27 October 2010
EMA/CVMP/529651/2009
Veterinary Medicines and Product Data Management

Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Derquantel (ovine species)

On 8 October 2010 the European Commission adopted a Regulation[1] establishing maximum residue limits for derquantel in ovine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Derquantel is intended for oral administration in ovine species for the treatment and control of gastrointestinal parasites.

Pfizer Limited submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 5 June 2009.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 19 May 2010 the establishment of maximum residue limits for derquantel in ovine species .

Subsequently the Commission recommended on 6 August 2010 that maximum residue limits in ovine species are established. This recommendation was confirmed on 27 August 2010 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 8 October 2010.

[1] Commission Regulation (EU) No 890/2010,O.J. L266, of 09.10.2010.

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Derquantel
Therapeutic class:	Antiparasitic agents/Agents against endoparasites
Procedure number:	EU/09/168/PFZ
Applicant:	Pfizer Limited
Target species:	Ovine
Intended therapeutic indication:	Treatment and control of gastrointestinal parasites
Route(s) of administration:	Oral use

1. Introduction

Derquantel (2-desoxoparaherquamide, CAS# 187865-22-1) is a semi-synthetic anthelmintic, intended for use in sheep for the treatment and control of gastrointestinal parasites at a single oral dose of 2 mg/kg body weight.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Derquantel is active against parasitic nematodes, its mode of action is blockade of cholinergic neuromuscular transmission.

No stand-alone neurotoxicity studies were conducted, but in several semi-chronic toxicity studies special tests for neurotoxicity were included. A limited study was performed to characterize the antagonistic properties of derquantel (there are many more varieties of nicotinic acetylcholine receptors (nAChR) known). From this study it appears that dog is more sensitive than rat to the neurotoxic properties of derquantel due to differences in nAChRs.

Derquantel given orally to rats showed peak plasma levels at 0.5-4 hours after administration. The C_{max} and AUC were higher in females than in males. These differences were seen consistently in all studies and were most pronounced at lower dosages. In dogs, oral administration of derquantel showed peak plasma levels at approximately 0.5 hours. No differences between males and females were noted for C_{max} and AUC. The $AUC_{0\text{-}24h}$ and C_{max} increased at a rate greater than proportional with dose, in particular in the higher dose ranges. The $t_{1/2}$ in dogs was in the range of 3 to 4 hours.

2.1.2. Calculation of pharmacological ADI, if relevant

The primary and secondary pharmacological effects are expected to be related to neurotoxic responses. Because these responses are extensively studied in the repeated dose toxicity studies and hence included in the establishment of the toxicological ADI, it is not deemed necessary to derive a separate pharmacological ADI.

2.1.3. Overview of toxicology

Single-dose toxicity

After oral administration, no mortality was seen in rats up to doses of 1000 mg/kg bw. Clinical signs in rats such as jerky movements, piloerection, and reduced somatomotor activity were noted at single doses of 350 mg/kg bw and higher. The clinical signs observed are indicative of effects on the nervous system.

Mice were more sensitive than rats to the acute neurotoxic properties of derquantel; signs like jerky movements were noted in mice dosed at the lowest level tested (12.5 mg/kg bw). However it is plausible that the vehicle contributed to the toxicity.

Acute effects noted in the preliminary acute toxicity dog study included injection of sclera, mydriasis, ptosis and relaxed nictitating membranes in all dose groups (5 mg/kg bw and higher). In a neurobehavioural study in a limited number of dogs acute effects noted at the lowest dose level of 1 mg/kg bw included sclera injection, partial/slow constriction of the pupils, slightly drooping eyelids, and a dose related increase in heart rate.

The horse is very sensitive to derquantel. Doses of 20 and 2 mg/kg bw once orally were lethal in studies (2 horses per study).

Derquantel was non toxic by the dermal route of administration in rats.

Repeated dose toxicity

Repeated dose oral toxicity was extensively studied in rats (studies with durations of 3, 12, 28, 90, and 365 days) and in dogs (studies with durations of 28 and 90 days).

Two 28-day oral toxicity studies in rats (doses in the range of 0.5 to 200 mg/kg bw/day) showed effects on the liver, thyroid gland, and on behaviour (CNS). The NOEL was 0.5 mg/kg bw/day, based on decreased rearing activity and thyroid follicular hypertrophy.

In a 90-day oral toxicity study, rats were given derquantel at doses of 0, 1, 5, 50, and 150 mg/kg bw/day. The histopathological findings in this study (in liver in particular) were of limited value because of the poor quality of the slides. Other main findings in this study were CNS effects. Palpebral closure was observed at all dose levels, therefore the lowest dose of 1 mg/kg bw/day was considered a LOAEL.

Two 1-year oral toxicity studies in rats were performed. In contrast to the 28- and 90-day studies, these studies did not include behavioural assessments. In the first study, rats were given derquantel at doses of 0, 1, 5, and 50 mg/kg bw/day. Several effects were observed at the highest dose, including lower body weights, lower thyroid weights, higher liver and kidney weights, hypospermia, and cataracts. The most pronounced effect was bile duct hyperplasia, observed in a dose-related fashion in both sexes, starting at the lowest dose of 1 mg/kg bw/day. This effect was in many cases accompanied by periportal and periductal fibrosis or infiltration of mononuclear cells. Based on biliary hyperplasia, the LOAEL in this study was 1 mg/kg bw/day.

In the second 1-year oral toxicity study, rats were given derquantel at doses of 0, 0.01, 0.03, 0.1 and 0.3 mg/kg bw/day. This dose range is much lower than in the first study. Histopathology revealed that biliary hyperplasia was present in the control group. However, in males there seemed to be a trend of increased incidence in the exposure groups. Within the exposed groups the incidence did not increase with increasing dose in the males, but the severity did. The differences with controls were not statistically significant. In females both the incidence and severity seemed to be comparable with the control group. Although biliary hyperplasia can be found spontaneously in ageing rats, the totality of

repeated dose studies in rats showed that this effect has a clear dose-effect relationship in males and females and is accompanied by fibrosis. Moreover, derquantel induced this effect also in immature rats (28-day study). Therefore, biliary hyperplasia is considered an adverse effect, relevant for the consumer risk assessment. However, the occurrence of biliary hyperplasia in control groups makes it difficult to conclude on the significance of the finding. Historical control data on biliary hyperplasia from the laboratory that performed the studies showed that the observed effects in control groups were well within historical ranges. An analysis of the pooled biliary hyperplasia data from the two 1-year studies was performed and subjected to different dose-response models for the derivation of a benchmark dose (BMD). Most suitable models gave $BMDL_{10}$'s (the lower bound of the confidence interval for a benchmark dose representative of 10% extra risk compared to placebo) in the range of 2-6 mg/kg bw/day, but one equally suitable model gave a BMDL 4 orders of magnitude lower. This difference might indicate that the data are not suitable for use with the BMD approach. In conclusion, the two studies revealed a NOAEL for biliary hyperplasia of 5 mg/kg bw/day based on statistical analyis, whereas the BMD approach was inconclusive.

A 28-day oral toxicity study in dogs with derquantel doses in the range of 0.01 to 0.1 mg/kg bw/day showed no behavioural or neurological effects. No necropsy was performed.

Two 90-day oral toxicity studies were performed in dogs. In the first study doses of 0, 0.1, 0.5, 1 and 10 mg/kg bw/day were used, in the second and pivotal study the doses were 0, 0.1, 0.5, 1 and 5 mg/kg bw/day. Derquantel did not induce histopathological changes. The most prominent effect was protruding nictitating membranes at 5 mg/kg bw/day and higher. The NOAEL was 0.1 mg/kg bw/day.

The NOAEL of 0.1 mg/kg bw/day established in the 90-day dog studies was considered sufficiently low to address any concerns relating to the effects on the bile ducts observed in rats.

Reproductive toxicity, including developmental toxicity

In a two-generation reproductive toxicity test, rats were given derquantel at doses of 0, 1, 5 and 25 mg/kg bw/day. The lowest dose produced increased thyroid weights in the P1 and F1 females, therefore no NOAEL was established. The substance did not produce any effects on reproduction at the dose levels tested.

Developmental toxicity was studied in rats at derquantel doses of 0, 20, 70 and 120 mg/kg bw/day. Maternal toxicity was evidenced by clinical signs and reduced feed intake at 70 mg/kg bw/day. Fetal development was retarded at 120 mg/kg bw/day (lower weights and delayed ossification). There were no teratogenic effects.

Developmental toxicity was also studied in rabbits at derquantel doses of 0, 0.1, 1 and 10 mg/kg bw/day. Maternal toxicity was observed at 10 mg/kg bw/day (mortality and decreased bodyweight). As a consequence, live fetal bodyweight was also reduced at the highest dose. Some skeletal variations were observed at the highest dose. The NOAEL for maternal and developmental toxicity was 1 mg/kg bw/day. Derquantel is not considered to be teratogenic.

Genotoxicity

The genotoxic properties of derquantel were tested in an *in vitro* test for gene mutations in bacteria (Ames test), an *in vitro* test for chromosomal aberrations in mammalian cells, and two *in vivo* tests for chromosomal aberrations in mice.

In relation to the gene mutation test, a number of deviations from OECD guideline 471 were noted (eg, 2-amino anthracene was used as the sole indicator of efficacy of the S9-mix and concurrent strain specific positive controls for the assessment of assay performance without metabolic activation were not used). Nevertheless, the results indicate that derquantel is not mutagenic in bacteria.

In human peripheral lymphocytes exposed to derquantel *in vitro*, increased chromosomal aberrations were observed. The substance is therefore considered clastogenic *in vitro*.

A bone marrow micronucleus test in mice (single derquantel doses up to 200 mg/kg bw, by gavage) was negative. Although the highest dose produced toxicity, the polychromatic/normochramatic erythrocyte (PCE/NCE) ratio was unchanged, therefore it is not certain that the bone marrow was actually exposed to the substance.

A second micronucleus test was performed in rats (doses up to 200 mg/kg bw/day for 4 days by gavage), but in this study micronuclei in livers were examined. The test was negative. As liver is a target organ for toxicity, it is evident that the liver cells were adequately exposed. It can be concluded that the substance is not clastogenic *in vivo*.

Overall, derquantel is not considered to possess genotoxic potential *in vivo*.

Carcinogenicity

No studies on carcinogenicity were performed. However, based on the fact that the substance is not considered to be genotoxic *in vivo* and has no structural alerts, no carcinogenicity studies are required.

2.1.4. Calculation of the toxicological ADI or alternative limit

Using the overall NOAEL of 0.1 mg/kg bw/day, based on the effects in the 90-day oral toxicity studies in dogs (protruding nictitating membrane observed at 0.5 mg/kg bw/day), and applying an assessment factor of 100, an ADI of 0.001 mg/kg bw was established, equivalent to 60 µg/person.

2.1.5. Overview of microbiological properties of residues

The substance has no antimicrobial activity.

2.1.6. Calculation of microbiological ADI

As the substance has no antimicrobial activity the establishment of a microbiological ADI is not relevant.

2.1.7. Observations in humans

No relevant data are available.

2.1.8. Findings of EU or international scientific bodies

Not available.

2.1.9. Overall conclusions on the ADI

The toxicological ADI of 0.001 mg/kg bw (60 µg/person) was established as the overall ADI.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

Derquantel is well absorbed, with an oral bioavailability of about 56% and a maximum plasma concentration reached about 4 hours after oral administration. From intravenous dosing studies it can be concluded that derquantel is widely distributed in the body with a volume of distribution at steady

state (Vss) of 3220 ml/kg; the clearance (CL) of derquantel is moderate, 11.1 ml/kg/min. From oral studies (using derquantel as well as the final formulation) it can be concluded that the C_{max} varied between 92.8 and 108 ng/ml; the t_{max} varied between 2.60 and 4.17 hours; and the $t_{1/2}$ varied between 9.3 and 13.1 hours (5.7 hours after intravenous administration). Based on the pharmacokinetic data and the intended use of derquantel bioaccumulation is not expected.

After oral administration, the majority of derquantel was eliminated in the faeces and a smaller part in the urine. By 48 hours 78.3% and 9.6% of the total dose was excreted in faeces and urine (plus cage rinse) respectively. After 6 days, the last measured time point, the total radioactive residue in faeces and urine (plus cage rinse) accounted for 85% and 6.7% of the dose administered, respectively.

It was also shown that the pharmacokinetic parameters of derquantel are not significantly affected when co-administered with abamectin.

In vitro metabolism studies of [^{14}C]-2-desoxoparaherquamide in rat, sheep, dog and human liver hepatocytes demonstrate that derquantel is subject to extensive metabolism in all species (hepatocytes) with human hepatocytes displaying the slowest metabolism. The similarity of the metabolic profiles between the rat and sheep confirm that the rat is an appropriate species for toxicological testing. Also the metabolites formed in human microsomes are covered by the metabolites formed and tested (by auto-exposure) in rat. Overall, the major metabolites found for sheep in this hepatocyte metabolism study are comparable to those found in the microsome metabolism study.

The metabolism of derquantel is extensive and complex. Derquantel undergoes biotransformation to a large number of metabolites over a short time period. As a result qualitative and quantitative variation in metabolites is found over tissues and time periods, and isolation and identification of the metabolites proved not to be possible. Derquantel comprised only a small percentage of the total residues in tissue extracts.

2.2.2. Residue depletion studies

In a residue study in the target species using a formulation of derquantel and abamectin in combination at a dose of 3 mg derquantel/kg bw + 0.3 mg abamectin/kg bw (which equates to 1.5 times the proposed dose) and including 6 animals per time point and several slaughter time points, highest concentrations of derquantel were found in fat, followed by liver, kidney and muscle. A rapid decline in derquantel levels was observed up to 6 days after product administration, after which levels in liver remained measurable above the limit of quantification of 0.1 µg/kg up to 35 days.

The inability to isolate and identify the metabolites of derquantel led to the decision to propose the parent compound as the marker residue for purposes of residue surveillance and withdrawal period determination. It is recognized that derquantel only comprises a small part of the residues.

In a study in the target species, performed according to GLP, radiolabelled derquantel was administered orally at a dose of 2.1 mg/kg bw (approximately the intended dose). Liver was found to be the target tissue, with the majority of derquantel being eliminated via the faeces and a smaller part via the urine, as also observed in the other studies. The marker to total residues ratio was calculated by comparing the parent derquantel amounts calculated from the distribution percentages of tissue extracts to the total recovered radioactivity in the tissue samples. The tissues collected in this study were used for metabolic profiling after extraction with acetonitrile. Sample storage at -20 °C caused some degradation of the parent compound. This was addressed by the inclusion of a correction factor in the calculation of the marker to total ratios, with the following values established: 0.25 in muscle, 0.24 in fat, 0.06 in liver, and 0.2 in kidney.

2.2.3. Monitoring or exposure data

An analytical LC-MS/MS method has been developed and validated. The method is considered appropriate for the determination of derquantel in ovine tissues. The method is presented in an internationally recognised format and has been validated in line with the current requirements of Volume 8 of The Rules Governing Medicinal Products in the European Union.

The detection is based on mass spectrometry (MS/MS technique) which is specific with regard to possible interferences of tissue matrix or interferences of other commonly used veterinary drugs.

The data showed that the method is acceptable for derquantel in ovine tissue in the concentration range 1 – 500 µg/kg, with limits of detection between 0.04 and 0.07 µg derquantel/kg and a limit of quantification of 1 µg derquantel/kg.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

No data were provided but as microbiological effects are not expected for this type of substance such data are not required.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

No data were provided but none are expected for this type of substance.

3.3. Elaboration of MRLs

Based on the tissue distribution of the marker residue derquantel, the following MRLs were elaborated:

Muscle: 2 µg/kg

Fat: 40 µg/kg

Liver: 20 µg/kg

Kidney: 5 µg/kg

Calculation of theoretical daily intake of residues

The theoretical maximum daily intake was calculated as follows:

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product (µg)
Muscle	0.30	2	0.25	2.4
Fat	0.05	40	0.24	8.3
Liver	0.10	20	0.06	33.3
Kidney	0.05	5	0.2	1.3

Based on these values, the theoretical maximum daily intake is 45.3 µg, i.e. 76% of the ADI. Detailed calculation of theoretical daily intake of residues- table to be included, if applicable

3.4. Considerations on possible extrapolation of MRLs

In line with the CVMP Note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMEA/CVMP/187/00-Final) the MRLs for ovine tissues could be extrapolated to tissues from caprine species provided that it can be confirmed that the proposed analytical method would be applicable and the marker residue is confirmed. Such data were however not provided.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- an ADI of 0.001 mg/kg bw was established, equivalent to 60 µg for a 60 kg person;
- fat and liver were the main target tissues for derquantel derived residues in sheep, with the highest concentration of the proposed marker residue, derquantel, found in fat, followed by liver, then kidney and then muscle;
- although derquantel, the parent compound, represents only a small part of the total residues, it was established as the marker residue in sheep;
- ratios of marker to total residues were 0.25 in muscle, 0.24 in fat, 0.06 in liver, and 0.20 in kidney;
- an analytical procedure for the determination of derquantel in edible ovine tissues (liver, kidney, muscle and fat) is available and has been validated according to current requirements of Volume 8 and may be used for monitoring purposes;

the Committee recommends the establishment of maximum residue limits for derquantel in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Derquantel	derquantel	ovine	2 µg/kg 40 µg/kg 20 µg/kg 5 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Antiparasitic agents/Agents against endoparasites

4. Background information on the procedure

Submission of the dossier

Steps taken for assessment of the substance

Application validated:	18 June 2009
Clock started:	19 June 2009
List of questions adopted:	16 September 2009
Consolidated response to list of questions submitted:	20 April 2010
Clock re-started:	21 April 2010
CVMP opinion adopted:	19 May 2010