LEVAVETO 750 mg/g powder for use in drinking water for pigs	BE/V/0034/001/MR
V.M.D. v.n.	MRP
	Publicly available assessment report

FEDERAL AGENCY FOR MEDICINES AND HEALTH PRODUCTS

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(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

LEVAVETO 750 mg/g powder for use in drinking water for pigs

Date created: September 2019

MODULE 1

PRODUCT SUMMARY

EU Procedure number	BE/V/0034/001/MR
Name, strength and pharmaceutical form	LEVAVETO 750mg/g powder for use in drinking water for pigs
Applicant	V.M.D. n.v. Hoge Mauw 900 2370 Arendonk Belgium
Active substance(s)	Levamisole hydrochloride
ATC Vetcode	Anthelmintics, imidazothiazoles ATC vet code: QP52AE01
Target species	Pigs
Indication for use	For the treatment of infections by *Ascaris suum* (L3, L4, L5 and adult stage).

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MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Veterinary Medicines Agencies website (http://www.HMA.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Well established veterinary use in accordance with Article 13a of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	03/05/2018
Date product first authorised in the Reference Member State (MRP only)	14 June 2010
Concerned Member States for original procedure	Germany, Estonia, France, Hungary, Lithuania, Latvia, The Netherlands, Poland and Romania

I. SCIENTIFIC OVERVIEW

The product is indicated in pigs for the treatment of infections by *Ascaris suum* (L3, L4, L5 and adult stage), with a recommended dose of 10 mg levamisole/kg bodyweight, administered on a single occasion.

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product released on the market.
It has been shown that the product can be safely used in the target species; the tolerance profile is adequately reflected in the SPC.
The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.
The efficacy of the product was demonstrated according to the claims made in the SPC.
The overall risk/benefit balance was assessed as positive.

II. QUALITY ASPECTS

A. Qualitative and quantitative particulars

The product contains 884 mg levamisole hydrochloride per gram powder, which amounts to 750 mg levamisole (base) as active substance. The product contains also the following excipients: Colloidal silica anhydrous and lactose monohydrate.

The container/closure systems are:

- multilayer bag of polyester (outer layer) – polyethylene low density/aluminium/ polyethylene low density – polyethylene low density (inner layer).

- multilayer bag of polyester (outer layer) – aluminium – polyethylene low density (inner layer).

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines>.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice at a licensed manufacturing site.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

The active substance is levamisole hydrochloride, an established active substance described in the European Pharmacopoeia. The active substance is supplied by two manufacturers for which the QP declarations - manufacture in accordance with the principles of good manufacturing practice - (Annex 5.19 of Part I), are present.

The active substance specification is considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided.

Certificates of suitability issued by the EDQM have been provided for the two suppliers and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

D. Control on intermediate products

Not applicable

E. Control Tests on the Finished Product

The finished product specification controls the relevant parameters for the pharmaceutical form. The tests in the specification, and their limits, have been justified and are considered appropriate to adequately control the quality of the product.

Satisfactory validation data for the analytical methods have been provided.

Batch analytical data from the proposed production site have been provided demonstrating compliance with the specification.

F. Stability

Stability data on the active substance have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions.

Conclusions on the shelf life for this products are:

- Shelf life of the veterinary medicinal product as packaged for sale: 3 years.
- Shelf life after first opening the immediate packaging: 6 months.
- Shelf life after dilution or reconstitution according to directions: 24 hours.

The claim of a 6 months stability after broaching is based on the demonstration of stability for a batch broached and stored 6 months at 25°C.

III. SAFETY AND RESIDUES ASSESSMENT (PHARMACO-TOXICOLOGICAL)

III.A Safety Testing

Pharmacological Studies

The applicant has provided bibliographical data which show that levamisole binds to acetylcholine receptors of the nicotinic type, which results in an increase in the permeability of the post-synaptic membrane for small cations and in prolonged depolarization. It acts principally at the level of the helminth somatic muscle cells, where it causes uncontrolled neuromuscular stimulation, leading to spastic paralysis of the parasite.

Levamisole was also found to be a potent inhibitor of fumarate reductase in several immature and adult nematodes, both *in vitro* and *in vivo*; it could thus interfere with the parasite's energy metabolism.

Levamisole shows activity against a broad range of gastro-intestinal and lung nematodes, adults and immature.

The applicant has provided bibliographical data which describe the general pharmacokinetic properties of levamisole in different animal species. These show that levamisole is readily absorbed by the oral route. After extensive metabolisation in the liver it is mostly eliminated within a few days. The main part of the dose is eliminated in urine, but a significant part is also eliminated via the bile.

Toxicological Studies

- Single Dose Toxicity

The applicant has provided bibliographical data which show that levamisole toxicity relates to its activity as a nicotinic agonist. The acute toxicological profile is essentially described through signs reported in cases of intoxication in several species, and through a series of reported LD50 in laboratory animals. No reference NOEL or LOEL value is presented. Acute oral toxicity of levamisole is moderate in laboratory rabbits and rodents, the lowest LD50 recorded being in the order of 200 mg/kg by the oral route, 80 mg/kg by the subcutaneous

route, and 20 mg/kg by the IV route. Two LD50 values are available for dermal acute toxicity: 252 mg/kg in rats and >2000 mg/kg in rabbits.

- Repeated Dose Toxicity

The effects of long-term administration of levamisole, by the oral route, have been examined in laboratory rats and dogs, for periods varying between 1 and 18 months. Repeated oral administration of levamisole may decrease weight gain in rats from doses of 10 mg/kg/day; at 160 mg/kg/day, a significant reduction in feed consumption was observed in addition.

- Reproductive Toxicity, including Teratogenicity:

The applicant has provided bibliographical data which show that levamisole is not teratogenic or embryotoxic/foetotoxic, and does not impair fertility at therapeutic levels. Several reproductive toxicity studies have been conducted in sows. It comes out that levamisole administered repeatedly at the recommended dose (10 mg/kg) by the IM route, during various periods of the pregnancy, did not induce teratogenic or embryotoxic/foetotoxic effects.

- Carcinogenicity and Mutagenicity

The applicant has provided bibliographical data which show that levamisole may induce chromosome abnormalities in human lymphocytes; however no mutagenic potential was revealed by several other test systems.

Levamisole is generally recognized as not carcinogenic, although the underlying rat and mice studies bear some deficiencies, mainly in relation to the reduced number of appropriate tissues available for examination. It should be noted that this product is not intended for long term treatment.

User Safety

The applicant has provided a user safety assessment presenting the known toxicological properties of the product components and the relevant exposure scenarios. The user can be exposed systemically, mainly by the dermal route, but also additionally by the oral route, through hand-to-mouth contact and through swallowing of inhaled, non-respirable particles. The user risk may also consist in local effects (skin, eye, or airways irritation, and skin sensitization).
The risk characterization was not formally conducted in accordance with the current CVMP guideline on user safety (EMEA/CVMP/543/03-Rev.1), since the initial national application was submitted before that guideline came into effect. Notably, the URA contains no formal quantitative risk assessment; the applicant has directly proposed user warnings in order to prevent exposure in each identified scenario.
Also, no local effect studies are available for the final formulation, but the available studies show that levamisole can be mildly irritating to the skin and consequently, to the eye.

The presented User Safety Assessment was considered as sufficient, taking notably into account the well-established use of the product components.

User warnings and precautions as listed in the product literature were considered adequate to ensure safety to users of the product.

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Environmental Risk Assessment

Phase I

The environmental risk assessment can stop in Phase I since the PECsoil initial values are under the threshold of 100 µg/kg, and the product is not for use on pasture. It can be concluded from the phase I ERA that there is no risk for undesirable effects on the environment foreseen.

III.B Residues documentation

Residue Studies

Four residue depletion studies using the final formulation have been conducted in pigs. Samples of kidney, liver, muscle, skin+fat were taken from animals at several time points.

The analytical method, consisting in an HPLC method with MS/MS detection and ESI interface, has been fully validated.

MRLs

Levamisole is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 as follows:

LEVAMISOLE					
Marker residue	**Animal Species**	**MRL**	**Target Tissues**	**Other Provisions**	**Therapeutic Classification**
levamisole	Bovine, ovine, porcine, poultry	10 µg/kg 10 µg/kg 100 µg/kg 10 µg/kg	Muscle Fat Liver Kidney	For porcine and poultry species the fat MRL relates to 'skin and fat in natural proportions'. Not for use in animals from which milk or eggs are produced for human consumption.	Antiparasitic agents/ Agents against endoparasites

The excipient anhydrous colloidal silica is classified as "No MRL required" in virtue of the same regulation and lactose monohydrate is part of the list of substances considered as not falling within the scope of that regulation ("Out of scope list").

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Withdrawal Periods

A total of four residue depletion studies have been requested sequentially, because residue levels exceeding the MRL at the last time point, or conflicting results, prevented the calculation of a fully reliable WP.

The first study (LEV-RES.01) did not allow to establish a reliable WP since not all residue concentrations were below the corresponding MRL at the last time point of 8 days, and the value resulting from the application of the statistical method was based on an inacceptable extrapolation.
In the second study (LEV-VMD.02), only skin+fat and liver (the tissues showing the slowest residue depletion) were analysed; at the last time point of 16 days some residue levels were still above the MRL, potentially due to an error in dosing resulting in a considerable overdosage.
A further study was conducted (LEV-VMD.05), with the analysis of skin+fat and liver, but this was not considered satisfactory because the observed residue levels were surprisingly low, potentially due to the administration by gavage.
The results of the fourth residue depletion study (LEV-VMD.06), investigating only skin+fat (the limiting tissue), led to a WP 8 days when taken on its own. This is based on the alternative method, consisting in adding a safety span of 30% to the time point where all residue levels fell below the MRL (6 days).

All available residue data were taken into account in an overall assessment of consumer safety, eventually leading to a WP of **21 days** for pig meat and offal, which adequately covers the high variability within and between depletion studies.

IV. CLINICAL ASSESSMENT (EFFICACY)

IV.A Pre-Clinical Studies

Pharmacology

The general PK properties of levamisole are described through several literature references addressed under part III.

Four main PK studies in pigs were provided, documenting Absorption, Distribution, Metabolism and Excretion, and covering the intended mode and dose of administration as well as other routes. Two of those studies are own, GLP-compliant, product-specific PK studies, and two are literature studies concerning other formulations and using a radio-labelled marker. In addition, blood levels of levamisole were measured in the first residue study LEV-RES.01.

Levamisole is readily absorbed by the oral or parenteral route. It is mostly eliminated within a few days after extensive metabolization in the liver through complex pathways. The main part of the dose is eliminated in urine, but a significant part is also eliminated in bile. Levamisole is notably distributed to the gastro-intestinal tract, even after parenteral administration. After administration of the candidate formulation to pigs, levamisole is rapidly absorbed from the gastro-intestinal tract, with a mean Cmax of 1.32 ± 0.38 µg/L (SD) and a

mean Tmax of 2.57 ± 1.87 h. The calculated absolute bioavailability is 85.3 ± 13.9%. The mean elimination half-life is 9.54 ± 3.80 hours. From the residue data, it appears that liver is the tissue with the highest levamisole concentrations.

Tolerance in the Target Species of Animals

The applicant provided several literature studies investigating tolerance of levamisole in the pig. Their results are quite variable in terms of no effect levels and in terms of levels associated to serious adverse events. However it appears overall, that levamisole presents a relatively narrow safety margin. Transient salivation and vomiting may be observed following single treatment at therapeutic levels. Severe nervous side effects can be observed from unique oral doses of 40 mg/kg, and even at lower parenteral doses.

One own TAS study, where the candidate product was administered via the intended route (orally in the drinking water), at up to 12.5 times the intended dose, was also submitted.That study shows no adverse events at 1x and 2x the recommended dose (10 mg levamisole/kg bw), except a possibly reduced feed intake on the day following the treatment day.Adverse effects consisting of less active behaviour, reduction of feed intake up to 50% were seen following administration of 12.5 times the recommended dose in all animals. Those animals also presented some alterations in biochemical and haematological parameters and a significantly lower heart and respiratory rate.

It is of note that in one of the own PK studies provided, anaphylactic shocks were observed in animals dosed by the IV route, or dosed orally after previous IV exposure, which was attributed to the immunostimulant, sensitizing properties of levamisole. This is not relevant to the intended conditions of use.

The product literature accurately reflects the type and incidence of adverse effects which might be expected.

Resistance

The applicant described the levamisole resistance mechanisms and resistance status of pig worms, through literature references.

The information provided suggests that cross-resistance exists between levamisole and tetrahydropyrimidines (pyrantel/morantel), which however can be partial (conferring side resistance), reflecting that the site of action for the two classes is not exactly identical.

In regard of resistance prevalence in pigs, surveys carried out in Germany and Denmark conclude that most of the anthelmintic resistance (to levamisole and other classes of anthelmintics) has been observed in *Oesophagostomum* spp., although it has also been reported for *Ascaris suum*, in an old and unclear reference and with no adequate confirmation.
Consequently, in the SPC, only the standard text recommended by the SPC guideline for anthelmintics (EMEA/CVMP/EWP/170208/2005) was mentioned.

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IV.B Clinical Studies

Laboratory Trials

The applicant performed no dose determination study;the recommended dose of 10 mg/kg has been set based on literature data and on dosages already approved in the EU, with a view to target also larval stages.

. The recommended dose of 10 mg/kg was compared *a posteriori* to that of 7.5 mg/kg in one of the dose confirmation studies targeting L3 (Geldhof, 2016a); both doses resulted in sufficient efficacy and no difference was evidenced. Besides, a higher dose is in general considered as less likely to select for resistance. Moreover, the tolerance profile of the candidate product at the recommended dose was shown to be favourable. In conclusion, it was considered that the choice of the 10 mg/kg dose was appropriately justified.

Literature data show that levamisole administered orally at doses inferior to the recommended one is more than 90% effective against adult *A. suum*. This was confirmed in a product-own dose confirmation study with experimental infection, conducted in accordance with the current guidance, and showing in addition more than 90% efficacy against the L4 and L5 stages.
Furthermore, two product-specific dose confirmation studies with experimental infection, conducted in accordance with current guidance, show more than 90% efficacy against the migrating, L3 stage, which may be regarded as the dose-limiting stage.

Field Trials

The applicant has conducted one field study in Belgium in May 2005. 57 fattening pigs, females and castrated males, were enrolled based on their *A. suum*EPG count in faeces.

This was a well-designed field study performed in accordance with VICH GL 7 (Efficacy of anthelmintics: general requirements) and VICH GL 16 (Efficacy of anthelmintics: specific requirements for porcines). The number of involved animals was relatively low, and the study was monocentric; it should be reminded however that it was conducted in the context of a well-established used application, which is supported in addition by an abundant body of literature and by the long history of use of levamisole as a pig anthelmintic in the EU.

The efficacy results are based on EPG counts 8 and 15 days post-treatment, in regard to a negative control group. With efficacy percentages of 99.82 and 99.95%, respectively, they clearly allow to conclude to overall efficacy in field conditions against *A. suum*.

No safety issue was reported while the animals were monitored daily for general health for 15 days post-treatment.

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V . OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The therapeutic benefit of the product has been well demonstrated and the target parasite is clearly of medical importance in pig breeding. It was considered that this outweighs the identified risks. Indeed the tolerance profile in the target animal was deemed favourable. The risk to the user was considered as acceptable, provided adequate risk mitigation measures are implemented in the form of user instructions in the product literature. No unacceptable risk has been identified for the environment. The withdrawal period set ensures consumer safety. No risks in terms of resistance emergence has been identified.

The formulation and manufacture of Levaveto 75% is well-described and the specifications set ensure that a product of consistent quality will be produced.

Based on the dossier presented it was considered that the quality, safety and efficacy of Levaveto 75% are overall in accordance with the requirements of Directive 2001/82/EC, as amended, and that the product has been shown to have a positive benefit-risk balance.