College ter Beoordeling van Geneesmiddelen / Medicines Evaluation Board

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MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Intra Dysovinol

NL/V/0304/001/MR

May 2025

MODULE 1

PRODUCT SUMMARY

EU Procedure number	NL/V/0304/001/MR
Name, strength and pharmaceutical form	INTRA DYSOVINOL 499 mg/ml solution for use in drinking water
Applicant	Intracare B.V. Voltaweg 4 5466 AZ Veghel The Netherlands
Active substance(s)	Zinc disodium edetate
ATC Vetcode	QA07XA92
Target species	Pigs (fattening pigs)
Indication for use	For the treatment and metaphylaxis of dysentery due to *Brachyspira hyodysenteriae* infection in fattening pigs (25-125 kg).

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	Full application in accordance with Article 12 (3) of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	03 July 2019
Date product first authorised in the Reference Member State (MRP only)	18 December 2018
Concerned Member States for original procedure	AT, BG, CY, EE, EL, HR, HU, IE, LT, LU, LV, MT, PL, PT, RO, SK
Concerned Member States for subsequent recognition procedure	2nd wave: ES 3rd wave: IT

I. SCIENTIFIC OVERVIEW

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; pigs (fattening pigs).

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 analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Qualitative and quantitative particulars

The product conatins 499 mg/ml zinc disodium EDTA and the following excipients: sodium methyl parahydroxybenzoate, tartrazine E102, brilliant blue FCF (E133) and purified water.

The container/closure system consists of a 5, 10 or 20 liter HDPE container closed with HDPE screw cap with seal ring. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the formulation is justified. The concentration of preservative sodium methyl parahydroxybenzoate in the drug product is demonstrated to be effective to preserve the drug product

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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 from 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 zinc disodium EDTA, a novel active substances that is not described in the European/British Veterinary Pharmacopoeia. The active substance is manufactured in-house by the applicant in accordance with the principles of good manufacturing practice.

The active substance specification include appearance, color, density, pH, and control of identity and assay of the active substance. Batch analytical data demonstrating compliance with this specification have been provided on three batches of the drug substance.

There are no substances within the scope of the TSE Guideline present or used in the manufacture of this product.

D. Control on intermediate products

Not applicable since no intermediate is formed.

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.

Where relevant, 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 substances have not been provided. The active substance is tested prior to use and not stored as an individual substance before further use. For this reason, a retest period for the active substance is not applicable and no stability data is required.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product up to 24 months. Based on the provided stability data the proposed shelf life of 36 months can be granted.

The storage condition “This veterinary medicinal product does not require any special temperature storage conditions” and “Do not refrigerate or freeze” is justified.

The claim of 2 month stability after broaching is granted based on the demonstration of a stability study for an in-use batch broached and stored 61 days at room temperature.

Shelf life of the medicated drinking water is set at 24 hours, in accordance with the 'Guideline on quality aspects of pharmaceutical veterinary medicines for administration via drinking water'. However, the applicant should still conduct in-use stability studies with medicated drinking at the lowest concentration (based on bodyweight) and using soft and hard water as required.

III. SAFETY AND RESIDUES ASSESSMENT (PHARMACO-TOXICOLOGICAL)

III.A Safety Testing

Pharmacological Studies

Dermal bioavailability is very low for EDTA(salts). Also very low dermal absorption values have been observed for zinc compounds.

Toxicological Studies

The applicant has provided bibliographical data, which show that the toxicity of the formula is determined by its active substance, zinc disodium EDTA. Toxicity data for this compound are very scarce, so toxicity data for zinc (salts) and EDTA (salts) are taken into account.

- Single Dose Toxicity

Based on the oral LD_{50} values for zinc diammonium EDTA (>5000 mg/kg bw) and zinc salts, the acute oral toxicity of zinc disodium EDTA is expected to be low.

- Repeated Dose Toxicity
- No information on repeated toxicity of zinc or zinc disodium EDTA is available. For disodium EDTA, a NOAEL of 500 mg/kg bw/d (highest dose tested) is identified based on a chronic toxicity study in rats and mice.
- For zinc, a 6-month study in rats receiving oral doses of 600 mg zinc chloride per day gave rise to gastrointestinal erosions. Human subjects tolerated 660 mg zinc sulphate per day for 10 weeks without any evidence of heamatologic, hepatic or renal toxicity.
- Developmental toxicity
- EDTA seems to have reproductive and embryotoxic effects, though above the NOAEL reported in the repeated dose study. There are no indications that zinc is of concern for developmental effects based on the results of developmental toxicity studies in different species (mice, rats, hamster and rabbits) and several studies in which pregnant women were exposed to soluble zinc compounds.
- Mutagenicity
- Zinc was not mutagenic in a number of bacterial and mammalian systems. For EDTA and its sodium salts it was concluded that they were not mutagenic for human and there is no concern for a carcinogenic potential.
- Irritation and sensibilisation

EDTA is irritating to the eye and incidentally may cause hypersensitive reactions. Eye irritation cannot be excluded for Zinc.

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Observations in Humans

No observations with zinc disodium EDTA in humans are available. Zinc sulphate is tolerated up to doses of 660 mg zinc sulphate per day for 10 weeks without any evidence of heamatologic, hepatic or renal toxicity. A maximum tolerable daily intake for man of 0.3-1.0 mg/kg was set by JECFA for zinc. The Scientific Committee on Food set tolerable upper intake levels varying from 7 mg per day for a child aged 1-3 year to 25 mg per day for adults. The acceptable daily intake for EDTA was set to 1.9 mg/kg/day by EFSA.

User Safety

The applicant has provided a user safety assessment in compliance with the relevant guideline, which shows that the relevant exposure routes are dermal and ocular exposure.

After dermal exposure, systemic exposure is not expected, given the low dermal absorption. Local effects (irritation) on the skin is not anticipated.

EDTA is irritating to the eye and for zinc no definite conclusion can be drawn with respect to eye irritation. Eye irritation may occur after ocular exposure. Hypersensitivity reactions cannot be fully excluded.

Warnings and precautions as listed on the product literature are adequate to ensure safety to users of the product.

Environmental Risk Assessment

A Phase I and Phase II environmental risk assessment (ERA) was provided according to the CVMP/VICH guidelines.

Phase I:

A Phase II ERA is required as the Phase I assessment showed that the initial predicted environmental concentration in soil (PECsoil initial = 400 µg/kg) is greater/equal to 100 µg/kg and no mitigations exist that alter the PECsoil.

Phase II:

A Phase II data set was provided according to the requirements of the CVMP/VICH guideline GL38 and the CVMP guideline on the Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1), The data were considered to be complete and acceptable.

Physical-chemical properties			
Study type	**Test protocol**	**Result**	**Remarks**
Water solubility	OECD 105	575900 mg/L	at 30°C
Dissociation constants in water pKa	OECD 112	pKa ≈3	
n-Octanol/Water Partition Coefficient logP_{ow}	OECD 107	log D_{ow} < -2	at pH 5, 7 and 9

Environmental fate			
Soil Adsorption/Desorption	OECD 106	Koc = • 13.8 L/kg (loamy sand, pH 5.5, 1.7% oc) • 12.6 L/kg (sandy loam, pH 6.8, 1.0% oc) • 9.9 L/kg (loam, pH 7.1, 2.4% oc) • 8.5 L/kg (clay loam, pH 7.7, 2.3% oc)	
Aerobic and Anaerobic Transformation in Soil	OECD 307	DT50 for 20°C= • 292 d, 4.4% mineralisation, 17% bound residue (loamy sand) • 199 d, 22.1% mineralisation, 11% bound residue (sandy loam) • 71 d, 22.8% mineralisation, 35% bound residue (clay loam)	DT50 is a dissipation half live since bound residue was not included as parent in the calculations.

Effect studies					
Study type	**Test protocol**	**Endpoint**	**Result**	**Unit**	**Remarks**
Algae, growth inhibition test/*Pseudokirchneriella subcapitata*	OECD 201	EC50	3900	mg/l	nominal, static
Daphnia sp., immobilisation/*Daphnia magna*	OECD 202	EC50	>10 000	mg/l	nominal, static
Fish, acute toxicity/*Cyprinus carpio*	OECD 203	LC50	>10 000	mg/l	nominal, static
Soil microorganisms: Nitrogen transformation test (28 days)	OECD 216	% effect	15.6 16.2	%	maximum effect over 7-14 days, <25% from the control.
Soil micro-organisms: nitrogen transformation test (100 days)	OECD 216	% effect	16.2	µg/kg	maximum effect over 7-14 days
Terrestrial Plants, growth test	OECD 208	EC50	698 2149 >8100	mg/kg	*Brassica pekinensis* *Solanum lycopersicum* *Avena sativa* The applicant has committed to test three additional plant species.
Earthworm/*Enchytraeidae* subacute reproduction/ *Eisenia fetida*	OECD 222	NOEC	≥8	mg/kg	

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Risk characterisation

The Predicted Environmental Concentration (PEC) for each compartment was calculated in accordance with VICH guideline GL6 and the CVMP guideline on the Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1)

Using the assessment factors (AF) in these VICH guidelines, predicted no effect concentrations (PNEC) were calculated and compared with the PEC values. This results in a risk quotient (RQ) for each compartment as follows:

Compartment	**PNEC**	**PEC**	**RQ**
surface water	39000 µg/L	189 µg/L	0.005
groundwater	3900 µg/L	59.8 µg/L	0.015
soil microorganisms: Nitrogen transformation test	<25% difference in N transformation	NA	NA
soil	800 µg/kg	689 µg/kg	0.86

The risk characterisation resulted in risk quotients (RQs) below 1 for the surface water, groundwater and soil compartments indicating that the product will not pose a risk to those compartments when used as recommended.

The conclusions for the soil compartment are preliminary awaiting the outcome of additional plant testing.

PBT assessment

The substance is considered very persistent (vP) because of DT50 values exceeding 180 days. The substance is however not PBT nor vPvB because the log Dow is < -2.

III.B Residues documentation

Residue Studies

No residue depletion studies were conducted because all constituents of the product are included in table 1 of the MRL Regulation 37/2010 with ‘No MRL required’.

MRLs

The active substance of the product is included in table 1 of the MRL regulation 37/2010, as follows:

Substance	**Species**	**MRL status**
The following zinc salts: zinc chloride, zinc gluconate, zinc oleate, zinc oxide, zinc stearate and zinc sulphate	All food producing species	No MRL required
Ethylenediaminetetraacetic acid and salts	All food producing species	No MRL required

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Zinc disodium EDTA is considered an EDTA-salt.

All excipients in the formulation are food additives with a valid E number and are therefore included in table 1 of MRL regulation 37/2010 with 'No MRL required'.

Withdrawal Periods

Based on a worst case intake estimate, it was concluded that consumption of meat from pigs treated with the product following the recommended dose is not expected to result in biological significant levels in human. A withdrawal period of 0 days for meat in pigs is therefore justified.

IV. CLINICAL ASSESSMENT (EFFICACY)

IV.A Pre-Clinical Studies

Pharmacology

The applicant has conducted studies and provided bibliographical data to demonstrate that the positive effect is caused by zinc (disodium) EDTA as a total compound. EDTA (Ethylenediaminetetraacetic acid) is used as a chelating agent in order to form a complex with zinc. Results of the provided studies demonstrated that ZnEDTA may act by prevention of adhesion of pathogens to intestinal cells and by decreasing the damaging effect of toxins to the cells.

For current product, a precise pharmacokinetic analysis of the plasma concentration profile was not performed. Also, kinetic studies were not performed for different doses of the product.

However, results of the presented studies demonstrated that systemic absorption of the compound is poor, as demonstrated in animals dosed much higher than currently proposed dose. Based on the presented literature data and the results obtained in the pharmacokinetic study, zinc disodium EDTA appears to be poorly absorbed after oral administration, and is mostly passed through faeces (roughly 90% of the product was not absorbed from the gut). In addition, no adverse events were recorded in all of the studies provided. In the light of this application, it was therefore not considered appropriate to demand for additional pharmacokinetic analysis and additional kinetic studies.

Tolerance in the Target Species of Animals

The toxicological profile of the product is largely determined by its active ingredient, since the veterinary medicinal product consists for 99.9% of an aqueous solution of zinc disodium EDTA. The applicant has provided information on acute and chronic zinc intoxication and EDTA toxicity.

A separate target animal tolerance study using multiples of the recommended dose in the target species was not performed. However, the nature of product in combination with the specific pharmacokinetic characteristics of the product (zinc disodium EDTA appears to be poorly absorbed after oral administration), combined with the extremely low dose that is administered (medicated water will contain no more than 0.040% of the veterinary medicinal product, corresponding with 0.016% active substance, for the prescribed duration of 6 consecutive days) and the absence of any adverse events in all of the *in vivo* studies provided (including the PK study, in which the currently prescribed dose was administered up to 6.5 times). Also, in the feasibility study, pathological evaluation of the gut following

administration of the product did not reveal any abnormalities that could be attributed to the product.

If administered according to label, tolerance of the product is considered fully acceptable.

Resistance

Use of ZnO, especially when used widespread and at a considerable dose, is potentially harmful to the ecosystem. In the past, CVMP therefore concluded that zinc oxide as a VMP demonstrated an unacceptable risk for both animal- and public health. However, current product contains Zinc disodium EDTA and not zinc oxide. Therefore, this product does not fall within the scope of the referral, and the conclusions related to use of ZnO as a VMP are considered not applicable to this product.

Also:

- The proposed dose of zinc is much lower than the dose of zinc in products intended as dietary supplementation for the prevention of diarrhoea. In fact, the proposed dose complies to the maximum level of zinc that is allowed in feed of fattening pigs and piglets/sows, respectively.

- Duration of treatment according to current label is limited to six days only, and since the indication is for treatment and metaphylaxis and not for prevention, only a limited number of animals will be treated.

- Studies presented by the applicant demonstrated that no antimicrobial effects against *Escherichia coli* were found for zinc disodium EDTA in concentrations of 200 – 4000 µM, in line with currently proposed dose.

In conclusion, given current knowledge and the proposed dose and duration of treatment, resistance-related problems are unlikely to occur. A significant increase of exposure is unlikely to result from treatment with this product. It is therefore accepted that the product does not significantly contribute to zinc release to the environment.

IV.B Clinical Studies

Laboratory Trials

The applicant performed a small scale dose determination study on naturally infected animals. Results supported the final dose of 11.3 mg zinc disodium EDTA per kg of bodyweight. In the dose determination study, of the group (n=5) treated with 50% of the dose, excretion of *Brachyspira hyodysenteriae* could still be determined in one animal, and one animal of this group died, demonstrating clinical signs considered attributed to swine dysentery (SD) i.e. very thin, diarrhoea and low energy in an animal that was demonstrated to be infected with *Brachyspira hyodysenteriae* by PCR. None of the animals treated with the final dose (n=2), or 150% dose (n=4) excreted *Brachyspira hyodysenteriae*, indicating that the 50% dosage does not provide an optimal effect.

In addition, the applicant has conducted (non-GCP/non-GLP) developmental laboratory studies (one *in vitro* study, evaluating zinc disodium EDTA activity on porcine and human intestinal cell lines, and one *in vivo* pilot study) to demonstrate safety and efficacy of the product.

The non blinded, negatively controlled feasibility study was performed in eight SPF boars, of appropriate age. All pigs were inoculated with *Brachyspira* by spontaneous oral ingestion of a

total of $3.1*10^{10}$ cfu /pig. Inoculation of the animals resulted in severe SD infections in 6 out of 8 animals. After observation of a faeces quality score of 2 or higher in at least two animals, treatment was initiated in half of the animals (D13). Animals were treated with 15.7 mg active ingredient / per kg bw, administered through feed, during nine days. Results of this study demonstrated that animals with severe SD infections, were capable of complete recovery (healthy faeces, no *Brachyspira* present in faeces, no abnormaities on micro- and macroscopic inspection of the colon after treatment). Faecal consistency was normal 3-6 days after initiation of treatment. In the control group, *Brachyspira hyodysenteriae* was still present at that time, faecal scores deteriorated and the pigs did not recover spontaneously during the study period. No adverse events were observed throughout this Study.

This Study is considered to support safety of the product, when administered according to label.

In the feasibility study, duration and dose of treatment was not according to label. Dose and duration was changed to lower the total quantity of active substance used during treatment for the environmental risk assessment. Also, it was preferred to minimize the quantity of zinc used in light of the recent zinc(oxide) discussions with respect to ecotoxicity. Current dose still falls within the range that was demonstrated to have a positive effect in the *in vitro* study. Also, results of this feasibility study demonstrated that animals with severe SD infections, were capable of complete recovery, with normal fecal consistency 3-6 days after initiation of treatment. Overall, the justification for currently proposed dose and duration of treatment was accepted.

Initially, the product was administered through feed. This was changed, since sick pigs show a drop in feed intake, while the reduction in drinking water consumption is relatively less, and moreover, adding a medication to drinking water is a more flexible method than feed medication. Since the molecule has been demonstrated to be highly soluble in water, both methods are however considered to be representative for oral application of the product. The decision to administer the product via water is therefore considered justified.

Field Trials

To confirm safety and efficacy of the treatment at currently proposed dose, the applicant has conducted one GCP compliant, multi-site, double blinded, partially randomised field study with a complete block design.

Study objective was to evaluate the efficacy of the product in the treatment of clinical signs due to *Brachyspira hyodysenteriae* infection in pigs. The Study included 58 commercial-breed recently weened pigs (24 female and 34 male; 28 investigational product (IP) treated (n=15 animals from farm A, n=13 animals from farm B) and 30 placebo treated pigs; weight ranged from 10 to 80 kg), distributed over 16 pens at two representative farms in the Netherlands. Both farms had a recent history of clinical disease due to *Brachyspira hyodysenteriae*.

Treatment (according to label) was initiated in case at least 1 pig in a pen showed abnormal faeces (this triggered faecal sampling for PCR analysis to confirm the next criterion), and also at least 10% of the pigs, or at least 2 pigs, excreted *Brachyspira* in their faeces (confirmed by PCR analysis).

Results demonstrated that shedding of *Brachyspira hyodysenteriae* (primary efficacy criterion) was significantly less (p-value < 0.001) in the IP group compared to the control group. Treatment also significantly improved the faecal consistency scoring (secondary efficacy criterion) both for individual pigs and at pen level. No adverse events were observed throughout the study period. Administration of the product did not negatively influence the uptake of water. There was no evidence of relapse of clinical *Brachyspira hyodysenteriae* infection in IP treated pigs. These results support the efficacy and safety of the IVP for treatment of *Brachyspira hyodysenteriae* in weaned pigs.

In addition a double-blinded, randomized GCP field trial to further support metaphylaxis and therapeutic claim for the product at the approved dose and treatment period was conducted.

Study objective was to assess the product's efficacy at a geographic area within the EU, different form the Netherlands. The study was performed at 2 farms (2 different locations, 2 different farm types) in Spain. The farms had a known history of the clinical disease and met the inclusion criteria as defined in the study protocol. 100 sick and 100 healthy commercial-breed pigs, between 40 and 60 kg, were included.

The experimental unit in this study was a 'a row of adjacent pens in 1 barn', while the analytical/statistical unit was the pig. Treatment was started if the number of suspected pigs was at least 10% of the total number of pigs in that experimental unit, as determined by a faecal score; and at least 80% of the faecal samples of the individual pigs, collected in the experimental unit, were PCR positive for *B. hyodysenteriae*. The investigator administered a blind treatment (either IVP or placebo) to the complete experimental unit for 6 successive days through the animals' drinking water, according to a randomization plan.

From those animals which started the trial in sick conditions, 20% of them (treated with placebo) were still sick on day 9 of trial, while pigs treated with the IVP were completely recovered (0% of sick animals, p=0.0008). However, many pigs, especially in placebo group, required an antibiotic intervention to be cured (60.0% vs 2.0%, p=0.0022) between days 6 and 9. Therefore, recovery of animals in the placebo group was mostly due to the rescue treatment. Metaphylaxis evaluation: from those animals which started the trial in healthy condition, 14% in placebo-group and 0% in the IVP group were sick on day 9 ($p<0.001$).

These results further support the efficacy and safety of the IVP for treatment and metaphylaxis of *B. hyodysenteriae* in fattening pigs.

V. OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Heads of Veterinary Medicines Agencies website (www.HMA.eu).

This section contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

Summary of change	**Section updated**	**Approval date**
Addition of field study to further substantiate the indication (NL/V/0304/001/II/001)	Module 3, section IV.B	21 APR 2022
SRP to add Spain as CMS (NL/V/0304/001/E/001)	Module 3	12 SEP 2022
implementation of changes to PI as agreed during SRP + update of PI to QRD v9.0 (NL/V/0304/001/A/002/G)	Not applicable	13 JAN 2023
SRP to add Italy as CMS (NL/V/0304/001/E/002)	Module 3	05 MAY 2025