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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Bravecto CombiUNO (EMEA/V/C/006358/0000)

INN: Fluralaner/Milbemycin oxime

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 4 March 2024, an application for a marketing authorisation to the European Medicines Agency (The Agency) for Bravecto CombiUNO, through the centralised procedure under Article 42(4) of Regulation (EU) 2019/6 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 16 May 2023, as no other marketing authorisation has been granted for the veterinary medicinal product within the Union.

At the time of submission, the applicant applied for the following indications:

"*For dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm. The veterinary medicinal product is only indicated when used 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 on dogs providing immediate and persistent flea (Ctenocephalides felis and C. canis) killing activity and immediate and persistent tick (Dermacentor reticulatus, Ixodes hexagonus, I. ricinus, and Rhipicephalus sanguineus) killing activity for 1 month.

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

For reduction of the risk of infection with Babesia canis via transmission by D. reticulatus for 1 month. The effect is indirect due to the product's activity against the vector.

For reduction of the risk of infection with Dipylidium caninum via transmission by C. felis for 1 month. The effect is indirect due to the product's activity against the vector.

Treatment of infections with gastrointestinal nematodes of the following species: roundworms (immature adult (L5) and adult stages of Toxocara canis, and adult stages of Toxascaris leonina), hookworms (immature adult (L5) and adult stages of Ancylostoma caninum) and whipworm (adult stage of Trichuris vulpis).

Prevention of heartworm disease (Dirofilaria immitis).

Prevention of angiostrongylosis (by reduction of the level of infection with immature adult (L5) and adult stages of Angiostrongylus vasorum) with monthly administration."

The active substances in Bravecto CombiUNO are fluralaner and milbemycin oxime (MBO), which belong to the isoxazoline and milbemycins class of parasiticides, respectively. Both substances exert parasiticidal activity by interacting with ligand-gated ion channels in the nervous system of various parasites such as insects, acari and helminths. The target species is dogs.

Bravecto CombiUNO chewable tablets for dogs contain 25/1.875, 50/3.75, 100/7.5, 200/15, 400/30 or 600/45 mg fluralaner and milbemycin oxime, respectively, and are presented in packs containing 1, 3 or 6 tablets.

The rapporteur appointed is Ricardo Carapeto García and the co-rapporteur is Els Dewaele.

The dossier has been submitted in line with the requirements for submissions under Article 20 of Regulation (EU) 2019/6 – a combination veterinary medicinal product application.

On 12 June 2025, the CVMP adopted an opinion and CVMP assessment report.

On 30 July 2025, the European Commission adopted a Commission Decision granting the marketing

authorisation for Bravecto CombiUNO.

Scientific advice

The applicant received scientific advice (EMA/SA/0000056753, EMA/SA/0000064418, EMA/SA/0000074222 and EMA/SA/0000084580) from the CVMP on 15 April 2021, 7 October 2021, 16 February 2022 and 14 July 2022, respectively. The scientific advices pertained to the safety and clinical development of the dossier.

The approach taken is consistent with the scientific advice provided to the applicant on the combination of fluralaner with milbemycin oxime and its safety profile.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant has provided a summary of the pharmacovigilance system master file, which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided, the applicant has in place a pharmacovigilance system master file (PSMF) with reference number PSMF5527014338, has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

Active substance

Fluralaner

Manufacture and quality control testing of the active substance fluralaner and its intermediate take place outside the EEA.

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

Milbemycin oxime

Manufacture and quality control testing of the active substance milbemycin oxime take place outside the EEA.

A 'Qualified Person' (QP) declaration for all the active substance manufacturing sites is provided from the QP at the EU batch release site. This declaration states that the active substance is manufactured in compliance with EU GMP.

Finished product

Batch release of the finished product takes place at Intervet GesmbH, Vienna, AT. The manufacturing authorisation was issued on 26 June 2020 by the Competent Authority of Austria. A GMP certificate

issued by the Competent Authority of Austria is provided. The certificate was issued on 13 September 2023, referencing an inspection on 31 May 2023.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file was considered to be in line with legal requirements.

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

Part 2 - Quality

Composition

The finished product is presented as a chewable tablet containing fluralaner (5.00% w/w) and milbemycin oxime (0.375% w/w) as active substances and aspartame, butylhydroxytoluene (antioxidant), citric acid monohydrate, glycerol, triglycerides, medium-chain, sucrose, macrogol 3350, sodium lauryl sulfate, sodium starch glycolate, disodium pamoate monohydrate, Natural Beef flavour and maize starch.

Six different strengths are proposed: 25 mg/1.875 mg, 50 mg/3.75 mg, 100 mg/7.5 mg, 200 mg/15 mg, 400 mg/30 mg and 600 mg/45 mg fluralaner and milbemycin oxime, respectively, to cover a dog bodyweight range between 1.27 kg and 60 kg. For simplicity, they have been named according to the weight of the tablets (0.5 g, 1 g, 2 g, 4 g, 8 g and 12 g).

Containers and closure system

The tablets are packaged on a 5-ply aluminium blister sealed with an aluminium lidding foil. Each blister strip contains one pocket. The blisters are packed into a carton box with 1, 3 or 6 tablets. The tablets are packaged on a 5-ply aluminium blister sealed with an aluminium lidding foil. The blisters are packed into a carton box with 1, 3 or 6 tablets.

Adequate specifications have been proposed for packaging materials, and certificates of analysis demonstrating compliance with these specifications have been provided. Additionally, appropriate data on holding containers used to package bulk tablets have also been submitted.

Product development

The pharmaceutical development report submitted is based on the development of the products 'Bravecto chewable tablets for dogs' (authorized since 2014 and with 13.64% of fluralaner) and 'Fluralaner Intervet chewable tablets for dogs' (under registration and with 5.46% of fluralaner). The marketing authorisation holder and the manufacturer responsible for batch release of both products are the same as proposed in this application. Therefore, the development strategy for Bravecto CombiUNO was to leverage the formulation and manufacturing experience achieved during the development of these two products.

The components and the manufacturing processes of Bravecto CombiUNO and the other two products containing fluralaner are very similar and the differences have been adequately explained and justified. For this purpose, pilot scale batches were manufactured in the proposed manufacturing site and tested successfully.

This section includes appropriate information about development of the final composition, manufacturing process, container closure system and stability of the product.

Measures to avoid potential dosing mistakes have been implemented, and an appropriate justification with respect to the description of the pharmaceutical form as chewable tablet has been provided.

This section also includes a report on the development of the dissolution test which is performed through two separate methods: one for smaller tablets (0.5, 1.0 and 2.0 g) and one for the larger tablets (4.0, 8.0 and 12.0 g). Both methods are satisfactorily evaluated using varying surfactant concentrations, paddle rotation speeds, vessel types and degassed media.

Description of the manufacturing method

The manufacturing process consists of the preparation of a mass which enables the manufacture of chewable tablets using a forming machine. All the tablet sizes are formed from identical bulk mass.

The batch formula and the detailed description of the manufacturing process, including in-process controls (IPCs) are included.

The IPCs are appropriately described.

The applicant considers the manufacturing process as standard. The overall documentation provided supports this claim and, therefore, the submission of the process validation scheme for commercial scale batches only can be accepted. Full process validation will be performed on commercial scale batches and relevant information should be available at the manufacturing site for inspection.

Control of starting materials

Fluralaner

Information on the control of starting materials has been provided. The active substance fluralaner is a non-compendial material. The supporting data for the active substance is provided in the form of an ASMF. The version of the ASMF supplied with this application has already been approved in relation to other fluralaner-containing products.

Milbemycin oxime

The active substance milbemycin oxime has a monograph in the Ph. Eur. The manufacturer of the active substance has been granted a 'Certificate of Suitability' (CEP) of the Ph. Eur. for milbemycin oxime for veterinary use, a copy of which has been provided within the application. The relevant information has been assessed by the EDQM before issuing the CEP.

Batch analysis data of the active substance have been provided from both the active substance and finished product manufacturers. The results are within the specifications and consistent from batch to batch.

Excipients

The excipients aspartame, butylhydroxytoluene, citric acid monohydrate, glycerol, triglycerides, medium-chain, sucrose, macrogol 3350, sodium lauryl sulfate, sodium starch glycolate and maize starch are well known excipients controlled in accordance with their respective Ph. Eur. monographs.

Disodium pamoate monohydrate is a non-pharmacopoeial excipient. Sufficient information has been provided.

The flavour 'Natural Beef Flavour' is considered a novel excipient since it is not yet used in any registered product. The data provided is also satisfactory.

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

The product contains no materials of animal or human origin, and no material of human or animal origin is used in the manufacturing process.

Control tests on the finished product

Finished product specifications for release include the following tests: appearance, identification of fluralaner, identification of milbemycin oxime, identification of butylhydroxytoluene, assay of fluralaner, assay of milbemycin oxime, assay of BHT, fluralaner degradation products, milbemycin degradation products, water content, uniformity of dosage units of fluralaner and milbemycin (UPLC), texture analysis, dissolution of both active substances and microbiological quality (Ph. Eur. 2.6.12 and 2.6.13).

These specifications are adequate for the dosage form.

The analytical methods have been appropriately described and validated in line with VICH GL1 and VICH GL2.

Batch data for pilot batches and production batches have been provided. All results are within the specifications proposed at release and are comparable between batches.

An adequate characterisation of the potential impurities that may be present has been provided. A summary of the elemental impurities risk assessment performed on the finished product is presented.

Information provided to justify the specifications for the finished product is adequate and appropriate data has been provided regarding reference standards.

Stability

Stability studies have been performed on a number of strengths from pilot batches, applying a bracketing design and according to VICH conditions. These batches were manufactured according to the manufacturing process proposed for commercial batches and packed in the primary packaging proposed for marketing. The studies cover a period of 36 months at 30 °C/65% RH and 6 months at 40 °C/75% RH.

Compliance with the 'Note for guidance on start of shelf-life of the finished dosage form' (EMEA/CVMP/453/01) is confirmed and the applicant has confirmed that the first three batches produced for commercial release will be placed in a stability study. Any confirmed out-of-specification result, or significant negative trend, should be reported to the Agency.

A shelf-life of 24 months, stored below 30 °C, is acceptable for all presentations.

Additionally, no changes have been observed in the photostability study and temperature cycling and temperature excursion studies have been also included with satisfactory results.

Overall conclusions on quality

The finished product is presented as a chewable tablet containing fluralaner (5.00% w/w) and milbemycin oxime (0.375% w/w) as active substances. Six different strengths are proposed 25 mg/1.875 mg, 50 mg/3.75 mg, 100 mg/7.5 mg, 200 mg/15 mg, 400 mg/30 mg and 600 mg/45 mg fluralaner and milbemycin oxime, respectively, equivalent to the following tablet weights 0.5, 1, 2, 4, 8 and 12 g.

The composition of the different strengths is qualitatively identical and quantitatively proportional, and it has been described appropriately in composition table. The tablets are packaged on a 5-ply aluminium blister sealed with an aluminium lidding foil. The blisters are packed into a carton box with 1, 3 or 6 tablets.

The pharmaceutical development report submitted is based on the development of other products of the Bravecto range. The development strategy for this product was to leverage the formulation and manufacturing experience achieved during the development of these other products.

The pharmaceutical development section includes appropriate information about composition, manufacturing process, container closure system and stability of the product.

The batch formula, a description of the manufacturing process and the IPCs established are included.

The applicant considers the manufacturing process as standard and only a process validation scheme for commercial scale batches has been provided. This has been accepted. Full process validation will be performed on commercial scale batches before commercialisation.

Information on the control of starting materials has been provided. The active substance fluralaner is a non-compendial material. The supporting data for the active substance is provided in the form of an ASMF. The version of the ASMF supplied with this application has already been approved in relation to other fluralaner-containing products.

The active substance milbemycin oxime is monographed in the Ph. Eur. and the proposed supplier has a valid Ph. Eur. CEP. The control specifications for the active substance include the test of the Ph. Eur. monograph.

The excipients aspartame, butylhydroxytoluene, citric acid monohydrate, glycerol, triglycerides, medium-chain, sucrose, macrogol 3350, sodium lauryl sulfate, sodium starch glycolate and maize starch are well known excipients controlled in accordance with their respective Ph. Eur. monographs. Regarding non pharmacopoeia excipients, appropriate information has been provided about disodium pamoate monohydrate.

The flavour 'Natural Beef Flavour' is considered a novel excipient since it is not yet used in any registered product. The data provided is satisfactory.

Finished product specifications for release and shelf life have been provided which are appropriate for this dosage form.

The analytical methods have been appropriately described and validated in line with VICH GL1 and VICH GL2.

Batch data for pilot batches and production batches have been provided. All the results are acceptable.

An adequate characterisation of the potential impurities that may be present has been provided. A summary of the 'Elemental Impurities Risk Assessment' performed on the finished product is presented.

Regarding reference standards and container closure system, appropriate data has been provided.

The specifications of the finished product at the end of shelf life are the same at release except for the test uniformity of dosage units of fluralaner and milbemycin.

Stability studies have been performed on pilot batches of each of the tablet sizes 0.5, 4 and 12 g, applying a bracketing design and according to VICH conditions. The studies cover a period of 36 months at 30 °C/65% RH and 6 months at 40 °C/75% RH.

A shelf-life of 24 months, stored below 30 °C, is acceptable for all presentations.

Additionally, no changes have been observed in the photostability study and temperature cycling and temperature excursion studies have been also included with satisfactory results.

Overall, data provided in part 2 of the dossier are considered to be satisfactory.

Part 3 – Safety documentation (Safety tests)

Bravecto CombiUNO is a new fixed combination for dogs containing fluralaner and milbemycin oxime (MBO).

The safety-related studies provided for fluralaner have previously been evaluated by the CVMP in the frame of the original marketing authorisation applications for 'Bravecto chewable tablets for dogs' and 'Bravecto spot-on solution for dogs/cats' as well as with the application for the establishment of Maximum Residue Limits (MRLs) for fluralaner in chickens.

Milbemycin oxime is a well-established substance that has been used in veterinary medicine for more than 10 years and is currently used alone or in combination in veterinary medicine as an anthelmintic and miticidal treatment in companion animals.

Reference is therefore made to published data concerning the toxicity of MBO and structurally related other macrocyclic lactones.

Albeit no new toxicology studies have been conducted with MBO, new target animal safety studies with the combination (fluralaner and MBO) have been performed and are described in more detail in part 4 of this report.

Additionally, a new in vivo skin irritation study and a sensitisation study were conducted with Bravecto CombiUNO by the applicant and are described in more detail in the respective section below.

Safety tests

Pharmacology

See part 4.

Toxicology

Most of the toxicity studies provided for fluralaner were assessed by the CVMP in the frame of the marketing authorisation applications for 'Bravecto chewable tablets for dogs' and 'Bravecto spot-on solution for dogs/cats' or in the frame of the MRL establishment procedure for use of fluralaner in laying hens. The conclusions, which remain applicable, are summarized below.

No new toxicology studies with MBO have been submitted and reference is made to published data concerning the toxicity of this compound and other structurally related macrocyclic lactones. This is considered acceptable.

Single-dose toxicity

Fluralaner

From an acute oral and dermal toxicity study in rats, an LD_{50} of > 2000 mg/kg bw for fluralaner was derived. Based on these data, it can be concluded that the substance has low acute toxic potential.

Milbemycin oxime

Single-dose toxicity was evaluated in acute oral and subcutaneous toxicity studies conducted in mice and rats.

Following oral treatment, MBO led to median lethal dose levels (LD_{50}) of 946 mg/kg bw (male) or 722 mg/kg bw (female) in mice and 863 mg/kg bw (male) or 532 mg/kg bw (female) in rats.

Following subcutaneous treatment to mice and rats, the lethal dose levels of MBO were higher than the dose level tested (3000 mg/kg bw).

Repeat-dose toxicity

Fluralaner

Repeated dose toxicity was extensively studied in rats (including studies with durations of 14, 28 and 90 days, oral and dermal administration) and dogs (28 and 90 days, and 52 weeks, oral administration). The liver was the most sensitive organ; changes noted include an increased organ weight and hepatocellular fatty change as well as effects in related blood parameters.

From the subacute oral toxicity studies in rats (14 and 28 days duration), a NOAEL of 60 mg/kg bw/day was derived. From the subacute oral dose toxicity studies in dogs (28 days duration), a LOAEL of 20 mg/kg bw/day was set, as effects were observed at all dose levels.

From the subacute dermal toxicity studies in rats (14 and 28 days duration), a NOAEL of 50 mg/kg bw/day was derived.

From the subchronic dermal dose toxicity study in rats (90 days duration), a NOAEL of 50 mg/kg bw/day was derived.

From the subchronic oral toxicity study in rats (90 days duration), a NOAEL of 40 mg/kg bw/day was derived.

From the subchronic oral toxicity study in dogs (90 days duration), a NOEL of 2 mg/kg bw/day was derived.

From the chronic oral dose toxicity study in dogs (365 days duration), a NOEL of 1 mg/kg bw/day was derived.

Milbemycin oxime

Oral 28- and 90-day repeated dose toxicity was studied in rats. MBO-induced haematological changes at 15 mg/kg bw/day and above as well as increases in liver weights, fatty change and hepatocyte swelling at 100 mg/kg bw/day. From a 28-day study, a NOEL of 10 mg/kg bw/day was derived. From a 90-day oral study, a NOEL of 3 mg/kg bw/day was derived.

Fluralaner and milbemycin oxime

No repeat-dose toxicity studies for fluralaner and MBO in combination in laboratory animals (rats, mice) were provided. However, repeat-dose studies were conducted with the combination in the target species, investigating target animal tolerance. This is considered acceptable.

The repeat-dose studies did not identify signs indicative of carcinogenicity, immunotoxicity or neurotoxicity.

The available in vivo data with the combination administered to the target species do not indicate an altered safety profile of the fixed combination compared to the safety profile of the individual substances when administered alone.

Tolerance in the target species

See part 4.

Reproductive toxicity, including developmental toxicity

Study of the effect on reproduction

Fluralaner

The potential systemic effects of fluralaner on reproduction were investigated in two pivotal one- and two-generation studies in rats.

From the one-generation reproductive toxicity study in rats, a parental and foetal LOAEL of 50 mg/kg bw/day was derived.

From the two-generation reproductive toxicity study in rats, a LOAEL of 8 mg/kg bw/day was derived for parental toxicity. The NOEL for reproductive toxicity was set at 50 mg/kg bw/day based on post-implantation, post-natal and breeding loss at the next higher dose. The NOEL for pup toxicity was set at 50 mg/kg bw/day based on reduced bodyweight, clinical signs, pathological findings, and delayed physical and sexual development.

Milbemycin oxime

The potential systemic effects of MBO in reproduction were not investigated, which is acceptable for an active substance used in a companion animal product.

Study of developmental toxicity

Fluralaner

The potential toxicological effects of fluralaner on pregnant females and embryo-foetal development were investigated via oral exposure in one pivotal study in rats and two pivotal studies in rabbits as well as in one pivotal prenatal development dermal study in rabbits.

From the oral developmental toxicity study in rats, a NOEL for maternal and foetal toxicity of 100 mg/kg bw/day was derived.

From the oral developmental toxicity study in rabbits, a NOAEL for maternal toxicity of 50 mg/kg bw/day was derived, based on reduction of food consumption and increased post-implantation loss.

From the 2nd oral developmental toxicity study in rabbits, a NOAEL for developmental toxicity of 10 mg/kg bw/day was derived, based on the increase in fusions of cervical vertebra 2 at 25 mg/kg bw/day.

Additionally, a dermal developmental toxicity study was performed in rabbits, from which a NOAEL of 100 mg/kg bw/day was derived, based on foetal malformations at the next higher dose.

Milbemycin oxime

The potential toxicological effects of MBO on pregnant rats and rabbits as well as embryo-foetal development were investigated via oral exposure in one study in rats and one study in rabbits.

From the oral developmental toxicity study in rats, a maternal and foetal NOEL of 30 mg/kg bw/day was derived.

From the oral developmental toxicity in rabbits, a maternal and foetal NOEL of 30 mg/kg bw/day was derived.

Genotoxicity

Fluralaner

The potential genotoxic effects of fluralaner were investigated in three in vitro tests (bacterial reverse mutation test, mouse lymphoma thymidine kinase locus test, chromosomal aberration test in human lymphocytes) and one in vivo test (micronucleus test in bone marrow cells of the mouse).

The results of all four mutagenicity tests were negative, and it was therefore concluded that fluralaner does not have genotoxic potential.

Milbemycin oxime

The potential genotoxic effects of MBO have been investigated in two in vitro tests (bacterial reverse mutation [Ames] test and chromosome aberration in cultured Chinese hamster lung cells) and it was concluded that MBO has no genotoxic potential in vitro. The existing battery of studies, along with the extensive history of safe use of MBO and related substances, supports the absence of genotoxic potential.

Carcinogenicity

Fluralaner

Studies investigating the carcinogenic potential of fluralaner were not submitted. This is considered

acceptable considering the absence of effects in all genotoxicity assays as well as the absence of pre-neoplastic lesions in repeated dose toxicity studies. Based on this, fluralaner is unlikely to have carcinogenic potential.

Milbemycin oxime

Studies investigating the carcinogenic potential of MBO were not submitted. This is justified by the negative results in in vitro genotoxicity tests as well as the absence of pre-neoplastic lesions in repeat-dose toxicity studies. Furthermore, there is a lack of a carcinogenic potential emanating from other macrocyclic lactones.

Other requirements

Special studies

Immunotoxicity

Fluralaner

No signs of immunotoxicity were observed in the repeat-dose toxicity studies provided. Therefore, no specific studies were performed. This is considered acceptable.

Milbemycin oxime

No signs of immunotoxicity were observed in the available studies. Therefore, no specific studies were performed. This is considered acceptable.

Local tolerance

Fluralaner

Studies on skin and eye irritation as well as sensitisation previously submitted in the frame of the marketing authorisation applications for 'Bravecto chewable tablets for dogs' and 'Bravecto spot-on solution for dogs/cats' were also considered for the current evaluation.

The dermal irritation potential of fluralaner was evaluated in vivo in New Zealand White rabbits. The substance was found to be non-irritant.

The eye irritation potential of fluralaner was evaluated in vivo in New Zealand White rabbits. The substance was found to be non-irritant.

In a study in guinea pigs, fluralaner tested at concentrations up to 25% did not show any skin-sensitising potential.

Milbemycin oxime

No data are available for MBO in the publicly available literature regarding its skin/eye irritation and skin sensitisation potential.

Considering the data available for other milbemycins and chemically related avermectins, it is accepted that MBO is a mild eye-irritant and negative for skin sensitisation.

Fluralaner and milbemycin oxime

The dermal irritation potential of the combination was evaluated in vivo in New Zealand White rabbits, and the test item was found to be non-irritant.

The combination also tested negative for skin sensitization using the Buehler method in vivo in guinea pigs.

The use of in vivo methods to test the above-mentioned toxicity endpoints has been scientifically justified by the applicant in this case.

Neurotoxicity

Fluralaner

No effects on the nervous system have been reported for fluralaner in the toxicity tests provided. The absence of additional neurotoxicity studies is therefore justified.

Milbemycin oxime

Macrocyclic lactones (MLs) such as MBO potentiate the receptor response to glutamate which is considered the main mechanism for their anthelmintic and insecticidal activity. Even though the insect GABA receptor was reported to be several orders of magnitude more sensitive than the mammalian GABA receptor, binding of macrocyclic lactones to this receptor is considered to explain their well-known neurotoxic potential in mammals (i.e. tremors, mydriasis, ataxia, and CNS depression).

Signs of intoxication with macrocyclic lactones generally are related to the CNS. The signs seen are similar in both dogs and cats for all the MLs. Depending on the dose and the breed involved and due to the long half-life of these agents, toxicosis may persist for days to weeks.

The therapeutic dosages of MBO for heartworm prevention are 0.5 mg/kg in dogs and 2 mg/kg in cats. However, mild clinical signs of ataxia, hypersalivation, mydriasis, and lethargy have been documented in ivermectin-sensitive dogs dosed at 5 to 10 mg/kg.

Fluralaner and milbemycin oxime

No specific studies on the immunotoxicity or neurotoxicity of the combination of fluralaner and MBO were provided. However, the assessment of tolerance in the target species did not identify changes indicative of immunotoxicity or neurotoxicity.

Observations in humans

Fluralaner:

Fluralaner has been developed exclusively for veterinary use. No data are available on health effects of fluralaner in humans. The isoxazoline class of parasiticides are currently not used in human medicine. However, post-authorisation safety data have shown that sensitivity reactions are observed in humans when exposed to fluralaner-containing products, even though studies to investigate the skin sensitisation potential of fluralaner and other Bravecto formulations were negative. Indeed, based on the post-authorisation safety data, the product information for 'Bravecto chewable tablets for dogs' and 'Bravecto spot-on solution for dogs/cats' has been updated with the following user safety warning: "*Hypersensitivity reactions in humans have been reported*". It is proposed that a similar warning be included for the present candidate product.

Milbemycin oxime:

Milbemycin oxime has been developed exclusively for veterinary use. No data are available on health effects of MBO in humans. However, moxidectin, another substance from the group of milbemycins, was approved by the United States (US) Food and Drug Administration (FDA) in 2018 for the treatment of onchocerciasis (river blindness) in humans. This allows for an extrapolation of effects in humans to MBO.

Based on data from the published literature provided by the applicant, it appears that moxidectin is well tolerated by human subjects when administered at doses in the range of 3–36 mg/kg bw. Moxidectin appears to be generally safe and well tolerated, with a slightly higher incidence of transient, mild, and moderate central nervous system adverse events as the dose increased compared to the placebo. No severe adverse effects were noted, with the main findings being mild-moderate transient events such as headache.

Excipients

The applicant has provided information on the common use and safety profile of the individual excipients in the final formulation of Bravecto CombiUNO, as intended for marketing.

Based on the information presented, noting the reported current and historical safe use, it can be considered that the excipients of Bravecto CombiUNO are unlikely to have a potential for adverse systemic effects. It is accepted that they will not pose a concern to the user and that any risk to the user due to exposure to the final product will be determined by the active substances.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with the CVMP 'Guideline on user safety for pharmaceutical veterinary medicinal products (EMEA/CVMP/543/03-Rev.1).

Bravecto CombiUNO is supplied in (an) aluminium foil blister(s) sealed with a PET aluminium foil lid stock. Pack sizes will contain 1, 3 or 6 tablets per blister card. The maximum strength tablet contains 600 mg fluralaner and 45 mg MBO. Bravecto CombiUNO is to be administered up to once monthly. The main potential routes of exposure are considered to be dermal contact by adult users (owners/professionals) during administration of Bravecto CombiUNO to dogs and accidental oral ingestion by children.

Adults can become dermally exposed every time they administer the tablets. Dog owners and veterinarians that may treat more than one dog per day, may additionally be exposed. Considering the worst case where the highest strength of Bravecto CombiUNO is used to treat two animals, and assuming an adult bodyweight of 60 kg, the user would be exposed to 0.6 x 2 mg fluralaner via dermal contact (0.02 mg/kg bw/day), 0.045 x 2 mg MBO via dermal contact (0.0015 mg/kg bw/day). Considering the dermal NOAEL of 50 mg/kg bw/day for fluralaner and the oral NOEL of 10 mg/kg bw for MBO, this would result in an estimated margin of exposure (MOE) higher than 100.

It is considered that the greatest risk would be accidental ingestion of the largest tablet by a child (600 mg fluralaner and 45 mg MBO). The calculated exposure resulting from accidental intake of a large size tablet by a 12.5 kg child would equal to 48 mg/kg bw/day fluralaner and 3.6mg/kg bw/day MBO. Considering the oral NOAEL of 10 mg/kg bw/day for fluralaner and the oral NOEL of 10 mg/kg bw for MBO, the estimated MOE for oral exposure in a child is less than 100 (0.2 and 2.8, respectively). Appropriate risk control options are therefore required. The risk will be mitigated, in part, by the primary packaging, which, as demonstrated by the applicant, is child-resistant in accordance with ISO 14375:2018, as well as by the inclusion of appropriate warnings and safety measures in the product information. Information on how to proceed in case of accidental ingestion has been added.

In addition, in vivo skin irritation and sensitisation studies with Bravecto CombiUNO were submitted and Bravecto CombiUNO assessed to be non-irritant and non-sensitising to skin.

Section 3.5 of the SPC reflects the risk of hypersensitivity and follows the ABCD format.

Environmental risk assessment

An environmental risk assessment (ERA) was provided according to the relevant CVMP/VICH guidelines. Based on the data provided, the ERA can stop at Phase I, as the Bravecto CombiUNO will only be used in non-food producing species. Bravecto CombiUNO is not expected to pose a risk for the environment when used according to the SPC. Consequently, the applicant proposes not to include specific warnings in the SPC.

Nevertheless, given the nature of the substances contained in Bravecto CombiUNO and the potential unspecific effects to non-target species, the applicant has proposed to add an additional recommendation in section 5.5. of the SPC ('Special precautions for the disposal of unused veterinary medicinal products or waste materials derived from the use of such products'), mentioning that the active substances may be dangerous for fish and aquatic organisms and that the product should not enter surface waters. This is considered acceptable.

Overall conclusions on the safety documentation

Fluralaner is currently used in veterinary medicinal products as an insecticidal and acaricidal treatment in companion and food-producing animals. Milbemycin oxime is a well-established substance that has been used in veterinary medicine for more than 10 years and is currently used alone or in combination in veterinary medicines as an anthelmintic and miticidal treatment in companion animals.

A full safety file in accordance with Article 20 of Regulation (EU) 2019/6 has been provided.

Pharmacology:

The CVMP had previously assessed the pharmacological particulars of fluralaner and MBO. The same conclusions are retained for the present application. Pharmacodynamics and pharmacokinetics are addressed in detail part 4 of this report.

Toxicology:

Fluralaner:

The main toxicological findings can be summarised as follows:

- The acute oral and dermal LD_{50} for fluralaner in rats was estimated to be > 2000 mg/kg in rats.
- The potential systemic effects following subchronic and chronic exposure (oral and dermal) have been comprehensively investigated in the rat. The studies conducted meet GLP requirements and were conducted according to relevant OECD guidelines. The liver was the most sensitive organ for adverse 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 in the oral studies and above 100 mg/kg bw 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, 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.
- The oral NOAEL for maternal toxicity and NOEL for embryo-foetal development were determined to be 10 mg/kg bw/day, derived from developmental toxicity study in rabbits. In rats, the oral

maternal and foetal NOEL was determined to be 100 mg/kg bw/day.

- Studies on carcinogenicity were not conducted for fluralaner. This is justified by the negative results in all genotoxicity tests and the absence of any pre-neoplastic lesions in the multiple repeat-dose studies, conducted up to chronic duration and at a wide range of dose levels.
- Fluralaner was non-irritating in an ocular irritation study and non-irritating in a dermal irritation study. It is not considered a sensitiser based on results of a maximization test conducted in albino guinea pigs. However, hypersensitivity reactions to fluralaner have been reported in periodic safety assessments for similar products. Therefore, a relevant warning has been included in the product information. No effects on the nervous system have been reported for fluralaner in the toxicity tests provided.

Milbemycin oxime (MBO):

The available toxicological data from published information can be summarised as follows:

- The acute oral LD_{50} values of MBO in rats and mice were reported to be between 532 and 946 mg/kg, respectively.
- In repeat dose toxicity studies, an oral NOEL of 3 mg/kg bw/day was established based on a 90-day toxicity study in rats. At higher doses, increases in liver weights, fatty change and hepatocyte swellings were noted. Similar findings were reported in a 4-week oral study toxicity study in rats and a NOEL of 10 mg/kg bw/day derived.
- No studies on reproductive toxicity were presented. In teratogenicity studies in rats and rabbits, the maternal and foetal NOEL was 30 mg/kg bw/day in both species and no effects on foetal development were observed.
- Milbemycin oxime is not considered to be of mutagenic concern based on results from in vitro studies. An in vivo genotoxicity study was considered unnecessary in this case, based on results from the existing battery of studies, along with the extensive history of safe use of MBO and related substances, which supports the absence of genotoxic potential.
- Carcinogenicity studies were not presented. The absence of a carcinogenicity study is justified by the negative results obtained from the in vitro mutagenicity tests performed, the absence of pre-neoplastic lesions in the repeat-dose toxicity studies and lack of carcinogenic potential in other structurally related macrocyclic lactones.
- Milbemycin oxime may be a mild skin irritant, and a moderate eye irritant based on the data available for other macrocyclic lactones.

User safety:

A user safety assessment in line with the relevant guidance document has been presented. The potential health risk of Bravecto CombiUNO to adult users is considered to be low and acceptable when used in accordance with the SPC. The worst-case scenario for user safety would be the ingestion of a tablet by a child, with an estimated margin of exposure (MOE) of 0.2 (fluralaner) and 2.8 (MBO). The risk identified is mitigated in part by the inclusion of appropriate safety advice/warning statements in the SPC and package leaflet, as well as by the child-resistant packaging. Moreover, the product literature has been updated to adapt the risk management measures to the ABCD format and reflect on the risk of hypersensitivity reactions.

Environmental risk assessment:

The use of Bravecto CombiUNO is not expected to pose an unacceptable risk for the environment

when used in accordance with the SPC.

Part 4 – Efficacy

Pre-clinical studies

Pharmacology

Pharmacodynamics

Fluralaner belongs to the class of isoxazolines and has parasiticidal activity against a range of external parasites but lacks efficacy against nematodes. A total of three published papers were provided to describe the pharmacodynamic activity of the active substance fluralaner. Of these data, two studies and one publication focus on the activity on the relevant receptors.

In addition to these references from published literature, the applicant also provided eleven pilot studies investigating the effect of fluralaner in ticks and fleas using a well-established experimental model. One study investigated if the activity on ticks and fleas is exerted by contact or via feeding, two studies investigated the action on immature stages of ticks and fleas, and one study compared the action on *C. felis* with the action on *C. canis*.

Additionally, susceptibility to fluralaner of tick isolates from different geographical locations were compared: two studies compared *R. sanguineus* isolates from the EU and the US on a molecular and functional level; one study compared the GABA-receptor binding sites of *H. longicornis* isolates from the US and AU; and further studies compared fluralaner's flea activity for *C. felis* isolates from the US, AU and EU.

Furthermore, an in vitro study compared the acaricidal activity of fluralaner against three tick species (*A. americanum, R. sanguineus* and *H. marginatum*). In addition, the applicant provided four preliminary studies to demonstrate the lack of endoparasitic activity of fluralaner. These studies evaluated respectively the efficacy of fluralaner against different species of nematodes (families: Ascaridiidae, Strongylidae, Trichostrongylidae), *Trichostrongylus axei* and *Trichostrongylus colubriformis* in jirds, *Haemonchus contortus* in sheep and *D. immitis* in dogs.

The four studies concluded a lack of effectiveness of fluralaner against the named endoparasite species.

Based on the data provided, the CVMP can conclude that:

- Fluralaner has a high potency against ticks and fleas by exposure via immersion (although this is not the predominant in vivo route of exposure).
- Adult ticks were identified to be the least susceptible tick stage.
- Fluralaner had an impact on the inhibition of reproduction calculated from flea mortality and control of oviposition, pupae and flea emergence.
- Fluralaner demonstrated very similar potency in unfed adult fleas of *C. felis* and *C. canis*.

- Fluralaner demonstrated a lack of endoparasitic activity.
- Laboratory clinical data using strains of United States (US) and Australian (AU) origin are considered representative of the EU.
- Clinical laboratory data collected for *R. sanguineus* in the US were considered as sufficiently representative for the EU.

Generally, pharmacodynamics for fluralaner are adequately described in section 4.2 of the SPC.

Milbemycin oxime is a well-established pharmaceutically active substance with a known anthelmintic activity, so the applicant has not presented pre-clinical studies on pharmacodynamics. This is considered acceptable.

The mode of action of the active substance is based on the binding of ligand gated chloride channels (glutamate-receptor (R) and GABA-R) which leads to an increased membrane permeability of nematode and arthropod nerve and/or muscle cells for chloride ions and results in hyperpolarization, paralysis, and death of the parasites.

The applicant provided sixteen bibliographic references, which support that MBO has activity against a range of internal and external parasites. In addition, one reference was submitted to show the lack of efficacy against trematodes and cestodes.

Finally, four other references were provided which support the large therapeutic window and overall good compatibility in target animals due to the selectivity to invertebrates over mammals. Glutamate gated chloride channels are exclusively expressed in invertebrates.

Nevertheless, significant overdoses of MBO cannot be ruled out, although, under normal conditions of use, MBO is generally well tolerated by target animals and provides sufficient safety margins even in susceptible sub-populations (i.e. dogs with a mutation in the gene encoding for P-glycoprotein [Pgp]).

Generally, pharmacodynamics for MBO are adequately described in section 4.2 of the SPC.

Pharmacokinetics

The applicant provided three PK studies in order to characterise the pharmacokinetic profile of BRAVECTO CombiUNO chewable tablets for dogs.

The conduct and evaluation of the pivotal pharmacokinetic study, the pilot pharmacokinetic study and the pivotal margin-of-safety study complied with current guidelines and quality standards for the respective kind of study (pivotal and pilot).

The pivotal pharmacokinetic study was carried out to obtain information on the time-course of unchanged plasma concentrations following an oral or intravenous administration. Three different oral doses (10, 20, or 40 mg fluralaner + 0.75, 1.5, or 3 mg MBO/kg bw) were used, with the middle dose (20 mg fluralaner + 1.5 mg MBO/kg bw), being the maximum recommended clinical dose of BRAVECTO CombiUNO, as specified in the SPC. BRAVECTO CombiUNO was the final formulation produced according to GMP. In addition, a fluralaner-only and an MBO-only oral tablet were administered at the maximum recommended clinical doses to compare their pharmacokinetic parameters with those following administration of BRAVECTO CombiUNO. These formulations were similar to the formulation intended to be marketed but contained only one active substance. The pilot pharmacokinetic study was conducted to administer fluralaner and MBO in combination at the minimum recommended clinical dose (10 mg fluralaner + 0.75 mg MBO/kg bw). In the pivotal margin-of-safety study, BRAVECTO CombiUNO was administered orally at 20, 60, or 100 mg fluralaner + 1.5, 4.5, or 7.5 mg MBO/kg bw, respectively.

The oral route of administration used in all studies is the anticipated route in clinical use. The number of blood samples and the timing of sampling are considered appropriate to allow an adequate determination of absorption, distribution, elimination, and achievement of steady state of fluralaner and MBO in canine plasma.

The bioanalytical methods used were either adequately validated according to current guidelines (pivotal and efficacy studies) or sufficiently qualified (pilot studies). A difference in PK parameter values between males and females was observed. Nevertheless, it was concluded that the high inter-individual variability for fluralaner PK parameters (R-, S- and total fluralaner) as determined by high coefficients of variation (CV) and ranges could be the main reason for this difference.

The pilot pharmacokinetic study determined the blood plasma PK profiles of R-fluralaner, S-fluralaner and total fluralaner as well as milbemycin oxime (MBO) A3 and A4 oxime after single oral administration of a fluralaner plus MBO combination formulation (fluralaner [10 mg/kg bw] and MBO [0.75 mg/kg bw]) and a reference combination formulation (afoxolaner [2.50 mg/kg bw] and MBO [0.50 mg/kg bw]) to healthy young Beagle dogs. Plasma samples were collected and the content of fluralaner (R-fluralaner, S-fluralaner) and MBO (A3 and A4) was measured.

The pivotal margin-of-safety study was conducted to characterise the target animal safety and to demonstrate the safe monthly administration of BRAVECTO CombiUNO during seven monthly administrations. Besides the assessment of safety, a detailed toxicokinetic assessment was conducted. This study determined the accumulation ratio of both active substances.

These studies were correctly designed and conducted, and the number of animals was sufficient to allow for assessment of the pharmacokinetic data. According to the results of these studies, the following conclusions about the pharmacokinetic profile of BRAVECTO CombiUNO are drawn:

Absorption

Following oral administration, fluralaner was readily absorbed, reaching individual maximum concentrations in plasma between ~1 and 7 days after administration. Systemic exposure to fluralaner tended to be slightly less than dose proportional. Systemic bioavailability after oral administration at the anticipated dose range in clinical use (10 to 20 mg fluralaner/kg bw) was 47.4% to 55.1%.

Following oral administration, MBO A3 and A4 were readily absorbed, reaching individual maximum concentrations in plasma between 1 and 6 hours (A3) or 1 and 4 hours (A4) post administration. Systemic exposure to MBO A3 and A4 was generally dose proportional. Systemic bioavailability after oral administration at the anticipated dose range in clinical use (0.75 to 1.5 mg/kg bw) was 68.7% to 75.6% and 66.5% to 72.9% for MBO A3 and A4, respectively.

Distribution

Considering the total body water of 604 ml/kg bw in a 10 kg dog, fluralaner distributed well into tissues. This is not unexpected, based on the physico-chemical properties of fluralaner, with a molecular weight of 556.29 g/mol, a unionised state, and a high log P_{ow} value of 5.35. The mean apparent volume of distribution (V_z) was 2040 and 1400 ml/kg bw in males and females, respectively.

Considering the total body water of 604 ml/kg in a 10 kg dog (), MBO A3 and A4 distributed well into tissues. This is not unexpected based on the physicochemical properties of MBO, with a molecular weight of 541.68 g/mol (A3) and 555.70 g/mol (A4), respectively, a unionised state, and a high XlogP3 value of 4.1 (A3) and 4.7 (A4), respectively.

Metabolism

No quantifiable phase I or II metabolite concentrations were analysed in faeces of dogs. In vitro, fluralaner was metabolically stable in all species tested (mouse, rat, dog and cat).

Publicly available information is utilised to demonstrate that MBO is mainly metabolised to hydroxylated metabolites in vivo.

Elimination

Fluralaner concentrations declined slowly in plasma, with quantifiable concentrations until the last sampling time point 71 days after treatment. Fluralaner had a low systemic clearance; accordingly, mean elimination half-life was long. The major route of excretion of fluralaner is via faeces (~ 90%) and, to a lesser extent, via the renal route. The pharmacokinetic profile of fluralaner was comparable when administered alone or in combination with MBO.

Milbemycin oxime A3 and A4 concentrations declined readily in plasma, with quantifiable concentrations until 8 and 16 days after treatment, respectively. Milbemycin oxime A3 and A4 had a relatively low systemic clearance; accordingly, mean elimination half-life was relatively long (14.0 to 37.9 hours). Excretion of MBO is primarily via faeces and, to a lesser extent, via urine. The pharmacokinetic profile of MBO A3 and A4 was highly comparable when administered alone or in combination with fluralaner.

Steady state

Based on the results of the pivotal target animal safety (TAS) study, the accumulation ratios were determined in the three treatment groups (1x, 3x, 5x the maximum recommended dose during 7 administrations at 30-day intervals).

For fluralaner at the recommended dose, the R values calculated with the first treatment interval as the reference, appear to increase regularly up to the 4th treatment (day 91), then are stable at the 5th treatment, and increase again at the 6th treatment. The values range from 1.27 to 1.89.

For MBO A3 and A4, all plasma values, except for single events, dropped below the lower limit of quantification towards the middle of each dosing interval indicating that there is no ongoing accumulation and that 4 treatment intervals were sufficient to demonstrate the achievement of steady state.

However, although it is agreed that no concern arises from the accumulation of the active substances over consecutive administration intervals in this study, the accumulation ratios obtained after the 6th dosing do show an increase and, hence, further accumulation following more dosing intervals cannot be excluded.

Finally, the applicant has submitted an expert report to justify the lack of interaction between both active substances, fluralaner and MBO, from a pharmacodynamic, pharmacokinetic and possible clinical perspective.

Bearing in mind the mode of action of both substances, together with the results of the main PK parameters when fluralaner is administered solely or in combination, the data provide evidence for the absence of interference with MBO. The justification for the observed discrepancies, an overall high inter-individual variability, is considered the most likely cause for the discrepancies observed.

Considering the pharmacokinetic data provided, the pharmacokinetics of the active substances fluralaner and MBO have been summarised in the proposed SPC. The information as proposed for inclusion in section 4.3 of the proposed SPC is supported by the data presented and can be accepted.

Justification of the fixed combination

Based on the 'Guideline on pharmaceutical fixed combination products' (EMA/CVMP/83804/2005-Rev.1), any particular combination of active substances should be justified based on valid therapeutic principles. The therapeutic advantages offered by the combination of fluralaner and MBO over its individual components when used as single active substance products have been described by the applicant.

The combination of these two active substances is intended for an effective and safe treatment of mixed ecto- and endoparasitic infections in dogs. In addition, a number of warnings have been included in the SPC to ensure the proper use of the product, that is, dogs at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm.

In addition, according to the applicant, the product presents a number of additional benefits based on the reduction of vector borne diseases, the time of administration (monthly treatment) and the feasibility for the owner to administer the product at home.

Taking into account these considerations, the CVMP agrees that the combination is justified. In addition, it is noted that there are other products authorised with similar combinations.

Development of resistance and related risks in animals

Fluralaner

A bibliographical review was provided to support the lack of resistance to fluralaner. This review included bibliographical references about the in vitro efficacy of fluralaner against some tick isolates resistant to common antiparasitic substances. In addition, a new search on peer-reviewed literature concluded that the current resistance situation of fluralaner in the EU remains favourable.

Additionally, current data from in vitro studies indicated that fluralaner is able to overcome the most important resistances observed in the field.

In in vitro bioassays, fluralaner was not affected by proven field resistances against amidines (tick), organophosphates (tick, mite), cyclodienes (tick, flea, fly), macrocyclic lactones (sea lice), phenylpyrazoles (tick, flea), benzophenyl ureas (tick), pyrethroids (tick, mite), and carbamates (tick, mite). The efficacy of fluralaner against fleas was not affected by dieldrin resistance demonstrated in a molecular on-target study on insect GABA receptors of fleas and flies.

Finally, some references showed the in vitro efficacy of fluralaner against some ticks and isolates of *C. felis* resistant to common antiparasitic substances. The applicant has summarised the laboratory studies conducted in different countries. All parasite isolates used in laboratory studies originated from the field and were multiplied in vivo in the laboratory, as recommended in 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.4).

Fluralaner demonstrated efficacy for the tick and flea isolates in all these studies but only one study was conducted in Europe.

Milbemycin oxime

The applicant has provided a bibliographical review to state the current situation of resistance to MBO against heartworms (*D. immitis*), lungworms (*A. vasorum*) and gastrointestinal nematodes proposed in the indications (*T. canis, T. leonina*, L5 and adult stages of *Ancylostoma caninum* and adult stages of *T. vulpis*).

This bibliographical review showed that MBO is effective only against *D. immitis* L3, L4, and microfilariae. Therefore, the use of MBO against adult forms is not recommended since it may lead to the selection of ML-resistant heartworm populations.

Notably, one reference concluded that *D. immitis* resistance to macrocyclic lactones (MLs) has only been confirmed in dogs in the USA (lower Mississippi area) and, although the extent of the problem is not known, it seems currently not yet a known problem in other countries or regions of the USA. Despite the documentation of MBO and other ML-resistant *D. immitis* strains in the USA, MLs are still widely accepted as safe and highly effective in preventing heartworm infections in dogs.

Bravecto CombiUNO is also indicated against gastrointestinal helminths including hookworms, roundworms, and whipworms. According to the applicant, no reports of roundworm and whipworm resistance to MBO are available in the public domain. A possible explanation could be the low number of resistant organisms cycling in dogs due to refugia of parasite populations in the environment.

The information included in section 4.2 of SPC is in accordance with the references submitted and other authorised products with the same active substance.

Finally, the applicant reviewed the in vivo efficacy studies of MBO against some worms for which the product is indicated. It is noted that most of the studies where the threshold of efficacy was not reached were conducted in the USA (*R. sanguineus*, adult and larval stages of *A. caninum*, larval stages of *T. canis*, *D. immitis* and *A. vasorum*).

Taking into consideration the totality of the in vivo data provided, the efficacy of Bravecto CombiUNO was demonstrated in laboratory and field studies against ticks, fleas, gastrointestinal nematodes and for the prevention of heartworm and lungworm disease (*A. vasorum*).

Dose determination and confirmation

Dose determination

Fluralaner

During the global product development program, studies conducted by the applicant to demonstrate the effectiveness of fluralaner for an intended 12-week treatment interval (Bravecto chewable tablets and Bravecto spot-on solution) showed that, for both products, *A. americanum* was the least susceptible US prevalent tick species at all assessment timepoints (48 or 72 hours).

Additionally, fleas were more susceptible to fluralaner than ticks. Therefore, to determine the oral fluralaner dose required for an intended one-month treatment interval for fleas and ticks, three studies were conducted with fluralaner doses ranging from 2.5 to 10.0 mg fluralaner/kg bw against adult stages of *A. americanum* by using a fluralaner-only formulation. The studies submitted were generally well designed and conducted.

In addition, as *A. americanum* is not prevalent in the EU and the parasite species chosen for dose determination (DD) studies should be evaluated in relation to the EU indications for the product, two

speed-of-kill studies against EU prevalent tick species (i.e. *D. reticulatus* and *R. sanguineus*) were conducted to further substantiate the dose determination conducted with *A. americanum*.

Regarding the dose determination studies, the results from one study indicated that a dose of 7.5 mg fluralaner/kg bw was effective (93.5%) in reducing live *A. americanum* ticks for one month using 48-hour counts and for 5 weeks (96.4%) using 72-hour counts. However, it is acknowledged that efficacy at 3 weeks was 89.3% and, therefore, lower than the 90% effectiveness threshold required in the guidelines.

In one study, a dose of 5.0 mg fluralaner/kg bw resulted in a 92.6% reduction in live *A. americanum* ticks using 48-hour counts for one month. However, the higher dose of 7.5 mg fluralaner/kg bw was only 82.6% effective at one month. Neither dose was effective at 5 weeks using 72-hour counts, but the same pattern was observed for the lower dose of 5.0 mg fluralaner/kg bw, resulting in a higher reduction (89.3% vs 78.5%) in live tick counts than the higher dose of 7.5 mg fluralaner/kg bw.

The inconsistency in the *A. americanum* efficacy data observed in these two studies may be due to a combination of several factors, including the use of arithmetic means vs. geometric means, the use of 48-hour vs 72-hour counts for assessing efficacy against *A. americanum* and the possibility that the doses of 5.0 and 7.5 mg fluralaner/kg bw were not high enough to result in consistent efficacy in all dogs.

Therefore, another study was subsequently conducted to evaluate fluralaner efficacy at 5.0, 7.5, and 10 mg fluralaner/kg bw against *A. americanum* as determined with 72-hour counts. The results of this study indicated that a dose of 10.0 mg fluralaner/kg bw provides consistent efficacy above 90% for at least one month following treatment. It is noted that only one dose determination study was conducted with the 10 mg/kg bw dose. Although it is noted that this is a deviation from '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' (EMA/CVMP/EWP/005/2000-Rev.4), it is accepted taking into account the results shown in the dose confirmation studies.

The EU-prevalent tick species *D. reticulatus* was chosen for the EU-specific dose determination study, as speed-of-kill properties against *D. reticulatus* are considered a critical characteristic of fluralaner activity against the transmission of *Babesia canis*.

Regarding sample size and study animals, these can be considered largely adequate in terms of both guideline requirements and representativeness of the target population. With regards to housing, it is noted that animals were individually housed for the duration of the study, which is neither in accordance with Directive 2010/63/EU nor 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.4), which recommends that housing of animals in groups should be considered, where feasible/appropriate.

Bravecto CombiUNO, at a dose of 10 mg fluralaner/kg bw showed the fastest speed-of-kill against *D. reticulatus* within 12 to 24 hours depending on the time point, and its efficacy was confirmed for a 32-day period following treatment.

An additional speed-of-kill study using Bravecto CombiUNO against a European strain of the tick *R. sanguineus* was conducted, since this species is widespread in Europe. Bravecto CombiUNO was declared effective against *R. sanguineus* at 12 hours after chewable tablet administration and 24 hours after infestation for 32 days, following a single administration at the 3 fluralaner doses under evaluation (2.5 mg fluralaner/kg bw and 0.1875 mg MBO/kg bw, 5 mg fluralaner/kg bw and 0.375 mg MBO/kg bw or 10 mg fluralaner/kg bw and 0.75 mg MBO/kg bw). Since no clear differences

were observed among different doses, the results didn't provide useful information to determine the adequate dose of fluralaner in Bravecto CombiUNO.

Nevertheless, the 10 mg fluralaner/kg bw dose is justified by its efficacy against *A. americanum* and additionally by reducing the risk for transmission of *B. canis* via *D. reticulatus* 24 hours after attachment.

Milbemycin oxime

Four studies were carried out to justify the proposed dosage for MBO. Two studies were conducted against *A. vasorum* and two against *A. caninum*.

The minimum effective dose for MBO against endoparasites was primarily selected based on the dose determination study for the 5th stage larvae of *A. caninum*, which is considered the dose-limiting species.

One study tested three different monthly doses of MBO for the prevention of lungworm infection: 0.375 mg MBO/kg bw, 10 mg fluralaner + 0.75 mg MBO/kg bw and 1.5 mg MBO/kg bw. Although, treatment with 0.375 mg MBO/kg bw showed a lower reduction in the weekly mean larvae count when compared with the other two treated groups, all three MBO doses, i.e. 0.375, 0.75 and 1.5 mg MBO/kg bw, were effective in the prevention of angiostrongylosis caused by *A. vasorum* (92.8%, 97.7%, and 98.9%, respectively).

In another study, Bravecto CombiUNO was not efficacious against *A. caninum* larval stages (64.2%). However, the study animals received a lower-than-targeted dose of MBO. The same *A. caninum* isolate was used in two other studies (adult and larval stages, respectively), where the minimum efficacy required (90%) was also not achieved. Thus, it is speculated that the resistance of this strain caused the lack of efficacy of Bravecto CombiUNO and consequently the inconsistencies across study results.

To corroborate the dose determination of MBO, a study was conducted to evaluate Bravecto CombiUNO's efficacy against the L5 stage of *A. caninum* (European isolate). This study showed that the two higher doses of MBO (0.75 mg/kg bw and 1.5 mg/kg bw) were efficacious (98.4% and 99.1%, respectively).

Therefore, the dose of 0.75 mg MBO/kg bw was selected based on the results of this last study and in line with the well-established dose of MBO for the targeted indications of Bravecto CombiUNO. This is supported by two references, which refer to *A. caninum* larval stages as the dose limiting parasite stage for MBO.

The CVMP agrees with the applicant's conclusion to select the minimum recommended dose intended for treatment.

Dose confirmation studies

Dose confirmation fluralaner

Various GCP-compliant dose confirmation studies were conducted. Thus, the conduct and evaluation of the studies complied with current quality standards. Dose confirmation studies provided were conducted using the final formulation of Bravecto CombiUNO.

Three dose confirmation studies against *R. sanguineus* were conducted using US isolates, which are representative for EU isolates (see pharmacodynamics part). Concerning housing, it is noted that the animals were individually housed for the study duration, which is neither in line with Directive 2010/63/EU nor 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.4). In addition, for some of the studies, animals were selected at a very young age (7–8 weeks).

In addition, Bravecto CombiUNO's efficacy against *R. sanguineus* with the dose of 10 mg fluralaner and 0.75 mg MBO/kg bw was confirmed in the dose determination study conducted using an EU isolate.

Conducting laboratory dose confirmation studies in the dose-limiting tick species is in line with the principle of clinical equivalence recommended by VICH GL7, although this does not directly apply to combinations and to ectoparasites.

In addition, supportive dose confirmation laboratory data against *A. americanum, I. holocyclus* and *H. longicornis* have been provided that further prove the safe and efficacious use of Bravecto CombiUNO in other tick species currently not prevalent in the EU.

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.3), the arithmetic mean is preferred to calculate efficacy. Although the 90% efficacy was not achieved in one study by using the arithmetic mean through day 31, the efficacy was above 90 % when the geometric mean was used. In addition, in two further studies, an efficacy of > 99% (using the arithmetic mean) was achieved.

Finally, two supportive studies were submitted to support the efficacy of Bravecto CombiUNO against *R. sanguineus*. It is agreed that these studies support the indications for the treatment of tick (*R. sanguineus)* and flea infestations (*C. felis)* until day 35 following administration.

Therefore, dose confirmation laboratory data obtained against *R. sanguineus* is considered representative, and conclusions can be extrapolated to the following tick species relevant for the EU: *D. reticulatus, I. hexagonus and I. ricinus.*

As fluralaner demonstrated very similar potency for both flea species, *C. felis* and *C. canis*, the results from laboratory studies conducted in *C. felis* can be extrapolated to the flea species *C. canis.*

The dose confirmation studies confirmed a 1-month efficacy of the selected dose of 10 mg fluralaner/kg bw against *R. sanguineus* and *C. felis*, and therefore also against *D. reticulatus, I. ricinus, I. hexagonus* and *C. canis.*

Dose confirmation milbemycin oxime

All dose confirmation studies were conducted according to the principles of GCP, except for one heartworm study. They were also conducted in accordance with the general principles of VICH GL7 and VICH GL19, the recommendations of which are discussed below in detail. The final formulation of Bravecto CombiUNO was used as recommended in VICH GL7.

Animals used in the studies were adequately selected and purpose-bred. Male and female animals were included. Young puppies of an age of 8 to 17 weeks (for *A. caninum* studies), 8 to 15 weeks (for *T. canis* studies), 8 to 12 weeks (for *T. leonina* studies) and 10 to 11 weeks (for *T. vulpis* studies) which were worm-free before experimental infection were used. In the studies using *D. immitis* and *A. vasorum*, adult dogs (> 6 months) were used.

Although VICH GL19 recommends younger dogs for effectiveness studies using *A. caninum* and roundworms (*T. canis*/*T. leonina*), some of the *A. caninum* studies and the *T. canis* and *T. leonina* studies used older puppies due to the unavailability of dogs or the difficulty of using dogs that are so young and might present adverse events due to their early age. Although these studies did not follow

the aforementioned guideline, the infections in the control groups prove the adequacy of the study dogs. The study animals were housed under routine husbandry conditions compliant with relevant animal welfare regulations (Directive 2010/63/EU and Regulation (EU) 2019/6).

Most of the studies targeting adult stages were carried out with induced infections with recent isolates less than 10 years. The dose confirmation studies in larval stages were all performed using induced infections as recommended in VICH GL7 and GL19.

However, according to these guidelines, at least one study should be conducted in naturally infected animals. Consequently, one study used naturally infected animals and was designed to cover all four nematode species. However, no *A. caninum* and *T. leonina* isolates were found. Naturally infected animals were selected based on faecal egg count using the Mini-Flotac method, with a sensitivity of 5 eggs/g (EPG) faeces. Dogs needed to have an EPG > 100 for at least one of the target nematodes to be included in the study and co-infected animals were allowed. *T. canis* egg counts of enrolled animals ranged from 10 to 1360. For *T. leonina*, egg counts ranged from 145 to 320, and for hookworms from 40 to 2160.

The studies in *D. immitis* used induced infections due to the complexity of the disease, as recommended in VICH GL19. Dogs were inoculated subcutaneously with 50 infective larvae (L3) of *D. immitis*, isolates were 1–3 years old. In the studies against *A. vasorum*, animals were orally infected with 200–250 L3 larvae, isolates were 4 and 10 years old.

At least six animals per treatment group should be adequately infected, and this was previously defined in the study protocols. In all studies, eight to ten animals were used to achieve a minimum of six adequately infected dogs.

The evaluations of effectiveness were considered valid if the total number of recovered worms from the small intestine and large intestine was a minimum of 5 per dog and counted in at least 6 control animals, either at necropsy or after diagnostic deworming. This requirement was achieved in all studies, with the exception of the following: in one study only 5 of 10 dogs were adequately infected with *A. caninum*, in one study no animals in the control group were properly infected with *T. canis* and in one study the efficacy for *A. caninum* and *T. leonina* could not be calculated because no isolates were found.

For *D. immitis* and *A. vasorum*, adequacy of infection in terminal studies was defined if six out of eight control animals harboured at least five worms. This requirement was achieved in the studies with worm counts of 13 to 42 *D. immitis* specimens and 34 to 160 *A. vasorum* specimens, respectively. In the non-terminal *A. vasorum* study, an adequate infection was achieved, as 10 out of 10 control dogs shed L1 larvae.

At least two successful controlled dose confirmation studies per individual claim are required. These were provided for adult *T. canis*, adult *A. caninum*, adult *T. vulpis, T. canis* larvae (L5), *A. caninum* larvae (L5), *D. immitis* and *A. vasorum* (one study was conducted as dose determination study as permitted according to GL7 and confirmed in a Scientific Advice). In the natural infection study, no *T. leonina* was found, and a second strain for induced infection was not available. Thus, only one study was provided. Considering that *T. canis* infections were successfully treated with MBO, the efficacy results seen for this roundworm can also be extrapolated to the second roundworm species *T. leonina*. Additionally, no evidence of genetic differences in *T. leonina* populations affecting dogs and no reduced efficacy of anthelmintics against *T. leonina* is reported.

At least one of the studies should be conducted in the geographic location where registration is being pursued. The study with natural infections was conducted in the EU (Spain and Greece), whereas the sites for induced infection studies were split between US and European isolates.

The only exception to this were the studies in *D. immitis*, as the three studies were conducted in the US using US isolates. This is justified, as there is very little genetic variation between two sequenced isolates from Europe and the US. Additionally, a genotype analysis of eleven European *D. immitis* clinical samples indicates that all genotypes are consistently susceptible to MLs. The evaluated EU isolates were genotypically similar to two susceptible US isolates for the concerned marker 'Single Nucleotide Polymorphic' sites (SNPs). A genotypic analysis of European *D. immitis* isolates indicated that there was no evidence of resistance at any of the European sites sampled and that, so far, there is no evidence of *D. immitis* resistance to MLs in Europe. This approach was agreed upon in a Scientific Advice procedure.

The dose confirmation studies were conducted with the goal of confirming the minimum effective dose. This was achieved by administering one or more whole tablets to dose as close as possible to the target dose. The final formulation was used in all studies. The recommended times of treatment after induced infection as detailed in VICH GL19 were followed.

For *D. immitis*, Bravecto CombiUNO was administered at 1, 2, or 3 monthly doses beginning 1 month after infection with L3 larvae to evaluate the effectiveness in preventing heartworm disease in dogs. In the *A. vasorum* studies, Bravecto CombiUNO was administered 28–31 days after experimental infection with L3 larvae to assess the efficacy in preventing angiostrongylosis. Treatment was performed before the larvae matured to adults. According to VICH GL7, the evaluation of effectiveness should be based on parasite counts (adults and larvae, respectively).

In terminal gastrointestinal nematode (GIN) studies with experimental infestation, necropsy was performed at 7 days after treatment. However, as agreed upon in a Scientific Advice procedure, one of two dose confirmation studies could be conducted using a non-terminal study design.

In each study protocol, the efficacy criteria were pre-specified as follows: after diagnostic deworming with a licensed anthelminthic, the control animals needed to show an adequate infection (≥ 5 worms in at least 6 dogs) and the reduction in worm counts after diagnostic deworming in Bravecto CombiUNO-treated group needed to be ≥ 90%.

In addition, secondary efficacy criteria were evaluated, such as faecal egg count reduction, copro-antigen ELISA and PCR-testing. The same study design was used for the studies targeting larval stages.

Thus, the following studies were conducted:

- *A. caninum* adult, two terminal (of which one failed) and two non-terminal (of which one with inadequate infection) studies,
- *A. caninum* larvae (L5), 3 terminal studies (of which one failed) and one non-terminal study (with inadequate infection),
- *T. canis* adult, one terminal and one non-terminal study,
- *T. canis* larvae (L5), three terminal (of which one failed and one with inadequate infection) and one non-terminal study,
- *T. leonina* adult, two non-terminal studies (one with no isolates),
- *T. vulpis* adult, one terminal and one non-terminal study.

For *T. leonina*, no second laboratory strain was available to perform a terminal study, thus, only one non-terminal study was performed. In the natural infection study, no isolates of *T. leonina* were found after diagnostic deworming and eggs of *T. leonina* were only found in one animal. This underlines the

rare occurrence of this species. However, susceptibility of *T. leonina* to MBO and gross morphology of adult worms is comparable to *T. canis*, for which both study designs were provided.

In the heartworm studies, animals were necropsied approximately 5 months after infection, which corresponds to the duration of development from L3 larvae to adult *D. immitis*.

In the terminal lungworm studies, the animals were necropsied when patency of *A. vasorum* had been reached (64–66 and 159–161 days post inoculation, respectively). This was confirmed by the fact that all control animals were shedding L1 larvae. The studies used additional diagnostic methods to determine efficacy such as faecal larvae count and serology (antigen and antibody tests). The results of these diagnostic tools correlated very well with the necropsy results. Thus, in the second non-terminal study, these diagnostic tools were used (faecal larvae count as primary efficacy parameter), in addition to computed tomography (CT) imaging.

As discussed in a Scientific Advice procedure, two different isolates of *A. vasorum* (one from Denmark and one from Switzerland) were used in the studies.

All studies were conducted as controlled studies, using a negative untreated group. Geometric means were used for calculation of percent effectiveness as required. Furthermore, VICH GL7 and GL19 require an efficacy of at least 90% for a compound to be declared effective and a statistically significant ($p<0.05$) difference in parasite numbers between control and treated animals. This requirement was achieved *for A. caninum*, *T. canis*, *T. leonina*, *T. vulpis* and for *A. vasorum*. There were exceptions where the efficacy did not reach the 90% threshold for adult and larval stages of *A. caninum*, larval stages of *T. canis.* However, at least two other studies provided > 90% efficacy for all mentioned nematodes.

VICH GL19 specifies that, for *D. immitis*, higher efficacy standards (i.e. up to 100%) may be imposed because of the severity of heartworm disease. For *D. immitis*, Bravecto CombiUNO achieved an efficacy < 100% in one study, where a genotyped ML-resistant strain was used. However, in another study, where a genotyped non-ML resistant strain was used, efficacy against *D. immitis* after one, two or three treatments was 100%. In another study, Bravecto CombiUNO administered at the target doses of 10 mg/kg bw fluralaner + 0.75 mg/kg bw MBO, provided 100% prevention of heartworm disease caused by infections with *D. immitis* in dogs following a single dose or multiple doses administered at 30-day intervals beginning 30 days after infection.

For *A. vasorum*, the first terminal study revealed that a single treatment 1 month after inoculation leads to insufficient efficacy (67.5%). A second, similar terminal study used 5-monthly treatments and showed 97.7% efficacy. A third, non-terminal experimental study, showed efficacy above 90% based on larval counts from the second treatment, with, however, an increase in larval counts between the third and fourth administration, associated with an increase in lung lesions. Overall, efficacy of 99.82% and an improvement of lung lesions were obtained after the fourth treatment. These results support the inclusion of the following recommendation in the SPC: "*Lungworm prevention should be continued until at least 4 months after the last exposure to slugs and snails*".

Reduction of the risk of flea and tick-borne disease transmission

Several speed-of-kill studies were provided to demonstrate the prevention of transmission of flea-born (*C. felis*) diseases like *D. caninum* infections and of tick-borne (*D. reticulatus*) diseases like *B. canis* infections.

Two studies showed a rapid speed-of-kill (4 and 12 hours respectively) of *C. felis* for the intended monthly treatment interval at the minimum recommended dosage of 10 mg fluralaner + 0.75 mg MBO/kg bw.

Regarding the indication related to *Babesia canis*, data were provided from different studies, with Bravecto CombiUNO and Bravecto chewable tablets. For Bravecto chewable tablets, data from two speed-of-kill studies against *D. reticulatus*, one dose confirmation laboratory study for the prevention of experimental transmission of *B. canis* to dogs by *D. reticulatus* ticks and one field study for the prevention of transmission of *B. canis* in dogs were presented in the dossier.

One speed-of-kill study concluded that the optimal dose of fluralaner when combined with MBO in Bravecto CombiUNO was 10 mg fluralaner + 0.75 mg MBO/kg bw, and its efficacy following a single treatment based on the speed-of-kill was confirmed for a 32-day period following treatment.

This speed-of-kill study against *D. reticulatus* showed a rapid speed-of-kill (24 hours) of *D. reticulatus* for the intended monthly treatment interval. An interrupted feeding effect and by that early transmission events are not documented under field conditions.

For the rest of studies, while similar efficacy was observed, it should be noted that the studies were carried out at a higher dose than that recommended for Bravecto CombiUNO, that is, a dose of 25 mg/kg bw of fluralaner was administered to support Bravecto CombiUNO's preventive efficacy against the transmission of *B. canis* by infected *D. reticulatus* ticks. The recommended dose is of 10–20 mg/kg of fluralaner and 0.75–1.5 mg/kg of MBO. Noting that fluralaner's efficacy relative to the indication is against the vector *D. reticulatus*, that an approximate period of feeding of 24–48 hours is required for *D. reticulatus* prior to transmission of *B. canis* sporozoites and that > 90% efficacy against *D. reticulatus* is achieved within 24 hours, the data is considered adequate to support the indication for 'reduction of risk' for *B. canis* transmission when fluralaner is administered at a the minimum recommended dose of 10 mg/kg bw. The same approach is applicable to the dose confirmation studies conducted to demonstrate the reduction of the risk of infection with *Dipylidium caninum* via transmission by *C. felis*.

In conclusion, the dose confirmation studies have adequately confirmed the efficacy of the selected minimum dose of 10 mg fluralaner/kg bw in the treatment of tick (*R. sanguineus, D. reticulatus, I. ricinus, I. hexagonus*) and flea (*C. felis, C. canis*) infestations for 1 month, which will be the intended dosing interval for Bravecto CombiUNO. The studies further confirmed the safety of Bravecto CombiUNO. Further, Bravecto CombiUNO rapidly kills *C. felis* fleas and *D. reticulatus* ticks and thus can be considered effective in the prevention of *D. caninum* transmission by *C. felis* as well as in the prevention of B*. canis* transmission by *D. reticulatus.*

Tolerance in the target animal species

The safety of BRAVECTO CombiUNO in the target species is based on two pivotal TAS studies, and on one pilot safety study. In some of these target animal safety studies, dogs were single housed (without necessity and/or without adequate enrichment/compensation) and in cages that were either smaller than stipulated in Directive 2010/63/EU or inadequately equipped.

The margin-of-safety of BRAVECTO CombiUNO was assessed in puppies, as puppies are considered to be the most sensitive subpopulation of the target species. This study was conducted according to FDA GLP regulations. The final formulation intended to be marketed and produced according to GMP was used. Dogs were orally treated with either BRAVECTO CombiUNO at doses of 20/1.5, 60/4.5, or 100/7.5 mg fluralaner/MBO per kg bw on seven occasions or were sham-dosed (control animals) according to the requirements outlined in VICH GL43, i.e. that doses 0X, 1X, 3X and 5X the maximum expected clinical doses should be administered. 32 Beagle puppies (16 male and 16 female) 56 to 58 days of age (age on day 1 [initiation of dosing]) and weighing 1.4–1.8 kg on day -1 (day prior to initiation of dosing) were enrolled in the study and were allocated to four groups of eight puppies (four per sex) per group.

The response to treatment was assessed by clinical observations, monitoring of bodyweight development, monitoring of individual food consumption (daily), ophthalmology (pre-dose and on day 179), faecal examinations (on days -12, 4, 16, 33, 63, 93, 123 and 153), evaluation of changes in clinical pathology parameters (haematology, clinical chemistry, coagulation, C-reactive protein and urinalysis [pre-dose and on Days 2, 32, 62, 92, 122, 152, and 182], qualitative bone marrow evaluation [at the end of the study]), macroscopic examination at necropsy (on day 182), determination of weights of selected organs and histopathological examination of tissues. Further, blood plasma samples were analysed for fluralaner (R- and S-fluralaner isomers) and MBO (MBO A3 and A4). All animals survived until the scheduled necropsy.

A non-compartmental analysis was used to estimate toxicokinetic parameters. No test article-related effects were observed for the following endpoints: clinical observations, bodyweight development, food consumption, ophthalmoscopic examinations, faecal examinations, clinical pathology parameters, organ weights, macroscopic and microscopic findings during necropsy. Lacrimation was noted in both sexes at ≥ 20/1.5 mg/kg bw (1X). This was not considered test article-related due to lack of dose-response relationship, occurrence at a similar frequency in control group animals and a lack of histological correlates.

Neurological evaluations were normal in all animals. Carpal laxity disappeared with minimal veterinary intervention (application of splints only) after a few days. Therefore, this was not considered test article-related due to sporadic nature of occurrence and low frequency, as well as occurrence in the control group and was thus considered to be due to some unknown nutritional deficiencies.

Abnormal faeces and thin body condition were observed across all groups at the beginning of the study and were due to adaption problems to the new food and environment, and due to the parasitic cysts and ova which were observed (including Coccidia and Giardia). This is a common finding in animals of this age when transferred to a new environment. These findings resolved during the progress of the study when animals grew older. Additionally, these findings were also observed in the control group.

No treatment-related adverse effects were observed following oral administration at up to 100 mg fluralaner/kg bw and 7.5 mg MBO/kg bw on seven occasions thirty days apart to Beagle dogs (56 to 58 days of age and weighing 1.4 to 1.8 kg at first administration). Therefore, the oral administration of BRAVECTO CombiUNO is confirmed to be safe and shows a sufficient margin-of-safety (5X the maximum recommended clinical dose) when monthly administered.

In avermectin-sensitive Collie dogs, results from the pilot and pivotal target animal safety study differed from each other. In the pilot study, the incidence of neurological findings in dogs treated with BRAVECTO CombiUNO appeared to be higher. This could potentially be explained by the lack of blinding in the pilot study, which may have led to a certain bias, as the investigator was aware of the potential effect of MBO overdosing. This was confirmed by the pivotal target animal safety study in avermectin-sensitive Collie dogs, in which neurological signs were transient, were also present in animals that had been sham-dosed, and no obvious dose-relationship was observed. Overall, any signs of neurotoxicity that were observed after administration of BRAVECTO CombiUNO at 1X, 3X, or 5X the maximum dose intended in clinical use on three occasions thirty days apart, were transient and were also present in sham-dosed animals. It seems that no obvious relationship between doses was observed and there was no evidence of seizures or convulsions during the neurologic examinations. However, according to the results provided by the applicant, it was difficult to determine if the neurological adverse events observed were related to the test-article or not. In order to clarify if possible neurological signs were related to the test-article, the applicant provided further clarification in relation to the pivotal target safety study. Although the results of this study *a*

priori do not seem to suggest a concern of neurological symptoms, it cannot be totally ruled out. The applicant has proposed to include appropriate warnings in the SPC with specific statements for ivermectin-sensitive dogs. This is acceptable and similar to the information included in the SPC of other similar authorised VMPs.

Overall, it can be concluded that the administration of BRAVECTO CombiUNO in accordance with the product information recommendations is well tolerated in the target animal species. The proposed information in the SPC is considered adequate. The reproductive safety of Bravecto CombiUNO has not been tested in dogs.

Neither local effects, nor systemic adverse events have been observed after oral administration (up to the 5X dose group). This is correctly reflected in section 3.10 of the SPC as follows:

"*No adverse reactions were observed following oral administration to puppies aged 56 to 58 days and weighing 1.4 to 1.8 kg treated with overdoses of up to 5 times the maximum recommended dose (20 mg fluralaner + 1.5 mg milbemycin oxime, 60 mg fluralaner + 4.5 mg milbemycin oxime and 100 mg fluralaner + 7.5 mg milbemycin oxime/kg BW) on 7 occasions.*

In a laboratory study the veterinary medicinal product was administered on 3 occasions monthly at 1-, 3- and 5-times the maximum recommended dose to dogs with a deficient multidrug-resistance protein 1 (MDR1-/-). After repeated administration of 3- and 5-times the maximum recommended dose, mostly within 24 hours, ataxia and emesis were observed. Overall, the veterinary medicinal product was tolerated in MDR1-/- dogs following oral administration".

Results of the pivotal margin-of-safety study demonstrate that the repeated oral administration of the VMP is safe in puppies when administered at doses up to 5 times the maximum recommended dose at the recommended treatment intervals. In conclusion, the safety margin in the target species of the VMP is acceptable and no signs of intolerance have been observed.

Furthermore, and also based on these results, the applicant has appropriately reflected aspects not tested in this pivotal study in section 3.5 of the SPC as follows:

"Use with caution in dogs with pre-existing epilepsy.

In the absence of available data, treatment of puppies less than 8 weeks of age and/or dogs less than 1.27 kg bodyweight (BW) should be based on a benefit-risk assessment by the responsible veterinarian.

In (MDR1-/-) dogs, safety of the veterinary medicinal product has been investigated after multiple monthly administrations in a laboratory study. The recommended dose should be strictly observed in MDR1 mutant (-/-) dogs with a non-functional P-glycoprotein, which may include, but not necessarily be limited to, Collies and related breeds. Please see also section 3.10 'Symptoms of overdose (and where applicable, emergency procedures and antidotes)'.

The veterinary medicinal product should not be administered at intervals shorter than 1 month as the safety at shorter intervals has not been tested".

In conclusion, the safety margin in the target species of BRAVECTO CombiUNO is acceptable and no signs of intolerances have been observed.

Furthermore, the safety of BRAVECTO CombiUNO in the target species was studied in the 57 clinical and preclinical studies conducted by the applicant. A total of 1473 dogs were treated with BRAVECTO CombiUNO at recommended label doses in the range of 10 mg fluralaner + 0.75 mg MBO/kg bw and 20 mg fluralaner + 1.50 MBO/kg bw.

Gastrointestinal signs with a potential relationship to BRAVECTO CombiUNO administration were observed in a total of 41 dogs. The most common gastrointestinal signs were emesis (28 dogs) and diarrhoea (10 dogs), followed by hypersalivation (3 dogs), retching (2 dogs) and blood in faeces (1 dogs). General signs were also observed such as lethargy and decreased appetite. Lethargy was assessed as an uncommon non-serious and transient adverse event (8 of 1473 dogs).

Decreased appetite is assessed as an uncommon and transient adverse event (4 of 1473 dogs). Other signs (pruritus) also were observed but it seems that this symptom was caused by the flea infestation in the study since no animal from other studies conducted with Bravecto CombiUNO presented this symptom. This information is correctly reflected in section 3.6 of the SPC.

Clinical trial(s)

Ectoparasites

The applicant conducted one multi-centre field efficacy study in the EU according to current quality standards (i.e. GCP) and following the relevant specific guidelines, including 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.4) and the 'Guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats' published by World Association for the Advancement of Veterinary Parasitology (WAAVP; 2nd edition).

The investigational veterinary products were 5.00% (w/w) fluralaner + 0.375% (w/w) milbemycin oxime chewable tablet (Bravecto CombiUNO) and 12.5% (w/w) fluralaner + 0.03% (w/w) moxidectin + 6.25% (w/w) pyrantel chewable tablet (Bravecto TriUNO), whilst the control product (CP) was Simparica Trio chewable tablets for dogs. Information about batches, expiry dates and receipt, storage, return and disposal was provided. Certificates of analysis were also included. In this assessment, the focus is on Bravecto CombiUNO.

Bravecto CombiUNO was the final product formulation in the final packaging material intended to be marketed and produced according to GMP. Six different tablet sizes are available. Thus, the tablets were administered according to 6 different weight bands, taking into account that individual doses ranged from 10 to 20 mg fluralaner/kg bw and 0.75 to 1.50 mg MBO/kg bw.

Simparica Trio chewable tablets for dogs, which is authorised centrally in the EU, was selected as the positive control. It contains three active ingredients: sarolaner (an isoxazoline), moxidectin and pyrantel. Its indications are similar to those proposed for the authorisation of Bravecto CombiUNO.

The study was conducted in four European countries, representing at least two geographic regions (i.e. Southern Europe and Northern Europe) as required by 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.4*).

Of the 651 dogs in the 'Full Analysis Set' (FAS) population, which represented a range of bodyweights (1.4 to 61.4 kg [mean 20.0 kg]) and hair lengths, females made up 53.2% of the dogs (36.9% spayed, 16.3% intact) while 46.9% were male (36.1% castrated, 10.8% intact). The age at inclusion ranged from 9 weeks to 17 years (mean 5.3 years). Included animals were kept under different husbandry conditions: 301 dogs were living inside and outside, 73 only inside, 277 only outside.

356 dogs were included as primary dogs (PDP population). Of the 356 households, 191 households had only 1 dog, 76 had 2 dogs, 48 had 3 dogs and 41 had 4 dogs. Treatment groups were well balanced for all demographic parameters at inclusion and considered as representative for the

European canine patient population. The PDP population comprised all primary dogs that were enrolled into the study and received a treatment.

Dogs were randomly allocated to either the Bravecto CombiUNO group, the Bravecto TriUNO or the control group (CP) in a 2:2:1 ratio.

Included animals were confirmed to be infested with ticks and/or fleas by a veterinarian who was adequately experienced in the reliable and well-established comb-counting procedure described in the aforementioned WAAVP guideline. Homogeneity of study groups was demonstrated for FAS and per-protocol (PP) populations at inclusion (on day 0) for flea and tick counts. Numerical counts as recommended by the aforementioned WAAVP guideline were used with at least 5 live fleas or 4 live ticks required for inclusion.

Mean flea counts (PP) for the primary dogs were 10.2 in the group treated with Bravecto CombiUNO and 13.7 in the CP-group. Overall, the flea-infested primary dogs had a flea burden ranging from 5 to 58 fleas at inclusion. Mean tick counts (PP) for the primary dogs were 6.9 in the group treated with Bravecto CombiUNO and 15.0 in the CP group. The tick-infested primary dogs had tick burdens ranging from 4 to 248 ticks at inclusion. Thus, the infestation level for both ticks and fleas was considered adequate for this study.

The recommended schedule of tick and/or flea counting after treatment given in 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.4) proposes weekly intervals for ticks and bi-weekly intervals for fleas. However, in this study, both parasites were counted at day 7 ± 1 to assess the efficacy at start of the study and on day 31 ± 2 to assess the efficacy at the end of the treatment interval. The assessment timepoints are justified, as the day 7 and day 31 are critical for the products immediate and persistent killing effect.

Tick and flea counts were performed using the comb-counting method described in the WAAVP guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of fleas and tick infestation on dogs. This is agreed.

Very high levels of efficacy were observed after treatment with Bravecto CombiUNO. For ticks, the percentage reduction was 99.8% at visit 2 and 100% at visit 3, using geometric means (GM). As no dogs with ticks were found, a statistical comparison of in Bravecto CombiUNO group or the CP group was not possible. For fleas, the percentage reduction was 99.9% and 100% (GM) at visit 2 and 3, respectively, and the Bravecto CombiUNO-group was significantly non-inferior to the CP group (p = 0.0167).

Considering the flea lifecycle, only about 5% of the total flea population lives and feeds on the dog while the remaining 95% (eggs, larvae, pupae) are spread around the indoor habitat, thus exhibiting strong re-infestation pressure. Given the high efficacy rates and the fast onset of action, it can be concluded that the product effectively controls existing environmental flea populations in areas to which the dog has access.

The relevant European tick species were present in the study, as outlined in '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.4). The most abundant species was *I. ricinus*, followed by ticks of *R. sanguineus complex*, *D. reticulatus* and *I. hexagonus*. Although only few ticks or dogs with ticks were observed as concerns *D. reticulatus* (and *I. hexagonus),* the results observed in the Bravecto TriUNO group (100% of efficacy) can be also considered since no interaction between active substances was shown. In addition, the results shown against *R. sanguineus* which is considered the dose-limiting tick species in Europe supported the results. Fleas were not specifically identified to species in this field study, which is compliant with 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.4), but it is assumed that the predominant species was *C. felis*.

The presence of FAD at inclusion was assessed by the veterinarians at visit 1. They examined skin lesions (size, location, type) and presence of marked itching and evaluated if these were related to FAD. FAD was present at visit 1 in 14 dogs treated with Bravecto CombiUNO. FAD related skin lesions resolved to normal by visit 2 (day 7) in 6 dogs (42.9%) and by visit 3 (day 31) in the other 8 dogs (57.1%). Thus, Bravecto CombiUNO can be used as part of a treatment strategy to control FAD.

Bravecto CombiUNO was administered according to the instructions given in the proposed SPC, i.e. one tablet was administered according to the respective weight band. Palatability (voluntary consumption of the entire dose within 5 minutes; FAS) was 74.3% in the Bravecto CombiUNO group, 82.6% in the Bravecto TriUNO group and 85.6% in the CP group. It is noted that according to the 'Guideline on the demonstration of palatability of veterinary medicinal products' (EMA/CVMP/EWP/206024/2011), to be granted a palatability claim, the overall voluntary acceptance rates within 2 minutes should at least reach the threshold of 80% in dogs and 70% in all other species. In consequence, the applicant adapted the wording in the SPC to these results, i.e. no palatability claim is made and section 3.9 of the SPC includes the following statements: "*The veterinary medicinal product is a flavoured chewable tablet. Tablets can be offered to the dog, given with food or placed directly into the mouth. The dog should be observed during administration to confirm that the full chewable tablet is swallowed*".

During this field study, no interactions between Bravecto CombiUNO and routinely used VMPs were observed.

The assessment of all adverse events observed in this field study is presented under the 'Target Animal Safety' section of this scientific overview.

The applicant has further conducted six GCP-compliant field efficacy studies in dogs naturally infested with *C. felis* in Australia with monthly parasite counts and *R. sanguineus* and *C. felis* in Brazil with weekly parasite counts. The studies were conducted as unblinded, single-arm studies. In all studies high levels of efficacy over 90% in treatment and control of natural flea and tick infestations in dogs were observed for up to 30 days. In these six studies the product was well tolerated and safe at the tested dose.

In conclusion, the results of the European field study confirm the efficacy and safety of Bravecto CombiUNO in the treatment of flea and tick infestations in dogs under European field conditions. Bravecto CombiUNO contributes towards the control of the environmental flea populations in areas to which treated dogs have access. Bravecto CombiUNO can be used as part of a treatment strategy to control FAD. Australian and Brazilian field studies support the safety and efficacy of Bravecto CombiUNO.

Endoparasites

Gastrointestinal nematodes

The applicant conducted one multi-centre field efficacy study for gastrointestinal nematodes in the EU according to current quality standards (i.e. GCP) and following the relevant guidelines, i.e. VICH GL7, VICH GL19, the 'Guidelines for evaluating the efficacy of anthelmintics for dogs and cats' published by the " WAAVP and the 'Guideline on statistical principles for veterinary clinical trials' (EMA/CVMP/EWP/81976/2010).

Another new combination product, Bravecto TriUNO, was included in the study as a second investigational product. However, in this assessment, the focus is on Bravecto CombiUNO.

Bravecto CombiUNO was the final product formulation in the final packaging material intended to be marketed and produced according to GMP. Six different tablet sizes are available. Thus, the tablets were administered according to 6 different weight bands, taking into account that individual doses ranged from 10 to 20 mg fluralaner/kg and 0.75 to 1.50 mg MBO/kg bw.

The study design included Nexgard Spectra chewable tablets for dogs as a positive control (CP). Both the WAAVP guideline and VICH GL7 require either an untreated control group or a group treated with a registered anthelmintic. Nexgard Spectra chewable tablets for dogs is authorised centrally, thus it is commercially available in all countries where the field study was performed. It is given by the same route of administration as Bravecto CombiUNO, which is favourable for a blinded study. Its indications include those proposed for the authorisation of Bravecto CombiUNO.

Dogs were randomly allocated to the three treatment groups in a 2:2:1 ratio for Bravecto CombiUNO, Bravecto TriUNO and CP. This is compliant with both the WAAVP guideline and VICH GL7, which require at least 25% control animals.

The study was conducted in four European countries, representing different geographic regions as required by the WAAVP guideline. Consequently, the results can be considered representative of expected efficacy within the European community.

In total, 171 dogs were treated with the Bravecto CombiUNO, 172 with Bravecto TriUNO and 86 with the CP. This complies with the WAAVP guideline, which requires data from at least 300 dogs.

Dogs included in the study were representative of the different breeds and gender. The mean bodyweight was 20.0 kg (2.6–58.3 kg) and the mean age was 4.0 years (3 months–12 years). Dogs were maintained in the home environment by owners or stayed in their kennel. When needed, dogs were housed individually to ensure correct allocation of faecal samples obtained. The inclusion criteria are considered acceptable. In addition, non-inclusion criteria were defined. It is noted that dogs with known epilepsy were not included. The applicant included the warning " *Use with caution in dogs with pre-existing epilepsy*" in section 3.5 of the SPC.

Parasite infections in the dogs were confirmed by detection and identification of nematode eggs by coproscopic examination. Counting of nematode eggs in the faeces, which is the preferred method to evaluate effectiveness according to VICH GL7, was done by Mini Flotac method with saturated sodium chloride flotation solution and a sensitivity of 5 eggs/gram. Identification of parasites was based on the distinct morphology of their faecal forms. However, distinction between the two hookworm species (*A. caninum* and *U. stenocephala*) was only done for some cases, as the egg morphology of these species is very similar and distinction is difficult.

According to a recent multi-site study in Europe, *A. caninum* and *U. stenocephala* have an approximate proportion of 3:7. Therefore, with 110, 110 and 58 hookworm-positive animals in the Bravecto CombiUNO, Bravecto TriUNO and CP-groups, respectively, it is likely that both hookworm species were represented in at least 10 infected dogs per group (Bravecto CombiUNO and Bravecto TriUNO).

Mean faecal egg counts before treatment in the group treated with Bravecto CombiUNO were 359.55 (range: 5–3,500) for *T. canis*, 278.57 (range: 5–1550) for *T. vulpis,* 302.00 (range: 50–825) for *T. leonina* and 510.45 (range: 5–3125) for hookworms. Mean faecal egg counts before treatment in the CP group were 455.00 (range: 40–1550) for *T. canis*, 362.78 (range: 5–1800) for *T. vulpis*, 321.43 (range: 40–925) for *T. leonina* and 471.64 (range: 5–1700) for hookworms. Thus, it can be concluded that the dogs were adequately infected.

Efficacy was determined by quantitative examination of faeces for eggs, in compliance with the WAAVP guideline and VICH GL7. Geometric means were used as recommended in VICH GL7, however, arithmetic means were given for additional information. Faecal samples were examined before treatment (maximum 7 days) and 14 ± 2 days after treatment as recommended by the WAAVP guideline.

A significant difference between pre- and post-treatment faecal egg counts was shown ($p < 0.0001$). Efficacy was evaluated if at least 10 animals initially positive for a nematode species were identified. Efficacy could be shown for *T. canis* and *T. leonina* as well as for *T. vulpis* and hookworms (all > 99%). This is well above the threshold of 90% recommended in VICH GL7.

The percentage of nematode-free dogs was higher in the group treated with Bravecto CombiUNO (82.46%) compared to the CP group (72.09%). With the lower confidence limit well above the non-inferiority margin of -0.15, significant non-inferiority of Bravecto CombiUNO group in relation to the CP group was demonstrated. As the lower confidence limit is greater than 0, the percentage of nematode-free dogs in the Bravecto CombiUNO group can even be considered statistically superior to the results obtained in the CP group.

Bravecto CombiUNO was administered according to the instructions given on the proposed SPC, i.e. one tablet was administered according to the respective weight band. 89.5% of the dogs consumed the tablet voluntarily within 5 minutes. Nevertheless, according the 'Guideline on the demonstration of palatability of veterinary medicinal products' (EMA/CVMP/EWP/206024/2011), to be granted a palatability claim, the overall voluntary acceptance rates within 2 minutes should at least reach the threshold of 80% in dogs and 70% in all other species. In consequence, the applicant adapted the wording the SPC to these results, i.e. no palatability claim is made, and section 3.9 of the SPC includes the following statements: "*The veterinary medicinal product is a flavoured chewable tablet. Tablets can be offered to the dog, given with food or placed directly into the mouth. The dog should be observed during administration to confirm that the full chewable tablet is swallowed*".

There was one adverse event reported in the group treated with Bravecto CombiUNO. The investigator recorded a tick infestation that resolved within one day. Consequently, Bravecto CombiUNO demonstrated excellent tolerance among the dogs under field conditions.

In conclusion, the results of the European field study confirm the efficacy and safety of Bravecto CombiUNO in the treatment of gastrointestinal nematode infections (*T. canis*, *T. vulpis*, hookworms and *T. leonina*) in dogs, under European field conditions.

Two Brazilian field studies were performed to evaluate the efficacy of Bravecto CombiUNO against the gastrointestinal nematodes *Trichuris* spp., *Ancylostoma* spp., *Toxascaris* spp. and *Toxocara* spp. Both studies were single-arm studies, with no control group. Both studies showed 100% efficacy against these gastrointestinal nematodes. The safety of Bravecto CombiUNO was also confirmed.

Heartworm

The applicant conducted one multi-centre field efficacy study against heartworm (*D. immitis*) in the US according to GCP standards. Thus, the conduct and evaluation complied with current quality standards.

Bravecto CombiUNO was the final product formulation in the final packaging material intended to be marketed and produced according to GMP. Six different tablet sizes are available. Therefore, the tablets were administered according to 6 different weight bands, taking into account that individual doses ranged from 10 to 20 mg fluralaner/kg bw and 0.75 to 1.50 mg MBO/kg bw. The study design included Interceptor as a positive control product (CP), which is in line with WAAVP guideline, in which it is indicated that a negative control group should not be included for animal welfare reasons.

Interceptor contains one of the active ingredients of Bravecto CombiUNO, namely MBO. It is given by the same route of application, which is favourable for a blinded study. The treatment interval is also the same (monthly).

Dogs were randomly allocated to the two treatment groups in a 1:1 ratio for Bravecto CombiUNO and the CP, which is compliant with both the WAAVP GL guideline and VICH GL7, both of which require at least 25% control animals. Treatment groups were well balanced for all demographic parameters at inclusion and considered as representative for the US canine patient population.

Client-owned dogs represented a variety of breeds and aged ≥ 8 weeks, and weighing ≥ 1.25 kg were enrolled in the study. Dogs were negative for heartworm microfilaria and antigen test at inclusion as required in the WAAVP guideline. All dogs had monthly study visits with the investigator (visits 1 to 12), conducted either in person or, due to the restrictions related to the Covid pandemic, remotely using video or voice technology, when allowed. Physical examinations were performed at each in-person visit.

Additionally, heartworm testing, through antigen testing and microfilaria detection, was performed for all dogs on visits 1, 5, 7, 9, and 12. The primary effectiveness was determined based on the day 330 heartworm microfilaria and antigen test results. The study design is considered adequate to assess the efficacy in preventing heartworm disease.

In the CP group, 1 of 125 (0.80%) dogs was positive for heartworm on day 330, while in the Bravecto CombiUNO group, 0 of 124 (0%) dogs were positive for heartworm, and non-inferiority of Bravecto CombiUNO was established. However, a single positive result was noted at the day 240 visit in the IVP group, albeit this finding didn't have an impact on the final results.

The assessment of all adverse events observed in this field study is presented. During this field study, no interactions between Bravecto CombiUNO and routinely used VMPs were observed.

The study was conducted in the US as agreed in a Scientific Advice procedure. As detailed in the Scientific Advice, the data should meet the following criteria:

a) The US data should be representative of European conditions.

b) As the US field trial will be positively controlled, evidence is needed that the infection pressure was sufficiently high.

Both aspects were correctly addressed.

The enrolled animals in the field trials in the US were compared to the enrolled animals in the EU field trials. Overall, the dog breeds as well as the age and the weight were considered similar. Climatic conditions regarding the presence and activity of competent mosquito vectors were comparable. In addition, the infection pressure was comparable. Nine study sites were located in heartworm-endemic areas as defined by the American Heartworm Society and enrolled the majority of the cases in the study. Finally, the prevalence of *D. immitis* in the US and the EU was compared using to two bibliographical references and regarded as similar.

Lungworm

Although no field study for *A. vasorum* was conducted (due to low prevalence, even in endemic areas), it is noted that MBO is already in in-field use for the prevention of angiostrongylosis at the same route of administration in the target species, and the omission of a field study can be accepted as agreed in a Scientific Advice procedure.

Overall conclusions on efficacy

Pharmacodynamics

Fluralaner is an ectoparasitic substance with killing activity against fleas and ticks.

The mode of action has been sufficiently described.

Fluralaner is a potent inhibitor of parts of the arthropod nervous system by acting antagonistically on ligand-gated chloride channels (GABA receptor and glutamate receptor).

Milbemycin oxime is an antiparasitic endectocide acting on invertebrate neurotransmission by hyperpolarization of the neuromuscular membrane.

Bravecto CombiUNO contains two active substances with an ectoparasitic and an anthelmintic-fungal component. The applicant justified the combination by the need to widen the spectrum of activity. The main pharmacodynamic characteristics have been suitably described in the SPC.

Pharmacokinetics

The pharmacokinetic characteristics of Bravecto CombiUNO are generally well documented and have been satisfactorily evaluated in the target species (i.e. dogs).

After oral administration, fluralaner and MBO are readily absorbed, reaching individual maximum plasma concentrations between approximately 1 and 7 days or between 1 and 6 hours post administration, respectively. Fluralaner is quantifiable until the last sampling timepoint, 71 days post dosing, i.e. fluralaner declines slowly from canine plasma, whereas MBO declines readily from dog plasma and is quantifiable until 8 to 16 days post administration. Fluralaner and MBO show a relatively high volume of distribution, a low systemic clearance accompanied with a long elimination half-life for fluralaner and a relatively long elimination half-life for MBO, thus demonstrating persistent effects in the dog during the intended treatment intervals. Fluralaner and MBO are mainly excreted via faeces. The information included in the proposed SPC is suitably supported by the data presented.

Justification of the fixed combination

A satisfactory justification for the combination product, in accordance with the CVMP 'Guideline on pharmaceutical fixed combination products' (EMEA/CVMP/83804/2005) was provided. The principal advantage claimed for the combination is its efficacy in the treatment of flea and tick infestations combined with its efficacy against infections with gastrointestinal nematodes. Moreover, Bravecto CombiUNO is intended for the prevention of heartworm and lungworm disease and for reduction of the risk of infection by *B. canis canis* via transmission by *D. reticulatus* and *D. caninum* by *C. felis*.

Development of resistance and related risks to animals

Fluralaner

A bibliographical review was provided to support the lack of resistance to fluralaner. This review included references about the in vitro efficacy of fluralaner against some ticks isolates resistant to common antiparasitic substances. Nevertheless, it is noted that these references are more than ten years old. Additionally, the applicant has summarised the laboratory studies conducted in different countries. Fluralaner demonstrated efficacy for the tick and flea isolates in all these studies although only one study was conducted in Europe which could make it more difficult to evaluate comparability among different geographical areas.

Milbemycin oxime

A bibliographical review was provided to state the current situation of resistance to MBO against heartworms (*D. immitis*), lungworms (*A. vasorum*) and gastrointestinal nematodes proposed in the indications (*T. canis, T. leonina*, L5 and adult stages of *Ancylostoma caninum* and adult stages of *T. vulpis*).

This bibliographical review shows that MBO is effective only against *D. immitis* L3, L4 and microfilariae. Therefore, the use of MBO against adult forms is not recommended since it may lead to the selection of macrocyclic lactones-resistant heartworm populations. One reference concludes that *D. immitis* resistance to macrocyclic lactones has only been confirmed in dogs in the USA.

The applicant reviewed the in vivo efficacy studies of MBO against some worms for which the product is indicated. It is noted that most of the studies where the threshold of efficacy was not reached were conducted in the USA.

Dose determination and confirmation

The dose of fluralaner/MBO was established based on a number of dose-finding studies with a suitable range of dosage according to the relevant guidelines and supported by two dose confirmation studies performed under experimental conditions in each indication. No specific dose confirmation studies were provided for *B. canis canis* and *D. caninum.*

Tolerance in the target animal species

Results of the pivotal margin-of-safety study demonstrate that the repeated oral administration of BRAVECTO CombiUNO is safe in puppies when administered at doses up to 5-times the maximum recommended dose at the recommended treatment intervals. The CVMP concludes that the safety margin of BRAVECTO CombiUNO in the target species is acceptable and no signs of intolerances have been observed.

Overall, it can be concluded that the administration of BRAVECTO CombiUNO in accordance with the product information recommendations is well tolerated in the target animal species. The applicant has proposed to include some warnings in the SPC with specific statements for ivermectin-sensitive dogs. This is acceptable and similar to the information included in the SPC of similar authorised medicinal products.

Field studies confirmed that BRAVECTO CombiUNO was well-tolerated at the recommended dose of 10 mg fluralaner + 0.75 mg MBO/kg bw and the adverse events observed were reflected adequately in the SPC. Administration of the product in accordance with SPC recommendations is generally well tolerated.

Clinical trials

The applicant conducted one pivotal multi-centre field efficacy study in the EU according to current quality standards (i.e. GCP) and following the relevant guidelines to evaluate the efficacy and safety of the fixed combination chewable tablets administered once orally against natural infestations with fleas and/or ticks for 1 month in client-owned dogs at multiple sites under field conditions.

The data show that the product is effective for the treatment of tick and flea infestations on dogs providing immediate and persistent flea (*C. felis* and *C. canis*) killing activity and immediate and persistent tick (*D. reticulatus, I. hexagonus, I. ricinus,* and *R. sanguineus)* killing activity for 1 month at a minimum recommended dose of 10 mg/kg bw of fluralaner in dogs.

The applicant has further conducted six GCP-compliant field efficacy studies in dogs naturally infested with *C. felis* in Australia with monthly parasite counts and *R. sanguineus* and *C.* in Brazil with weekly parasite counts.

In addition, one pivotal study to evaluate the efficacy of Bravecto CombiUNO for dogs administered once orally at dose rates of ≥ 10 mg fluralaner and ≥ 0.75 mg MBO/kg bw against natural infections with gastrointestinal nematodes (*T. canis*, *T. leonina*, hookworms, *T. vulpis* and others) in client-owned dogs at multiple sites was conducted under field conditions according to GCP standards and following the relevant guidelines.

A significant difference between pre- and post-treatment faecal egg counts was shown ($p < 0.0001$). Efficacy could be shown for *T. canis* and *T. leonina* as well as for *T. vulpis* and hookworms (all > 99%). The percentage of nematode-free dogs was higher in the group treated with Bravecto CombiUNO (82.46%) compared to the control group (72.09%). Significant non-inferiority of Bravecto CombiUNO group in relation to the control group was demonstrated.

In addition, the applicant conducted one multi-centre field efficacy study against heartworms in the US according to GCP standards. The primary effectiveness was determined based on the day 330 heartworm microfilaria and antigen test results. In the control group, 1 of 125 dogs was positive for heartworm on day 330, while in the Bravecto CombiUNO group, 0 of 124 dogs were positive for heartworm and non-inferiority of Bravecto CombiUNO was established.

Part 5 – Benefit-risk assessment

Introduction

Bravecto CombiUNO is presented as chewable tablets containing a fixed combination of two active substances: fluralaner and MBO. Both active substances are well-known.

The active substances belong to the isoxazoline and milbemycins class of parasiticides, respectively, and exert parasiticidal activity by interacting with ligand-gated ion channels in the nervous system of various parasites. The product is intended for the treatment of dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm.

The proposed effective oral dose of 10–20 mg/kg bw fluralaner and 0.75–1.5 mg/kg bw MBO administered at 1-month intervals remains to be confirmed.

The application has been submitted in accordance with Article 20 of Regulation (EU) 2019/6 (combination veterinary medicinal product application).

Benefit assessment

Direct benefit

The proposed benefit of Bravecto CombiUNO is its proposed efficacy in dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm.

The combination of these two active substances allows for an effective treatment of mixed ecto- and endoparasitic infections in dogs. Bravecto CombiUNO is only intended for use in situations where fluralaner and MBO are required, e.g. in situations when use against ticks or fleas and one or more of the other target parasites is indicated at the same time.

The indication for Bravecto CombiUNO is as follows:

"*For dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and gastrointestinal nematodes is indicated at the same time. The veterinary medicinal product also provides concurrent efficacy for the prevention of heartworm disease and angiostrongylosis.*

For the treatment of tick and flea infestations on dogs providing immediate and persistent flea (Ctenocephalides felis and C. canis) killing activity and immediate and persistent tick (Dermacentor reticulatus, Ixodes hexagonus, I. ricinus, and Rhipicephalus sanguineus) killing activity for 1 month.

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

For reduction of the risk of infection with Babesia canis canis via transmission by D. reticulatus for 1 month. The effect is indirect due to the product's activity against the vector.

For reduction of the risk of infection with Dipylidium caninum via transmission by C. felis for 1 month. The effect is indirect due to the product's activity against the vector.

Treatment of infections with gastrointestinal nematodes of the following species: roundworms (immature adult (L5) and adult stages of Toxocara canis, and adult stages of Toxascaris leonina), hookworms (immature adult (L5) and adult stages of Ancylostoma caninum) and whipworm (adult stage of Trichuris vulpis).

Prevention of heartworm disease (Dirofilaria immitis).

Prevention of angiostrongylosis (by reduction of the level of infection with immature adult (L5) and adult stages of Angiostrongylus vasorum) with monthly administration."

These indications were investigated in a large number of well-designed laboratory and field studies conducted to an acceptable standard. The CVMP is of the opinion that the claimed efficacy against target parasites has been adequately supported.

Additional benefits

Bravecto CombiUNO increases the range of available treatment possibilities for dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm.

An effective control strategy for Bravecto CombiUNO's proposed indications will not only treat a clinical or subclinical disease but also decrease the risk of transmission of a disease and/or the underlying parasites to other animals and man. Infected dogs can act as the reservoir for infective parasite stages leading to the spread of the parasites within households or greater environments, increasing field contaminations (e.g. larval/egg shedding if dogs are infected with gastrointestinal nematodes).

The consistent monthly treatment interval for each indication of Bravecto CombiUNO improves the ability of veterinarians to offer a comprehensive flea and tick treatment option in dogs suffering from or which are at risk from endoparasitic infections.

In dogs where a repeated monthly treatment is required based on the epidemiological situation, a monthly treatment interval greatly contributes to treatment compliance. Owner compliance is highly important for the continuous prevention of heartworm and/or lungworm disease in enzootic areas.

Bravecto CombiUNO can be administered by the animal owner at home. Bravecto CombiUNO is easy to administer orally, as it is accepted by the majority of dogs and can also be administered with food.

This results in decreased stress for the animals when treated in their usual environment.

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

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

Risks for the target animal

Overall, it can be concluded that the administration of Bravecto CombiUNO in accordance with the product information recommendations is well tolerated in the target animal species (i.e. dogs).

Risk for the user

The most severe risk is considered to be accidental ingestion by a child. To mitigate this risk, Bravecto CombiUNO is intended to be marketed in child-resistant packages, and the product information includes a warning advising of the potential for adverse effects in case of accidental ingestion, in addition to specific instructions to remove tablets from the packaging only when required, and to store the product out of the sight and reach of children.

The user safety for Bravecto CombiUNO is acceptable when used as recommended and taking into account the safety advice in the SPC.

Risk for the environment

Bravecto CombiUNO is not expected to pose a risk for the environment when used following SPC recommendations. Given the nature of the active substances and the potential undesired effects, specific warnings are included in the product literature to avoid the disposal in surface waters.

Resistance

Fluralaner is a molecule that was exclusively developed for use in veterinary medicines. It has been on the Community market since April 2014.

It is not used as plant protection product or human medicine. Therefore, no resistance data from arthropods in the field is yet available. However, current data from in vitro studies strongly indicate that fluralaner is able to overcome the most important resistances in the field. In in vitro bioassays, fluralaner is not affected by proven field resistances against amidines (tick), organophosphates (tick,

mite), cyclodienes (tick, flea, fly), macrocyclic lactones (sea lice), phenylpyrazoles (tick, flea), benzophenyl ureas (tick), pyrethroids (tick, mite) and carbamates (tick, mite). The efficacy of fluralaner against fleas is not affected by dieldrin resistance demonstrated in a molecular on-target study on insect GABA receptors of flea and fly. In addition, fluralaner is highly effective against fleas proven to be less susceptible to phenylpyrazole insecticides in vivo.

Despite reports of MBO and other macrocyclic lactone-resistant *D. immitis* strains in the USA, macrocyclic lactones are still widely accepted as safe and highly effective in preventing heartworm infections in dogs. So far, resistant strains have only been confirmed in the USA. As macrocyclic lactones are the only compounds available to prevent canine heartworm disease, selection pressure from the use of macrocyclic lactones will likely continue to increase the frequency of resistant strains in the USA and possibly other parts of the world.

Label compliance is critical to prevent under-dosing and exposure of immature heartworms to subtherapeutic compound levels, thereby leading to patient infections in dogs with potentially resistant, reproducing adult worms. Indications of hookworm infections in dogs with resistant parasites has been mounting in the USA over the past few years, which was recently confirmed experimentally.

Resistant *A. caninum* strains have not been reported in other parts of the world. Instances of milbemycin oxime-resistant roundworms and whipworms have not been reported in the public domain, presumably due to vast refugia of susceptible populations in the environment.

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

User safety risks have been identified, mainly the risks associated with exposure in children. These risks are mitigated by the presentation of Bravecto CombiUNO in a child-resistant packaging.

Environmental safety

No specific risks have been identified for any of the active substances.

Conditions or restrictions as regards the supply or safe and effective use of the VMP concerned, including the classification (prescription status)

Bravecto CombiUNO is subject to a veterinary prescription.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication:

"*For dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm. The veterinary medicinal product is only 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 on dogs providing immediate and persistent flea (Ctenocephalides felis and C. canis) killing activity and immediate and persistent tick (Dermacentor

reticulatus, Ixodes hexagonus, I. ricinus, and Rhipicephalus sanguineus) killing activity for 1 month.

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

For reduction of the risk of infection with Babesia canis canis via transmission by D. reticulatus for 1 month. The effect is indirect due to the product's activity against the vector.

For reduction of the risk of infection with Dipylidium caninum via transmission by C. felis for 1 month. The effect is indirect due to the product's activity against the vector.

Treatment of infections with gastrointestinal nematodes of the following species: roundworms (immature adult (L5) and adult stages of Toxocara canis, and adult stages of Toxascaris leonina), hookworms (immature adult (L5) and adult stages of Ancylostoma caninum) and whipworm (adult stage of Trichuris vulpis).

Prevention of heartworm disease (Dirofilaria immitis).

Prevention of angiostrongylosis (by reduction of the level of infection with immature adult (L5) and adult stages of Angiostrongylus vasorum) with monthly administration."

The product has been shown to be generally efficacious for these indications, and the CVMP agreed to the following indication(s):

"*For dogs with, or at risk from, mixed parasitic infestations by ticks or fleas, gastrointestinal nematodes, lungworm and/or heartworm. The veterinary medicinal product is exclusively indicated when use against ticks or fleas and gastrointestinal nematodes is indicated at the same time. The veterinary medicinal product also provides concurrent efficacy for the prevention of heartworm disease and angiostrongylosis.*

For the treatment of tick and flea infestations on dogs providing immediate and persistent flea (Ctenocephalides felis and C. canis) killing activity and immediate and persistent tick (Dermacentor reticulatus, Ixodes hexagonus, I. ricinus, and Rhipicephalus sanguineus) killing activity for 1 month.

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

For reduction of the risk of infection with Babesia canis canis via transmission by D. reticulatus for 1 month. The effect is indirect due to the product's activity against the vector.

For reduction of the risk of infection with Dipylidium caninum via transmission by C. felis for 1 month. The effect is indirect due to the product's activity against the vector.

Treatment of infections with gastrointestinal nematodes of the following species: roundworms (immature adult (L5) and adult stages of Toxocara canis, and adult stages of Toxascaris leonina), hookworms (immature adult (L5) and adult stages of Ancylostoma caninum) and whipworm (adult stage of Trichuris vulpis).

Prevention of heartworm disease (Dirofilaria immitis).

Prevention of angiostrongylosis (by reduction of the level of infection with immature adult (L5) and adult stages of Angiostrongylus vasorum) with monthly administration."

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

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

The product information has been reviewed and is generally considered to be acceptable.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for Bravecto CombiUNO is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) No 2019/6).

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.