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EMA/377159/2017
Committee for Medicinal Products for Veterinary Use

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CVMP assessment report for Exzolt (EMEA/V/C/004344/0000)

International non-proprietary name: fluralaner

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Intervet International B.V. submitted on 22 July 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Exzolt, through the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 December 2015 as the product would be in the interest of animal health at the Community level.

The indication is: "Treatment of poultry red mite (*Dermanyssus gallinae*) infestation in pullets, breeders and layer hens."

The active substance of Exzolt is fluralaner, a systemically active ectoparasiticide belonging to the isoxazoline group, which is a potent inhibitor of parts of the arthropod nervous system by acting antagonistically on ligand-gated chloride channels (GABA-receptor and glutamate-receptor). The target species is chickens.

Exzolt solution for use in drinking water contains 10 mg/ml fluralaner and is presented in bottles containing either 1 litre or 4 litres.

The rapporteur appointed is Peter Hekman and the co-rapporteur is Judita Hederová.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC (full application).

On 15 June 2017 the CVMP adopted an opinion and CVMP assessment report.

On 18 August 2017, the European Commission adopted a Commission Decision granting the marketing authorisation for Exzolt.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system has been provided, which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, it is accepted that the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the dosage form takes place in the EEA. Batch release takes place at Intervet Productions, Rue de Lyons, 27460 Igoville, France. The site has a manufacturing authorisation issued on 27 April 2015 by l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du

travail – l'Agence nationale du médicament vétérinaire (ANSES-ANMV) in France. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by a third party.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of both the active substance and finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

Part 2 - Quality

Composition

Exzolt 10 mg/ml solution for use in drinking water is a non-aqueous solution containing 10 mg/ml of the active substance fluralaner, for oral use in drinking water for chickens.

The other ingredients are all-*rac*-*α*-tocopherol (alpha-tocopherol, an antioxidant), diethylene glycol monoethyl ether (solvent) and polysorbate 80 (surfactant) and all are detailed in section 6.1 of the SPC.

The product is available in either 1 litre (1 l) or 4 litres (4 l) colourless high density polyethylene (HDPE) bottles, closed with aluminium/polyester foil seals and blue child-resistant polypropylene (PP) screw caps. There is no additional outer packaging. The packaging is described in section 6.5 of the SPC.

Containers

The primary packaging is either a 1 litre or 4 litres natural (colourless) HDPE bottle closed with an aluminium/polyester foil seal and a blue child-resistant polypropylene (PP) screw cap. There is no additional outer (secondary) packaging.

The HDPE bottle, the child-resistant PP screw cap and the foil seal all comply with the relevant EU requirements and sufficient details have been provided. The screw caps also comply with the relevant ISO standards (ISO 8317) for child-resistance.

The choice of the container-closure system has been validated by stability data and is adequate for the intended use of the product. The container sizes have been justified in relation to number of animals likely to be treated.

Development pharmaceutics

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.

The development of the formulation was explained and was based on the physicochemical characteristics of the fluralaner, and the intended administration route. A solution formulation was developed, which necessitated the use of a non-aqueous solvent and surfactant. Alpha-tocopherol was selected after development studies with several antioxidants.

It was not necessary to include a preservative in the formulation as an anti-microbial preservative efficacy test demonstrated it to be self-preserving.

The formulation is suitable with respect to its intended use. Compatibility between the components of the formulation and with the container-closure system, as well the construction materials of the medicated water distribution system, have all been demonstrated.

Method of manufacture

The manufacturing process is well-described, simple and consists of the preparation of a solution of fluralaner in a mixture of the three non-aqueous excipients. The bulk solution is then filled into the final packaging. The process is considered to be a standard manufacturing process. No complex manufacturing processes are used and no critical steps have been identified.

The in-process controls are adequate for this type of manufacturing process.

Batch analyses data from three pilot scale GMP batches of the product, manufactured at the proposed manufacturing site and used for clinical and stability studies, were provided. All batches comply with the proposed specifications and confirm the consistency of the manufacturing process and its ability to manufacture the product to the intended specification.

The manufacturing process will be validated using three commercial scale batches prior to commercialisation. Since the manufacturing process is a standard process, this is acceptable and in line with EU guidance. The process validation protocol has been provided and is acceptable.

Control of starting materials

Active substance

The active substance fluralaner is not a new active substance as it has been included in a previous EU authorised veterinary medicinal product. It is a white to pale yellow powder. Fluralaner possesses one chiral centre, resulting in the formation of a racemate. The substance exhibits polymorphism.

The active substance master file (ASMF) procedure has been used to provide details of its manufacture and control. Detailed information on the manufacture of the active substance has been provided in the restricted part of the ASMF and was considered satisfactory.

The synthesis comprises five steps. The starting materials are acceptable as they are non-complex structures, comprising elements of the structure of fluralaner and there are at least three subsequent synthetic steps where covalent bonds are formed or split. Adequate characterisation data have been provided for all starting materials.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

There is no monograph for fluralaner in the Ph. Eur. or in the pharmacopoeia of any EU Member State. The characterisation of the active substance and its impurities are in accordance with the current EU guidance on the chemistry of new active substances. Potential and actual impurities are well discussed with regards to their origin and characterised. The active substance specification includes justified tests. The analytical methods used have been adequately described and the non-compendial methods appropriately validated in accordance with the relevant VICH guidelines.

Batch analysis data demonstrating compliance with the proposed active substance specification have been provided for several production batches of fluralaner, both non-micronised and micronised.

Stability results have been provided for both real time (36 months) and accelerated (6 months) stability studies on fluralaner. No real trends were observed in the stability results. Stress testing under thermal, acidic, basic, oxidative, reductive and photolytic conditions demonstrates that fluralaner is stable to both heat and light. The proposed re-test period for fluralaner has been accepted.

Excipients

There are no novel excipients used in the finished product formulation. All the three excipients, diethylene glycol monoethyl ether, polysorbate 80 and all-*rac*-α-tocopherol, are well known pharmaceutical ingredients and their quality is compliant with the requirements of the respective Ph. Eur. monographs with additional tests for residual solvents. The additional tests for all three excipients and limits are in line with the VICH GL18 on Residual solvents in new veterinary medicinal products, active substances and excipients.

The list of excipients is included in section 6.1 of the SPC.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

The product does not contain any materials derived from human or animal origin. The product is therefore out of scope of the relevant Ph. Eur. monograph and the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01-Rev.3).

Control tests on the finished product

The specifications proposed for use at release and end of shelf life for the finished product include tests for appearance, identity (HPLC and UV) and assay (LC) of the active substance, unspecified and total impurities, identity of and limits for the antioxidant α-tocopherol, microbial limits (Ph. Eur. 2.6.12), fill volume and density, and are considered appropriate to control the quality of the finished product. The shelf life specification differs from that used for release purposes in that the lower limits for both the active substance and antioxidant are wider and the limit for total impurities (degradation products) is higher. The differences have been justified.

The analytical methods used have been adequately described and appropriately validated in accordance with the VICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for three pilot scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. A commitment has been provided to submit batch data of batches manufactured according to the proposed manufacturing process at the commercial scale in accordance with the agreed final specifications and this is acceptable.

Stability

Stability data from six pilot scale batches and one laboratory scale batch of the finished product stored under long term conditions for 24 months at 30 ºC/65% RH, and for up to 24 months under accelerated

conditions at 40 ºC/75% RH, according to the VICH guidelines, were provided. The batches of product used were representative of those proposed for marketing, and were packed in the primary packaging, in both pack sizes (1 l and 4 l) proposed for marketing.

Samples were tested for appearance, fluralaner assay, identity, degradation products, α-tocopherol assay and identity, water content, microbial limits and additionally for opalescence, colour, density and enantiomeric ratio. The analytical procedures used were stability indicating.

In addition, samples from two batches were exposed to light as defined in the VICH GL5 on photostability testing of new veterinary drug substances and medicinal products. No major differences were observed compared to the reference test article (stored in the dark). The results show that the product is not sensitive to light and therefore the absence of an outer box/carton is justified (for both two pack sizes).

A freeze–thaw study on samples from two batches demonstrated that all parameters tested remained stable and within the shelf life specification.

Based on the available stability data the proposed shelf life of the veterinary medicinal product as packaged for sale of 3 years, without any special storage conditions, as stated in the SPC, is acceptable.

An in-use stability study (with both the 1 l and 4 l pack sizes) simulated nine treatments over a 360-day testing period by withdrawing volumes simulating normal use of the product on each of the nine occasions. All results obtained complied with the shelf life specification. Therefore, a 1 year in-use shelf life after first opening the product, without any special storage conditions, is acceptable for both pack sizes.

A stability study was conducted in line with the CVMP guideline on quality aspects of pharmaceutical veterinary medicines for administration via drinking water (EMEA/CVMP/540/03-Rev.1) to evaluate the stability of the medicated drinking water. The stability was evaluated over at least 24 hours, in compliance with the CVMP guideline using different qualities of water. Three fluralaner concentrations were tested in the study, and all three were tested for fluralaner content. The fluralaner medicated drinking water is considered stable for at least 24 hours and no special conditions of use were considered necessary for the end user of the product.

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical aspects relevant to the performance of the product have been investigated and are controlled.

The manufacturing process is a standard one and therefore no validation data on commercial scale batches is required prior to authorisation. However, a validation protocol has been submitted and the validation of three pilot scale batches showed that the manufacturing process appeared to be satisfactorily controlled and capable of producing consistent batches of the appropriate quality.

The finished product specifications are acceptable and the test methods have been described and appropriately validated.

Based on the stability data provided, a shelf life of 3 years, with no special storage precautions, has been justified.

An in-use shelf life of 1 year, with no special storage precautions, has also been justified.

Stability of the product in drinking water of various qualities has been investigated and a shelf life in drinking water of 24 hours justified.

The applicant is recommended to provide the following information post-authorisation:

- Process validation studies should be performed on the first three commercial batches.
- The first three batches produced for commercial release should be placed in a stability study (for which the protocol has already been approved).
- Batch data should be provided from batches manufactured at the commercial scale according to the proposed manufacturing process (process validation batches), in accordance with the agreed specifications.

Part 3 – Safety

The product 'Exzolt 10 mg/ml solution for use in drinking water for chickens' contains the active substance fluralaner, an inhibitor of the arthropod GABA receptor and glutamate receptor. The substance has been previously assessed by the CVMP in the context of the application for the establishment of maximum residues limits for fluralaner in chicken. The safety of fluralaner has also been assessed during the marketing authorisation of products containing the same active substance for use in dogs and cats. For the authorisation of the current product, new studies were provided.

Safety documentation

Pharmacodynamics

Fluralaner belongs to the group of isoxazolines. Isoxazolines have been found to be potent inhibitors of GABA receptor function through the inhibition of GABA ligand-gated chloride channels in insects.

Detailed information on pharmacodynamics in the target species can be found in part 4.

Pharmacokinetics

Absorption

Two GLP compliant studies were performed to investigate the pharmacokinetic profile of fluralaner in blood plasma of chickens. When administered to laying hens by oral gavage at a single dose rate of 0.5 mg/kg bw, fluralaner was rapidly and extensively absorbed with an oral bioavailability of 91%. Maximum plasma concentrations were obtained at 2 hours.

Distribution

Fluralaner is well distributed to tissues. Radiolabelled studies in dogs, rats and layer hens demonstrated that the highest concentrations were found in fat and liver, followed by kidney and muscle. Fluralaner accumulates after daily oral administration and steady state fluralaner plasma concentrations were reached after approximately 30 days in rats and 90 days in dogs indicating a long half-life; no actual half-lives could however be calculated due to the study design. Fluralaner appears to undergo enterohepatic recirculation.

The plasma half-life of fluralaner in chickens, determined after intravenous administration, was about 5 days. In addition, dose linearity was demonstrated following single oral doses of 0.25, 0.5 and 1 mg fluralaner/kg bw. Following oral administration, layer hens receiving the final product twice, 7 days apart in their drinking water at a dose rate of 0.5 mg fluralaner/kg bw, showed the C_{max} at 36 hours after the first dose, and 12 hours after the second dose. The C_{max} was higher after the second dose, 7 days after the first, showing a slight accumulation of fluralaner.

Fluralaner is highly bound to plasma proteins, equal to or higher than 99.90%.

Metabolism

Unmetabolised fluralaner is the major component present in all analysed organs and tissues in dogs, rats and layer hens. Fluralaner is metabolised to many metabolites.

Excretion

The main excretion route is faeces: up to 49% in rats and 17% in dogs over 148 hours (4 hours post last dose when administered for 7 consecutive days). Urinary elimination was limited, up to 3.7% in rats as well as in dogs.

Toxicological studies

Single dose toxicity

Acute oral and dermal toxicity studies using the active substance and the final formulation were conducted in rats. Fluralaner and the final formulation are of low acute oral and dermal toxicity (LD_{50} > 2000 mg/kg bw; limit test). No adverse systemic effects were observed. However, some local effects (erythema, scaling and scabs) were observed after dermal administration, and slightly ruffled fur after acute oral dosing of the active substance.

Repeat dose toxicity

Oral:

Repeated dose oral toxicity was extensively studied in rats (studies with durations of 2-, 4-, 13-weeks) and dogs (4-, 13- and 52-week studies).

In the 2-week and a 4-week toxicity study in rats, rats were given fluralaner by oral gavage at doses of 0, 30, 60, and 600 mg/kg bw/day. The main target organ in the repeated dose toxicity studies was the liver, which is the main organ for the elimination of fluralaner. Effects (increased organ weight, hepatocellular fatty change, effects in related blood parameters) were observed at all dose levels, though considered mild at the lower doses. Decreased thymus and increased adrenal weight was observed at the highest dose. A no-observed adverse effect level (NOAEL) of 60 mg/kg bw/day was established by the CVMP.

The 13-week oral toxicity study in rats, dosed 0, 20, 40 and 400 mg/kg bw/day, confirmed the effects on the liver. In addition, at the dose of 400 mg/kg bw/day effects on thymus and adrenal weight and microscopic changes in lung and thymus were observed. As the effects were mild at lower doses, a NOAEL of 40 mg/kg bw/day was established by the CVMP.

Two 4-week toxicity studies in dogs were provided with respective oral (by capsule) dose levels of 0, 100, 250, 750 mg/kg bw/day and 0, 20, 40, 100 mg/kg bw/day. Reductions in cholesterol, phospholipid and triglyceride levels were observed at all dose levels, in both sexes and at different time points. Although no histopathological changes of the liver were observed, it cannot be firmly concluded

that the observed effects are considered non-adverse. A lowest-observed-adverse-effect level (LOAEL) of 20 mg/kg bw/day was therefore established.

In a 13-week oral toxicity study, dogs were given fluralaner orally (by capsule) at doses of 0, 2, 4 and 8 mg/kg bw/day. Reductions in cholesterol and phospholipids were observed at 4 and 8 mg/kg bw per day in both males and females. In addition, reduction in triglycerides levels were observed at 4 and 8 mg/kg bw per day in males. Based on this study, the CVMP concluded on a no-observed effect level (NOEL) of 2 mg/kg bw per day over 13 weeks.

Similar results were observed in the 52-week study in dogs, when dosed 0, 1, 2, or 4 mg/kg bw/day. Reductions in cholesterol and phospholipids were observed in males at 2 and 4 mg/kg bw per day, and in females at 4 mg/kg bw per day. Reduction of triglycerides concentration were observed at 2 mg/kg bw per day in males and in females at 4 mg/kg bw per day and the derived NOEL was set at 1 mg/kg bw per day.

Dermal:

The potential subacute effects of fluralaner were investigated in one 2-week (dose range finding) and two 4-week dermal (6 hour semi-occlusive) toxicity studies in the rat. From the first of the 4-week studies, fluralaner was dosed at 0, 100, 200 or 1000 mg/kg bw/day, no NOAEL could be established. Treatment-related effects were observed at all doses and included: fatty changes in the liver, effects on serum liver enzymes, triglyceride, albumin and globulin, and moderately increased liver weights. In addition, at all dose levels spleen weights were increased in males, though not correlated with histopathological findings. A further 4-week study was conducted using the doses 0, 25, 50 or 100 mg fluralaner/kg bw/day. At 100 mg/kg bw/day, microvesicular fatty change, periportal or diffuse, was observed in the liver of three males and three females. However, there was no other indicator of liver injury. No effects were observed at the other doses. Taking into account all three studies (the effects are considered to be mild and comparable to the effects observed in the oral studies) and taking account of the oral NOAEL from the rat studies (it is not conceivable that the dermal NOAEL will be lower than the oral NOAEL), CVMP decided that a NOAEL of 100 mg/kg bw/day was appropriate when considering repeated dose toxicity (to be used for the assessment of subacute exposure).

A 90-day dermal toxicity study was performed in rats, administered doses of 0, 25, 50 or 500 mg/kg bw/day. At 500 mg/kg bw/day liver effects were observed, which were similar to the effects observed in the 2-, 4-, and 13- week oral studies as well as the 2- and 4-week dermal studies. At the highest dose alveolar histiocytosis was observed in females, in some animals accompanied by (multi)focal interstitial lobular inflammation and intra alveolar amorphous material. Therefore a dermal NOAEL of 50 mg/kg bw/day was derived from this study (to be used for the assessment of subchronic exposure).

Tolerance in the target species of animal

See part 4.

Reproductive toxicity

Study of the effect on reproduction

In a one-generation study, rats were given fluralaner at a dose level of 0, 50, 100 or 500 mg/kg bw/day. Liver, thymus, lung and the adrenals appeared to be affected in parents at the lowest dose of 50 mg/kg bw/day, resulting in a LOAEL of 50 mg/kg bw/day. The effects are consistent with the adverse effects observed in the repeated dose studies. The reproduction NOEL was set at 100 mg/kg

bw/day, based on reduced litter size due to reduced implantation rate and increased post-implantation losses at the higher dose of 500 mg/kg bw/day. Statistically significant reductions in thymus weight and lymphoid atrophy in the thymus was observed in pups at all doses, showing a clear dose response and resulting in a LOAEL of 50 mg/kg bw/day.

In a two-generation study, rats were given fluralaner at a dose level of 0, 8, 50 or 500 mg/kg bw/day. In the parental and/or first generation (F1) generation, peribronchial inflammatory lesions in the lungs and increased hypertrophy of the adrenal cortex and atrophy/involution of the thymus were observed at all dose levels, resulting in a LOAEL of 8 mg/kg bw/day for parental toxicity, though the effects were considered marginal at the lowest dose. The reproduction NOEL was set at 50 mg/kg bw/day, based on higher post-implantation, post-natal and breeding losses at the higher dose of 500 mg/kg bw/day. The pup NOEL was set at 50 mg/kg bw/day based on reduced body weight, clinical signs, pathological findings, and delayed physical and sexual development at 500 mg/kg bw/day.

Study of developmental toxicity

Oral:

Developmental toxicity was studied in the rat at doses (oral gavage) of 0, 100, 300 or 1000 mg fluralaner/kg bw/day. Food consumption was significantly reduced in the two higher dose groups; in the highest dose group, body weight and body weight gain were also reduced. In the foetuses of rats in the two highest dose groups, a higher incidence of dilated renal pelvis/ureter and supernumerary ribs were observed at both the foetus and litter level. Both the maternal and foetal NOELs were 100 mg/kg bw per day.

Developmental toxicity was studied in rabbits at doses (oral gavage) of 0, 50, 250 or 1000 mg fluralaner/kg bw/day. The NOAEL for maternal toxicity was 50 mg/kg bw/day, based on reduction in food consumption at 250 mg/kg bw per day. No NOAEL for foetal toxicity could be established, the LOAEL was 50 mg/kg bw/day based on adverse embryo-foetal developmental effects observed at the lowest dose of 50 mg/kg bw/day. A complementary prenatal developmental toxicity study using lower oral doses of 10, 25 and 250 mg/kg bw/day was therefore conducted. Fatty changes of the liver and related changes in blood biochemistry were observed at all doses in dams, however considered mild at the lowest dose level, resulting in a NOAEL of 10 mg/kg bw/day. Based on the increase in fusions in cervical vertebra 2 at 25 mg/kg bw/day, the developmental NOEL was set to 10 mg/kg bw/day.

Dermal:

Developmental toxicity was studied in rabbits administered fluralaner suspended in 0.5% carboxymethylcellulose aqueous solution (w/v) containing 0.1% v/v polysorbate 80 at doses of 0, 50, 100 and 1000 mg/kg bw/day. A maternal dermal NOAEL of 1000 mg/kg bw/day was set (the highest dose tested in the pivotal study). However, it is noted that the liver (blood biochemistry), which appeared the most sensitive target organ and also appeared to be the basis for the maternal NOAEL in the rabbit oral study, was not investigated in the dermal study. Based on adverse effects observed at 1000 mg/kg bw/day, including external and visceral abnormalities and skeletal abnormalities, such as fusion of cervical vertebra 2 and sternebrae, and decreased ossification of the humerus and femur of fore- and hindlimbs, the NOEL for foetal toxicity was set to 100 mg/kg bw/day.

Genotoxicity

The potential mutagenic effects of fluralaner have been investigated in three in vitro tests (Ames-test, mouse lymphoma thymidine kinase locus assay, chromosomal aberration test in human lymphocytes

in vitro) and one in vivo test (micronucleus assay in bone marrow cells of the mouse) on genotoxicity. The results of all four tests were negative. It was concluded that fluralaner does not have any mutagenic potential.

Carcinogenicity

Studies on carcinogenicity were not conducted. This was justified by the negative results in all the genotoxicity tests and the absence of pre-neoplastic lesions in the oral (sub-)chronic repeated dose toxicity studies. There is no evidence for any carcinogenic potential of fluralaner.

Studies of other effects

The active substance fluralaner was considered to be non-irritating to rabbit skin. However, a dermal irritation study in rabbits using the final formulation demonstrated mild skin reactions at the application site at 24, 48 and 72 hours after application. Slight effects were still observed after 7 days.

The active substance fluralaner was considered to be non-irritating to the rabbit eye. However, an eye irritation study in rabbits using the final formulation demonstrated slight to moderate eye irritating effects, which were fully reversible within 72 hours.

The active substance fluralaner did not have sensitising potential when tested in the guinea pig maximisation test of Magnusson and Kligman. In addition, the final formulation was tested for its sensitising potential. No adverse effects were observed, however it is doubted whether the immune system was adequately triggered during the test as no skin reactions were observed during the induction phase. Nevertheless, as it was concluded that the active substance has no sensitising potential and the excipients are not considered to have sensitising properties, skin sensitisation and/or allergic reactions when exposed to the formulation are not expected.

Thymus atrophy was observed in several studies, but was mostly associated with high doses and/or not accompanied by significant or consistent adverse effects on other organs of the immune system or haematology. The repeated dose toxicity studies were considered sufficient to cover potential effects on the immune system. No effects on the nervous system have been reported in the toxicity tests provided. The absence of additional neurotoxicity studies is therefore justified.

Excipients

The product contains the excipients diethylene glycol monoethyl ether, polysorbate 80 and α-tocopherol, which are all included in table 1 of the annex to Commission Regulation (EU) No 37/2010 indicating that no MRLs are required and are considered safe as used in current product. The toxicity of this product will be determined by its active substance, fluralaner.

User safety

A User Safety Risk Assessment has been conducted in accordance with CVMP Guideline (EMEA/CVMP/543/03-Rev.1).

This product will be administered by professional users, i.e. veterinarians and farmers. As it is used only in a professional setting and is not expected to be stored in the household, accidental exposure by children is considered to be negligible; though this exposure scenario was included in the risk assessment.

The most likely exposure situation is the dermal route during either the pre-application phase when the

solution is prepared and/or during application when the medicated drinking water system is handled. In addition, ocular exposure due to splashing of the product during preparation of the diluted product may occur; therefore exposure to the undiluted product may occur. Oral and ocular exposure due to hand-to-mouth and hand-to-eye contact is negligible when personal hygiene measures are taken.

This product is used in large poultry farms, and after absorption by the chicken fluralaner and/or metabolites may be found in the excreta. A poultry farm can be very dusty, therefore inhalation of dust particles including fluralaner and/or metabolites cannot be excluded when working with treated chickens.

(Accidental) exposure by adults:

Dermal:

A worst case calculation for dermal exposure is performed assuming exposure to both the diluted product as well as the undiluted product in a single day. Moreover, it is a worst case assumption that part of the product (14%) remains on the user's skin after the first administration occasion (which seems unrealistic as the user is expected to maintain personal hygiene measures). This results in a worst case exposure estimate of 1.25 mg fluralaner/kg bw/day after the second administration (assuming a bodyweight of 60 kg).

When compared to the 'adjusted dermal NOEL' of 185 mg/kg bw/day derived from the NOEL of the 28 day study in rats, i.e. 50 mg/kg bw/day, adjusted with a skin penetration factor of 3.7 for rat versus human skin, the margin of exposure (MOE) would be 148. Although this does not take into account the presence of the penetration enhancer diethylene glycol monoethyl ether (DGMEE) in the formulation, it can be concluded that no risk is expected for the user as the CVMP decided on an overall dermal NOAEL of 100 mg/kg bw/day to be used for the risk characterisation of short-term dermal exposure; and the exposure level is considered rather a worst case when considering exposure to the undiluted product as well as to the diluted product and accumulation of the product on the user's skin. Moreover, prolonged exposure will be mitigated by personal hygiene measures such as washing hands.

Also no risk for the pregnant user is expected after incidental dermal exposure, when compared to the 'adjusted dermal NOEL' of 620 mg/kg bw/day derived from the NOEL of the developmental study in rabbits, i.e. 100 mg/kg bw, adjusted with a skin penetration factor of 6.2 for rabbit versus human skin, the MOE would be 496, however this does not take into account the presence of the penetration enhancer DGMEE. Alternatively, when compared to the oral NOAEL of 10 mg/kg bw/day (foetal NOAEL, derived from the developmental study in rabbit; to be used as a dermal reference value considering that oral bioavailability is at least as high as dermal bioavailability), and also adjusting with a skin penetration factor of 6.2, a MOE of 50 is calculated, which would be slightly below the acceptable MOE of 60 (interspecies safety factor of 2.4 x 2.5 = 6 for rabbit vs human, based on allometric scaling and an intraspecies safety factor of 10). Again, the exposure level is considered rather worst case and prolonged exposure will be mitigated by personal hygiene measures such as washing hands.

Additionally, no reproductive effects are expected. When the estimated exposure level is compared to the oral NOEL of 100 mg/kg bw/day derived from the one-generation study in rats to be considered as a dermal reference value, adjusting with a skin penetration factor of 3.7 for human versus rat skin, a MOE of 296 is calculated. Even when using the NOAEL of 50 mg/kg bw/day, as derived in the 2-generation study for reproductive toxicity, the MOE would be acceptable.

Inhalation:

Based on a very worst case situation, assuming the total dose to be found in excreta and turned into dust resulting in 0.033 µg fluralaner/mg dust and an occupational exposure level of 10 mg/m^3,

inhalation rate of 2.13 m^3/h and exposure duration of 4 hours per day, the farmer would maximally inhale 2.8 µg fluralaner per day or 0.05 µg/kg bw for a 60 kg adult. This demonstrates a large safety margin, even when compared to the ADI of 10 µg/kg bw/day, even when including an extra safety factor to correct for inhalatory bioavailability versus oral bioavailability. It can therefore be concluded that the inhalation of dust containing fluralaner and/or its metabolites will not present a risk for the user when the occupational exposure level for dust is not exceeded.

Local effects:

Ocular and eye irritation studies using the final formulation have demonstrated that the product may be slightly irritating to the skin and eye; therefore appropriate warnings should be in place. The product is not expected to cause skin sensitisation and/or allergic reactions.

Accidental exposure by children:

With regard to accidental exposure by children, the ingestion of 15 ml of product was considered, equivalent to 150 mg of fluralaner and a dermal exposure to 35 mg of fluralaner, resulting in an estimated oral exposure of 10 mg fluralaner/kg bw and an estimated dermal exposure of 2.3 mg fluralaner/kg bw for a 15 kg child.

The estimated dermal exposure level of 2.3 mg/kg bw is compared to the 'adjusted dermal NOEL' of 185 mg/kg bw/day derived from the NOEL of the 28 day study in rats, i.e. 50 mg/kg bw/day, adjusted with a skin penetration factor of 3.7 for rat versus human skin. This results in a MOE of 80, however, it does not take into account that the formulation contains a penetration enhancer, DGMEE.

The estimated oral exposure level of 10 mg/kg bw is compared to the oral NOEL of 10 mg/kg bw/day derived from the developmental study in rabbits (maternal effects; fatty acid changes of the liver and the related reduction in blood chemistry parameters). This results in a MOE of 1. In fact, exposure should be compared to the oral LOAEL of 20 mg/kg bw/day as observed in the 28-day repeated dose toxicity study in dogs, including a safety factor of 3–10 for extrapolating from a LOAEL to a NOAEL.

Both the dermal and oral MOEs as calculated are below the acceptable level, though it is acknowledged that the MOEs were calculated using NOELs derived from repeated dose toxicity studies. However, as the product is to be used in a professional setting and not expected to be stored in the household, accidental exposure by children is considered to be negligible. In addition, the product is presented in HDPE bottles with child-resistant screw caps, which would further mitigate accidental exposure by children. Based on the above risk assessment it is concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the CVMP/VICH guidelines. The predicted environmental concentration for soil was calculated in accordance with VICH GL6 and the CVMP Guideline on the Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1-Corr.).

The product is not intended to be used in broilers, since during their relatively short lifetime no colonisation of red blood mites is expected to occur. Therefore no assessment was provided for this production type of chickens.

Phase I:

The calculated PEC_{soil} values are presented below.

Exposure assessment	Value (µg/kg)
PEC soil laying hen	1.0
PEC soil replacement layer	2.0
PEC soil broiler breeder	0.56

Even though the PEC_{soil} values in phase I are below the trigger value of 100 µg/kg (which would usually exempt the substance from further testing), the CVMP considered the need for further data taking into account the specific nature of the active substance ('however clause').

Concerning the use of fluralaner as a veterinary medicinal product, there are two particular concerns, namely that the active substance could have persistent, bioaccumulative and toxic (PBT) properties, and the high toxic potency for invertebrates, in particular mites and insects. Because of these particular concerns, two further assessments were performed in phase II: an assessment of PBT properties (hazard assessment) and a tailored hazard and risk assessment of the compound to the environment due to its specific toxicity profile.

Phase II:

PBT assessment

The applicant has submitted all the studies required for a PBT assessment.

Persistence studies:

Results show that in soil, fluralaner disappears slowly under aerobic conditions with DT_{50} values of 989 days for sandy loam, 404 days for loam, 717 days for clay, and 697 days for silt loam. Corresponding DT_{90} values were 3286 days, 1342 days, 2382 days, and 2315 days, respectively at a temperature of 18–23 °C. In aquatic systems, fluralaner disappears quickly under both, aerobic and anaerobic conditions (DT_{50} values in fresh water sediment were determined with 85.9 days and 71.1 days in river and pond sediment under aerobic conditions and with 8.53 days and 49.1 days, respectively, under anaerobic conditions). DT_{50} values in fresh water were determined with 3.35 days for the river water while in pond water a DT_{50} value was not calculated due to the fact that the test item had essentially completed dissipated prior to the sampling interval of 7 days. Under anaerobic conditions, the DT_{50} values in water were determined with 3.62 days for river water and 3.19 days for pond water. The study was conducted at a temperature of 20.8 °C. Accordingly, the respective highest DT_{50} values for water (3.35 days aerobic, 3.62 days anaerobic) and sediment (85.9 days aerobic, 49.1 days anaerobic) were extrapolated to a temperature of 12 °C (which represents the environmental temperature in Europe) following Arrhenius equation. The corrected DT_{50} values for water amount to 7.7 days (aerobic) and 8.3 days (anaerobic). For sediment the corrected DT_{50} values for amount to 196.2 days (aerobic) and 112.1 days (anaerobic).

Considering these findings, fluralaner has to be classified as persistent/very persistent (P/vP) in soil and aerobic freshwater sediment, while it is clearly not persistent in freshwater and anaerobic freshwater sediment.

Toxicity studies:

A *D. magna* reproduction study was conducted and the lowest NOEC determined, which was for the endpoint growth, was 47 ng/l (0.047 µg/l). Considering these findings, fluralaner has to be classified as toxic (T; NOEC <0.01 mg/l).

Bioaccumulation studies:

The bioconcentration factor at steady state was reported with 79.4 l/kg, the lipid normalised bioconcentration factor at steady state with 48.5 l/kg. Considering these findings, fluralaner clearly does not fulfil the bioaccumulation criterion, which is a bioconcentration factor in aquatic species >2000 l/kg.

Fluralaner is considered to be very persistent (vP) and toxic (T) but is not bioaccumulative. Therefore fluralaner is not considered to be a PBT substance.

Based on these findings, the following additional SPC statement in Section 6.6 was proposed by the applicant:

"Fluralaner should not enter water courses as this may be dangerous for aquatic invertebrates."

Additionally, a warning on the persistence of fluralaner is included in section 5.3.

Risk assessment for the soil compartment

A phase II assessment has been performed.

The toxicity to the soil compartment was assessed conducting a collembolan reproduction toxicity test, according to OECD guideline 232. The results of this study were used to characterise the risk for soil organisms, and concluded that a risk to soil is not anticipated (with a calculated RQ from the PNEC derived from this study being 0.65).

A study on adsorption and desorption to soil has been submitted according to OECD guideline 106, with deviations. This study has been performed with a high (5% v/v) concentration of organic solvent in the aquatic phase and is therefore considered unreliable as the presence of solvent potentially underestimates the actual sorption to the soil. Additionally, the applicant has provided a study in which the Koc was estimated with the HPLC method, according to OECD guideline 121. Given the substance characteristics and the reported difficulties in performing a reliable test according to OECD guideline 106 and the absence of specific guidance on how to handle in this situation, the outcome of the HPLC study is considered sufficiently reliable for use in the risk assessment. Therefore, the PEC calculations were performed with the reported log Koc of 4.3 from the OECD guideline 121 study.

The initial assessment identified a risk for all compartments, excluding groundwater.

To allow for the refinement of the calculated PECs, metabolism data was considered. On the basis of a metabolism study it could be concluded that only 36.6% of the applied dose of fluralaner is excreted as the sum of unchanged parent + major metabolites in the excreta. Therefore, data on metabolism were considered for further refinement of the $PEC_{soil,}$ and consequently the PECs for surface water, groundwater and sediment, could be reduced with a factor 0.366. The recalculated RQs are presented below.

Refined PEC and risk quotients for soil

Target animal	PEC [µg/kg]	PNEC [µg/kg]	RQ
Laying hen	4.82	7.47	0.65
Replacement layer	4.56	7.47	0.61
Broiler breeder	2.60	7.47	0.35

Refined PEC and risk quotients for surface water

Target animal	PEC [µg/L]	PNEC [µg/L]	RQ

Laying hen	0.00101	0.0047	0.21
Replacement layer	0.000953	0.0047	0.20
Broiler breeder	0.000542	0.0047	0.12

Refined PEC and risk quotients for sediment

Target animal	PEC [µg/kg]	PNEC [µg/kg]	RQ
Laying hen	1.00	4.7	0.21
Replacement layer	0.952	4.7	0.20
Broiler breeder	0.541	4.7	0.12

Conclusions on the environmental risk assessment

An ERA was provided according to the CVMP/VICH guidelines. Because of concerns on the PBT/vPvB characteristics of the active substance and the high toxic potency for invertebrates, a targeted phase II assessment has been performed. The PBT assessment resulted in the conclusion that the substance is not a PBT. In regard to toxicity to the soil, sediment, surface water compartments and groundwater it could be concluded that the available data do not indicate a risk.

Overall it could be concluded that no risk to the environment is to be anticipated from Exzolt when used as recommended in the Summary of Product Characteristics.

Residues documentation

MRLs

Fluralaner is included in table 1 of the annex to Regulation (EU) No 37/2010, as follows:

Pharmaco-logically active substance	Marker residue	Animal Species	MRL (µg/kg)	Target Tissues	Other provisions	Therapeutic classification
Fluralaner	Fluralaner	Poultry	65 650 650 420 1300	Muscle Skin and fat Liver Kidney Eggs	NO ENTRY	Antiparasitic agents / Agents against ectoparasites

The excipients listed in section 6.1 of the SPC are allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required when used as in this product.

Residue studies

Pharmacokinetics

Two GLP compliant studies were performed to investigate the pharmacokinetic profile of fluralaner in blood plasma of chickens. (see part 3)

Two radiolabelled residue studies were performed using [^{14}C]-fluralaner in laying hens administered the final product at the recommended dose (two single doses of 0.5 mg fluralaner/kg bw, 7 days apart) by oral gavage. The highest total radioactive residue (TRR) concentration in tissues was found in liver, followed by skin with fat, kidneys and then breast muscle. In tissues and eggs the major component is assigned as unchanged fluralaner. Numerous metabolites were detected in all tissues and eggs, but

these were generally present at low concentrations with only one metabolite (the carboxylic acid of fluralaner) detected (slightly) above 10% of the TRR. A large portion of the total dose is excreted via eggs, i.e. a mean of 28.8% of the total dose. Fluralaner and/or metabolites were measured at high concentrations in bile. From excreta samples, analysed in one of the studies, it could be estimated that 60% of the dose could be recovered from the excreta, during the 7 days following the first of the two administrations.

Depletion of residues

Three pivotal residue studies in chickens were performed to assess the depletion of the marker residue fluralaner in edible tissues and eggs. Two studies were performed in laying hens; one to assess the residues in eggs and one to assess the residues in tissues, which have been evaluated during the MRL assessment. A new study was provided for male and female breeder chickens.

To study the residues in eggs, a GLP compliant residue depletion study was provided using a sufficient number of laying hens and administering the final formulation at the recommended dose (two single doses of 0.5 mg fluralaner/kg bw, 7 days apart) and by the recommended administration route (orally, in drinking water). Eggs were analysed for marker residue fluralaner using a sufficiently validated LC-MS/MS method, with a limit of quantification (LOQ) of 400 µg/kg. The highest concentrations of fluralaner in eggs were observed 7 days after administration of the product, resulting in the highest average concentration observed at day 14 (7 days after second dose), i.e. 828.4 µg/kg (range 636.7–1065 µg/kg).

To study the residues in chicken tissues, two GLP compliant residue depletion studies were provided using a sufficient number of chickens and administering the final formulation at the recommended dose (two single doses of 0.5 mg fluralaner/kg bw, 7 days apart) and by the recommended administration route (orally, in drinking water). Tissue samples (liver, muscle, kidney and skin and fat) were obtained at days 1, 2, 4, 7 and 10 after last administration and were analysed using a sufficiently validated LC-MS/MS method with respective LOQs of 20 µg/kg for kidney, 30 µg/kg for liver, 5 µg/kg for muscle and 40 µg/kg for skin and fat.

In one study, only layer hens (Lohman LSL-Classic; 7 per slaughter group) were included. The highest concentrations of fluralaner were observed 1 day after administration of the product with the highest residue levels observed in liver (range 906.60–1963.00 µg/kg) and skin and fat (range 977.80–1526.00 µg/kg), followed by kidney (range 582.10–1203.00 µg/kg). Lower concentrations were found in muscle (range 75.60–152.20 µg/kg). The residues depleted in all tissues at a comparable rate. At day 10 post last dose, concentrations had declined to 133.10–348.60 µg/kg in liver, to 122.10–418.10 µg/kg in skin and fat, to 83.71–253.50 µg/kg in kidney and 16.32–40.39 µg/kg in muscle.

Considering the potential use of the product in breeders, where about 8% of the animals are male, the second study included male and female chickens (Lohman LSL-Classic; 4 males and 4 females per group). The highest concentrations of fluralaner were observed 1 day after administration of the product with the highest residue levels observed in liver (range 1250.00–2060.00 µg/kg), followed by kidney (range 774.80–1260.00 µg/kg) and skin and fat (range 696.40–1724.00 µg/kg). Lower concentrations were found in muscle (range 154.70–285.20 µg/kg). The residues depleted in all tissues at a comparable rate. At day 10 post last dose, concentrations had declined to 110.30-509.90 µg/kg in liver, to 68.00–289.40 µg/kg in kidney, to 69.67–516.40 µg/kg in fat and skin and 13.25–70.61 µg/kg in muscle. Higher concentrations of fluralaner were observed in males compared to females.

Withdrawal periods

The provided residue depletion studies were suitable for determination of the withdrawal periods.

No CVMP Guidance is available for the calculation of withdrawal periods for eggs; however, the tolerance limit calculation per time point is often used. The highest residue level is found 14 days after the first administration; at this time point a 95/95 tolerance limit of 1174.72 µg/kg is calculated. The residue depletion study in eggs demonstrates, by calculating the 95/95 tolerance limits for each time point, that residues are below the MRL of 1300 µg/kg at all time points during the administration period. The zero day withdrawal period for eggs after administration of the product at the recommended dose of 0.5 mg fluralaner/kg bw/day twice 7 days apart, is acceptable.

The withdrawal period for meat and offal is 14 days when administering the product at the recommended dose of two single doses of 0.5 mg fluralaner/kg bw, 7 days apart. This withdrawal period is the worst case calculated on the basis of male animals which have a slower residue depletion compared to female chickens.

The proposed withdrawal periods are justified:

Meat and offal: 14 days.

Eggs: zero days.

Overall conclusions on the safety and residues documentation

Pharmacokinetics

Following oral administration to dogs, fluralaner appears to be readily absorbed. Fluralaner is well distributed to tissues. Radiolabelled studies in dogs, rats and laying hens demonstrated that the highest concentrations were found in fat and liver, followed by kidney and muscle. Fluralaner appears to be highly bound to plasma proteins. Fluralaner accumulates and steady state fluralaner plasma concentrations were reached after approximately 30 days in rats and about 90 days in dogs indicating a long half-life. Unmetabolised fluralaner is the major component present in all analysed organs and tissues in dogs, rats and laying hens. Fluralaner is metabolised to many metabolites. The main excretion route is faeces; urinary elimination was limited.

Toxicology

A battery of toxicity tests have been provided using fluralaner or the finished product.

From the repeated dose toxicity studies it appeared that liver was the most sensitive organ, resulting in increased organ weight, hepatocellular fatty change and effects in related blood parameters (reduction in blood cholesterol, phospholipid and triglyceride levels). Post-implantation, post-natal and breeding losses were observed in rats at the higher dose (500 mg/kg bw/day). At this dose also reduced body weight, clinical signs, pathological findings, and delayed physical and sexual development were observed in the pups. Developmental toxicity was studied in the rat and rabbit. A higher incidence of dilated renal pelvis/ureter and supernumerary ribs was observed in rats (NOEL 100 mg/kg bw per day). Increased fusions in cervical vertebra 2 were observed in rabbits (NOAEL 10 mg/kg bw/day).

Fluralaner is not genotoxic. Carcinogenicity studies have not been performed and are not requested.

Other effects

The product was shown to be slightly irritating to the skin and eyes. The product has no skin sensitising properties. The data presented are considered adequate to characterise the toxicity profile of both the active substance and the finished product.

User safety

This product is to be administered by professional users. Accidental exposure by children is considered to be unlikely as the product is to be used in a professional setting. In addition, the product is presented in HDPE bottles with child-resistant screw caps.

The most likely exposure situation is during the pre-application phase when the bottle is opened, the product is diluted and transferred to the drinking water medication tank and the measuring devices are cleaned. The main contact route for this product is therefore the dermal route. In addition, ocular exposure may occur. Also, as this product is used in large poultry farms, it may end up in dust and then be inhaled.

Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. Appropriate warnings for the user have been included in the SPC and other product literature.

Environmental risk

Fluralaner is considered to be very persistent (vP) and toxic (T) but is not bioaccumulative. Therefore fluralaner is not considered to be a PBT.

After assessment of the available information, for the soil compartment, the available data do not indicate a risk to soil organisms, with a risk quotient close to 1 (0.65). Also for the aquatic and sediment compartments, no risk is anticipated when the product is used as suggested in the Summary of Product Characteristics.

Residues

In a non-radiolabelled residue depletion study in chicken, fluralaner concentrations were the highest in liver, and lower in kidney, skin and fat. Lower concentrations were found in muscle. Residues were below the MRL at day 7 for skin and fat and at day 10 for liver, kidney and muscle, except for one male which still had a residue level above the muscle MRL. Fluralaner was also excreted in eggs, resulting in the highest residue levels found 7 days after administration of the product; though residue levels were below the MRL at all time points.

Based on the marker residue data and the MRLs established by the CVMP, withdrawal periods for edible tissues of 14 days for meat and offal in chickens and zero days for eggs were calculated.

Part 4 – Efficacy

Pharmacodynamics

Fluralaner belongs to the isoxazoline class, showing both acaricidal and insecticidal activity. Information on pharmacodynamics has been provided in an article describing the inhibitory action of fluralaner on the arthropod ligand-gated chloride channel (GABACl) channel (by using cell models that express gene subunits). From this study it could be concluded that the ectoparasiticidal activity of fluralaner was not affected by dieldrin resistance mutation in the gene encoding for GABACl (*C. felis*). In addition, fluralaner did not show any inhibitory action on the rat GABACl, expressed in a cell model.

The acaricidal effect of fluralaner on the poultry red mite (*Dermanyssus gallinae*) has been investigated

in three studies. It was demonstrated that fluralaner has both contact and feeding acaricidal activity against poultry red mites, and nymphs are more susceptible to the contact efficacy than adult stages. The LC_{90} is 2.378 mg/l of fluralaner for a mixture of nymphs and adult stages after feeding on fluralaner-spiked blood. A reduction in oviposition might be due to the lethal effect of treatment on affected female mites. No effect of treatment was observed in larval hatchability and nymphal conversion in mites (moulting).

Development of resistance

Since fluralaner is not yet authorised for use in chickens (and was only first authorised in 2014, for use in dogs), the risk of resistance development with regard to the use of this product is currently not expected to pose a risk to the population.

The susceptibility of eleven poultry red mite field isolates and one laboratory strain to fluralaner was tested in four studies. The field isolates for the studies were collected from commercial poultry farms in Germany, France and Spain in 2014 or 2015. The laboratory strain was collected from a German commercial layer farm in 2001. All studies used a "mite package test" to determine susceptibility. This in vitro test was developed based on the FAO-recommended resistance test for tick larvae, which is meant to assess lethal concentration following contact exposure, but not for oral exposure of the ectoparasite.

In the studies, phoxim, spinosad, propoxur and deltamethrin were used as reference active substances. The field isolates of *Dermanyssus gallinae* (*D. gallinae*) as well as the laboratory isolate were highly susceptible to fluralaner.

As a result, in all field isolates the mortality rate of mites was more than 90% at fluralaner concentrations of < 31.25 mg/l. The laboratory strain also had a mortality rate of over 90% at the concentration of < 31.25 mg/l. The LC_{90} (lethal concentration, mortality rate of 90%) against the other tested substances (i.e. phoxim, spinosad, deltamethrin and propoxur) was substantially higher. All studies had the same study design.

The results of the studies conducted between 2014 and 2016 showed a higher rate of resistance to the organophosphate phoxim than in a study conducted in 2011. Poultry red mite strains had developed an acquired resistance to phoxim, propoxur and to deltamethrin. Based on the LC_{90} values of spinosad in all studies, it was not demonstrated that the observed high tolerance of mites to spinosad was acquired resistance or that poultry red mites have an innate resistance to spinosad. However, the following sentence is accepted in section 5.1 of the SPC: "In vitro bio-assays show that fluralaner is effective against parasites having proven field resistance, including organophosphates, pyrethroids and carbamates."

Since all mobile mite stages will be exposed to the veterinary medicinal product (VMP) and the treated chicken is the single host during at least one life cycle, there is a risk that resistance might develop. Therefore prudent use of the VMP is of great importance. Warning sentences have been added in section 4.4 of the SPC to minimise the risk of resistance development (e.g. frequency of use, use according to the recommended dosing scheme).

Pharmacokinetics

See part 3.

Dose determination / finding studies

Two non-GLP dose determination studies were carried out in Germany to establish the optimum dose of fluralaner to treat infestations of chicken with *Dermanyssus gallinae*. In one pilot study, the VMP was administered as a single dose of 0.5, 1.0 or 2.0 mg fluralaner per kg body weight (n=6 animals per group) on day 0, and a fourth group was treated twice at 0.5 mg/kg one week apart. In another study, the VMP was administered twice, with a one week interval between doses, at doses of 0.25, 0.5 and 1.0 mg/kg of fluralaner. In both studies a near-to-final formulation of the VMP was used. The slight difference in formulation (absence of α-tocopherol, an antioxidant) is not deemed to have affected the results of dose finding since the product was diluted in drinking water before administration. Mite mortality was investigated at D 1, 5, 8, 12, 15, 19, 22 and 26.

In order to evaluate efficacy in the dose determination studies, an infestation model with *Dermanyssus gallinae* had been developed. Considering the very small size and frailness of a red mite's body it was concluded that chickens needed to be infested with 200 mites per hen. Four of six hens per group were individually transferred to an infestation box, and infested with approximately 200 unfed *D. gallinae* per infestation on D1, D5, D8, D12, D15, D19 and D22. After removal of the chickens, 25 engorged mites remaining in the box were sampled and incubated for 24 hours. The sample size of 25 engorged mites per chicken to evaluate the efficacy was derived from the CVMP Guideline for the testing and evaluation of the efficacy of antiparasitic substances for treatment and prevention of tick and flea infestation in dogs and cats (EMEA/CVMP/005/00-Final-Rev.2). This guideline considers a tick infestation on dogs/cats adequate if 12–25 ticks are attached on a control animal. In contrast to ticks and fleas, mites are short-feeders which take a blood meal within an hour and then leave the host. Therefore, the sampled engorged mites were incubated in a tube for approximately 24 hours. As the mortality rate of the mites sampled from the untreated control groups was very low, the efficacy results are considered reliable and this infestation model was accepted. The parameter 'mite mortality' and the parameter 'mite inhibition' was used in both studies. This parameter was defined as the sum of dead and damaged red mites, but has not been taken into consideration since the term 'damaged' was classified as "uncoordinated movements of mites", which is not well-defined and is difficult to interpret.

Within 4 hours of starting to feed on treated chickens, dead mites (100%) were observed on Day 1 and Day 8, and mortality above 90% was observed at the other observation days (D 5 and 12) after the first and second treatment doses. Mite mortality (over 24 hours) was higher when the VMP at the dose rate of 0.5 mg fluralaner kg/bw was administered twice at one week interval (100%) compared to a single dose administration (97% and 55%) at Day 8 and 12, respectively. In the second study, satisfactory efficacy (>90%) was demonstrated after treatment twice, 1 week apart, and was shown to last up to day 12, 15 or 19 after dose rates of 0.25 mg/kg, 0.5 mg/kg and 1.0 mg/kg, respectively. The dose of 0.5 mg fluralaner/kg bw was selected as being the optimum dose since a persistent efficacy of 15 days covers two mite life cycles.

The mite strain used for the dose determination studies was the same as that used for the resistance study. The studies conducted between 2014 and 2016 used *Dermanyssus* field strains that had been obtained from several locations, which showed a susceptibility to fluralaner that was comparable to the susceptibility of the laboratory strain.

The applicant also provided another dose determination study that was carried out in the USA using hens naturally infested with *Ornithonyssus sylviarum,* which is rarely reported in commercial European poultry farms, but can be found in small numbers of hobby poultry (Jansson et al., 2014). Thus this mite species is considered as an exotic mite in Europe. However, since the pack sizes of the product are not considered suitable for use in individual birds (hobby poultry), and data were considered as being too limited to support the efficacy, reference to this mite species is therefore not part of the SPC.

Dose confirmation studies

The dose confirmation studies fulfilled all requirements of the CVMP guideline: Demonstration of efficacy of ectoparasiticides (7AE17a, 1994). Three GCP dose confirmation studies were carried out in naturally infested flocks of hens in farms in Germany, France and Spain, respectively. The studies confirmed the efficacy of the VMP at the proposed dose of 0.5 mg fluralaner/kg bw twice, 7 days apart, for the treatment of poultry red mite (*Dermanyssus gallinae*) infestation in layer hens. Farms with two similar houses were selected to allow the use of a negative controlled study design. The dose confirmation studies were carried out in farms because poultry red mites reside predominantly in the environment (cracks, corners, perches, etc) and it is therefore difficult to reproduce mite infestation in laboratory conditions. Furthermore, as mite infestations of houses had to be monitored for a long period, the trial could not be conducted in a clean laboratory setting.

Lammers et al. ("Experimental validation of AVIVET trap, a tool to quantitatively monitor the dynamics of *Dermanyssus gallinae* populations in laying hens") showed that the traps selected for the studies can be used to quantify *D. gallinae* infestation in hens as there was a significant correlation (93.8%) between the numbers of mites in the chicken cages and the numbers of mites collected in the traps. Although the correlation coefficient might be different under field conditions, the use of mite traps is considered to be a robust method to quantify mite infestations in a poultry house.

In the dose confirmation studies, mite traps were placed close to *D. gallinae* clusters and known mite hiding places. Traps were distributed exactly at the same place on days -7, -1 (pre-treatment) and on days 1, 3, 6, 9, 14, 21, and weekly or every second week until the end of the study. The traps were left for about 24 hours, then collected, deep frozen and sent to a laboratory for mite differentiation and counting (by a blinded person).

The animals in the most infested house of the farm were treated for animal welfare reasons. This is accepted since the Henderson Tilton formula, used to calculate the percentage of reduction of mites, is appropriate for non-uniform populations (i.e. mites/house).

The guideline "Demonstration of efficacy of ectoparasiticides" (7AE17a, 1994) recommends a threshold of more than 90% efficacy in mites, other than *Sarcoptes scabiei*. In 2 of the dose confirmation studies, the efficacy of treatment against *D. gallinae* infestation was higher than 98–99% from day 3 to day 119 and until day 89.

In the third dose confirmation study, the efficacy was above 95% on day 3 and above 99% from day 6 to day 77. The percent mite reduction in the house with the treated birds was also calculated in comparison to baseline values of mite counts on day -1, and this reduction was above 99% for 133 days.

No treatment-related adverse events were observed, the VMP was well tolerated. In the third dose confirmation study, a significant decrease in laying rate was observed in both the treatment and control groups between week -1 (96.6%) and week 0 (87.5%). This decrease in laying rate on the day of treatment was however considered to be related to the stress induced by the weighing of the hens.

In conclusion, the dose confirmation studies confirmed that the efficacy of the VMP against poultry red mite (*Dermanyssus gallinae*) infestation was higher than 90% in houses with layer hens for at least 77 days. Dose determination studies demonstrated that the efficacy was >90% for a minimum of 15 days after repeated artificial infestations of individual chickens with unfed mites.

The proposed indication 'control' was not demonstrated as the studies were all performed in already infested houses and the duration of the effect was variable between clinical sites. According to the CVMP Guideline: Demonstration of efficacy of ectoparasiticides (7AE17a, 1994), "it is the purpose of treatment with ectoparasiticides to eliminate or to reduce arthropod parasites or to protect animals from

them, in order to maintain animal health and to prevent losses in production". Moreover "where a claim for control of infestation is made, the period of time it takes to achieve control and the period over which control is achieved must be demonstrated". The CVMP therefore considered that the indication should

be limited to "treatment". In contrast to ectoparasite (re-)infestations of treated companion animals, new mite infestations of chicken houses are exceptional in layer farms due to lack of contact with chickens from outside providing that regular hygiene measures are taken. Re-infestations and parasite population regrowth during the production cycle can be controlled by implementing appropriate and strict biosecurity measures at house and farm level, including treating all infested poultry houses in proximity to each other.

Target animal tolerance

Two pivotal target animal safety studies were carried out, one in laying hens and one in 3 week old broilers. The pivotal target animal safety study in laying hens was carried out at the peak of their laying performance. Both studies were GLP compliant and designed according to VICH GL 43: Target animal safety for Veterinary Pharmaceutical Products. The safety of fluralaner was evaluated at the dose rate of at 0x, 1x, 3x or 5x the recommended therapeutic dose (RTD) in both studies. Laying hens were treated for three consecutive days over two periods, 5 days apart, i.e. on days 1, 2 and 3 and days 7, 8, and 9. Broilers were treated weekly for six consecutive weeks. Both studies demonstrated that fluralaner (10 mg/ml, final formulation) is in general well tolerated at the recommended dose and up to 5 times the recommended dose.

Although the product is not intended to be used in broilers, since during their relatively short lifetime colonisation of red blood mites is not expected to occur, the safety study in broilers was considered to be relevant. It should be noted that the term 'broiler' refers to a certain production type of chickens.

The safety study in hens was a thorough study including clinical observations, repeated blood sampling for chemistry and haematology measurements as well as daily assessment of the laid eggs and egg production. No clinically adverse events and no treatment related deviations were observed in the following egg parameters: egg production, shell thickness and Haugh unit rating. Significant differences were observed in monocyte levels. These differences, which were increased in treatment groups 2 and 3 (1x and 3x RTD), but not in the group receiving the highest dose (5x), cannot be considered as clinically relevant. The safety study in three week old broilers (Mills 2016) did not reveal any treatment related clinical adverse events or dose dependent abnormalities in haematology, clinical chemistry or pathology parameters.

An additional, pilot study with 18 layers also showed good tolerance at a 5 times overdose when administered weekly for 6 consecutive weeks. In two GLP studies the effect of a 3 times overdose (1.5 mg/kg bw/treatment) of oral fluralaner on reproduction on days 1 and 8 and on days 15 and 22 was studied in breeder layer hens and males. No significant effect was observed in egg production, fertilisation, hatchability and other reproduction parameters except for chick viability until 14 days. In the pilot study, a significantly higher mortality of chicks was observed in the first week of the treated group. However, this high mortality rate was only observed in this study which was conducted in a limited number of experimental units (6 pens with 3 hens and 1 male) and this higher mortality rate during their first week of age was not considered treatment related. In the other study conducted in a larger number of treated hens (8 pens with 24 females and 3 males per pen) and with 2 times the treatment duration (4 administrations), all chicks were viable.

It is concluded that the product is in general well-tolerated in male and female chickens and does not impair their reproduction i.e. egg production, fertilisation, hatchability and chick viability.

Clinical field trials

The pivotal field study investigated the safety and efficacy of Exzolt administered orally via drinking water at a dosage of 0.5 mg/kg body weight, twice, 7 days apart for the treatment of poultry red mites (*D. gallinae*) in natural infestations in commercial poultry farms. This was a negative controlled multicentre field study carried out in Spain, Germany and France in 9 farms (2 breeder farms, 2 replacement/pullet farms and 5 layer farms), which housed between 550 and 100,000 chickens of various breeds/hybrids per house. Each farm had two similar houses harbouring either the negative control flock or the treatment flock. The field trial was designed according to the CVMP guideline on demonstration of efficacy of ectoparasiticides (7AE17a, 1994).

Presence of the poultry red mite was confirmed prior to the trials, using mite traps. To evaluate infestation level, 8–24 mite traps per house, depending on the number of chickens and production type, were equally distributed over the entire house at time points: day -14, -11 and day -1, day 0, 3 and 6, day 9, 14 and then every second week.

No treatment related adverse events were observed.

The primary efficacy criterion was the percentage reduction of poultry red mites (mobile stages) in the treated house (were the chickens were treated) compared to the control house. The study was planned to be maximum of 6 months but ended when the production cycle of the chickens terminated earlier or if the control house also had to be treated, i.e. between 53 to 226 days. The secondary efficacy criterion was the percent mite efficacy against different *D. gallinae* stages. The weekly mortality rate and the weekly laying rate of the two flocks were also recorded.

For animal welfare reasons, the chickens in the more affected house of each farm were selected as the treatment group. This was accepted since the Henderson Tilton formula was appropriate to calculate the percentage of reduction of mite infestation in live animals and to compare non-uniform populations (i.e. mites/house).

In replacement chickens (pullets), the percentage mite reduction exceeded 99% from day 6 until day 28 and stayed over 98% until the end of the study (i.e. week 6).

Mite reduction in layer hens exceeded 99% from Day 6 (98.6% on day 3) at all 5 sites. This level of efficacy was maintained until treatment of the control house or until the end of the study (i.e. week 8 at the earliest). In week 24, the efficacy was above 94% in one site.

In breeders, the percentage mite reduction exceeded 99% from day 3 until day 98 (week 14).

Decreased efficacy (mite regrowth) was observed in three layer farms (01-A, 02-A and 09-A) and one breeder farm (04-A), approximately starting on days 113, 56, 126 and day 112, respectively. Decreased efficacy at one of these sites (02-A) from day 56 was considered to be due to an inadequate separation between the treated flock and the control house. Another farm (01-A) also did not have a fully hermetic separation between the two houses. Also, some control houses were treated with spinosad (farms 01-A (on day 64), farm 06-A (day 29) and farm 09-A (day 141), and the percentage reduction of poultry red mites was below 90% at many time points after treatment.

Tthe CVMP considered that overall these findings support the proposed indication for the treatment of *Dermanyssus gallinae* infestation at a dose of 0.5 mg fluralaner/kg bw administered twice, 7 days apart, in the chicken. The proposed indication for the "control" of poultry red mite (*Dermanyssus gallinae*) infestation was however not supported because the recommended treatment regimen does not control or provide protection against new mite infestations.

Overall conclusion on efficacy

Pharmacodynamics:

The mode of action has been sufficiently described. The main effects of fluralaner, which belongs to the isoxazoline class, are both acaricidal and insecticidal activity though blockage of GABA-gated chloride channels and L-glutamate-gated chloride channels. It was demonstrated that fluralaner has both contact and feeding acaricidal efficacy against poultry red mites at low concentrations (after feeding, LC_{90} was calculated to be 2.378 mg/l).

Resistance:

Fluralaner is not a new chemical entity but it has not been used in chickens before. The resistance development with regard to the use of this product is currently not expected to pose a risk. All tested field isolates of *D. gallinae* (collected in 2014 and 2015 in Germany, France and Spain) as well as the laboratory isolate were highly susceptible to fluralaner. However, since all stages of *Dermanyssus gallinae* will be exposed to the treated host (chicken), the development of resistance might occur and prudent use should be encouraged.

Pharmacokinetics:

The pharmacokinetic investigations in laying hens were properly conducted. Both studies characterising the plasma pharmacokinetic profile were conducted in accordance with GLP. The animals used were representative for the proposed target population. The number of animals per group was sufficient to allow a meaningful assessment of the pharmacokinetic data. The final formulation was used at the recommended treatment dose. Adequately validated analytical methods were used for the quantification of the test substance. The findings of those studies show that:

- fluralaner is rapidly absorbed after oral administration;
- dose linearity is demonstrated;
- slight accumulation of fluralaner is observed (higher second C_{max}) after two administrations of a 0.5 mg/kg dose with a-7 day interval between doses;
- plasma concentrations of fluralaner steadily decrease over time after reaching C_{max};
- the plasma clearance of 0.14 ml/min/kg is very low and the mean residence time is long (5 days);
- the absolute bioavailability is very high (91%) but was demonstrated with gavage administration.

Dose determination:

The dose of 0.5 mg fluralaner/kg bodyweight administered twice, one week apart, was determined in two dose finding studies, and confirmed in two dose confirmation studies performed under field conditions.

Tolerance:

Fluralaner was well-tolerated in a clinical field study at the recommended dose of 0.5 mg fluralaner/kg bodyweight administered twice, one week apart.

In two pivotal target animal safety studies, fluralaner was well-tolerated in layers and in three week old broilers at the recommended dose and up to 5 times the dose. It is concluded that the product is in general well-tolerated in breeder males and females and do not impair the reproduction i.e. egg production, fertilisation, hatchability and chick viability.

Efficacy:

The results of the dose confirmation studies and the clinical field trial show that the product is effective for the treatment of *Dermanyssus gallinae* infestation at the dose of 0.5 mg fluralaner/kg bw administered twice, 7 days apart, in the chicken.

Part 5 – Benefit-risk assessment

Introduction

Exzolt is a solution for use in drinking water. The active substance, fluralaner, is a systemically active ectoparasiticide belonging to the isoxazoline group, and is a potent inhibitor of the arthropod nervous system by acting antagonistically on ligand-gated chloride channels (GABA-receptor and glutamate-receptor). The target species are chickens. The product contains 10 mg/ml fluralaner and is presented in bottles containing 1 litre or 4 litres. The proposed withdrawal period is 14 days for meat and offal and zero days for eggs. The product is intended to be used in chickens for the treatment of poultry red mite (*Dermanyssus gallinae*) infestation at the dose of 0.5 mg fluralaner per kg body weight (equivalent to 0.05 ml of solution for use in drinking water) administered twice, 7 days apart.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The benefit of Exzolt is its efficacy in the treatment of chickens infested with *Dermanyssus gallinae.* The product's mite killing activity was investigated in pullets, breeders and layer hens in well-controlled laboratory and field studies conducted to an acceptable standard and from various European geographic regions, using different husbandry systems and production types.

Additional benefits

The product increases the range of available treatment possibilities against *Dermanyssus gallinae*.

Risk assessment

Quality:

Information on the development, manufacture and control of the active substance and the finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Measures to manage the risks identified below are included in the risk management section.

Risks for the target animal:

Administration of Exzolt in accordance with its SPC recommendations is generally well tolerated.

No adverse reactions were observed following the treatment of 3-week-old and adult chickens with up

to 5 times the recommended therapeutic dose (RTD) and for 3 consecutive days each week (instead of only once a week), for a total of 2 weeks.

Treatment of layer hens at up to 5 times RTD for 3 times the recommended duration of treatment had no negative effect on egg production.

No adverse effects on reproductive performance were reported when breeding chickens were treated at 3 times RTD for 2 times the recommended duration of treatment.

No treatment-related adverse reactions were observed in any of the clinical studies conducted under field conditions.

Risk for the user:

The user safety assessment has been conducted in accordance with the relevant CVMP guidance. Based on the information provided, the exposure scenarios for which a risk was identified were mild skin or eye irritation due to short-term exposure to small quantities of the product. The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment:

The environmental risk assessment has been conducted in accordance with the relevant CVMP guidance. Based on the information provided no risk for the environment is anticipated when Exzolt is used according to the SPC recommendations.

Risk for the consumer:

The consumer safety assessment has been conducted in accordance with the relevant CVMP guidance. Based on the information provided, the CVMP concluded that consumer safety for this product is acceptable when used according to the SPC recommendations. Standard advice on withdrawal periods is included in the SPC.

Risk management or mitigation measures

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

The withdrawal period is set at 14 days for meat and offal, and 0 days for eggs.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious for the treatment of poultry red mite (*Dermanyssus gallinae*) infestation in pullets, breeders and layer hens at the proposed dose of 0.5 mg/kg, administered twice with a 7-day interval.

Information on the development, manufacture and control of the active substance and finished product has been presented and leads to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal periods, have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Exzolt is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.