15 March 2018
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Bravecto Plus (EMEA/V/C/004440/0000)

International non-proprietary name: fluralaner / moxidectin

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 23 November 2016 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Bravecto Plus, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 21 April 2016 as Bravecto Plus contains a new combination of active substances which is not yet authorised as a veterinary medicinal product in the Union. The new active substance consists of a fixed combination of fluralaner and moxidectin.

The applicant applied for the following indications:

For cats suffering from, or at risk from, mixed ecto- and endo-parasitic infections:

For the treatment of tick and flea infestations in cats. This veterinary medicinal product is a systemic insecticide and acaricide that provides immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus*) killing activity for 12 weeks.

For the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 weeks.

For the treatment of infections with intestinal roundworm (*Toxocara cati*; 4th stage larvae, immature adults and adults) and hookworm (*Ancylostoma tubaeforme;* 4th stage larvae, immature adults and adults).

Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).

The active substances of Bravecto Plus are fluralaner and moxidectin. Fluralaner is an ectoparasiticide belonging to the isoxazoline group, which is systemically active against ticks and fleas. Moxidectin belongs to the milbemycin group of macrocyclic lactones and has parasiticidal activity against a range of internal and external parasites including various nematode species. The target species is cats.

Bravecto Plus spot-on solution for cats is available in three different strengths: 112.5 mg fluralaner/5.6 mg moxidectin, 250 mg fluralaner/12.5 mg moxidectin and 500 mg fluralaner/25 mg moxidectin per pipette and is presented in packs containing 1 pipette or 2 pipettes.

The rapporteur appointed is Gerrit Johan Schefferlie and the co-rapporteur is Rory Breathnach.

The dossier has been submitted in line with the requirements for submissions under Article 13b of Directive 2001/82/EC – a fixed combination application.

On 15 March 2018, the CVMP adopted an opinion and CVMP assessment report.

On 8 May 2018, the European Commission adopted a Commission Decision granting the marketing authorisation for Bravecto Plus.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 15 October 2016) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided 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 and packaging takes place outside the EEA. GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture of such veterinary dosage forms, has been provided.

Batch release within the EU takes place at Intervet UK Ltd, Milton Keynes, United Kingdom which holds a manufacturing authorisation issued by the Veterinary Medicines Directorate (UK).

A GMP declaration for the active substance manufacturing site of fluralaner was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release or a corporate representative.

A GMP declaration for the active substance manufacturing site of moxidectin was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release or a corporate representative.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Composition

The finished product is a single dose spot-on non-aqueous solution for topical administration. The spot-on solution is a clear colourless to yellow solution and contains 280 mg of fluralaner and 14 mg of moxidectin per ml of solution (i.e. 28% (w/v) fluralaner/1.4% (w/v) moxidectin).

Three strength combinations (fluralaner/moxidectin) are available covering cat body weight ranges between 1.2 kg and 12.5 kg. The three strengths are made from the same bulk solution containing

28% (w/v) fluralaner and 1.4% (w/v) moxidectin. The different strengths are obtained by filling the pipettes as single dose presentations with different volumes of the bulk solution.

The other ingredients are butylhydroxytoluene (BHT) and dimethylacetamide (DMA), glycofurol, diethyltoluamide (DEET) and acetone as described in section 6.1 of SPC.

The product is available in unit dose pipettes as described in section 6.5 of the SPC.

Containers

The primary packaging is a unit dose pipette made of laminated aluminium/polypropylene (PP) foil closed with a high-density polyethylene (HDPE) cap and packed in a laminated aluminium foil sachet. The materials in direct contact with the solution are PP and HDPE. Statements of compliance with EU Regulations on plastic materials intended to come into contact with food and relevant European Pharmacopoeia (Ph. Eur.) monographs have been provided.

One or two pipettes are individually packed into sachets which are packed into a cardboard box.

The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

The pack sizes are consistent with the dosage regimen and duration of use.

Development pharmaceutics

Active substances

Fluralaner:

Three polymorphic forms have been identified, but only one is manufactured. Either non-micronised or micronised fluralaner may be used. As the active substance is dissolved easily during the manufacturing process of the finished product, particle size and polymorphic form are not critical.

The quality of the active substance is documented in an ASMF which has been assessed and accepted already for use within another veterinary medicinal product belonging to the same company.

Moxidectin:

Moxidectin is an amorphous powder that is easily soluble in the solvent system of the finished product. Since moxidectin is susceptible to oxidation its Ph. Eur. monograph permits the inclusion of antioxidants. The moxidectin used in manufacture of this product contains BHT.

The quality of the drug substance is suitably controlled according to a certificate of suitability issued by the European Directorate for Quality of Medicines and Healthcare (EDQM).

Excipients

All excipients are well known pharmaceutical ingredients and their quality is compliant with to Ph. Eur., USP or in-house standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

The use of organic solvents in veterinary spot-on solutions is common. For the safety of these solvents in this product, see Part 3.

Finished Product

Bravecto Plus spot-on solution is a development of the previously approved Bravecto spot-on solution, containing 28% (w/v) fluralaner as active substance, by adding 1.4% of the active substance moxidectin.

Formulation development

The solubilities of fluralaner and moxidectin in regard to the final formulation and its manufacture are considered well addressed. A number of other considerations in the development of the Bravecto Plus formulation leveraged knowledge obtained from the Bravecto spot-on solution formulation development.

BHT is already present in the active substance moxidectin as supplied. Formulations with and without BHT in addition to that included in moxidectin were investigated. The use of additional BHT in the formulation as an excipient to prevent degradation of moxidectin during its shelf-life was adequately justified.

The formulation used during clinical studies is the same as that intended for marketing.

Manufacturing process development

The development of the compounding and the filling process was properly explained from laboratory scale via pilot scale to commercial scale manufacturing, taking into account adjustment of the filling volumes and testing of seal integrity of the pipettes and sachets.

Much of the development of the Bravecto Plus formulation at pilot scale level was leveraged knowledge obtained from the Bravecto spot-on solution formulation development. With respect to the similarity in formulation this is acceptable. As a consequence the proposed commercial manufacture of the Bravecto Plus formulation is very similar to the Bravecto spot-on solution formulation.

Container closure system

The proposed pipettes and sachets comply with the requirements of the EU Food Directive and where applicable with the Ph. Eur.

For potential adsorption of the active substance and the quality of the packaging over shelf-life reference is made to the stability studies. The results of the migration studies indicate that there is no relevant leaching. The identical solvent systems in Bravecto Plus compared to Bravecto spot-on solution justify the application of leachable studies done for Bravecto spot-on solution to Bravecto Plus.

Microbiological attributes

The preservative efficacy of Bravecto Plus is confirmed with the similar Bravecto spot-on solution formulation.

The formulation contains a number of solvents, is non-aqueous and does not support microbiological growth. The omission of limits for microbial quality on the finished product specification has been satisfactorily justified.

Stability of the drug product

Preliminary non-GMP and pivotal stability data on prototype Bravecto Plus formulations and the final formulation demonstrate the proposed veterinary medicinal product is sufficiently stable.

It can be concluded that the final formulation is suitable with respect to its intended use. Compatibility between the components of the formulation and of the formulation with the container closure system

has been adequately justified.

Method of manufacture

The manufacturing process is straightforward and comprises:

- dissolution of fluralaner, moxidectin and BHT in the solvents;
- mixing of the solution to ensure homogeneous distribution of all ingredients in the formulation;
- filling of the pipettes;
- production of a sachet around the filled pipette.

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

The residual volume in the pipettes after administration was determined during pharmaceutical development for all three strengths. Together with the residual volume, the filling volume can be calculated ensuring that the declared dose is administered to the animal.

In accordance with the Guideline on Process validation for finished products (EMA/CHMP/CVMP/QWP/BWP/70278/2012 Rev. 1), the product is classified as a specialised dosage form by virtue of the fact that it is a unit dose product where one of the active substances (moxidectin) comprises less than 2% of the formulation. In accordance with the guideline, process validation data for full scale batches should be provided in the dossier, unless otherwise justified. Given the nature of the product, being a solution, the low content of moxidectin in the formulation would not be expected to be very critical for the homogeneity of the product, especially not in comparison to a solid unit dose product. This is supported by the process validation data on three pilot scale batches and also by the presented data on a commercial scale-up batch showing consistent results for moxidectin assay in all samples. The execution of the production scale validation studies post-approval according to the enhanced validation plan can be accepted.

Control of starting materials

Active substance

Moxidectin

Moxidectin is a semisynthetic substance derived from a fermentation product. It may contain suitable stabilizers such as antioxidants. The moxidectin quality used in the manufacture of Bravecto Plus contains butylhydroxytoluene as antioxidant.

Moxidectin is an amorphous powder with sufficient solubility in the solvent system of the finished product, so particle size distribution is not critical.

There is a monograph of moxidectin for veterinary use in the Ph. Eur., and the manufacturer of the active substance has been granted a Certificate of Suitability of the European Pharmacopoeia (CEP), a copy of which has been provided within the application. The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability. The control tests were carried out to comply with the acceptance criteria and test methods of the Ph. Eur. monograph. The CEP includes additional control for BHT content and residual solvents.

Batch data for three moxidectin batches from the CEP holder and the corresponding data of these batches from the applicant are provided. The analytical results are comparable and well within the specification limits of the monograph or CEP.

Re-test period and storage conditions are not covered by the CEP. Stability data are provided that support a re-test period of 24 months, when stored at or below 25 °C.

Fluralaner

The active substance fluralaner is an existing chemical entity. It is a white to pale yellow powder. Fluralaner may be micronised.

Fluralaner possesses one chiral centre, resulting in the formation of a racemate. The substance exhibits polymorphism, however, only one polymorphic form is obtained with this manufacturing method.

There is no monograph of fluralaner in the Ph. Eur. The 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 it was considered satisfactory.

The characterisation of the active substance and its impurities is in accordance with the CVMP guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

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

The active substance specification includes the following justified tests: appearance, identity, colour of solution, assay, impurities, residual solvents, water content, heavy metals.

In addition to the test parameters above, particle size distribution is specified for micronised fluralaner.

The analytical methods used to control the active substance have been sufficiently adequately described and the non-compendial methods appropriately validated in accordance with the relevant VICH guidelines.

Batch analytical data demonstrating compliance with the proposed active substance specification have been provided for several production batches of fluralaner, non-micronised and micronised, tested by the supplier and by the finished product manufacturer.

Stability results have been provided for both real time (60 months) and accelerated (6 months) stability conditions. Tests are performed for appearance, identification, colour of solution, assay, related substances, and water content.

No real trends were observed in the stability studies with fluralaner. Stress testing demonstrates that fluralaner is stable against heat and light.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months if stored below 30 °C in the proposed container.

The applicant has provided additional stability data on batches of micronised fluralaner. Tests are performed for appearance, identification, colour of solution, assay, related substances, water content and particle size distribution. Limits and analytical procedures are the same as applied at release. This re-test period will be calculated from the manufacturing date of the non-micronised fluralaner.

No real trends were observed and the stability results indicate that the micronised active substance is

sufficiently stable. The stability results justify the proposed retest period of 24 months if stored below 25 °C in the proposed container.

Excipients

Dimethylacetamide is compliant with the Ph. Eur. Butylhydroxytoluene is compliant with the Ph. Eur. Acetone is compliant with the Ph. Eur. Diethyltoluamide is compliant with the USP. Where relevant the descriptions and validations of additional test methods have been provided.

Glycofurol is a non-compendial material, which is neither described in the Ph. Eur. nor in the USP. An in-house specification has been provided which is based on the specification from the manufacturer used during the development of the finished product. Scientific information to support this specification has been provided. The specification is acceptable. The re-test period and storage conditions proposed for glycofurol are acceptable.

Certificates of analysis for all excipients have been supplied demonstrating compliance with the proposed specifications.

There are no novel excipients used in the finished product formulation. 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

None of the starting materials used for the active substance or the finished product are risk materials as defined in the current version of 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). The product is therefore out of the scope of the relevant Ph. Eur. monograph and the Note for guidance.

Valid TSE declarations from the manufacturers of the active substances and the finished product have been provided.

Control tests on the finished product

The finished product release specification includes tests for appearance, identification and assay of fluralaner, moxidectin and BHT, impurities, and uniformity of dosage units by mass variation, density and moisture.

The specifications proposed are appropriate to control the quality of the finished product at release and shelf-life.

The analytical methods used have been adequately described. The test methods for appearance (visual) and the Ph. Eur. methods for uniformity of dosage units by mass variation, density and moisture need no further validation. The UPLC method used for identification and assay of fluralaner, moxidectin and BHT and impurities has been appropriately validated in accordance with VICH guidelines.

Information regarding the reference standards used for assay testing of fluralaner, moxidectin and BHT has been presented.

Certificates of analysis are provided for nine batches made from pilot scale bulk batches and three sub-batches made from a commercial scale bulk batch, representing the three pipette sizes of the three bulk pilot batches confirming the consistency of the manufacturing process and its ability to

manufacture to the intended product specification.

Stability

Three pilot scale batches of finished product, each split-filled into to obtain the 3 proposed strengths, were initially stored under accelerated (40 °C/75% RH) and long-term (30 °C/65% RH) conditions. Further studies at 25 °C/60% RH and at 2–8 °C were started at the 12 month time point. The stability studies are performed in accordance with VICH GL3 on stability testing. The applied bracketing design is acceptable.

Stability results up to 12 months at 5±3 °C and 40 °C/75% RH, 21 months at 25 °C/60% RH and 18 months at 30 °C/65% RH are available. The studies at 30 °C/65% RH, 25 °C/60% RH and refrigerated conditions will run for 36 months.

The stability batches were packed in the primary packaging proposed for marketing.

Samples were tested according to the proposed shelf life specification including tests for appearance, identification and assay of fluralaner, moxidectin and BHT, impurities , and uniformity of dosage units by mass variation, density and moisture. The wider shelf life limits regarding concentration of solution for fluralaner and moxidectin, content of BHT, some specified impurities, density and moisture are appropriately justified. The same analytical methods as for release of the batches were applied. The analytical procedures used are stability indicating.

No real trends could be observed for individual impurities. However, increasing trends were noticed for total impurities of moxidectin and density, all more pronounced at accelerated conditions than at the long term conditions. The contents of fluralaner and moxidectin show an increasing trend over time but stay well within limits at long term and intermediate conditions. At accelerated conditions fluralaner and moxidectin contents exceeded the upper specification limit with one sample of the strength 112.5 mg fluralaner / 5.6 mg moxidectin after 12 months of storage. This is likely due to some loss of volatile solvents from the formulation, and moisture. Therefore the pipettes should be kept in the sachets to protect from solvent loss or moisture uptake, as mentioned in the SPC.

The observed physical and chemical changes are not likely to have a significant effect on efficacy and safety of the product when used according to the directions in the SPC.

In addition, pipettes of the 112.5 mg fluralaner / 5.6 mg moxidectin and 250 mg fluralaner / 12.5 mg moxidectin strengths of one pilot batch were exposed to light as defined in the VICH GL5 on photostability testing of new veterinary drug substances and medicinal products, as well as freeze-thaw cycling. No significant physical and chemical changes were observed. The formulation is not photosensitive.

Based on the available stability data, the proposed shelf-life of 2 years without any special temperature storage conditions as stated in the SPC is acceptable.

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 can be 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 in a satisfactory way. Data has been presented to give reassurance on TSE safety.

In addition, the applicant is recommended to re-evaluate the wider shelf-life limit for density when stability data on full scale batches is available and to provide data from 3 batches manufactured at the commercial site prior to commercialisation.

The product can be approved from a chemical-pharmaceutical point of view.

Part 3 – Safety

Pharmacodynamics

Both active substances, fluralaner and moxidectin, have well known modes of action.

Fluralaner is an acaricide and insecticide belonging to the isoxazoline group. Isoxazolines act at the central nervous system or the neuromuscular junction of the insect, rather than directly on muscle fibres. Isoxazolines are potent inhibitors of the neurotransmitter gamma-aminobutyric acid (GABA) receptor and glutamate receptor function and work by blocking pre- and post-synaptic transfer of chloride ions across cell membranes. This results in uncontrolled activity of the central nervous system and death of insects or acarines.

The binding affinity of fluralaner to receptors of ligand-gated chloride channels was reported significantly lower in mammals than in arthropods. This was confirmed by a study investigating the activities of fluralaner on GABA-receptors of different parasites (ticks, fleas, flies) and rats *in vitro*, compared to other GABA-receptor antagonists (fipronil, picrotoxin and dieldrin). The studies demonstrated high activities on all tested arthropod GABA-receptors, including the dieldrin resistant variants of the GABA-receptors of *Ctenocephalides felis* and *Drosophila melanogaster*. However, no measurable activity was found against the sole subunit combination of the mammalian (rat) GABA-receptor tested.

Studies investigating interaction with other mammalian receptors have not been presented. It was argued that there is no evidence from a range of toxicity studies that fluralaner exerts immediate or delayed secondary pharmacodynamic activity in mammals or organs of general concern (neurological, cardiovascular, respiratory, renal or gastrointestinal system) despite high systemic exposure. The longest repeat dose toxicity study was a 52-week oral toxicity study in dogs (daily administration of fluralaner). The target animal safety (TAS) studies were conducted over several months (in the pivotal TAS study the product was administered on three occasions with an 8-week between treatment interval) with animals continually exposed to fluralaner for approximately 24 weeks.

Moxidectin belongs to the milbemycin group of macrocyclic lactones and has parasiticidal activity against a range of internal and external parasites including various nematode species. However, it lacks substantial efficacy against fleas and ticks.

The mode of action of milbemycins is based on the binding of ligand-gated chloride channels (glutamate receptor and GABA receptor). This leads to an increased membrane permeability of nematodes (and some arthropods) nerve and/or muscle cells for chloride ions, resulting in hyperpolarization, paralysis and death of the parasites. The glutamate receptors are specific for invertebrates and are not expressed in mammals. Moxidectin is highly selective for GABA receptors. A significant overdose may result in adverse neurological signs including tremors, mydriasis, ataxia and CNS depression. Since moxidectin appears to be a substrate for P-glycoprotein (p-gp), concomitant administration of other substances that can inhibit

p-gp (e.g. cyclosporin, ketoconazole, spinosad) should be handled with caution; this is mentioned in SPC section 4.8.

Moxidectin is currently used in veterinary medicine as an anthelmintic and insecticidal treatment in companion and food-producing animals.

Fluralaner and moxidectin interaction:

Fluralaner acts as an inhibitor of insect GABA-R and glutamate-R whereas moxidectin is a positive modulator of these receptors. A study carried out reported that the insect GABA-R has at least four different binding sites which are relevant to insecticidal activity. Fluralaner binding to house fly head membrane preparations was reported to be insensitive to channel blockers (e.g. fipronil) but the isoxazoline binding site appears to be directly coupled to the avermectin GABA/glutamate chloride channels activator site. However, the isoxazoline insecticidals and avermectins have a distinct and unique binding site around the chloride channel modulators, and both sites have no features in common that confer cross-resistance.

Pharmacokinetics

Detailed information on pharmacokinetics of the fixed combination product in the target species, cats, can be found in Part 4.

The studies provided concerning fluralaner were also assessed during the procedure of registration of Bravecto tablets/spot-on and/or MRL procedure and briefly described below. Moxidectin has been evaluated previously by JECFA (1995). It is noted that moxidectin has previously been evaluated by CVMP as well.

Fluralaner:

After oral administration to dogs, fluralaner is readily absorbed. The oral bioavailability of fluralaner was slightly decreasing with increasing oral dose in dogs: with mean values of 34%, 27% and 20% for 12.5, 25 and 50 mg/kg bw. Increases in exposure by C_{max} and AUC were statistically dose-proportional.

Skin permeation tests demonstrated that human skin was less permeable than rat or rabbit skin for fluralaner, resulting in skin penetration factors of 3.7 for rat versus human and 6.2 for rabbit versus human when using an aqueous formulation; respectively 5.7 and 7.8 when using the Bravecto-formulation.

Fluralaner accumulates, and steady state of fluralaner plasma concentrations were reached after approximately 30 days in rats and about 90 days in dogs indicating a long half-life; no actual half-lives could be calculated due to the study design. It appears that fluralaner undergoes enterohepatic recirculation.

Fluralaner appears to be highly bound to plasma proteins as observed in calf, sheep and chicken (broiler), cats and dogs, i.e. approximately 100%.

Once orally 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.

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. It is postulated that metabolism of fluralaner proceeds by hydroxylation of the dichlorophenyl ring, the dihydroisoxazole ring and/or the side chain to yield mono- and dihydroxylated metabolites. Subsequent conjugation reactions result in the

monohydroxy and dihydroxy sulphates. The metabolism also proceeds by N-dealkylation to the corresponding amide or by amide hydrolysis of the side chain to the corresponding carboxylic acid.

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

Moxidectin:

The pharmacokinetics and metabolism of moxidectin were studied in rats, sheep and cattle. After oral administration to the sheep, about 20% of the dose was absorbed. Moxidectin is very lipophilic, and was found at high levels in fat, but at much lower levels in other tissues. It was excreted in the milk. After oral administration to rats, the major compound recovered in faeces was the parent drug, while small amounts of hydroxylated metabolites were found in liver and faeces. Hydroxylated metabolites were observed in sheep and cattle following oral administration.

Skin permeation tests demonstrated that human skin was less permeable than rat or rabbit skin for moxidectin, resulting in skin penetration factors of 1.8 for rat versus human and 2.1 for rabbit versus human when using an organic formulation; respectively 8.5 and 10.5 when using the Bravecto Plus formulation.

Toxicological studies

The active substance fluralaner was previously assessed by the CVMP in the context of the establishment of MRLs (for chickens) and authorisation of veterinary medicinal products (Bravecto tablets and spot-on solution, Exzolt). The active substance moxidectin has a well-established use and has also been previously assessed by CVMP, as well as by JECFA.

Single dose toxicity

Fluralaner:

An acute oral GLP toxicity study using the active substance was performed in rats in accordance with OECD guideline 423. All animals survived until the end of the study period. No adverse effects were observed in this study, except for slightly ruffled fur in all animals. An LD_{50} of > 2000 mg/kg bw could be derived from this study.

An acute dermal toxicity study using the active substance (limit test: 2000 mg/kg bw) was performed in rats in accordance with OECD guideline 402. No adverse effects were observed in this study, except for some local effects in some of the animals (erythema, scaling and scabs). An LD_{50} of > 2000 mg/kg bw could be derived from this study.

In conclusion, fluralaner is of low acute oral and dermal toxicity (LD_{50} > 2000 mg/kg bw; limit dose).

Moxidectin:

An acute oral toxicity study with moxidectin resulted in an LD_{50} of 106 mg/kg bw for rats and 84 mg/kg bw for mice.

An acute dermal toxicity study in rabbits with moxidectin resulted in an LD_{50} of > 2000 mg/kg bw.

Bravecto Plus:

An acute oral toxicity study in rats using a fluralaner/moxidectin formulation (limit test: 2000 mg/kg bw) was conducted. An LD_{50} of > 2000 mg of the product/kg bw could be derived from this study.

An acute dermal toxicity study in rats using a fluralaner/moxidectin formulation (limit test: 2000 mg/kg bw) was conducted. The study was performed in accordance with OECD guideline 402, including the appropriate number of animals, test conditions, observation period and examinations. All animals survived until the end of the study period. No adverse effects were observed in this study, except for bodyweight loss in one female during the first week of observation. An LD_{50} of > 2000 mg of the product/kg bw could be derived from this study.

It is noted that the formulation used as test item contained 2.8% moxidectin instead of 1.4% w/v as present in 'Bravecto Plus Spot-on Solution for Cats'. This can be considered worst-case scenario.

Repeat dose toxicity

Fluralaner:

Oral

All the data provided have previously been assessed by the CVMP. Repeat dose oral toxicity was extensively studied in rats (studies with durations of 2, 4 and 13 weeks) and dogs (studies with durations of 4, 13 and 52 weeks).

In a 2-week and a 4-week toxicity studies in rats, rats were given fluralaner by oral gavage at doses of 0, 30, 60, and 600 mg/kg bw per day. The main target organ in the repeat dose toxicity studies was the liver, which is the main organ for 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 per day was established by CVMP.

A 13-week oral toxicity study in rats, dosed 0, 20, 40 and 400 mg/kg bw per day, confirmed the effects on liver. In addition, at the dose of 400 mg/kg bw per 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 per day was established by CVMP.

Two 4-week toxicity studies in dogs were provided with respective oral dose (by capsule) levels of 0, 100, 250, 750 mg/kg bw per day and 0, 20, 40, 100 mg/kg bw per 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 concluded that the observed effects should be considered non-adverse. A lowest-observed-adverse-effect level (LOAEL) of 20 mg/kg bw per day is therefore established by CVMP.

In a 13-week oral toxicity study, dogs were given fluralaner by capsule at doses of 0, 2, 4 and 8 mg/kg bw per 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 of triglycerides concentration 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 per 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 rats. From the first of those 4-week studies, fluralaner was dosed at 0, 100, 200 or 1000 mg/kg bw per day, no NOAEL could be established. Treatment related effects were observed at all doses and included: fatty change 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 per day. At 100 mg/kg bw per 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 per day was appropriate when considering repeated dose toxicity. The studies are performed using fluralaner in a carboxymethyl-cellulose aqueous solution.

A 90-day dermal toxicity study was performed in rat, administered doses of 0, 25, 50 or 500 mg/kg bw per 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 also 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 per day was derived from this study by CVMP.

The potential systemic effects following subchronic and chronic exposure (oral and dermal) have been comprehensively investigated in the rat. The studies conducted meet with requirements (GLP and relevant OECD guidelines). The liver appears to be the most sensitive organ for effects (increased organ weight, hepato-cellular fatty change, effects in related blood parameters). These effects were observed at dose levels above 20 mg/kg bw/day in the oral studies and above 100 mg/kg bw/day in the dermal studies.

Subchronic and chronic (up to 52 weeks) effects following oral exposure in dogs were also comprehensively investigated. Reductions in cholesterol, phospholipids and triglycerides were consistently observed at dose levels above 1 mg/kg bw/day in the oral studies, although no histopathological changes of the liver were reported. It can be concluded that the dog is more sensitive to the effects of fluralaner than the rat and that systemic exposure of fluralaner is greater in dogs than in rats.

Moxidectin:

The toxicity of moxidectin has previously been evaluated by CVMP, as well as by JECFA, with consistent conclusions, which are summarised below.

Oral

A 28-day mouse oral (dietary) toxicity study with moxidectin resulted in a NOEL of 6.9 mg/kg bw per day, based on tremors, hypersensitivity to touch and urine-stained fur at the next higher levels. No NOEL could be derived in a 28-day rat oral (dietary) toxicity study with moxidectin, as hypersensitivity to touch was observed at the lowest dose level of 12 mg/kg bw per day.

A 13-week rat oral (dietary) toxicity study with moxidectin resulted in a NOEL of 3.9 mg/kg bw per day, based on hypersensitivity to touch, depressed bodyweight and increased adrenals weights in females and increased testes weights as observed at the next level of 7.9 mg/kg bw per day.

A 4-week dog oral (dietary) toxicity study with moxidectin resulted in a NOEL of 0.5 mg/kg bw per day, mainly based on effects on the nervous system observed at the next higher dose level.

A 13-week dog oral (dietary) toxicity study with moxidectin resulted in a NOEL of 0.3 mg/kg bw per day, based on dose-dependent reductions in absolute body weights and food consumption at the next level of 0.9 mg/kg bw. It is noted that this NOEL was selected for establishing the ADI (by JECFA (1995) as well as CVMP (EMEA/MRL/139/96-final)).

A 52-week dog oral (dietary) toxicity study with moxidectin resulted in a NOEL of 1.15 mg/kg bw per day, the highest dose-level tested.

The lowest oral NOEL value (0.3 mg/kg) was observed in dogs. It is noted from the studies provided that a number of changes (decrease in heart weights, decrease in pituitary and pituitary to brain weight ratios, reduction in absolute body weights and food consumption, reduced testes weights and reductions in spermatogenic activity) have been reported to occur at dose rates lower than those proposed to be applied topically to cats (albeit following oral daily administration whereas the product is intended for topical application no more frequently than once every eight weeks).

Dermal

Repeated dose toxicity data for the dermal route are not identified for moxidectin.

Tolerance in the target species of animal

See Part 4.

Reproductive toxicity

The toxicity of moxidectin has previously been evaluated by CVMP, as well as by JECFA. Apart from the developmental study in rats where the CVMP concluded on a lower NOAEL value, consistent conclusions were reached and are summarised below.

Study of the effect on reproduction

Fluralaner:

In the one-generation study, rats were given fluralaner at a dose level of 0, 50, 100 or 500 mg/kg bw per day. Liver, thymus, lung and adrenals appear to be affected in parents at the lowest dose of 50 mg/kg bw per day, resulting in a LOAEL of 50 mg/kg bw per 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 per day, based on reduced litter size due to reduced implantation rate and increased post-implantation loss at the higher dose of 500 mg/kg bw per 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 per day as concluded by CVMP.

In the two-generation study, rats were given fluralaner at a dose level of 0, 8, 50 or 500 mg/kg bw per 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 per day for parental toxicity, though the effects are considered marginal at the lowest dose. The reproduction NOEL was set at 50 mg/kg bw per day, based on higher post-implantation, post-natal and breeding loss at the higher dose of 500 mg/kg bw per day. The pup NOEL was set by CVMP at 50 mg/kg bw per day based on reduced body weight, clinical signs, pathological findings, and delayed physical and sexual development at 500 mg/kg bw per day.

Moxidectin:

In a one-generation rat study with moxidectin, no parental effects were observed at dose levels up to and including 3.9 mg/kg bw per day. A pup NOEL of 0.4 mg/kg bw per day was derived based on reduced pup weight and reduced survival rate in the F1b pups.

In a three-generation rat toxicity study with moxidectin, significant reductions in pup survival indices were observed during day 0-21 for the F1a litters, and during day 0-4 for the F2a litters at the highest dose level tested (0.83 mg/kg bw per day). A pup NOEL of 0.41 mg/kg bw per day could be derived. Slight reductions in weight were observed in males during the pre-mating (F2), mating and post-mating (F1 and F2), resulting in a parental NOEL of 0.41 mg/kg bw per day. No factual reproduction effects were observed.

Study of developmental toxicity

Fluralaner:

Oral: Developmental toxicity was studied in the rat at doses (oral gavage) of 0, 100, 300 or 1000 mg fluralaner/kg bw per 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 foetus and litter level. The NOEL for maternal and foetal organisms was set to 100 mg/kg bw per day by CVMP.

Developmental toxicity was studied in rabbit 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 per 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 per day based on adverse embryo-foetal developmental effects observed at the lowest dose of 50 mg/kg bw per day. A complementary prenatal developmental toxicity study using lower oral doses of 10, 25 and 250 mg/kg bw per day was therefore conducted. Fatty changes of the liver and the 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 per day. Based on the increase in fusions in cervical vertebra 2 at 25 mg/kg bw per day, the developmental NOEL was set to 10 mg/kg bw per day by CVMP.

Dermal: Developmental toxicity was studied in rabbits administered fluralaner suspended in 0.5% (w/v) carboxymethylcellulose aqueous solution containing 0.1% (v/v)polysorbate 80 at doses of 0, 50, 100 and 1000 mg/kg bw per day. A maternal dermal NOAEL of 1000 mg/kg bw per day was set (the highest dose tested in the pivotal study). However, it is noted that liver (blood biochemistry), which appeared the most sensitive target organ, and 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 per day including external and visceral abnormalities and skeletal abnormalities such as fusion of cervical vertebra 2 and sternebrae, decreased ossification of the humerus and femur of fore- and hindlimbs, the NOEL for foetal toxicity was set to 100 mg/kg bw per day by CVMP.

Moxidectin:

A rat prenatal developmental toxicity study with moxidectin resulted in an oral NOEL of 2.5 mg/kg bw per day, based increases in the total number of foetuses with abnormalities (increased incidences of cleft palate and wavy or incompletely ossified ribs) at 5, 10 and 12 mg/kg bw per day. It is noted that these effects were observed in the presence of maternal toxicity (reduced bw and food consumption).

In a rabbit developmental toxicity study with moxidectin, no embryo-foetal developmental effects were observed (highest dose tested: 10 mg/kg bw per day orally). A maternal NOEL of 1 mg/kg bw per day was derived, based on reduced body weight gain.

Genotoxicity

Fluralaner:

The potential genotoxic 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 previously concluded by CVMP and JECFA that fluralaner does not have genotoxic potential.

Moxidectin:

Moxidectin was reported to be non-mutagenic in the Ames test, did not induce mutations in a bacterial/microsome or a forward mutation assay, did not induce unscheduled DNA synthesis and was negative in a chromosome aberration test. Moxidectin is considered not to be genotoxic, as previously concluded by both CVMP and JECFA.

Carcinogenicity

Studies on fluralaner for carcinogenic potential were not submitted. This is justified by the negative results in all genotoxicity assays and the absence of pre-neoplastic lesions in repeated dose toxicity studies (tested up to 365 days, i.e. there is no evidence for a carcinogenic potential of fluralaner, at the relevant exposure levels).

CVMP concluded that moxidectin is unlikely to have carcinogenic potential.

Studies of other effects

Skin irritation

Fluralaner:

The active substance fluralaner was considered to be non-irritating to the rabbit skin.

Moxidectin:

A dermal irritation study in rabbits showed mild signs of skin irritation up to 72 hours. The CVMP concluded that moxidectin may be slightly irritating to the skin.

Bravecto Plus:

A dermal irritation study using a fluralaner/moxidectin formulation was performed in rabbits and demonstrated mild skin reactions at the application site at 24, 48 and 72 hour after application. Slight

effects were still observed after 7 days. Although it would not be classified as a skin irritant, the CVMP concluded that the formulation may be slightly irritating to the skin. It is noted that the formulation used as test item contained 2.8% moxidectin instead of 1.4% w/v. This can be considered the worst-case scenario.

Eye irritation

Fluralaner:

The active substance fluralaner was considered to be non-irritating to the rabbit eye.

Moxidectin:

An eye irritation study in rabbits demonstrated moderate signs of eye irritation, though effects were no longer present at 48h and 72h after treatment. The CVMP concluded that moxidectin may be slightly irritating to the eye.

Bravecto Plus:

An eye irritation study using a fluralaner/moxidectin formulation was performed in rabbits. The test item did elicit eye irritation reactions (corneal opacity, iridial inflammation, conjunctival redness and conjunctival chemosis). Effects were reversible within 10 days. The CVMP concluded that the product may be considered as eye-irritating. It is noted that the formulation used as test item contained 2.8% w/v moxidectin instead of 1.4% w/v. This can be considered the worst-case scenario.

Sensitisation and other effects

Fluralaner:

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. Absence of additional neurotoxicity studies is therefore justified.

Moxidectin:

A skin sensitisation study in guinea pigs was conducted with no evidence of skin sensitisation. The CVMP concluded that moxidectin is non-sensitising to the skin.

Bravecto Plus:

A mouse local lymph node assay using a fluralaner/moxidectin formulation was conducted. The test item did not elicit a sensitisation reaction. The CVMP concluded that the product is not considered a skin

sensitizer. It is noted that the formulation used as test item contained 2.8% w/v moxidectin instead of 1.4% w/v. This can be considered the worst-case scenario.

Excipients

The excipients dimethylacetamide (DMA), glycofurol, diethyltoluamide (DEET) and acetone are also included, and in similar concentrations (except for acetone) in the product 'Bravecto spot-on solution' which was previously assessed by the CVMP. Butylhydroxytoluene (BHT) is present in Bravecto Plus at a very low concentration (0.1%). Considering the low concentration, the risk of adverse effects will not be determined by BHT.

Based on the physico-chemical properties of the formulation (particularly acetone), CVMP concluded that the formulation of 'Bravecto spot-on solution' posed a high flammability risk.

For the excipient DEET, it was previously concluded by CVMP that when considering the acute neurotoxic effects serious adverse reactions cannot be excluded after accidental ingestion. In such a case, the packaging material has to be child resistant. Moreover the warnings and safety measures should include the warning 'This product is harmful after ingestion'. No risk was anticipated due to hand-to-mouth contact when stroking a treated animal.

During the assessment of the application for 'Bravecto spot-on solution', the CVMP concluded that DMA may cause damage to the unborn child when exposed to a significant amount and adverse systemic effects after prolonged dermal exposure. Based on a risk characterisation for dermal exposure however, it was concluded that no risk for the unborn child is expected when the user is exposed to a spilled volume (100 µl) of the product. Incidental ingestion was not expected to result in adverse effects.

Inhalation of acetone may cause e.g. CNS depression. Similar symptoms may be seen after ingestion of acetone. Systemic toxicity may occur after extensive or prolonged skin exposure. Acetone is absorbed through the lungs and from the gastrointestinal tract, and poorly through the skin. Therefore CVMP concluded during the procedure of 'Bravecto spot-on solution' that when considering the volume to be applied and concentration of acetone in the product, the risk of adverse effects after inhalation or dermal contact is considered to be negligible. In the candidate formulation, acetone is present however the risk of adverse effect after inhalation or dermal contact is still considered negligible.

User safety

The user safety assessment presented has been conducted in accordance with CVMP guideline on user safety for pharmaceutical veterinary medicinal products (EMEA/CVMP/543/03-Rev.1).

User exposure due to dermal contact with the treated animal is considered the most likely exposure route for adults and children. Moreover, adults may become exposed every time they administer the spot-on, which is at 12-week intervals according to the treatment schedule. The product will be administered by pet owners or professionals (including veterinarians and breeders).

In addition, eye contact (splashed or due to hand-to-eye contact) and oral contact (due to hand-to-mouth contact) may occur if personal hygiene measures (i.e. wash hands after administration) are not maintained. If the product is left unattended, accidental access to the product may result in dermal and/or oral exposure of children.

Hazard

Fluralaner:

The CVMP concluded that the following NOAELs should be used for the quantitative risk characterisation when considering active substance fluralaner:

For acute oral exposure due to accidental ingestion or hand-to-mouth contact (acute exposure) by children: a NOAEL of 10 mg/kg bw (maternal NOAEL based on liver changes in the developmental study in rabbits). It is noticed that this NOAEL is derived after repeated exposure; the NOAEL was based on liver effects which are not considered to occur and/or are not adverse after acute exposure. Moreover, in the oral acute toxicity (with fluralaner or product) no adverse effects were observed, although these studies are limited in examined parameters. This can be taken into account when performing the risk characterisation.

For repeated oral exposure (due to hand-to-mouth contact after stroking): a NOAEL of 2 mg/kg bw per day (a 90-day oral toxicity study in dogs).

For acute dermal exposure due to spillage: a NOAEL of 10 mg/kg bw. This is the oral NOAEL for acute toxicity. It is noticed that a dermal NOAEL of 50 mg/kg bw was derived, however, in all dermal studies fluralaner was suspended in 0.5% (w/v) carboxymethylcellulose aqueous solution containing 0.1% (v/v) polysorbate 80. These dermal NOAELs cannot be used because the test formulation was not representative of the candidate formulation which contains a penetration enhancer expected to affect dermal absorption. Based upon *in vitro* percutaneous absorption study data and to correct for a difference in percutaneous absorption between rabbits and humans, the NOAEL is multiplied by a factor 7.8 (rabbit versus human) as was done during the assessment of 'Bravecto spot on solution', resulting in a dermal NOAEL of 78 mg/kg bw.

For repeated dermal exposure (after stroking): a NOAEL of 50 mg/kg bw per day (90 day dermal toxicity study in rat). For repeated exposure it is expected that the penetration enhancer(s) in the formulation are no longer present in significant concentrations on the skin and therefore the derived NOAEL is acceptable to be used for chronic dermal exposure from the hair/skin of the animals. Based upon *in vitro* percutaneous absorption study data and to correct for a difference in percutaneous absorption the NOAEL was multiplied by a factor of 3.7 (rat versus human), resulting in a dermal NOAEL of 185 mg/kg bw per day.

For assessment of developmental toxicity: a NOAEL of 10 mg/kg bw per day was derived based on the oral developmental study in rabbit.

Moxidectin:

The following NOAELs should be in principle used for the quantitative risk characterisation when considering active substance moxidectin:

For acute oral exposure due to accidental ingestion or hand-to-mouth contact (acute exposure) by children: a NOAEL of 0.5 mg/kg bw per day (28-day oral toxicity study in dog).

For repeated oral exposure (due to hand-to-mouth contact after stroking): a NOAEL of 0.3 mg/kg bw per day (90-day oral toxicity study in dog).

No dermal repeated-dose toxicity studies for moxidectin were provided. Instead the oral NOAEL can be taken using route-to-route extrapolation.

For acute dermal exposure due to spillage: an oral BMDL of 5 mg/kg bw per day (from developmental study in rabbit). This value is corrected for route-to-route extrapolation (oral bioavailability of 9%, dermal

bioavailability of 63%). Further correction for the skin penetration ratio for rabbit versus human of 10.5 results in a human dermal NO(A)EL of 7.5 mg/kg bw per day.

For repeated dermal exposure (after stroking): a NOAEL of 0.4 mg/kg bw per day (reproductive toxicity study in rat). This value is corrected for route-to-route extrapolation (oral bioavailability of 19%, dermal bioavailability of 18%). Further correction for the skin penetration ratio for rat versus human of 1.8 results in a human dermal NO(A)EL of 0.8 mg/kg bw per day.

For assessment of developmental toxicity: a NOAEL of 2.5 mg/kg bw per day was derived based on oral developmental study in rat.

Exposure by adults and risk characterisation

During application the spillage of 100 µl (2 drops) is considered a realistic scenario for this product, especially as the product has to be administered to the skin, which may require parting of the hair and subsequently a risk of squeezing some of the product onto the fingers. This will result in subsequent exposure of 467 µg fluralaner/kg bw and 23 µg moxidectin/kg bw for the average 60 kg person. Unintentional oral exposure due to hand-to-mouth contact after applying the product by the adult is estimated to result in 47 µg fluralaner/kg bw and 2.3 µg moxidectin/kg bw (i.e. 10% of dermal exposure).

For dermal exposure to fluralaner, the proposed point of departure is 78 mg/kg bw. When compared to this NOAEL, the margin of exposure (MOE) is calculated to be 167 (78000/467).

For oral exposure to fluralaner, the proposed point of departure is 10 mg/kg bw. When compared to this NOAEL, the MOE is calculated to be 213 (10000/47).

For dermal exposure to moxidectin, the proposed point of departure is 7.5 mg/kg bw. When compared to this NOAEL, the MOE is calculated to be 326 (7500/23).

For oral exposure to moxidectin, the proposed point of departure is 0.5 mg/kg bw. When compared to this NOAEL, the MOE is calculated to be 217 (500/2.3).

Therefore, all are above 100. Moreover, washing hands will further reduce exposure. The foetal NOAEL is 10 mg/kg bw per day for fluralaner and 2.5 mg/kg bw per day for moxidectin. Therefore, there is also no unacceptable risk for the pregnant user. As the excipient DMA may cause damage to the unborn child following significant oral or dermal exposure, the estimated dermal exposure of two drops of formulation (equating to 0.6 mg DMA/kg bw) was compared with the lowest oral NOAEL for DMA (65 mg/kg bw/day) to derive a MOE value of 108. As this value exceeds 100, it can be accepted that no unacceptable risk from DMA in terms of reproductive toxicity has been identified. The same conclusion was reached by the CVMP when assessing the application for 'Bravecto spot-on solution' containing the same concentration of DMA.

Local effects:

Fluralaner was considered to be non-irritating to skin and/or eye. Moxidectin may be slightly irritating to the skin and eye.

Skin- and eye irritation-studies using a formulation similar to the final formulation, except for a higher concentration of moxidectin (2.8% w/v instead of 1.4% w/v), were provided for the current registration of 'Bravecto Plus'. Based on these studies, it is concluded that the formulation may be slightly irritating to the skin and irritating to the eye. Appropriate warnings and safety measures with respect to skin- and eye irritation should therefore be included in the product information.

Fluralaner was considered to be non-sensitising. It appears that moxidectin is also non-sensitising. The formulation similar to the final formulation, except for a higher concentration of moxidectin (2.8% w/v instead of 1.4% w/v), was also tested in a local lymph node assay in the mouse, from which it can be concluded that 'Bravecto Plus' did not cause hypersensitivity reactions. Therefore, the product 'Bravecto Plus' is not considered to have sensitising properties.

Accidental exposure by children and risk characterisation:

With regard to accidental exposure by children, the ingestion of 10% of the total contents of the product (largest pipette) was considered, equivalent to 50 mg of fluralaner and 2.5 mg moxidectin, i.e. respectively 4000 µg/kg bw and 200 µg/kg bw assuming a 12.5 kg child.

The estimated oral exposure level of 4000 µg/kg bw for fluralaner 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), resulting in a MOE of 2.5.

The estimated oral exposure level of 200 µg/kg bw for moxidectin is compared to the oral NOEL of 0.5 mg/kg bw/day derived from the 28 day oral toxicity study in dogs (effects on the nervous system), resulting in a MOE of 2.5.

As the excipient DEET may cause acute neurotoxicity after significant oral exposure, the oral exposure from ingesting DEET was estimated as half of that calculated for fluralaner (given that DEET is included as half the concentration of fluralaner). The potential oral exposure to DEET is estimated to be 2000 µg/kg (following accidental oral ingestion by a child). This has then been compared with the lowest oral repeated-dose toxicological NOEL (75 mg/kg bw/day) resulting in a MOE of 38. This was the lowest MOE for DEET for all the exposure scenarios examined. However, this assumes that a child only ingests 10% (as opposed to 100%) of the contents of the largest pipette volume. Even assuming only 10% is ingested, an unacceptable risk has been identified, which will not change if the calculations are repeated assuming the entire contents of the pipette are ingested. This risk is addressed by including appropriate warnings and safety measures in the product information to mitigate the risk. Moreover, each pipette is individually sealed in a sachet (flowrap pouch) which has been demonstrated to be child-resistant in accordance with EN 14375:2016 for non-reclosable packing.

Stroking the treated animal:

With regard to stroking treated animal, children are considered the worst-case. A risk characterisation was therefore performed for children.

A wipe test (as described in the Draft "Guideline on user safety of topically administered veterinary medicinal products" (EMA/CVMP/SWP/721059/2014)) was provided to determine the dislodgeable fraction from the treated animal. Dogs were used, which is acceptable as dogs exhibit less self-grooming than cats, resulting in worst-case dislodgeable residues. The highest (individual) dislodgeable fractions were found at the first time point measured (2 hours after application), i.e. 8.5% for fluralaner and 6.4% for moxidectin. The mean chronic dislodgeable fractions over 84 days of respectively 0.37% for fluralaner and 0.28% for moxidectin was calculated using the time weighted average (TWA) method, including time points from 8 hours (excluding the 2 and 4 hour time point) as proposed risk mitigation measures will reduce the likelihood during the acute phase, i.e. the product information states 'do not contact, or allow children to contact the application site until it is dry; it is therefore recommended to treat the animal in the evening'.

The dermal exposure when contacting a treated pet was calculated using the method as described in the Draft "Guideline on user safety of topically administered veterinary medicinal products" (EMA/CVMP/SWP/721059/2014). This resulted in the following exposure concentrations:

Dermal exposure:

Fluralaner acute phase: 1420 µg/kg bw. When compared to the NOAEL of 78 mg/kg bw, this would result in a MOE of 55.

Fluralaner subchronic phase: 62 µg/kg bw per day. When compared to the NOAEL of 185 mg/kg bw per day, this would result in a MOE of 3000.

Moxidectin acute phase: 53 µg/kg bw. When compared to the NOAEL of 7.5 mg/kg bw, this would result in a MOE of 142.

Moxidectin subchronic phase: 2.4 µg/kg bw per day. When compared to the NOAEL of 0.8 mg/kg bw per day, this would result in a MOE of 333.

Oral exposure due to hand-to-mouth contact:

Fluralaner acute exposure: 44 µg/kg bw. When compared to the NOAEL of 10 mg/kg bw, this would result in a MOE of 225.

Fluralaner subchronic exposure: 1.9 µg/kg bw per day. When compared to the NOAEL of 2 mg/kg bw per day, this would result in a MOE of 1050.

Moxidectin acute exposure: 1.7 µg/kg bw. When compared to the NOAEL of 0.5 mg/kg bw, this would result in a MOE of 300.

Moxidectin subchronic exposure: 0.07 µg/kg bw per day. When compared to the NOAEL of 0.3 mg/kg bw per day, this would result in a MOE of 4286.

All MOEs after stroking are above the acceptable value of 100, except for acute dermal exposure to fluralaner residues when stroking the treated animal. However, the toxicological reference value of 78000 µg/kg bw per day was derived from a repeated exposure study; the NOAEL was based on liver effects which are not considered to occur and/or be adverse after acute exposure.

The MOEs calculated for subchronic exposure provide sufficient margin when taking into account simultaneous dermal exposure and hand-to-mouth contact. For acute exposure the margins appear to be more limited. However, these MOEs are calculated using dislodgeable fractions measured 2 hours after application, while in the product information it is included that children should not be allowed to contact the application site until it is dry; the animal should be treated in the evening and treated animals should not be permitted to sleep in the same bed as their owner, especially children.

Therefore, it can be concluded that the risk when stroking a treated animal is sufficiently mitigated when the product is used in accordance with the information provided in the SPC.

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. However, the CVMP agreed that the user safety warnings to be approved should be consistent with those of the mono-active product Bravecto spot-on solution.

Environmental risk assessment

An environmental risk assessment was provided in the dossier, in accordance with the relevant CVMP/VICH guidelines (VICH GL6, Environmental impact assessment (EIAS) for veterinary medicinal products - Phase I, and 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 product Bravecto Plus spot-on solution for cats is only used in non-food producing species. Consequently, the ERA can stop in Phase I, and a Phase II assessment is not required.

Bravecto Plus is not expected to pose an unacceptable risk for the environment when used according to the SPC. Based on the known risk for adverse effects of moxidectin on fish and other aquatic organisms, a warning to avoid the product entering water courses is included in the SPC and product literature.

Conclusions on the environmental risk assessment

An ERA was provided according to the CVMP/VICH guidelines. Based on the data provided the ERA can stop at Phase I. The product is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

The active substances in 'Bravecto Plus' are fluralaner and moxidectin.

Pharmacodynamics:

Fluralaner is an ectoparasitic substance with acaricidal and insecticidal activity through blockage of GABA-gated chloride channels and L-glutamate-gated chloride channels.

Moxidectin expresses mostly anthelmintic activity, based on the binding of the ligand-gated chloride channels (glutamate-R and GABA-R). This leads to an increased membrane permeability of nematode nerve and muscle cells, resulting in hyperpolarization, paralysis and death of the parasites.

Pharmacokinetics:

Following oral administration, fluralaner is rapidly absorbed, reaching maximum plasma levels within 1 day. Volume of distribution is moderate and plasma clearance very low, with fluralaner plasma concentrations quantifiable for up to 3 months after treatment. The main route of elimination is via faeces.

Toxicology:

A battery of toxicity tests have been provided for fluralaner, which were already assessed during the procedure of 'Bravecto spot-on solution' or 'Exzolt'. From the repeated dose toxicity studies it appeared that liver was the most sensitive organ, resulting in increased organ weight, hepatocellular fatty change, effects in related blood parameters (reductions in blood cholesterol, phospholipid and triglyceride levels). Post-implantation, post-natal and breeding loss was 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 was observed in the pups. Developmental toxicity was studied in the rat and the 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 rabbit (NOEL 10 mg/kg bw per day).

Moxidectin has a well-established use. It has been evaluated previously by CVMP, as well as by JECFA. From the repeated dose toxicity studies it appeared that neurotoxicity is the most sensitive endpoint. Also reduced food consumption and body weight was observed. In the reproduction studies in rats reduced body weight and reduced survival rate of the pups was observed (NOEL 0.4 mg/kg bw per day). An increased number of foetuses with abnormalities were observed at doses of 5 mg/kg bw and higher in rats. Developmental effects included increased cleft palate and wavy or incompletely ossified ribs (NOEL 2.5 mg/kg bw per day). In rabbits no embryo-foetal developmental effects were observed (highest tested dose 10 mg/kg bw per day).

Acute toxicity studies (oral and dermal) with the product demonstrated that it is of low acute toxicity.

Fluralaner and moxidectin are not genotoxic. Carcinogenicity studies have been performed for moxidectin only, and are not requested for fluralaner.

Other studies:

Fluralaner is considered to be non-irritating to skin and/or eye. It appears that moxidectin may be slightly irritating to the skin and eye. Skin and eye irritation-studies using a formulation similar to the final formulation, except for a higher concentration of moxidectin (2.8% w/v instead of 1.4% w/v), were provided for 'Bravecto Plus'. Based on these studies, it is concluded that the formulation may be slightly irritating to the skin and irritating to the eye.

Both active substances, fluralaner and moxidectin, are considered to be non-sensitising. The formulation similar to the final formulation, except for a higher concentration of moxidectin (2.8% w/v instead of 1.4% w/v), was also tested in a local lymph node assay in the mouse, from which it can be concluded that 'Bravecto Plus' did not cause hypersensitivity reactions.

User safety:

'Bravecto Plus' may be harmful after ingestion, due to both active substances and to the excipient DEET. However, the packaging has been demonstrated to be child-resistant. Accidental spillage of the product during application is not expected to result in adverse effects, especially when hands are washed after administration of the product. Exposure to treated animals (dermal as well as oral due to hand-to-mouth contact) is not expected to result in adverse effects, especially when animals are treated in the evening and treated animals are not allowed to sleep with owners including children.

Warnings for the user have been proposed. Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the product information.

Environmental safety:

An ERA was provided according to the CVMP/VICH guidelines, and the product is not expected to pose a risk for the environment when used according to the SPC. However, based on the known risk for adverse effects of moxidectin on fish and other aquatic organisms, a warning to avoid the product entering water courses is included in the SPC and product literature.

Part 4 – Efficacy

Pharmacodynamics

See Part 3.

Justification of combination

The fixed combination of fluralaner and moxidectin has been justified on the grounds that the combination will broaden the activity spectrum of the product 'Bravecto spot-on solution' (veterinary medicinal product containing fluralaner only). 'Bravecto spot-on solution' is authorised for the treatment of flea (*C. felis*) and tick (*I. ricinus*) infestations, and with the inclusion of the additional active substance moxidectin the proposed indications are extended to include infestations with specific helminths, i.e.:

- Prevention of heartworm disease caused by *Dirofilaria immitis.*
- Treatment of infestations with intestinal roundworm (*Toxocara cati*) and hookworm (*Ancylostoma tubaeforme*).

The need for a broadened spectrum was supported based on literature data of prevalence of helminths, fleas and ticks in the cat, as well as prevalence of these pathogens combined in cats in several European countries. Prevalence of the claimed helminths in cats in Europe was estimated to be up to 35% (depending on helminth species and geographical location): Prevalence of *Toxocara cati* ranges from 4% in Germany to 35% in Spain, and for *Ancylostoma tubaeforme* prevalence ranged from 0.3% to 1% in Western Europe to approximately 10.5% in Eastern Europe. Especially younger animals (up to 6 months) are affected. Prevalence of *D. immitis* in cats varies from 17.5% to 33% in hyperendemic areas in Southern Europe.

Prevalence of the claimed ectoparasites in cats in Europe was estimated up to 30% (*I. ricinus* in southern France) and co-infestations with ticks and fleas were seen with a prevalence of 9-16% (field studies).

Combined infestations with gastrointestinal helminths and ectoparasites are found in 12% of cats according to literature.

In further support of the combination of active substances, the applicant also considered that such a combination would increase owner compliance, and avoid possible interactions as the co-administration of additional veterinary medicinal products will not be required. This justification of the fixed combination is accepted by CVMP.

Resistance

Fluralaner:

No additional studies with the candidate formulation have been performed. However, an *in vivo* study investigating efficacy of oral fluralaner against the *C. felis* isolate 'Margate' (an isolate found to be resistant to fipronil) was conducted. Results indicate that fluralaner is efficacious against this isolate. Up to now, no resistance *in vitro* or *in vivo*, has been reported against fluralaner; however, fluralaner is a fairly new molecule and only on the market since 2014. The molecular targets of fluralaner are the ligand gated chloride channels of invertebrates, namely the GABA receptor and the glutamate receptor.

In *in vitro* bio-assays, fluralaner is not affected by proven field resistances against amidines (tick), organophosphates (tick), cyclodienes (tick, flea), phenylpyrazoles (tick, flea), benzophenyl ureas (tick), and pyrethroids (tick).

Moxidectin:

The CVMP is aware of reports of resistance development of *D. immitis* to macrocyclic lactone use in dogs in America. However, a similar resistance development in cats does not appear to have been reported to date. In the EU there are no reports available on resistance development in cats. The CVMP therefore

accepted that macrocyclic lactones continue to be effective in the vast majority of *D. immitis* infections in cats.

However, the SPC in section 4.4 (Special warnings for each target species) contains appropriate prudent warnings in regard to resistance development, which is considered acceptable:
"Parasite resistance to any particular class of anthelmintic may develop following frequent, repeated use of an anthelmintic of that class under specific circumstances. The use of this product should be based on the assessment of each individual case and on local epidemiological information about the current susceptibility of the target species in order to limit the possibility of a future selection for resistance. Parasite control is recommended throughout the period of potential infestation risk."

Pharmacokinetics

For information on the pharmacokinetics of the individual components, see Part 3.

Two GLP studies were carried out to obtain the pharmacokinetic profile of the fixed combination of fluralaner and moxidectin following a single topical spot-on solution to cats.

The first study is a well-designed pharmacokinetic study. Pharmacokinetics of fluralaner and moxidectin after topical administration of the combination (final formulation) were investigated in 3 groups of cats receiving either a single dose of 0.5x, 1x or 2x the recommended therapeutic dose (RTD), respectively (i.e. 20 mg fluralaner+1 mg moxidecting/kg bw, 40 mg fluralaner+2 mg moxidectin/kg bw or 80 mg fluralaner+4 mg moxidectin/kg bw), to study dose proportionality/linearity. In addition to these three groups of animals, two groups received a single active substance only (i.e. 40 mg fluralaner/kg bw or 2 mg moxidectin/kg bw) topically to study the possible pharmacokinetic interactions between both active substances. Finally, a last group received a 5 minute intravenous infusion of the combination (2.5 mg fluralaner+0.125 mg moxidectin/kg bw). Each group contained 3 adult females and 3 adult males.

The absolute bioavailability of topical administration was calculated based on comparison of the different doses administered topically versus administration of the intravenous combination. Bioavailability of fluralaner was calculated to be 25.7% ± 10.7, 44.8% ± 12.6 and 23.3% ± 9.3, respectively, for 0.5x, 1x and 2x RTD of the fixed combination. Bioavailability of moxidectin was 17.2%±8.36, 26.2% ±15.3 and 13.2%±1.13, respectively, for 0.5x, 1x and 2x dose groups receiving the combination. The individual bioavailability ranged from 7.95-49.1% in all animals at all dose rates.

Elimination profiles of fluralaner (35-77 days) and moxidectin (21-91 days) were slow after intravenous infusion with a half-life of 8.84 and 32.0 days, respectively. Total plasma clearance of fluralaner and moxidectin is low, fluralaner has a moderate and moxidectin a high volume of distribution (3034 and 26260 ml/kg, respectively). Following topical administration (at the recommended dose), half-life was calculated to be 14.7 days for fluralaner and 26.4 days for moxidectin. It should be noted that especially for moxidectin, C_{max} and AUC had a large variation.

Table of pharmacokinetic parameters of Bravecto Plus applied topically at the recommended dose.

40 mg fluralaner/kg bw + 2 mg moxidectin/kg bw	Fluralaner	Moxidectin
T_{max} (days)	10 (3-21)	3 (1-5)
C_{max} (ng/ml)	2435 ± 1064	36.7 ± 26.7
AUC_t (ng/ml.day)	74306 ± 20943	874 ± 234
$T_{1/2}$	14.7 ± 2.93	26.4 ± 4.53

Higher values for AUC_t were observed following application of fluralaner and moxidectin in combination, compared to application of each active substance on its own: AUC_t for fluralaner was 64096 ng/ml·day when applied on its own, but 74306 ng/ml·day when applied in combination with moxidectin. AUC_t for moxidectin was 558 ng/ml·day when applied on its own, but 874 ng/ml·day when applied in combination with fluralaner. This may suggest that the fixed combination of the active substances may result in greater systemic exposure, particularly of moxidectin. However, when comparing the total internal exposure (AUC) of the active substances in the different pharmacokinetic studies, the systemic exposure varied to a great extent, but was within comparable ranges for the active substance alone compared to the active substance in the combination product.

The second study was designed to investigate the pharmacokinetic profile, biodistribution and excretion of fluralaner and moxidectin in domestic cats after a single topical administration of Bravecto Plus. Four groups (n=4) of animals were topically treated on day 0 and sacrificed on day 8, 15, 43 and 85, respectively, to study the distribution of both active substances over tissues. Plasma samples were collected during the study and faeces and urine were weekly collected for determination of both active substances.

Fluralaner and moxidectin showed a profile in plasma, with quantifiable levels in all animals up to at least 84 days after topical administration. Fluralaner and moxidectin were distributed in liver, kidney fat and muscle, and depleted slowly over time in all tissues. Based on the high fluralaner and moxidectin concentration in liver and kidney found on day 8, it can be suggested that the highest absorption occurs mainly during the first week after treatment. Fluralaner was distributed to a major extent in visceral and subcutaneous fat tissues, followed by kidney and liver. Moxidectin was also particularly distributed in all fat tissues, followed by kidneys and liver. The highest concentrations of fluralaner and moxidectin were found in hair at the administration site, but especially for fluralaner, high concentrations were also found in hair collected from sternum and hind leg. It was shown that fluralaner was incorporated into the hair. For fluralaner and moxidectin high concentrations were also found in skin at the administration site, and both substances could be determined in skin of sternum and hind leg, though not as high as in liver or kidney. Concentrations of fluralaner and moxidectin were much higher in faeces than in urine.

Concentrations in faeces of both fluralaner and moxidectin were clearly the highest in week 1, and after a marked drop in week 2 slowly decreased over time and were still detectable in faeces on week 12.

Dose justification

Fluralaner:

The dose determination studies submitted and assessed to support the accepted dose of 40-94 mg fluralaner / kg bw for the treatment of tick and flea infestations in cats over 12 weeks when administered as single substance (‘Bravecto spot-on solution’) were accepted as dose justification for fluralaner used in the fixed combination product ("Bravecto Plus").

Moxidectin:

For the intended 12 weeks duration of moxidectin efficacy, the applicant identified *D. immitis* as the dose-limiting parasite. Three studies (two pharmacokinetic studies and one pilot heartworm efficacy study) have been provided in support of the selected dose of moxidectin in this fixed combination product (2-4.7 mg moxidectin/kg bw).

The applicant states, that in the absence of published data on adequate threshold plasma concentrations for moxidectin for reliable parasiticidal activity against larval stages of L3 and L4 of *Dirofilaria immitis,* a threshold concentration of 0.5 ng/ml was assumed as a conservative estimate. This value was chosen

based on the fact that 0.5 ng moxidectin/ml plasma is the lower limit of quantification (LLOQ) for moxidectin in published literature relating to prevention of heartworm disease and considered to be necessary to ensure sufficient efficacy (i.e. 100%). The study, however, was not performed in cats, but in growing puppies.

The suggested dosage of 2 mg moxidectin/kg bw was further justified by the applicant based on data publicly available from published literature on moxidectin as well as from an approved topical combination product that contains imidacloprid and moxidectin which has been authorised for the proposed indication, prevention of heartworm disease caused by *Dirofilaria immitis*, through the centralised procedure (Advocate for cats). A minimum moxidectin dose of 1 mg/kg bw for cats was previously determined to provide therapeutic efficacy of moxidectin against up to 30-day old infections with L3 and L4 larvae of *D. immitis*.

The pivotal pharmacokinetic study demonstrated a large variation in plasma moxidectin levels when cats were treated with the proposed dose of 2 mg moxidectin/kg bw, suggesting that, under field conditions, a number of animals may have plasma concentrations below (or above) the threshold concentration indicated as being required for reliable parasiticidal activity against larval stages of L3 and L4 of *Dirofilaria immitis* at the end of a 3-month treatment interval.

Consequently, in order to support the proposed treatment interval of 12 weeks and a minimum dose rate of 2 mg/kg bw, three dose confirmation studies were conducted.

However, one of the studies was not able to demonstrate accurate quantifiable plasma titres below the LLOQ of moxidectin (1 ng/ml) in the majority of cats. Another study was unable to demonstrate sufficient efficacy against *D. immitis* after 90 days (<100%). In the third GCP-compliant study the product was used as intended, and the study was performed following relevant CVMP and VICH Guidelines. The study demonstrated 90 days protection against heartworm disease; however, adequacy of infection was not achieved in the control group. Therefore, this study was not considered adequate to substantiate the indication "For prevention of heartworm disease over 12 weeks".

The applicant stated that a classical dose determination study for the proposed indication against *D. immitis* was not considered to be required or appropriate. CVMP agrees that, whether or not a dose determination study is present, the validity of any proposed dose needs to be confirmed in dose confirmation studies (even more so in this case; since dose determination studies are missing). CVMP, however, considered the results of these efficacy studies insufficient to support efficacy of the product for the originally proposed 90 days re-treatment interval against *D. immitis*, as insufficient efficacy has been demonstrated for the full 90-day period.

The SPC has been updated so that users are aware that, for the prevention of heartworm disease, 100% efficacy of the product for the duration of 12 weeks when only a single treatment is applied cannot be guaranteed. The veterinarian is therefore informed in section 4.2 that the preventative effect of the product (with level of efficacy equivalent to other approved products) is 8 weeks (Section 4.2; Indications for use, specifying the target species: *For the prevention of heartworm disease caused by Dirofilaria immitis for 8 weeks*).

In conclusion, results of the dose confirmation studies demonstrated that the proposed dose was acceptable, but considered that the 90 days re-treatment interval was not demonstrated in all the intended target helminths.

Dose confirmation studies

Thirteen laboratory studies were conducted in cats to evaluate safety and efficacy of Bravecto Plus spot-on solution for cats under controlled conditions.

Studies were performed between 2015 and 2017. All but one were performed according to GCP guidelines in various parts of the world; Australia, United States, South Africa and Europe. All but two studies used the final formulation; athough in one study a close-to-final formulation was used.

The laboratory studies were largely performed according to the CVMP Guideline for testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats (EMEA/CVMP/EWP/005/2000 - Rev.2), the CVMP guideline Demonstration of Efficacy of Ectoparasiticides (NtA 7AE17a), and the VICH GL7 (Efficacy Requirements for Anthelmintics: Overall guidelines) and VICH GL20 (Efficacy of anthelmintics: specific recommendations for felines). The number of cats used in the laboratory studies was according to the guidelines (a minimum of 6 cats per group). Cats were adequately infected prior to treatment.

Ectoparasites:

Four dose confirmation studies (according to GCP) were conducted to demonstrate efficacy against ectoparasites.

The following claims for ectoparasites are substantiated by the dose confirmation studies provided:

Fleas (*Ctenocephalides felis*):

Two new pivotal dose confirmatory studies using the fixed combination were provided, and both were performed outside the EU.
Cats were treated with a single dose of Bravecto plus (final formulation) and either a negative control (placebo or moxidectin alone) or a positive control (monthly administration of a fixed combination of fipronil and methoprene).

In one study , cats (n=50 per group) were (re)infested with fleas at D-1, 30, 60 and 90, and efficacy was evaluated over 90 days, at 12 and 24 hours on D0/D1, D30/D31, D60/D61 and D90/D91. Results showed immediate efficacy at D0 (12 h) and D1 (24 h) of 100%, and persistent efficacy (24 h) of 100%, 99% and 85% on Days 31, 61, and 91. The study did not support persistent efficacy of 90 days, as efficacy at D 90 was <95% 24 h after infestation, whereas an efficacy >95% for adult fleas is required according to the Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats (EMEA/CVMP/EWP/005/2000-Rev.2). One cat was observed with reflexive salivation on D0, and another cat showed serious adverse events approximately 4 hours after administration (pyrexia, tachypnea and mydriasis).

In another study, cats (n=10 per group) were (re)infested with fleas every 14 days (D7 – D91), and efficacy was evaluated over 90 days on days 2, 9, 16; 30, 44, 58, 72, 79, 86 and 93. Results showed 100% efficacy in the group treated with fluralaner + moxidectin except for one time point (D58 – 99.7%) and efficacy in the positive control group ranging from 30.6% - 65.6% (geometric means). The results of this study supported the proposed dose of fluralaner (40 mg/kg bw) in the fixed combination and the proposed duration of effect over 90 days, when applied topically to cats. Treatment was generally well-tolerated, local reactions (alopecia) recorded in some animals did not appear to be treatment-related.

The results of this study support the proposed claims for immediate efficacy and for persistent efficacy of up to 12 weeks, as well as the claimed speed of kill against *C. felis* of 48 hours. The study was conducted in Australia, however, the CVMP considered it sufficiently relevant for the EU and therefore acceptable.

In addition to these studies, another study was already assessed for the application for 'Bravecto spot-on solution', and demonstrated immediate and persistent efficacy for up to 16 weeks. Although the study was conducted with fluralaner only, it is accepted as an additional dose confirmation study, since administration of moxidectin in combination with fluralaner is not expected to reduce the systemic availability of fluralaner or interfere with the antiflea and antitick activity of fluralaner in the fixed combination (see: section 'Pharmacodynamics').

Ticks (*Ixodes ricinus*):

One dose confirmatory study was provided to demonstrate the acaricidal efficacy of the fixed combination administered once topically at the minimum recommended dose of 40 mg fluralaner/kg bw to cats against artificially induced infestations with ticks (*Ixodes ricinus*) over 12 weeks (84 days). Twenty cats were (re)infested with *I. ricinus* (genetically from Europe) at D-2, 28, 56 and 84, and treated once with the test product or a placebo at D0. Based on arithmetic means the product was determined to be 100% effective against *I. ricinus* ticks on D2, 30, 58 and 86.

No local reaction at the administration site or product related abnormalities were detected.
This study used the final formulation and was conducted within the EU (Ireland). The CVMP agreed that the acaricidal effect (100%) was sufficiently demonstrated against artificial infestations of *I. ricinus* in cats for a period of 12 weeks (84 days) following treatment.

In addition, another study using fluralaner as a single substance was submitted, which was already assessed for the application for 'Bravecto spot-on solution'. Based on the data provided, immediate and persistent efficacy against *I. ricinus* was confirmed for up to 12 weeks. The claim for persistent efficacy of up to 12 weeks and the claim for a speed of kill of 48 hours are considered to have been adequately supported.

Endoparasites:

Prevention of heartworm disease:

Three GCP-compliant dose confirmatory studies were provided in order to demonstrate the efficacy of a single topical dose of Bravecto Plus at the recommended dose (i.e. 2 mg moxidectin/kg bw) in the prevention of heartworm disease for a period of 12 weeks.

All studies followed the same design: 30 healthy young cats were either treated on Day 0 (n=10) or Day 30 (n=10) with the test product or a placebo (negative control, n=10), and at Day 90 subcutaneously inoculated with 100 *D. immitis* infective larvae (L3) (recent field isolate originating from Kentucky or Georgia, US). Blood sampling and heartworm check was undertaken before inoculation and at different time-points up to the end of the study when the cats were necropsied (approximately 6 months after inoculation). Studies were performed in the USA with isolates sourced from outside the EU; however, acceptable clarification was provided on the relevance for the EU (in terms of representativeness and susceptibility) of the *D. immitis* isolates that were used. Evaluation of disease was not performed.

In the first study, 3 (out of 10) animals from the control group tested positive for *D. immitis* antigen on Day 262 (172 days after infection). All other animals from the control group and all animals from the product treated groups (n=20) tested negative at all time-points. At necropsy, the geometric mean of total *D. immitis* counts in the control group was 3.8 (2 out of 10 animals were free of *D. immitis*), while *D. immitis* was not found in any of the treated groups. It was therefore concluded that the heartworm prevention rate for each treated group was 100%. Treatment was well tolerated by the cats, cosmetic changes were noted in some cats (wet coat at the application site for up to 1 day after administration).

Ninety days after administration moxidectin was not quantifiable in the plasma of the majority of cats evaluated. Nonetheless, the CVMP agreed that this study adequately supported the efficacy of Bravecto Plus in the prevention of heartworm disease caused by *D. immitis*, for a period of up to 12 weeks.

In the second study, 8 (out of 10) cats from the untreated control group tested positive for *D. immitis* antigen post-infection (earliest detection time-point: Day 210). One animal (out of 10) from the group treated with test product at D0 tested positive on the last day of the study (D273). All other animals from the two treated groups and two animals from the control group tested negative for *D. immitis* antigen at all time-points tested. At necropsy, one worm each was recovered from 3 of the 10 animals treated at Day 0 (90 days prior to infection), whilst no worms were recovered from any of the animals treated at Day 30 (60 days before infection).

It was concluded that efficacy of Bravecto Plus in heartworm prevention was demonstrated in this study to be 100% up to 2 months, but was 92.3% when treated 3 months before infection. According to VICH GL20 (Efficacy of Anthelmintics Specific Recommendation for Feline) an efficacy standard up to 100% is imposed for infections with *D. immitis* because of animal welfare/clinical implications of the infection. The CVMP therefore considered that this study did not support the proposed treatment schedule in the indication: For prevention of heartworm disease when treated every 12 weeks.

In the third study, 2 (out of 10) animals from the control group tested positive for *D. immitis* antigen on Day 271. At necropsy, 1 - 14 *D. immitis* live adult worms were recovered from 4 (out of 10) animals in the control group. There were only 2 cats with two or more worms each in the control group; thus, adequacy of infection was not achieved in the control animals for efficacy evaluation. No worms were recovered from any of the animals from the treated groups (treated 90 and 60 days before infection) at necropsy. Treatment was well tolerated, with no adverse reactions reported. Since adequacy of infection was not established to allow meaningful assessment, the CVMP considered that this study does not support the proposed indication: For prevention of heartworm disease.

In combination with appropriate adequacy of infection, 100% efficacy could only be demonstrated for a 12 week period in one study. The CVMP therefore considered the results of current efficacy studies insufficient to support efficacy of the product for over the originally proposed 90 days re-treatment interval, as insufficient efficacy has been demonstrated for the full 90 day period. Accordingly, the veterinarian is informed in section 4.2 of the SPC that the effect of the product in the prevention of heartworm disease caused by *D.immitis* is only 2 months.

Roundworm (*Toxocara cati*)

Toxocara cati (adult):

Three GCP-compliant dose confirmatory studies have been provided.

Two studies were conducted in the USA investigating the effectiveness of a topical solution of fluralaner in combination with moxidectin against natural infections with *Toxocara cati* (and/or *Ancylostoma tubaeforme)* in cats, both following the same design. All study animals (n=20) were positive for *A. tubaeforme* eggs and 18 for *T. cati* eggs before treatment. At Day 0, cats were either treated with Bravecto Plus (n=10) or placebo (n=10), and necropsied at Day 10. The primary effectiveness parameter was the number of adult *T. cati* (and/or *A. tubaeforme)* recovered at necropsy in the treated groups compared to the untreated control group. In both studies, adequacy of infection was confirmed (9.9 and 8.8 *T.cati* counts in the control groups, respectively). The treated groups had geometric means of total *T. cati* counts of 0.0 at necropsy, demonstrating an efficacy level of 100.0% for adult *T. cati.*

Another study was conducted in Ireland investigating the efficacy of fluralaner plus moxidectin spot-on solution for cats against experimentally induced infection with the intestinal roundworm *T. cati* in cats. At D -60, 20 cats were orally infected with 300 *T. cati* eggs. Faecal egg counts were conducted prior to treatment (D -10, -9 and -4) and daily after treatment until necropsy (D7). Sixteen animals were randomized in 2 groups, 8 animals per group, receiving test product or placebo at D0. The efficacy after treatment with product against adult worms was 99.7%. It can be accepted that the results of this study demonstrate an acceptable level of efficacy against adult *T. cati*.

CVMP concluded that the claim against adult *T. cati* has been adequately supported.

In addition, the immature adults that were identified during necropsy were also used to determine the percentage of efficacy of the product against immature adults. The applicant claimed that the results demonstrate a 95% effectiveness of Bravecto Plus against immature adults of *T. cati* in cats, since the geometric mean of total immature adults of *T. cati* counts in the control group was 7.8, and that of the group treated with Bravecto Plus was 0.4 at necropsy.

In this study, treatment with the test product was performed 60 days following oral infection and consequently, it was unclear whether the effect of the product was on adult stages or immature adult stages as according to the VICH GL20, treatment should have been administrated at D28 to evaluate efficacy against L4-5 stages of *T.cati*.

However, the CVMP accepted that the geometric mean of total immature adults of *T. cati* counts in the treated group was well below that of the control group and agreed that this study can be considered supportive for the claim against immature adults.

Toxocara cati (L3, L4, L5):

Two GCP-compliant dose confirmatory studies have been provided investigating the efficacy of treatment with Bravecto Plus in cats against third stage (L3), fourth stage (L4) larvae and pre-adult (L5) *T. cati* after experimentally induced infection. Both studies followed a similar design: Cats were allocated randomly to treatment groups, or an untreated control group, and infected at D0 with approximately 300 embryonated eggs of *T. cati,* treatment was administered at D5, D14 or D28, to allow efficacy assessment of different larval stages at the time of necropsy.

One study conducted in Ireland was considered unsuitable to definitively conclude upon efficacy against larval stages as the timing of necropsy (D70) was later than that recommended in the relevant guidelines. If necropsy is performed only at D70, it is not possible to determine whether the product is effective against earlier larval stages. Considering the persistent effect of the product, it is possible that only later stages of the worm are susceptible to the product.

Another dose confirmatory study was therefore performed.

On D38 all cats were necropsied. Results showed adequate efficacy levels of more than 90% efficacy against L4 and L5 stages of *T. cati*. For L3 stages efficacy was below the 90% efficacy level required by VICH GL 20. No serious adverse event which could be related to the treatment with the product occurred during the study.

The applicant has sufficiently substantiated the relevance for the EU of the *T. cati* isolate that has been used.

Based on the bibliographic information provided, and the results of the studies and the supportive information that was provided from another study, the CVMP concluded that effectiveness against stage L4, and L5 of *T. cati* has been sufficiently substantiated.

Hookworm (*Ancylostoma tubaeforme*)

Ancylostoma tubaeforme (adult):

Two dose confirmatory studies were provided.

The design of the first study is described above, as the study was combined with the testing against *T. cati* (adult). Adequacy of infection was confirmed for adult *A. tubaeforme*. The treated group had geometric means of total *A. tubaeforme* (adult) counts of 0.0 at necropsy (D10), concluding on an efficacy level of 100.0% for adult *A. tubaeforme.*

The second study was conducted in Ireland investigating the efficacy of Bravecto Plus in cats against experimentally induced infection with *A. tubaeforme* when compared to a negative (placebo) control group. At D -35, 20 cats were infected with 300 *A. tubaeforme* L3 larvae (isolates from Germany). Faecal egg counts were conducted prior to treatment (D -10, -8 and -4) and daily after treatment until necropsy (D7). Sixteen animals were randomised in 2 groups, 8 animals per group, receiving test product or placebo at D0. All control cats were adequately infected. The efficacy after treatment with product against adult worms was 100%. It can be accepted that the results of this study demonstrate an acceptable level of efficacy against adult *A. tubaeforme*. The product was well tolerated.

CVMP agreed that the claim against adult *A. tubaeforme* is adequately supported.

Ancylostoma tubaeforme (L4, L5):

Two studies were submitted to demonstrate efficacy against L4 and L5 stages of *A. tubaeforme* in cats.

Both studies had a similar design. On D0 healthy young cats were experimentally infected with *A. tubaeforme* infective L3 larvae (European isolate). On D6 they were randomised in 3 groups (10 cats per group one of the studies; 8 cats per group in the other). On D7 group 1 and on D11 group 2 were treated with the test product to evaluate efficacy against L4 and L5, respectively. Group 3 was treated with placebo and served as negative control. In both studies all control cats were adequately infected and no worms were recovered from the treated groups. Results indicated that the product was effective against larval stages of *A.tubaeforme,* however, given the design of the studies and the observation of L5 larvae in control animals, it was not considered possible to differentiate between effect of the product on L4 or L5 larvae.

In addition to these studies, substantial bibliographic information on efficacy of moxidectin was provided. Taking into account all the available information, namely the well-known larvicidal activity of moxidectin

against L4 larval stages and immature adults of *A. tubaeforme*, CVMP therefore agreed that sufficient data was provided to accept an indication against L4 and immature adult stages of *A.tubaeforme*.

Regarding *Ancylostoma tubaeforme,* CVMP agreed that adequate data had been provided to support a claim for effectiveness of Bravecto Plus against adult, L4 and immature adult stages of *A.tubaeforme*.

Conclusions (laboratory studies)

In line with VICH GL20, at least 2 laboratory dose confirmation studies per claimed parasite species and stage have been provided. Concluding, the CVMP considers the following claims sufficiently substantiated:

- Treatment of tick and flea infestations in cats, providing immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus*) killing activity for 12 weeks.
- Treatment of infections with intestinal roundworm (*Toxocara cati*; L4, L5 and adults).
- Treatment of infections with hookworm (*Ancylostoma tubaeforme*; immature adults and adults).
- For the prevention of heartworm disease caused by *Dirofilaria immitis* for 8 weeks.

Target animal tolerance

One GLP-compliant pivotal study was performed to investigate the target animal safety of 3 topical administrations of 1x, 3x, 5x the highest recommended dose at 8-week intervals. Additionally, two studies (one pilot study and one pivotal GLP study) investigated the safety after oral administration of the spot-on solution using the highest expected dose.

A negative controlled pivotal study demonstrated that three topical administrations of 1x, 3x and 5x the highest expected dose (i.e. 93 mg fluralaner + 4.65 mg moxidectin/kg bw) administered at intervals of 8 weeks to 3 groups of kittens (5 males/5 females per group) were well tolerated. A fourth group was administered a placebo. In this study the kittens were aged 61-89 days at Day 0. Clinical assessments were carried out by a veterinarian prior to administration and during the first hour after administration, up to 84 h after administration.

The dosing regimen did not result in adverse events, except for some cases of pruritis (pruritis at the application site: 1 animal 3x group. General signs of post-dosing scratching: 3 animals control group, 2 animals 1x group, 1 animal 5x group) and alopecia at the application site (in 2 animals of the 1x group) and incidentally hypersalivation, which was presumably caused by self-grooming. These side effects are mentioned in section 4.6 of the SPC. Abnormal faeces were observed in one animal of each dose groups including the control group, and considered to be unrelated to treatment. Some haematology (large unclassified cells, lymphocytes, monocytes, neutrophils, platelet count and white blood cell count) and clinical chemistry parameters (ALAT, ASAT, amylase, creatin phosphokinase, cholesterol, creatinine, glucose, LDH, magnesium, sodium, total protein, triglyceride and urea) as well as pathology parameters (absolute kidney weight: higher for males assigned to 1x and 3x group; absolute epididymis weight: lower for males in group 5x; absolute weight of the uterus and cervix: lower for animals assigned to groups 1x and 3x; relative thymus gland weight: lower for animals and the relative weights of ovaries (higher for animals assigned group 3x) differed significantly ($p<0.1$) between control group and (one of the) treatment groups. However, none of these were considered to be of clinical significance, since individual values were within the study reference range (determined by control animals) or findings were not associated with clinical signs and (histo)pathology, and/or without a dose-response relationship.

The C_{min} levels of fluralaner and moxidectin increased after each dosing and was the highest on day 167 compared to day 112 and day 56. This could be observed in all three dose groups (1x, 3x and 5x). The AUC of both active substances also increased each treatment period in all three groups. Although the treatment interval of 8 weeks was well tolerated over 167 days, a 'steady state' of the C_{min} levels and AUC was not observed in this study.

The possibility for continued accumulation of fluralaner and moxidectin following repeated topical application in cats and its significance in terms of target animal tolerance was considered. The use of the product in fast-growing kittens that grew substantially throughout the length of the study, caused a "false" impression of continued accumulation. It is agreed by the CVMP that the use of growing animals can indeed result in such 'false' impression. Bibliographical information was provided on the occurrence of this effect, and a correction for its occurrence was performed. Based on this additional data, the CVMP agrees that the initial results of this study have to be corrected for this so-called 'growth effects'. The CVMP considered that the correction and explanation provided is sufficient to demonstrate that significant accumulation is not expected to occur when the product is applied according to the SPC.

When administrated according to the SPC, the CVMP considers tolerance after long-term use to be sufficiently demonstrated.

The applicant also suggested to include the sentence: "*Use at shorter intervals only according to the benefit risk assessment by the responsible veterinarian*" in section 4.5 of the SPC, but the CVMP disagree with this proposal, since this sentence could encourage undesirable off-label use of the product and is considered incompatible with the recommendation to re-administer the product at 12 week intervals.

In addition, two studies investigating the tolerance of Bravecto Plus after oral administration were provided.

One study was a negative controlled pivotal GLP study in six cats investigating the tolerance of Bravecto Plus after oral administration of the highest expected dose (i.e. 93 mg fluralaner + 4.65 mg moxidectin/kg bw) according to the SPC. Observed adverse events were hypersalivation (2 out of 6 cats, 10-15 minutes after administration) and vomiting (2 out of 6 cats, 2-8 h after administration). No clinically relevant changes in haematology, clinical chemistry and urinalysis parameters were observed 8 days after administration. Fluralaner and moxidectin plasma levels developed a C_{max} at day 2, and the relative variation in moxidectin levels was smaller than observed after spot-on application. It was concluded that after single oral administration, Bravecto Plus is generally well- tolerated; the only observed adverse events were transient vomiting and hypersalivation.

The second study is a negative controlled pilot study to investigate tolerance of the spot-on solution after oral administration (0.5x or 1x dose). After administration of 0.5x or 1x the highest expected dose (i.e. 93 mg fluralaner + 4.65 mg moxidectin/kg bw) a transient hypersalivation and transient reduction of food intake was observed. Bravecto Plus was otherwise orally well tolerated, without other test article related adverse events. However, one cat showed some transient lacrimation from the left eye during the first 15 minutes after treatment possibly due to direct contact with the test item. Section 4.5 of the SPC has a warning to avoid contact with eyes of the animal.

Target animal tolerance was also evaluated in the clinical studies. In addition to evaluating local and systemic safety by evaluating adverse events, one study also evaluated clinical pathology (complete blood count, chemistry screen, and urinalysis). No abnormal pathology changes were observed and treatment-related side-effects were mostly topical (hair loss and skin changes at the application site). A (temporary) change in local cosmetic appearance likely related to the treatment (e.g. wetness, greasy, sticky hair, slight smell) was also registered frequently throughout most of the other studies. The following adverse events in clinical and field studies were observed:

- Hypersalivation and pyrexia, tachypnoea and mydriasis (n=1), dyspnoea after licking the application site (n=1), itching at application site (n=1) and local side effects such as selective alopecia in the area of the spot on administration (n=10) alopecia and change of fur at application site (n=2); are included in the SPC.
- In addition, dizziness (n=1), diarrhoea (n=4), emesis (n=16), lethargy (n=4) and systemic disorders (NOS) (n=4) bloody vomiting (n=1), ptyalism, drooling and lethargy (n=1) were observed. However, the applicant considered these cases to be non-serious, and having an unknown relation to treatment with the product, and diarrhoea, emesis, haematemesis, hypersalivation, lethargy were added to section 4.6 of the SPC.

A number of warnings are already included in the SPC in regard to common effects such as mild and transient skin reactions at the application site (alopecia, flaking skin and pruritus) and uncommon observations such as dyspnoea after licking the application, hypersalivation/lethargy, hypersalivation, and pyrexia/tachypnoea/mydriasis. Based on the findings in the target animal safety and clinical studies, the adverse events 'emesis (or vomiting)', 'diarrhoea' and 'hematemesis, are included in section 4.6 of the SPC. These adverse events are noticed in the most recent studies, in which the product was applied according to the SPC.

Field studies

Four clinical studies to support the efficacy of Bravecto Plus under field conditions were submitted, three studies, evaluating the efficacy against ectoparasites, and one study evaluating the efficacy against endoparasites. All studies were performed in 2015-2016 and according to VICH GL9: Good Clinical Practices (GCP). The final formulation was used in all studies.

The cats that were included in the studies were of various breeds; long- and shorthair, neutered and non-neutered, with an almost equal distribution between males and females. Cats were included if they had a suitable temperament, were in good health and were not lactating or pregnant.

Field studies were largely performed according to the CVMP Guideline for testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats (EMEA/CVMP/EWP/005/2000 - Rev.2), the CVMP guideline Demonstration of Efficacy of Ectoparasiticides (NtA 7AE17a), the VICH GL7 (Efficacy Requirements for Anthelmintics: Overall guidelines) and the VICH GL20 (Efficacy of anthelmintics: specific recommendations for felines).

Ectoparasites (ticks and fleas)

The pivotal field study investigating the efficacy of Bravecto Plus against ectoparasites was conducted in Germany and Spain in cats naturally infested with ticks and/or fleas. Cats were enrolled on the basis of their household, i.e. at least one cat in the household fulfilled the inclusion criteria of having a flea count of ≥ 2 and/or a tick count of ≥ 2. Subsequently, all cats in the household received the same treatment, either Bravecto Plus or a positive control (containing fipronil). 332 households with 707 cats with confirmed tick and/or flea infestations were included in the study (236 flea households and 257 cats with ticks). The most frequent tick species found was *I. ricinus* (n=684, 78.4%).

On day 0, tick and/or flea burden of all cats per household was assessed, also assessment of signs of flea allergy dermatitis (FAD) was conducted, and cats were treated topically with Bravecto Plus (40 mg fluralaner and 2 mg moxidectin/kg bw) or 50 mg fipronil/cat. Follow-up visits were conducted at day 14, 28, 56 and 84. Treatment with the control product was repeated at these occasions (D28 and D56). All cats were observed for adverse events after treatment throughout the whole study period.

Primary efficacy criterion was the percentage reduction in tick and flea count for both products at each follow-up visit, in comparison to the initial tick and flea burden. Secondary efficacy criterion was the percentage of parasite-free cases in the Bravecto Plus group in comparison to the control group for each follow-up visit. Presence of clinical signs of flea allergy dermatitis (FAD) and the improvement were evaluated descriptively.

Results showed a reduction in tick and flea counts (PP) of more than 97% at each timepoint. Non-inferiority of the Bravecto Plus group compared to the control group (fipronil) at each follow-up visit was shown. Regarding the percentage of households free of fleas and cats free of fleas, superiority of Bravecto Plus over the control product could be shown at all time points. Regarding the percentage of households free of ticks and cats free of ticks, superiority of the Bravecto Plus group over the control group could be shown at visits 2 and 4. FAD at inclusion was present in 36 out of 635 cats (30 were treated with product). 53.3% of cats treated with Bravecto Plus were clinically cured at the end of the study, compared to 33.3% treated with fipronil. Treatment with Bravecto Plus was generally well tolerated, adverse reactions associated with treatment were noted in with 14 out of 475 (3%) of treated cats (see above, target animal safety).

In addition, two Australian studies were provided further supporting the efficacy of Bravecto Plus against ectoparasites. Both studies were performed as single-arm studies in Australia during summer flea breeding season.

The first study was a field household study to confirm the efficacy of a single application of a topical fluralaner plus moxidectin spot-on solution against natural infestations of adult fleas (*Ctenocephalides* spp.) on cats.

The geometric means of live flea counts were 11.4, 0.0, 0.1 and 0.1 at pre-treatment, and Days 30, 60 and 90, respectively. The efficacy was 99.7%, 98.9% and 99.0% in Days 30, 60 and 90, respectively. The arithmetic means of live flea counts were 12.3, 0.1, 0.2 and 0.2 at pre-treatment, and Days 30, 60 and 90, respectively. The efficacy was 99.5%, 98.4% and 98.4% in Days 30, 60 and 90, respectively. Treatment of flea infested cats (n=40) with Bravecto Plus, resulted in significant improvements to cat appearance, health and demeanour.

The second study was a 3-month field (household) study to assess the persistent efficacy of a single administration of a topical fluralaner plus moxidectin spot-on solution for the control of fleas (*Ctenocephalides* spp.) on cats in Queensland.

The overall study group mean flea counts were reduced by 100.0%, 98.7% and 99.4% at weeks 4, 9 and 13 respectively, compared to the pre-treatment mean flea count. Treatment resulted in reduction of pruritus for all study cats. The overall study group arithmetic mean pruritus visual analogue scale (PVAS) scores were reduced by 77%, 87% and 87% at weeks 4, 9 and 13 respectively, compared to the pre-treatment mean score. No cats exhibited any adverse or unusual behaviour due to the product treatment in the five-minute posttreatment period.

Gastrointestinal nematodes (*Toxocara cati* and *Ancylostoma tubaeforme)*

The pivotal field study investigating the efficacy of Bravecto Plus in cats naturally infected with gastrointestinal nematodes was conducted in Germany, Albania, Bulgaria and Hungary. Cats with a faecal sample positive for gastrointestinal nematode infection (epg >0) were enrolled in the study and treated with either Bravecto Plus or a positive control (containing a fixed combination of emodepside and praziquantel). 273 cats with confirmed infections of *T. cati*, hookworms (*Ancylostoma tubaeforme, Uncinaria stenocephala), Toxascaris leonina* and *Capillaria* spp. were enrolled.

On day 0, cats were treated topically with Bravecto Plus (40 mg fluralaner + 2 mg moxidectin/kg bw) or the control product (3 mg emodepside + 12 mg praziquantel/kg bw). A second faecal sample was taken on day 14±4 days and analysed for the presence of nematode eggs. All cats were observed for adverse events after treatment throughout the whole study period. Primary efficacy criterion was the percentage of faecal egg count (FEC) reduction for each nematode species in the study groups. Secondary efficacy was based upon the percentage of nematode-free cats post-treatment (no egg shedding).

263 cats (176 Bravecto Plus and 87 control product) were eligible for statistical analysis. In the Bravecto Plus group, 142 cats were infested with *T. cati*, 55 cats with hookworms (*A. tubaeforme, U. stenocephalia),* 15 cats with *T. leonina* and 13 cats with *Capillaria* spp. In the reference product group, 73 cats were infested with *T. cati*, 22 cats with hookworms, 6 cats with *T. leonina* and 4 cats with *Capillaria* spp.

Faecal egg count reduction (PP) for *T. cati* based on geometric means was 99.97% in the Bravecto Plus group and 99.93% in the reference product group. For *T. leonina*, hookworms and *Capillaria* spp. FEC reduction was 100% in both groups. The percentage of nematode-free cats was 98.3% in the Bravecto Plus group, and significantly non-inferior (p=0.0005) to the 96.55% in the reference product group.

Conclusions

The following claims are substantiated by the field studies provided:

Ctenocephalides felis and *Ixodes ricinus*: One pivotal EU field trial has been provided that supports the claim against *C. felis* and *I. ricinus* for up to 12 weeks. In addition, two supportive studies, conducted outside the EU support the efficacy against fleas.

Given that a pulicidal effect has been demonstrated, and the fact that improvement in symptoms of flea allergy dermatitis was reported in the pivotal field study, the proposed indication for use of the product as part of a treatment strategy for flea allergy dermatitis is considered to be adequately supported.

Toxocara cati and *Ancylostoma tubaeforme:* One field trial conducted in the EU has been provided that supports the claim against adult *T. cati* and *adult Ancylostoma tubaeforme.*

Overall conclusion on efficacy

Justification of the fixed combination

The justification for the combination of active substances (fluralaner + moxidectin) is the widening of the spectrum of activity. It can be accepted that the endo- and ectoparasites targeted by this product are likely to co-exist in the same animal. Consequently, the broadening of spectrum of activity arising from the combined use of fluralaner and moxidectin in this fixed combination product is considered to satisfy the CVMP guideline requirements for fixed combination products (EMEA/CVMP/83804/05).

The active substance fluralaner is already authorised as a spot-on formulation, Bravecto spot-on solution for cats. The active substance moxidectin is also already authorised as a spot-on formulation for cats in combination with imidacloprid. Consequently, the combination of fluralaner and moxidectin satisfies the requirements for both active substances of having been already authorised as veterinary medicinal products.

The justification of the fixed combination is accepted.

No synergistic effect of fluralaner and moxidectin is claimed.

Resistance:

The risk of resistance development seems unlikely and not highly critical for a product used in individual companion animals and fluralaner is a relatively new active substance. The risk of resistance development with regard to the use of this product is currently not expected to pose a risk to the population.

Pharmacokinetics:

The pharmacokinetic parameters of the fixed combination have been evaluated in a PK study by comparing topical administration of 0.5x, 1x and 2x the recommended dose of the test product , 1x topical administration of either fluralaner or moxidectin alone and administration of the fixed dose intravenously. Bioavailability of fluralaner was 25.7 ± 10.7, 44.8 ± 12.6 and 23.3 ± 9.3%, respectively, for 0.5x, 1x and 2x dose groups. Bioavailability of moxidectin was 17.2±8.36, 26.2 ±15.3 and 13.2±1.13%.

Total plasma clearance of fluralaner and moxidectin is low, fluralaner has a moderate and moxidectin a high volume of distribution (3034 and 26260 ml/kg respectively).

Both fluralaner and moxidectin were distributed to a major extent in visceral and subcutaneous fat tissues, followed by kidney and liver. The primary elimination route of both active substances is via the faeces.

Dose justification:

Based on the pharmacokinetic profiles of fluralaner and moxidectin it can be concluded that there is no biologically relevant interaction between the two active substances. Therefore dose determination studies using a fluralaner-only spot-on solution are accepted as dose justification for fluralaner in Bravecto Plus.

Three pharmacokinetic studies (a pilot pharmacokinetic study, a pilot heartworm efficacy study and a pivotal pharmacokinetic study), bibliographical data and three dose confirmation studies have been provided in support of the selected dose and re-treatment interval of moxidectin in this fixed combination product.

In conclusion, results of the dose confirmation studies demonstrated that the proposed dose was suitable, but not for the originally proposed 90 days re-treatment interval in all the intended target helminths.

Tolerance:

In the TAS studies, the fixed combination was well tolerated after topical administration up to 5x the highest recommended dose when given to kittens three times at intervals of 8 weeks. After oral administration of a single dose of the spot-on solution, vomiting, transient reduction of food intake and hypersalivation were observed. The trough level (C_{min}) and AUC did not reach a steady state after three applications of the maximum recommended (1x) dose in kittens.

Findings from the pivotal target animal tolerance study indicate that trough plasma concentrations of both active substances were shown to increase after each dose application. These findings (also observed in the 3x and 5x RTD groups) initially suggest accumulation of both active substances following topical application of the final formulation in cats. However, when correcting for the 'growing effect' of the animals used in the pivotal target animal tolerance study, significant accumulation is not expected to occur when the product is applied and long-term tolerance is considered sufficiently demonstrated.

In the clinical studies, both systemic and local adverse events were observed at the recommended dose in a few animals as well as other adverse events probably due to licking, like hypersalivation, vomiting and other gastro-intestinal events. As a conclusion, the following observed adverse events were included in the SPC section 4.6: Diarrhoea, emesis, hematemesis and skin reactions at the application site. These are considered sufficiently reflected in section 4.6 of the SPC.

Based on the data provided, the adverse reactions reported include skin reactions at the application site. The potential for mild and transient adverse effects such as dyspnoea after licking the application site, hypersalivation, emesis, haematemesis, diarrhoea, lethargy, pyrexia, tachypnoea and mydriasis were observed to occur uncommonly (more than 1 but less than 10 animals in 1,000 animals treated) and have been included in the SPC.

Efficacy:

The indication is in line with that agreed for other fixed combination products targeting more than one parasite:

"For cats with, or at risk from, mixed parasitic infestations by ticks and fleas, gastrointestinal nematodes or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and one or more of the other target parasites is indicated at the same time.

For the treatment of tick and flea infestations in cats providing immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus)* killing activity for 12 weeks. Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).

For the treatment of infections with intestinal roundworm (4^{th} stage larvae, immature adults and adults of *Toxocara cati*) and hookworm (4^{th} stage larvae, immature adults and adults of *Ancylostoma tubaeforme*).

For the prevention of heartworm disease caused by *Dirofilaria immitis* for 8 weeks."

Ctenocephalides felis: Two dose confirmation studies (using non-EU isolates) and one pivotal EU field study have been provided that supports the claim of immediate efficacy and the claim of persistent efficacy for up to 12 weeks.

The initial claim for a speed of kill of 12 hours is considered to have been inadequately supported as this has not been demonstrated over the entire period for which pulicidal effect is claimed. A speed of kill of 48 hours is acceptable.

Given that a pulicidal effect has been demonstrated and the fact that improvement in symptoms of FAD was reported in the field efficacy study, the proposed indication for use of the product as part of a treatment strategy for flea allergy dermatitis (FAD) is considered to have been adequately supported.

Ixodes ricinus: Two dose confirmation studies and one pivotal EU field study have been provided that support a claim for immediate efficacy and a claim for persistent efficacy of up to 12 weeks. The claim for a speed of kill of 48 hours is considered to have been adequately supported.

Dirofilaria immitis: Three dose confirmatory studies have been provided in support of the proposed indication against *D. immitis.* Only one of these studies was able to demonstrate 100% efficacy against the prevention of heartworm disease when artificial infection was induced at either 60 or 90 days after treatment with Bravecto Plus. This study supports an indication for the prevention of heartworm disease caused by adult *D. immitis* for a period of 12 weeks. In the second study, 100% efficacy was demonstrated for a period of 8 weeks as at 12 weeks only 92.3% efficacy was demonstrated. The third study was unable to demonstrate adequacy of infection (1 to 14 live adult worms were recovered from 4 out of 10 animals in the control group).

CVMP considered the results of current efficacy studies insufficient to support efficacy of the product for the originally proposed 90 days re-treatment interval, as insufficient efficacy has been demonstrated for the full 90 day period. Section 4.2 of the SPC has therefore been updated, so that the veterinarian is properly informed that the effect of the product in the prevention of heartworm disease caused by

D.immitis is only 2 months.

Toxocara cati: In total, five dose confirmation studies, one EU field study and bibliographic information have been provided that support a claim for efficacy against adult and L4 and L5 stages of *T. cati.*

Ancylostoma tubaeforme: Two dose confirmation studies and one field study have been provided that supports a claim for efficacy against adult *A. tubaeforme*. Two dose confirmation studies (one conducted within the EU) have been provided that support a claim for efficacy against immature adult stages of *A. tubaeforme*. Taking into account all the available information, namely the well-known larvicidal activity of moxidectin against L4 larval stages, CVMP also accepted the efficacy against L4 stages of *A.tubaeforme.* CVMP therefore accepts that sufficient data has been provided to accept an indication against L4, immature adult and adult stages of *A.tubaeforme*.

Part 5 – Benefit-risk assessment

Introduction

Bravecto Plus is a spot-on solution for topical use in cats, containing as active substances a fixed combination of fluralaner (280 mg/ml) and moxidectin (14 mg/ml). It is presented in pipettes of three different strengths to allow dosing of cats with different weight ranges. The combination is considered a new fixed combination of active substances previously authorised within EU and is therefore considered a new active substance.

The product is intended for use in cats for the following indications:

For cats with or at risk from, mixed parasitic infestations by ticks and fleas, gastrointestinal nematodes or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and one or more of the other target parasites is indicated at the same time.

- For the treatment of tick and flea infestations in cats, providing immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus*) killing activity for 12 weeks. Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.
- The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).
- For the treatment of infections with intestinal roundworm (4^{th} stage larvae, immature adults and adults of *Toxocara cati*) and hookworm (4^{th} stage larvae, immature adults and adults of *Ancylostoma tubaeforme*).
- For the prevention of heartworm disease caused by *Dirofilaria immitis* for 8 weeks.

The application has been submitted in line with the requirements for submissions under Article 13(b) of Directive 2001/82/EC - fixed combination application.

Benefit assessment

Direct therapeutic benefit

Both active substances in the proposed fixed combination product, fluralaner and moxidectin, are already used in single-substance veterinary medicines with well-described modes of action, with fluralaner exhibiting ectoparasiticidal properties, and moxidectin mostly anthelmintic properties. The risk for

development of resistance towards the target parasites in cats appears to be low for both active substances.

The benefit of the product would be its efficacy over 12 weeks in the treatment of cats that need treatment of both infestations with ectoparasites (ticks and fleas) and also the treatment of infections with helminths, i.e. intestinal roundworm (*Toxocara cati*; 4th stage larvae, immature adults and adults) and hookworm (*Ancylostoma tubaeforme*; 4th stage larvae, immature adults and adults), or over 8 weeks for the prevention of heartworm disease caused by *Dirofilaria immitis*.

Additional benefits

The fixed combination would facilitate animal handling by reducing the total number of applications needed.

The product would increase the range of available treatment possibilities for concurrent ectoparasite infestations and single or mixed nematode infections in cats.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and 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

Risks for the target animal:

Administration of Bravecto Plus in accordance with SPC recommendations is generally well tolerated.

The main reported adverse reactions include skin reactions at the application site. The potential for mild and transient adverse effects such as dyspnoea after licking the application site, hypersalivation, emesis, haematemesis, diarrhoea, lethargy, pyrexia, tachypnoea and mydriasis have been observed to uncommonly occur.

Risk for the user:

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.

Bravecto Plus can pose a risk to children coming into contact with the animal shortly after treatment and if accidentally ingested. Specific measures are included in the product information to mitigate the risk. These measures were aligned with those of Bravecto Spot-on.

Risk for the environment:

Bravecto Plus is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC including a warning on the product entering watercourses.

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 and environment and to provide advice on how to prevent or reduce these risks.

User safety risks have been identified, mainly the risks associated with exposure in children. These risks are mitigated by including an appropriate warning in the product information related to the storage of the product and by a child-resistant packaging. The user safety warnings were aligned with those of a related mono-substance product containing fluralaner.

Evaluation of the benefit-risk balance

Based on the data presented, the overall benefit-risk is considered positive.

The applicant initially applied for the following indication:
For cats suffering from, or at risk from, mixed ecto- and endo-parasitic infections:

For the treatment of tick and flea infestations in cats. This veterinary medicinal product is a systemic insecticide and acaricide that provides immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus*) killing activity for 12 weeks.

For the prevention of heartworm disease caused by *Dirofilaria immitis* for 12 weeks.

For the treatment of infections with intestinal roundworm (*Toxocara cati*; 4th stage larvae, immature adults and adults) and hookworm (*Ancylostoma tubaeforme;* 4th stage larvae, immature adults and adults).

Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).

The product has been shown to be efficacious and the CVMP accepted the following indications:

For cats with, or at risk from, mixed parasitic infestations by ticks and fleas, gastrointestinal nematodes or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and one or more of the other target parasites is indicated at the same time.

For the treatment of tick and flea infestations in cats providing immediate and persistent flea (*Ctenocephalides felis*) and tick (*Ixodes ricinus)* killing activity for 12 weeks. Fleas and ticks must attach to the host and commence feeding in order to be exposed to the active substance.

The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD).

For the treatment of infections with intestinal roundworm (4th stage larvae, immature adults and adults of *Toxocara cati*) and hookworm (4th stage larvae, immature adults and adults of *Ancylostoma tubaeforme*).

For the prevention of heartworm disease caused by *Dirofilaria immitis* for 8 weeks.

Information on development, manufacture and control of the active substances and finished product has been presented and lead 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 and the environment when used as recommended. Appropriate precautionary measures 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) considers that the application for Bravecto Plus 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.