidance when proceeding to Tier B the most sensitive species from the above experiment, i.e. radish, should have been retested together with two additional species from the same category. This was not the case. Instead a second main experiment was begun using only two additional species, courgette and sunflower. This experiment began three weeks later than the first experiment. As a result it is considered that the design of the second experiment is seriously flawed as radish should have been included in this experiment. The requirements of the VICH guidelines were not fulfilled.

The lowest EC50 determined in the study was for radish, and was 149.9 mg/kg, and the lowest NOEC and LOEC values were determined for courgette at 45.4 mg/kg and 90.8 mg/kg, respectively.

Huvepharma NV provided the statistical analysis for the proposed LOECs and NOECs for the three species (courgette, radish and sunflower). No justification for omitting to establish LOECs and NOECs for wheat and French bean was provided. Huvepharma NV insisted on the use of the study NOECs and an AF of 10 for a tier B refinement. However, the proposed NOEC cannot be accepted as information on the statistical analysis was presented for only three species. Therefore, a PNEC of 1.499 mg/kg was established, based on the lowest EC50 of 149.9 mg/kg applying an AF of 100. Tier B refinement is therefore not feasible.

The resulting PEC/PNEC ratios are 1.16 (pigs), 2.986 (broilers), 1.47 (turkeys) and 2.13 (calves), i.e. resulting in a risk quotient (RQ) above 1 indicating a potential risk.

Huvepharma NV also proposed to refine the risk assessment for plants by considering the degradation during litter storage. A new study and several published studies were provided which investigate the degradation of tylosin in cattle, pigs and poultry manure and soil/poultry mixtures. However, what was shown in the degradation study was that tylosin A could not be detected. This may have been because of degradation of the substance, but could be due equally to the formation of bound residues which could become available in the soil.

A study in turkey litter describes a rapid dissipation of tylosin under aerobic and sterile conditions with a half-live (DT_{50}) of 4.9 days. Other degradation studies in manure suggest a significant role for biodegradation, resulting in differences between sterile and non-sterile conditions, which are not observed in this particular study. The DT_{50} in manure reported in literature and the dissipation study were largely similar. The DT_{50} value of 7.6 days for chicken excreta can be accepted as a realistic conservative value. However, based on the information provided on degradation in manure, the CVMP considers that the total residue approach cannot be abandoned for tylosin.

Even with the proposed refinement for degradation in manure, RQs higher than 1 are obtained when the effect assessment for plants is based on the EC50 with an AF of 100.

The risk for plants cannot be determined based on the submitted data.

The results of the study cannot be considered reliable and they cannot be used in the environmental risk assessment.

2.1.4 Summary of risk assessment to the terrestrial compartment

The data provided did not indicate a risk regarding soil invertebrates.

Due to the unreliability of the data provided on the effects of tylosin on soil nitrification no conclusion can be drawn on the effects of Pharmasin 100% W/W Water Soluble Granules on soil microorganisms. A new study would be required with assessment of nitrate production continuing until after day 28 in order to draw a conclusion on the risk for soil microorganisms.

Due to the failure of the study on plant emergence and growth to satisfy any of the validity criteria for such a study no PNECs are available for plants and hence no conclusion can be drawn on the effects of Pharmasin 100% W/W Water Soluble Granules on terrestrial plants. A new study would be necessary in order to draw a conclusion on the risk to plants.

At present a risk for the terrestrial compartment cannot be excluded.

2.2 Risk to aquatic organisms

2.2.1 Prediction of concentrations in surface water

Concentrations of tylosin in surface water and groundwater were estimated using methods described in the CVMP Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products (EMEA/CVMP/ERA/418282/2005). $PEC_{groundwater}$ values for pigs, broilers, turkeys and calves were calculated as 6.43, 16.4, 8.17 and 11.8 µg/l respectively. $PEC_{surfacewater}$ values for the same species were calculated as 2.14, 5.47, 2.72 and 3.93 µg/l respectively.

These PECs were considered acceptable.

2.2.2 Effects on algae (cyanobacteria)

A study performed to assess the effect of tylosin tartrate on the growth of the freshwater cyanobacteria *Anabaena flos-aquae* was provided. The study was performed in line with OECD guideline 201. *Anabaena flos-aquae* was exposed to different concentrations of an aqueous solution of tylosin tartrate for 72 hours. Test concentrations were seen to decline over the test period and consequently it was considered justifiable to base the results on the geometric mean of the measured test concentrations. EC50 values based on the geometric mean of test concentration

were 1.5 mg/l for inhibition of growth rate and 0.42 mg/l for inhibition of yield. Based on this EC50 value a PNEC of 4.2 µg/l was calculated (PNEC equal to EC50/100); the assessment factor of 100 is in line with the VICH GL38 guideline.

A previously reported endpoint for *Microcystis aeruginosa* was not used in the assessment as it was not in line with the relevant OECD guideline 201.

Using the above PNEC value and the estimated $PEC_{surfacewater}$ values, PEC/PNEC ratios derived for pigs, turkeys and calves were all below 1. However, the PEC/PNEC ratio derived using the PEC for broilers (5.47 µg/l) was 1.30, indicating a risk of toxicity for cyanobacteria (blue-green algae).

Due to this apparent environmental risk Huvepharma NV undertook further investigations with the aim of refining the PEC estimates, using higher tier modelling and manure degradation data.

Pharmasin 100% W/W Water Soluble Granules was assessed using the models developed by Forum for Co-ordination of Pesticide Models and their Use (FOCUS) as recommended in the CVMP Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products (EMEA/CVMP/ERA/418282/2005). For the majority of climate and water body scenarios investigated, tylosin concentrations were below 0.001 µg/l. The maximum predicted surface water concentration was 4.44 µg/l.

A comparison of the surface water concentrations obtained using the FOCUS models, with the established PNEC (4.2 µg/l) demonstrated that, for all but one of the scenarios investigated, predicted concentrations of tylosin were below the predicted no effect concentrations. However, using the maximum predicted surface water concentration for Pharmasin 100% W/W Water Soluble Granules (4.44 µg/l), the PEC/PNEC ratio was 1.1.

On the basis that the concentrations estimated using FOCUS modelling represent peak values and so overestimate the risks, Huvepharma NV calculated the time weighted average concentration (TWA PEC) and recalculated the PEC/PNEC ratio for the scenario where a risk had been indicated. The TWA PEC was calculated over 3 days as this corresponds to the duration of the blue-green algae (cyanobacteria) study. Using this approach Huvepharma NV concluded that the maximum predicted surface water concentration could be revised from 4.44 µg/l to 1.77 µg/l, leading to a TWA PEC/PNEC ratio of 0.42, indicating according to the applicant that Pharmasin 100% W/W Water Soluble Granules does not pose a risk for blue-green algae (cyanobacteria).

Huvepharma NV also considered that a further refinement could be made to account for degradation during manure storage, as discussed above in relation to phytotoxicity. This would have the effect of reducing the TWA PEC from 1.77 µg/l to 0.40 µg/l, and lead to a PEC/PNEC ratio of 0.10.

However, the CVMP considered that there are concerns regarding the refinements made to the PECs generated using FOCUS modelling; in particular on the use of the time weighted averages in relation to the acute cyanobacteria study and the use of a DT_{50} value of 19.15 days in the FOCUS models when the geometric mean DT_{50} presented by Huvepharma NV was 51 days. In addition, the refinement for the degradation in manure cannot be accepted and consequently, based on the available data, the Committee did not consider it appropriate to abandon the total residue approach in the assessment of Pharmasin 100% W/W Water Soluble Granules.

The Committee also noted that, with regard to the cyanobacteria (blue-green algae) study, a refinement following Tier IIB recommendations, using the study NOEC with an AF of 10, would represent a more logical approach.

2.2.3 Effects on aquatic invertebrates

Effects on *Daphnia magna* were determined based on data from published literature. The EC50 value for 48 hour survival of *Daphnia magna* exposed to tylosin tartrate was 680 mg/l. The PNEC

(EC50/1000) was therefore concluded to be 680 µg/l; the assessment factor of 1000 is in line with the VICH GL38 guideline.

Using this data and the estimated $PEC_{surface\ water}$ values, all PEC/PNEC ratios were below 1, indicating a low risk for aquatic invertebrates.

2.2.4 Effects on fish

A study performed to assess the acute toxicity of tylosin tartrate to rainbow trout (*Oncorhynchus mykiss*) was provided. The study was performed in line with OECD guideline 203. Test fish were exposed to an aqueous solution of tylosin tartrate at a single concentration of 100 mg/l for a period of 96 hours at a temperature of approximately 14 ºC under semi-static test conditions. The 96 hour LC50 based on nominal test concentration was greater than 100 mg/l and correspondingly the NOEC was 100 mg/l. The EC50 from this study was concluded to be greater than 100 mg/l and PNEC (EC50/1000) was concluded to be greater than 100 µg/l; the assessment factor of 1000 is in line with the VICH GL38 guideline.

Using the data from the above study and the estimated $PEC_{surface\ water}$ values, all PEC/PNEC ratios were below 1, indicating a low risk for fish.

2.2.5 Summary of risk assessment for aquatic organisms

A low risk for fish and aquatic invertebrates is expected. However, based on the information provided a risk for cyanobacteria cannot be ruled out. Without a tier II-B assessment, no further conclusions with regards to the risk for cyanobacteria can be drawn.

3. Risk mitigation measures

The consideration of restricting the SPC by removing either indications alone or target species and indications is currently inappropriate as a conclusive assessment of the risk to the terrestrial and aquatic compartments cannot be carried out for the reasons given above. The absence of reliable effects data for plants and microorganisms means that reducing exposure, i.e. lowering the PEC_{soil} by restricting indications and species does not alter the potential for an unacceptable risk.

GROUNDS FOR REFUSAL AND REVOCATION OF THE MARKETING AUTHORISATIONS

Considering that:

- no conclusion on long term influence on nitrogen transformation by soil microbes can be drawn;
- no conclusion on the effects on terrestrial plants can be drawn;
- a risk for cyanobacteria cannot be excluded on the basis of the data available;
- restricting the SPC by removing either indications alone or target species and indications is currently inappropriate as a conclusive assessment of the risk to the terrestrial and aquatic compartments cannot be carried out;

Therefore, the CVMP is of the opinion that the application for Pharmasin 100% W/W Water Soluble Granules and associated names does not satisfy the criteria for authorisation in respect of environmental risk as established in Article 12(3)(j) of Directive 2001/82/EC, as amended by Directive 2004/28/EC.