SCIENTIFIC DISCUSSION

Name of the medicinal product:	Advocate
Marketing Authorisation Holder:	Bayer HealthCare AG 51368 Leverkusen Germany
Active substances:	Imidacloprid & moxidectin
Pharmaco-therapeutic group: **(ATC-vet Code):**	Therapeutic antiparasitic agent QP54AB52
Therapeutic indication(s):	**Dogs:** For dogs suffering from, or at risk from, mixed parasitic infections: For the treatment and prevention of flea infestation (*Ctenocephalides felis*), prevention of heartworm disease (L3 and L4 larvae of *Dirofilaria immitis*) and treatment of infections with gastrointestinal nematodes (L4 larvae, immature adults and adults of *Toxocara canis, Ancylostoma caninum* and *Uncinaria stenocephala*, adults of *Toxascaris leonina* and *Trichuris vulpis*). The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD). **Cats:** For cats suffering from, or at risk from, mixed parasitic infections: For the treatment and prevention of flea infestation (*Ctenocephalides felis*), prevention of heartworm disease (L3 and L4 larvae of *Dirofilaria immitis*) and treatment of infections with gastrointestinal nematodes (L4 larvae, immature adults and adults of *Toxocara cati* and *Ancylostoma tubaeforme*). The product can be used as part of a treatment strategy for flea allergy dermatitis (FAD). **Ferrets:** For ferrets suffering from, or at risk from, mixed parasitic infections: For the treatment and prevention of flea infestations (*Ctenocephalides felis*) and the prevention of heartworm disease (L3 and L4 larvae of *Dirofilaria immitis*).
Target species:	Cats, dogs and ferrets

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1. INTRODUCTION

Advocate is a spot-on solution containing imidacloprid and moxidectin for use in dogs, cats and ferrets.

The product is indicated for the prevention and treatment of flea infestations and for the prevention of heartworm disease in dogs, cats and ferrets. In dogs and cats Advocate is also indicated for the treatment of mature and immature roundworms and hookworms. In dogs the product is also indicated for the treatment of whipworms. There are two formulations of Advocate. The pipettes for dogs contain 100 mg/ml of imidacloprid and 25 mg/ml of moxidectin. The pipettes for cats and ferrets contain 100 mg/ml of imidacloprid and 10 mg/ml of moxidectin.

All the pipettes are for single use. Advocate for dogs is available in pipettes of 0.4 ml, 1 ml, 2.5 ml and 4 ml. Advocate for(small) cats and ferrets is available in pipettes of 0.4 ml. Advocate for (large) cats is available in pipettes of 0.8 ml.

Imidacloprid has been marketed worldwide by the Applicant as Advantage (10 % imidacloprid), a spot-on treatment for the control of fleas on dogs and cats. Moxidectin was developed as a veterinary product by Fort Dodge. In the EU, moxidectin is registered in some member states for the prevention of heartworm infection in dogs after oral administration. For the treatment of cats it is a new active substance in the EU. Various medicinal products containing moxidectin are registered in the EU for the treatment of horses, sheep and cattle. It is evident from the application that Advocate is essentially Advantage spot-on with moxidectin added in place of a corresponding proportion of the inactive excipients.

As ferrets are not named as a major species in the CVMP Position Paper regarding availability of Products for Minor Uses and Minor Species (MUMS) (EMEA/CVMP/477/03-FINAL) and because the number of ferrets is considerable lower than that of dogs and cats, during its meeting in July 2008, CVMP decided that the variation to use Advocate for ferrets satisfied the criteria necessary for a “Limited Market”.

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PART II: CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL ASPECTS

In Advocate spot-on solutions, the active substances imidacloprid and moxidectin have been combined into a single endoectoparasiticide product. Imidacloprid is an ectoparasiticide belonging to the group of chloronicotinyl compounds. Moxidectin is a second generation macrocyclic lactone of the milbemycin family.

II.A QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

II.A.1 Composition of the Veterinary Medicinal Product

Imidacloprid is present in the formulations at 10.0 % w/v and moxidectin at 1.0 % w/v for cats and ferrets or 2.5 % w/v for dogs. The active substances are dissolved in an organic solvent mixture. All of the excipients have been employed previously in veterinary medicinal products. 0.1 % butylhydroxytoluene is used as an antioxidant to prevent the oxidation of the organic solvent mixture.

II.A.2 Containers, closures and dosing device

The spot-on solutions are packed into white polypropylene unit dose pipettes with white polypropylene screw caps. Three, four or six pipettes are sealed within outer blister packs made of polyvinyl chloride or polypropylene and aluminium foil. A single blister is placed into a cardboard carton, together with a package insert. No dosing device is used with this product, the solution is administered directly and completely from the tube. The accuracy and precision of pipette filling has been confirmed.

II.A.3 Clinical Trial Formulae

The formulations used in the clinical studies were the same as intended for marketing. The placebo formulations were comprised of the organic solvent system used in the actual formulations. Certificates of analysis have been provided for representative batches.

II.A.4 Development Pharmaceutics

In Advocate spot-on solutions, the active substances imidacloprid and moxidectin have been combined as a single endoectoparasiticide preparation. The aim of the pharmaceutical development was to produce a formulation of low concentration and small dosage volume, which could be applied topically as a spot-on solution.

Taking into account the recommended doses of the active substances, one formulation containing 10 % w/v imidacloprid with 2.5 % w/v moxidectin was developed for dogs, and a second comprising 10 % w/v imidacloprid with 1.0 % w/v moxidectin was developed for cats and later also authorised for ferrets. 0.1 % butylated hydroxytoluene was included as an antioxidant and was justified by accelerated stability studies.

No ingredients were identified as substances of bovine, ovine or caprine origin, therefore, there is no risk of transmission of TSE from the pharmaceutical use of these products.

As Advocate spot-on solutions are non-sterile unit dose preparations, there is no information on sterilisation cycles nor on any preservatives. The microbiological purity of the Advocate spot-on solutions has been investigated according to the Ph.Eur. for three batches of each filling size of both formulations both directly after production and after 6 months storage at 25°C/60%RH. The low microbial counts (total microbial count, yeasts and moulds, *Enterobacteriaceae*) found after manufacturing did not change during the storage period, and support the fact that no preservative is needed. The routine control of microbiological purity in the finished

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product specification is therefore considered unnecessary, however, the test for microbiological purity is included as a periodic test to the finished product specification.

The proposed packaging materials have been widely used throughout the EU for the topical application of small volume medicinal products. Successful use over many years for other similar small animal unit dose products confirms suitability for purpose and as a consequence no development studies have been carried out. The container material complies with the requirements of the Ph.Eur. for Plastic Containers and Closures. Furthermore, the primary packaging is confirmed as child-proof. As Advocate spot-on solutions are for single-use, no fragmentation nor self sealability data for the closure is needed.

There are no manufacturing overages included in the formulations, however, the unit dose containers do each contain a filling overage to ensure that the stated quantity of the product may be expelled from the pipettes.

II.B METHOD OF PREPARATION

II.B.1 Manufacturing Formula and Batch Size

A batch size range of 500-5000 l is proposed and has been justified, as the formulation is a homogeneous liquid, and is extremely stable to air, water and humidity. Furthermore, the process for production of the bulk product is conventional and unremarkable. The manufacturing formula for a typical 1000 l batch size is presented.

II.B.2 Manufacturing Process and In-process Controls

KVP Pharma + Veterinär Produkte GmbH, Kiel, Germany, is responsible for the whole manufacturing process including batch release.

The manufacturing process is a simple process, involving dissolution of the active substances and filling into the primary packs (unit-dose pipettes). Before manufacture, the temperature of all ingredients can be increased to temperatures between 15 and 30°C to improve the dissolution rate. The active substances are dissolved in a mixture of the solvents and antioxidant with mixing for at least 60 minutes. The bulk solutions are filtered (pore size 75 μm) and filled into 500 l bulk containers. The pipettes are filled from the bulk container within a period of 6 months after manufacture, and then sealed by heat or ultrasonics.

The in-process control tests check for complete dissolution of the constituents and specify the controls applied during the filling process.

II.B.3 Validation of Manufacturing Process

The critical steps in the manufacturing process that require control are the dissolution of the active substances and the homogeneity of the bulk solution. The reproducibility of the process has been demonstrated by the production of three batches of both formulations (two each of 329 kg and one each of 550 kg) and their subsequent filling at the proposed production site. Appearance (clarity, colour), identity (HPLC, TLC), density, water content and assay of imidacloprid, moxidectin and butylhydroxytoluene were evaluated and all the results were within the limits of the release specification. The in-process limits were also met.

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II.C CONTROL OF STARTING MATERIALS

II.C.1 Active Substances

Neither imidacloprid (manufactured by Bayer AG, Germany) nor moxidectin (manufactured by Fort Dodge Animal Health, Italy) are described in a pharmacopoeia accordingly monographs with which the substances comply are provided.

Imidacloprid

Specifications and routine tests with validation

In the absence of a pharmacopoeial monograph, imidacloprid is controlled by an in-house specification. Methods are described and limits are justified for the content of water, chloride content, free acidity or alkalinity, residual solvents (the limits for which correspond to those of the VICH guideline), heavy metals and sulphated ash. Appropriate validation data have been provided.

Scientific data

Nomenclature:	
Generic name	imidacloprid
INN	none assigned
Other names	Confidor
Chemical names	CAS name: 2-imidazolidinimine, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro IUPAC name: 1-(6-chloro-pyridin-3-yl-methyl)-N-nitroimi-dazolidin-2-ylideneamine
CAS number	138261-41-3
Description:	
Physical form	cream coloured, crystalline powder with a characteristic weak odour
Molecular formula	$C_9H_{10}ClN_5O_2$
Relative molecular mass	255.7
Aqueous solubility (g/1000 ml at 20˚C)	0.61
Potential isomerism	molecule does not show an asymmetric centre & is therefore optically inactive
Dissociation constant	pKa (H_2O) not possible to specify in pure aqueous systems, but a very weak base
Partition coefficient (octanol/water)	log Pow = 0.57 at 21°C
Melting point	143.8°C

Manufacture:
The data on the manufacture and control of the drug substance provided in the dossier is considered to be clearly presented, of a high standard and comprehensive. The synthetic route is chemically feasible under the specified reaction conditions and the product is obtained with a high degree of purity.

The synthesis is described in full detail in the dossier, and specifications for the starting material, reagents and solvents are presented.

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Development chemistry:
The structure elucidation has been carried out by elemental analysis, UV, IR, 1H -NMR, 13C -NMR and EI mass spectrometry. The results and spectra are in agreement with the suggested structure.

Impurities:
The possible impurities originating from the synthesis have been discussed and investigated. The specifications for residual solvents correspond to those of the VICH guideline for residual solvents and the levels measured in the batch analyses are well below the relevant limits specified in this guideline. The known decomposition products (formed under stress conditions) are also identified and discussed.

Analytical validation of the methods:
The methods for the determination of the active substance, impurities, and residual solvents are soundly validated.

Batch analysis:
Certificates of analysis from five batches of imidacloprid are presented. The results are reproducible and comply with the specifications. Results from batches manufactured by using different qualities of the reagents (fresh, recovered or both qualities mixed) are also presented. The results are comparable.

Moxidectin

Specifications and routine tests with validation

In the absence of a pharmacopoeial monograph, moxidectin is controlled by an in-house specification

Scientific data

Nomenclature:

Generic name	moxidectin
INN	moxidectin
Other names	moxidectin solid, moxidectin technical material, lab code CL 301423
Chemical name	(2a*E*,4*E*,5'*R*,6*R*,6'*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6'-[(*E*)-1,2-dimethyl-1-butenyl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethylspiro[11,15-methano-2*H*,13*H*,17*H*-furo [4,3,2-pq] [2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyrano]-4',17 (3'*H*)-dione,4'-(*E*)-(*O*-methyloxime)
CAS number	113507-06-5

Description:

Physical form	white to pale yellow powder
Molecular formula	$C_{37}H_{53}NO_8$
Relative molecular mass	639.84
Solution pH	6.6 in 70 % dioxane: 30 % water
Aqueous solubility:	0.51 mg/l
Melting point (liquification)	145-154°C
Partition coefficient (octanol/water)	58,300
Specific optical rotation (acetontrile/589nm/20°C)	+104.1° (±1°)

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Manufacture:
Fort Dodge Animal Health, Italy synthesizes moxidectin in a four step reaction sequence, full details for which are included in the dossier. Specifications for the solvents and reagents are also contained in the dossier, together with a detailed process description and reaction scheme, a flow chart, details of the in-process controls and the materials used in the purification. None of the ingredients used in the manufacture of the starting material or of the moxidectin itself are from animal origin.

Development chemistry:
The structure elucidation has been carried out with ^{1}H-NMR, ^{13}C-NMR, mass spectrometry (EI and CI), IR, circular dichroism, fluorescence and ultraviolet spectroscopy and x-ray crystallographic analysis.
Moxidectin contains 10 chiral centres, and the specific optical rotation is +104.1°. Although moxidectin is a chiral molecule, no stereoisomers or enantiomers are known. In part, this lack of enantiomers is due to the fact that the starting material (a fermentation product) is pure. Typically, microbes synthesize only one enantiomer. In addition, the chemical synthesis reactions and conditions performed to convert the starting material into moxidectin are not conducive to enantiomer formation.

Impurities:
All the potential impurities (starting materials and derivatives, process-related impurities, residual solvents, degradation products) are detailed and discussed in the dossier.

Analytical validation of the methods:
Four of the analytical methods used (assay, residual solvents, residual dioxane, moxidectin related minors) were validated for an older version of those methods. All the methods were soundly validated. Extensions of the validation were not considered necessary, as none of the changes were judged to affect the conclusions in the validation report.

Batch analysis:
Certificates of analysis for five consecutive moxidectin batches of 194 - 254 kg manufactured according to the commercial process are presented. The results are reproducible and comply with the specifications.

II.C.2 Excipients

C.2.1 Specifications and routine tests

Each of the components of the organic solvent mixture is tested in accordance with its respective monographs in the European Pharmacopoeia or USP-NF. Additionally, the finished product manufacturer performs tests for water content, and the methods and their corresponding validations are satisfactory.

C.2.2 Scientific data

Certificates of analysis for three batches of each of the excipients demonstrate their compliance with the specifications.

IIC.3 Packaging Material (Immediate Packaging)

C.3.1 Specifications and routine tests

The pipettes are made of polypropylene which complies with the requirements of the Ph.Eur. monograph (3.2.2. *Plastic containers and closures*) and Directive 90/128/EEC. The maximum value of 100 ppm of the sum of lead, cadmium, chromium-IV and mercury meets the requirements of Directive 94/62/EEC. The pipettes and the cap are coloured white by titanium dioxide (E 171), a colouring material authorised for use in medicinal products

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(Council Directive 78/25/EEC). The controls for containers include appearance, colouring, material identity (polymer type, polymer identification by IR) and wall thickness.

C.3.2 Scientific data

Certificates of analysis for three batches of each size of primary packaging material comply with the specifications. The suitability of the chosen packaging materials has been demonstrated by the stability studies presented in II.F. Concerning compatibility between the pipettes and the solvents, reference has been made in section II.A (pharmaceutical development) to an authorised topical 10 % solution of imidacloprid.

II D. CONTROL TESTS ON INTERMEDIATE PRODUCTS

The bulk solutions may be stored for up to 6 months under defined conditions before filling. Specifications for the bulk solutions are presented and fully justified by batch analyses data.

II E. CONTROL TESTS ON FINISHED PRODUCT

IIE.1 Product Specification and Routine Tests:

1.1. Product specifications and tests for release at time of manufacture (general characteristics, specific standard)

Full details of the test methods for the finished product and their validations are presented in the dossier. The specification includes tests for appearance, content of each of the active substances and BHT (all by HPLC), impurities, water content and appropriate physical parameters. The limits are fully justified by reference to batch analyses data.

IIE.2 Scientific Data

The HPLC methods have been validated with respect to specificity, linearity, range, accuracy, precision, robustness, limits for detection and quantitation satisfying the ICH guidelines for analytical method validation.

Batch data on three batches of both formulations (all pack sizes) complies with the specifications valid at that time. Since then, the assay of degradation products has been added to the release specification, and the test for withdrawable content has been replaced by the test for uniformity of content.

II F: STABILITY

II F.1.1 Stability Tests on the Active Substance(s)

Imidacloprid:
In forced degradation studies, imidacloprid proved to be stable at 90˚C in solid form and in the pH 2.0 buffer solution. A remarkable decomposition occurred in solution at pH 10 and under influence of light at pH 7.0. Hydrogen peroxide 3 % solution caused slight oxidation.

Stability results are presented from three batches stored in polyethylene lined paper bags (similar to the bags used for commercial storage and distribution) at 25˚C/60%RH for 24 months and at 40˚C/75%RH for 12 months. The parameters studied were appearance, identity (HPLC), water content, chloride content, acidity, alkalinity, organic impurities and assay. No remarkable changes occurred in these parameters.

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In a photo stability study solid imidacloprid was irradiated with a xenon lamp for 14 hours. The compound was spread out with a layer of thickness of approximately 2 mm. No change occurred in the concentration of imidacloprid and the impurities.

The proposed re-test period of 24 months (stored at 15 to 25°C and in uncontrolled relative humidity) is justified.

Moxidectin:
Three commercial batches of moxidectin have been stability tested in accelerated (25±2°C/ambient humidity) and long-term (2-8°C/ambient humidity) conditions, which are not in line with the VICH guideline with respect to the humidity. Moxidectin manufactured by Fort Dodge Animal Health, Italy was packed into HDPE bags and further into metal containers similar to those used for commercial storage and distribution. The batches were tested for appearance, moxidectin content, moxidectin related minors and BHT content. The methods for assay and BHT content are based on and equivalent to the methods presented in II.C, and the method for moxidectin related minors is the same presented in II.C, therefore no validation reports are presented in this section. No remarkable changes were observed.

The relative humidity was not considered to be an issue with the packaging materials used, however, all future studies will be performed at 4°C/ambient RH and 25°C/60%RH. Preliminary results of three batches of moxidectin stored at 25°C/60%RH are presented, and they show no difference in stability when compared to the samples stored at ambient humidity.

The recommended storage temperature for moxidectin is 5±3°C, and claimed re-test period of 24 months at these conditions has been justified.

II F.1.2 Stability tests on the intermediate products

The stability of the bulk solutions stored under ambient warehouse conditions (15 to 25°C and ambient relative humidity) was investigated. All the results comply with the specifications. There is no loss of antioxidant indicating that oxidation is not occurring in the bulk solution. Further stability data will be generated from samples, stored at 25°C/60%RH in containers of the same material as the storage containers.

II F.2 Stability Tests on the Finished Product

The shelf-life specifications and methods for the finished product have been presented in the dossier.

Stability data has been presented on three batches (300-500 l) of both the dog and the cat/ferret formulation packed into 1.0 ml, 2.5 ml and 4.0 ml polypropylene pipettes for 36 months at 25°C/60%RH, 30°C/50%RH and 30°C/80%RH, and for 6 months at 40°C/75%RH. The results comply with the proposed specifications with a few exceptions, for example, the sum of moxidectin degradation products exceed their limits, in all pack sizes, after 36 months at 30°C/80%RH. This is acceptable considering the proposed storage precautions of 3 years when stored up to 30°C. Based on the data, the short-term excursions outside the real-time storage conditions have no impact on the stability of the finished product.

Originally, the requirement for clarity was ‘clear’. However, after 6 months storage at 40°C/75%RH, fine, white particles were observed in the dog formulation. The same effect is also seen at lower temperatures after 6-12 months storage. The particles were identified by the use of IR, UV-Vis, LC, LC-MS, GC-MS, ICP, DSC and IC as magnesium sulphate originating from the active substance moxidectin. Magnesium sulphate is used in the final purification stages of moxidectin synthesis and it is postulated that either the magnesium sulphate is hydrating to a more insoluble form or that inorganic components of the matrix previously not visible to the naked eye are agglomerating with the increased moisture into fine particles. Additional testing of the clear

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supernatant and samples with re-suspended solid showed no difference in content of imidacloprid, moxidectin nor butylhydroxytoluene. As a result of this finding, the shelf-life specification for the clarity was changed to 'clear to slightly opalescent'.

The light stability trial with the dog formulation was performed according to the VICH guideline *Stability testing: photostability testing of new veterinary drug substances and medicinal products*. The results demonstrate that the formulation is not light sensitive and hence no special precautions are needed to protect the product from light.

Both formulations were subjected to stress testing against elevated temperatures (40°C and 70°C; humidity not controlled) and increased water content (cat/ferret formulation + 4.0 %, dog formulation + 5.0 %) for 20 weeks (dog formulation) and 33 weeks (cat formulation). The formulations were stored in glass vials, and the dog formulation also into 1 ml polypropylene pipettes (only at 40°C). No significant degradation of imidacloprid was observed, whilst the degradation of moxidectin was more prominent, especially with the additional water. The results from the assay of butylhydroxytoluene remained within the specification.

The dog formulation, in 4.0 ml pipettes, was stored at –18°C for 65 days. No crystallisation was observed.

The suggested shelf-life of 3 years when stored up to 30°C, has been supported.

OVERALL CONCLUSION ON PART II

The manufacture of the dosage form is adequately described and controlled. The proposed shelf-life for the finished product (2 years when stored below 30°C) is supported by the stability data presented. Overall the quality of the final product has been demonstrated.

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PART III: TOXICO-PHARMACOLOGICAL ASPECTS

III.A. SAFETY TESTING

III.A.1. Pharmacological Studies

2.1. Pharmacodynamics

Imidacloprid

Imidacloprid acts on the nicotinergic acetylcholine receptors in the nervous system of insects. Due to the similarity in these receptors in insects and vertebrates, the Applicant provided several studies in insects and/or vertebrates to support this.

Following administration of high oral and intravenous doses to mice, rats and rabbits the following observations were made: Oral doses of 30 and 100 mg imidacloprid/kg/day caused a decrease in alertness, motor activity and motor incoordination in mice and a decrease in spontaneous behaviour and pupil reflex in rabbits. Oral doses of imidacloprid caused a temporal increase in respiratory rate and heart rate, and later a decrease in respiration rate and persistent increase in heart rate was observed in the 100mg/kg/day animals. In anaesthetised rabbits, a dose of 10 mg imidacloprid/kg/day intravenously, resulted in functional changes on respiration, blood pressure and heartbeat. Imidacloprid induced mydriasis in rabbits and rats. At 100mg/kg/day p.o. and above, imidacloprid produced a slight decrease in urine volume and at 300 mg/kg/day p.o. changes in electrolyte levels were observed. A minimal prolongation of coagulation time was noted in rats receiving 30 and 100 mg/kg/day orally.

Moxidectin

The Applicant provided one study on the pharmacological properties of moxidectin in various species and by various routes of administration. The main findings were in the smooth muscle (weak contractions or relaxation of tracheal smooth muscle in guinea pigs), respiratory and cardiovascular system (slight increase in the respiration rate and a transient decrease in heart rate in rats) and the gastrointestinal tract (increased gastrointestinal motility).

2.2. Pharmacokinetics

2.2.1 Imidacloprid

The Applicant provided several GLP-compliant studies on the pharmacokinetics of (radiolabelled) imidacloprid in rats, mice, chickens and goats.

Absorption

Absorption following oral administration in the rat is rapid and almost total, approximately 94%. The maximum concentration in plasma in the rat was reached between 1 and 4 hours, depending on the dose administered. The maximum concentrations in plasma were relatively low, on average 0.73 times the equidistribution. In goats, the radioactivity level in the plasma reached the highest concentration at 2 hours after administration with the equivalent concentration of about 40% of the equidistribution concentration in the body.

Distribution

Imidacloprid is rapidly distributed from the plasma into to the tissues and organs of the rat with terminal half-lives ranged between 9 hours (150 mg/kg/day p.o.) and 25 hours (1 mg/kg/day p.o.). Elimination of the

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radioactivity from plasma is described by two exponential terms, one with an elimination half-life of between 2.6 and 3.6 hours and the other 26-118 hours. The relatively small mean residence time (MRT) recorded in one study in the rat indicates that redistribution into the plasma from tissues is also very rapid. In goats, radioactivity was eliminated from the plasma with a half-life of about 4.8 hours for the time period from 2 to 24 hours after oral administration of 10 mg/kg.

Initial high concentrations of residues in rats and goats are mainly observed in the liver and kidney; furthermore in muscle, walls of the aorta, the adrenals, salivary gland, thyroid, walls of the stomach and the small intestine. Significant lower radioactivity is found in the lungs, the brown and white fatty tissues, brain, testes and the mineral parts of the bone.

Following oral administration of 10 mg imidacloprid/kg/day for three consecutive days to hens, radioactivity was eliminated from the plasma with a half-life of approximately 14 hours for the time period 6-24 hours after the last administration of imidacloprid. Therefore, the metabolism of imidacloprid in laying hens is similar to that in mammals.

Metabolism

Following oral administration of radiolabelled imidacloprid to the rat, all identified metabolites were found in both sexes with male rats showing a higher tendency to metabolise imidacloprid. Sex dependent differences found were quantitative rather than qualitative. The main metabolites include 6-chloronicotinic acid and it's glycine conjugate WAK 3583, which were found only in the urine. The monohydroxylated metabolites were identified in similar amounts as the unchanged parent compound. All other biotransformation products were quantitatively of minor importance.

Excretion

In rats, more than 90% of intravenously or orally administered radiolabelled imidacloprid had been excreted within the 48 hours. The excretion ratio (urine/faeces) was in the range of circa 4:1 for both routes of administration. In the urine 5 metabolites were identified. Renal excretion occurred rapidly, approximately 6 hours more than 50% of the renal radioactivity had been eliminated and 24 hours after administration, renal excretion of the total radioactivity was almost complete. This is due to the good water solubility of the parent compound and the metabolites. Faecal excretion was of minor importance, representing 21% of the administered dose.

In goats, the excretion of radiolabelled imidacloprid (10 mg/kg/day p.o., 3 days) up to 2 hours after the last administration amounted to 58% of the total radioactivity administered. As in the rats, renal excretion was the predominant elimination route. Via the urine, 46% of the total dose was eliminated from the body. The faecal excretion was low with a value of 9.6% and a very low amount (0.4%) was found in the milk.

In hens, up to 50 hours after the oral administration of 10 mg radiolabelled imidacloprid/kg/day for three consecutive days, excretion of radioactivity was on average 32.9% of total radioactivity administered. Approximately 50% of the dose administered with the first dose was excreted in the first 24 hours with the main excretion route via the kidneys.

It is concluded that following oral administration, imidacloprid is rapidly and completely absorbed from intestinal lumen. It is readily distributed to the body from the central compartment. However, there is no indication of accumulation in any particular organ or tissue and elimination is rapid and mainly via the urine.

2.2.2. Moxidectin

Five GLP-compliant studies were provided on the pharmacokinetics of radiolabelled moxidectin administered orally, subcutaneously or intravenously to rats, sheep and cattle.

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Absorption

Moxidectin is rapidly absorbed following oral or subcutaneous administration.

In rats, following oral administration of 0.2 mg/kg, the mean C_{max} was obtained at 4.8 hours and the elimination half-lives ($t_{1/2}$) were 22.9 hours and 44.6 hours for male and female rats respectively. Oral administration resulted in a 19% absorption of radioactivity (based on AUC calculations), compared to intravenous administration.

In sheep, the calculated t_{max} in blood after oral dosing was 9 hours, with a C_{max} of 9 ppb (moxidectin equivalents) in the blood. The $t_{1/2}$ of 88.3 hours is similar to that for the intravenous dosing (74.6 hours) calculated over up to 120 hours post-treatment. The average absorption of the subcutaneous dose is 75.9%. The MRT (124 hours) and CL (0.22 L/kg.hr) following subcutaneous dosing were similar to those of intravenous dosing.

In cattle, the C_{max} in serum occurs an average of 8 hours after subcutaneous dosing. There were no apparent differences between total AUC, total AUMC, clearance rate, mean residence time or steady state volume of distribution after intravenous and subcutaneous administration. Moxidectin is completely absorbed from the injection site following subcutaneous administration.

Distribution

Maximum concentration in the blood is low and declines rapidly. Moxidectin is transported in the serum and not associated with the cellular components of the blood.

In rats, the mean terminal elimination half-lives after intravenous administration ($t_{1/2}$) were about 64 hours and the systemic clearances were 31.2 ml/hr (male) and 23.0 ml/hr (female). No significant differences between males and females were observed in peak blood concentrations, volumes of distribution at steady state and clearance rates.

In sheep, maximum blood levels are low and decline rapidly after peaking 8-9 hours post-dosing. In cattle, the elimination half-life of moxidectin from serum was 55.7 hours, based on parent compound, and 76.3 hours, based on total carbon-14 for the subcutaneous treatment. Similar values for intravenously dosed animals were 68.4 and 68.1 hours, respectively. After the first 24 hours post dosing, moxidectin accounts for approximately 50% of the total carbon-14 residue in serum. Moxidectin and related metabolites have a half-life of 3 days in the serum of cattle.

Residues were detected in fat, liver, kidney and muscle with the highest concentration in fat and the lowest in muscle. The residue levels in liver, kidney, muscle and fat were generally higher in females than in males and were dose dependent with fat being the target tissue.

The average depletion half-lives of the total residue in rats (1.5 mg/kg, p.o.) and cattle (0.2 mg /kg, s.c.) were in the liver 2.4 days (rat) and 11.4 days (cattle), in kidney 2.4 days (rat) and 11.8 days (cattle), in muscle: 3.9 days (rat) and 9.0 days (cattle) and in fat 11.5 days (rat) and 12.2-14.3 days (cattle). In rats, total mean residue levels in the tissues accounted for less than 2% of the administered dose. There was no bioaccumulation of moxidectin-related residues in edible tissues

Metabolism

The principle route of metabolism of moxidectin in rats, sheep and cattle is hydroxylation of alkyl substituents of moxidectin.

In rats, the only significant component of the residue was unchanged parent drug in faeces and tissues (24 hours post-dose). In urine, moxidectin accounted for only a small amount of the residue. Five and six metabolites were isolated from in vivo rat liver and faeces, respectively. Of the six metabolites isolated from faeces, one minor component identified in rats was as the 23-keto derivative of moxidectin. The remaining five isolates are all

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mono-oxygenated derivatives with the exception of the most polar metabolite, O-demethylated at the oximino oxygen. These data suggest that the principle route of metabolism of moxidectin in the rat is hydroxylation.

In sheep and cattle, the metabolic profile in faeces is qualitatively similar to that in liver. One major metabolite and at least six minor metabolites were identified in the faeces of sheep by mass spectroscopy. The major faecal metabolite was identified as a monohydroxylated derivative of moxidectin.

Excretion

Excretion of moxidectin is almost exclusively via the faeces. In rats, the average recovery of total radioactivity from urine, tissues/organs, carcasses and cage rinses 7 days after oral treatment of rats was about 85-90% of the administered dose. Faeces accounted for up to 91.3% of the administered radioactivity for rats after 7 days, with most excreted 2-3 days post-dose. Less than 2% of the administered dose was eliminated via urine for all animals during the 7 day post treatment period.

In sheep, faecal excretion was the major elimination pathway after oral administration (up to 52% of the applied dose) while urinary excretion accounted for <1% of the administered dose. In cattle, total radioactivity recovered accounted for up to 76.9% of the subcutaneously administered dose at 7, 14 and 28 days after treatment. These were distributed as follows: less than 2% in the urine; 32.2-58.1% in the faeces; 11.6-29.8% in the carcass and 4.2-10% in all other components sampled.

In summary, moxidectin is rapidly absorbed in rat and sheep following oral or subcutaneous administration. Maximum concentrations in the blood are low and decline rapidly. Moxidectin is transported in the serum and not associated with the cellular components of the blood. Excretion is almost exclusively via the faeces. Fat was identified as the target tissue. Moxidectin was the only major component of tissue residues. The principal metabolic pathway is hydroxylation.

III.A.3.Toxicological studies

3.1. Single dose toxicity

The Applicant has provided reports of a number of studies investigating the acute toxicity of imidacloprid and moxidectin by various routes in the rat and mouse.

Imidacloprid

The acute **dermal toxicity** was investigated in rats receiving a topical application of 5000 mg/kg of imidacloprid. No overt signs of toxicity in either male or female animals were observed and the dermal toxicity was considered being very low with an LD_{50} in both sexes was determined >5000 mg/kg.

Three GLP-compliant studies were provided in rats and one study in mice using single oral doses of 50 – 1800 mg imidacloprid/kg/day (rats) and 10 - 250 mg/kg/day (mice), respectively. Two further studies were provided investigating the acute **oral toxicity** of an imidacloprid spot-on formulation in rats (0 - 2620 mg imidacloprid spot-on formulation/kg).

First signs of toxicity were observed in rats at a dose of 100 mg/kg and more. The effects appeared relatively rapidly after administration and were reversible within a period of up to six days. At near lethal doses signs of toxicity included apathy, (transient) laboured or accelerated breathing, decreased motility, transient staggering gait, blepharophimosis, transient trembling and transient spasms, soft faeces and piloerection. In the test using the spot-on solution, non-specific signs such as oral staining, nasal staining and urine staining were also noted. In rats, no mortalities were observed in animals of the dose groups below 400 mg/kg, whilst all animals receiving

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≥900 mg/kg died. In mice, 100% mortality was observed at doses from 160 mg/kg in males and 250 mg/kg in females. All mortalities occurred up to one hour post-dosing.

The NOEL for both males and females were below 495 mg/kg and 600 mg/kg respectively the test using the spot-on solution. The LD_{50} values derived from the studies in rats and mice were:

Rats: Males: 424 mg/kg (fasted), 504 mg/kg, 642 mg/kg (active substance) and 1943 mg/kg (spot-on formulation)
Female: 379 mg/kg, 450-475 mg/kg (fasted) and 648 mg/kg (active substance) and 1732 mg/kg (spot-on formulation)

Mice: 131 mg/kg (male) and 168 mg/kg (female) (active substance)

One study was provided to evaluate the acute toxicity of 10 – 500 mg imidacloprid/kg following **intraperitoneal** administration in the rat (5 females and 5 males per group).

At near lethal doses overt signs of toxicity include apathy, reduced motility, laboured and accelerated breathing, spasms, periodic tremors, periodic narrowed and twitching eyelids, closed eyelids, spastic gait, laying on side, piloerection, dyspnoea and lacrimation. The effects were mainly moderate, started shortly after administration and had resolved by day 4 of the study. Effects on body weight, as a slight reduction in body weight gain, were observed at doses of 170 mg/kg and above. No mortalities occurred in male rats at a dose of 160 mg/kg or less and in females at a dose of 100 mg/kg or less. 100% mortality was observed at doses of 250 mg/kg (males) and 224 mg/kg (females). Death occurred between 22 minutes and 5 hours after administration.

The intraperitoneal LD_{50} values derived for NTN 33893 in this study were >160 <170 mg/kg for males and 186 mg/kg for females. Accordingly, NTN 33893 was classified as possessing moderate to low toxicity in the rat following intraperitoneal administration.

One study was provided on the acute **inhalation toxicity** in rats. The LC_{50} was determined >69 mg/m^3 (aerosol) and >5323mg/m^3 (dust), respectively.

The acute toxicity of imidacloprid has been well established in the studies provided. Imidacloprid is considered to be of low to moderate toxicity following oral administration, a possible route of exposure of pet owners.

Moxidectin

A GLP-compliant study on the **dermal toxicity** of 2000 mg/kg moxidectin was performed in albino rabbits. The material was moistened with water and applied for 24 hours to an area of intact skin representing approximately 10% of the body surface area. All test animals survived to the end of the study. No overt signs of toxicity and no treatment related gross lesions were observed. The dermal LD_{50} of moxidectin was greater than 2000 mg/kg for male and female rabbits.

Six GLP-compliant studies were provided investigating the **oral toxicity** of a single dose of moxidectin administered by oral gavage in rats (2 studies, dose range: 6 – 300 mg/kg), mice (3 studies, dose range: 15 – 240 mg/kg) and chicken (1 study, dose range: 25 – 400 mg/kg). Animals were observed for 14 days.

The first signs in rats were observed at doses of 30 mg/kg and above within 2 hours and up to day 9 of the study. The signs observed included piloerection, decreased activity, uncoordinated and spastic gait, increased reflexes, decreased/laboured respiration, prostration, tremors, chromodacryorrhea, hypersensitivity to sound and touch, epistaxis, temporary convulsions, narrowed palpebral fissure, moderate diarrhoea and dazed condition.

In mice, signs of toxicity were observed from 15 mg/kg and consisted of decreased activity, tremors and ataxia with a complete recovery after 4 days.

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In chickens, signs of toxicity were observed from 25 mg/kg and included decreased activity, depression, prostration and a red flushing of the skin with a recovery after 4 days.

Mortalities were observed in animals receiving doses from 75 mg/kg (rats), 30 mg/kg (mice) and 100 mg/kg (chicken) with deaths occurring within the first 24-72 hours post dosing. There were no gross pathological abnormalities observed in animals surviving to the end of the study. The oral LD_{50} for moxidectin in rats was determined as 97 mg/kg (female) and ≥100 mg/kg -122 mg/kg (male). The oral LD_{50} in the mouse was determined 118 mg/kg (males) and 42 - 72 mg/kg in female mice, respectively. The oral LD_{50} of moxidectin in chicken (male) was 283 mg/kg.

The **intraperitoneal toxicity** of moxidectin was investigated in two GLP-compliant studies in rats (160 – 640 mg/kg) and mice (40 – 230 mg/kg), respectively. Overt signs of toxicity were observed from 160 mg/kg and consisted of decreased activity, prostration, anorexia, epistaxis and chromodacryorrhea with a recovery by day 4. In mice, signs of toxicity were observed from 80 mg/kg and consisted of decreased activity, coma, tremors, loss of righting reflex and epistaxis with a recovery by day 5. Weight gains were generally reduced in rats and mice. Mortality was observed from 160 mg/kg (rats) and 40 mg/kg (mice) and generally occurred between post-dosing days 6-10 (rats) and days 1-3 (mice). Gross necropsy findings in rats dying during the study showed lesions in liver, intestinal tract and test material remaining in the abdominal cavity.

The intraperitoneal LD_{50} for moxidectin in males was 453 mg/kg (rats) and 86 mg/kg (mice) and 359 mg/kg (rats) and 87 mg/kg (mice) in females. The combined LD_{50} for both sexes was 394 mg/kg (rats) and 84 mg/kg (mice), respectively.

The **subcutaneous toxicity** of moxidectin was investigated in two GLP-compliant studies in rats (640mg/kg) and mice (80 – 320 mg/kg), respectively. In mice, mortality and signs of toxicity were only observed at the highest dose of 320 mg/kg. Signs of toxicity included decreased activity and tremors with a recovery by day 3 of the study. No overt signs of toxicity or mortality were observed in rats.

The subcutaneous LD_{50} of moxidectin in mice was 285 mg/kg (males) and 247 mg/kg (females); the combined LD_{50} for both sexes was 263 mg/kg. The study in rats is only of limited value and only indicates an LD_{50} for moxidectin of more than 640 mg/kg.

The acute toxicity of moxidectin has been well established in the studies provided. Moxidectin is considered to be moderately toxic, with the mouse being the most sensitive species. The signs of toxicity observed in these studies are mostly confined to the nervous system and reflect the mode of action of moxidectin on GABA mediated nerve transmission.

3.2. Repeated dose toxicity

Imidacloprid

The repeat dose toxicity of imidacloprid has been investigated in three species, rabbit, rat and dog, in GLP-compliant studies via the dermal and oral routes, and for varying periods of time.

Dermal exposure to a dose of 1000 mg imidacloprid/kg/day for three weeks (6 hours/day, 5 days/week) was well tolerated by male and female rabbits.

Repeat dose toxicity of imidacloprid after **oral administration** was investigated in six GLP-compliant studies in rats (three studies) and dogs (three studies).

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Rats were treated with doses of 150 – 2400 mg/kg feed (i.e. 14 - 422 mg imidacloprid/kg/day) in their diet for up to 13 weeks and 100 - 1800 mg/kg feed for up to 24 months. Dogs were treated with oral doses of 200 - 5000 mg imidacloprid/kg feed (7.3 - 49 mg/kg/day) for up to 4 weeks, 200-1800 mg imidacloprid/kg feed for 13 weeks and 200-1250(-2500) mg imidacloprid/kg feed (6.1, 15 and 41(-72) mg/kg/day) up to 52 weeks.

Doses of 600 mg imidacloprid/kg feed produced effects in rats and dogs, mainly reduced food consumption and decreases in body weight. At higher doses, changes were observed in clinical chemistry and histopathology that indicated adverse effects on the liver. These changes included a slight increase in plasma cholesterol levels and cytochrome P-450 levels and increased incidence of abnormal cells in the liver. In rats, slightly elevated liver weights and increased incidence of mineralisation in the colloid of the thyroid follicles were also recorded. In dogs, ataxia, tremor and occasionally vomiting were observed in the group treated with 49 mg imidacloprid/kg/day.

The NOELs derived from the oral rat studies are 150 mg imidacloprid/kg feed (14 mg/kg/day) for males and 600 mg imidacloprid/kg feed (83.3 mg/kg/day) for females in a 13 week oral dosing study and 100 mg imidacloprid/kg feed (5.7 mg/kg/day) for males and 300 mg imidacloprid/kg feed (24.9 mg/kg/day) for females in a 24 month feeding study.

In dogs, oral NOELs of 200 mg imidacloprid/kg feed (7.3 mg/kg/day) and 1200 mg imidacloprid/kg feed were derived from the 4 and 13 week feeding studies, respectively and a NOEL of 500 mg imidacloprid/kg feed (15 mg/kg/day) was derived from the 52 week feeding study.

Moxidectin

The repeat dose toxicity of moxidectin after **oral administration** was investigated in mice, rats and dogs in six GLP-compliant in-house studies over 4 to 52 weeks.

Mice were treated with 33.7 - 150 mg moxidectin/kg feed for 4 weeks. From the dose of 75 mg/kg/day feed mortality and other signs of toxicity were observed including tremors, hypersensitivity, urine stained fur and a slight depression of body weight gain.

Rats were treated with 100 - 600 mg moxidectin/kg feed for 4 weeks and 25 - 150 mg/kg feed for 13 weeks. Above a dose of 150 mg moxidectin/kg feed (12.2 mg moxidectin/kg/day) mortality and signs of toxicity were observed including hypersensitivity, ataxia, tremors, salivation, piloerection and diuresis, lethargy, aggressive behaviour and urine stained coat. Symptoms appeared to be more pronounced in females than in males. Also, food intake, body weight and weight gain were significantly depressed. Although a dose related increase in absolute and relative adrenal gland and kidney weights was noted in female rats from the 100 mg/kg/day feed treatment groups, no histopathological changes were observed in these tissues.

Three studies were provided in **dogs** the most sensitive species, treated with 20 - 160 mg moxidectin/kg feed (4 weeks), 10 - 60 mg moxidectin/kg feed (13 weeks) and 10 - 45 mg moxidectin/kg feed (52 weeks). Dose-dependent depression in mean absolute body weights and food consumption values was noted from 0.87 mg/kg/day and more. Other clinical signs observed in different dose groups including lacrimation, thin and languid appearance, tremors, slight salivation, slight ataxia, anorexia, weight loss, prostration and mydriasis. Histopathology revealed decreased spermatogenic activity and a decrease in colloid in the thyroid glands in males dosed with 80 mg/kg/day feed or more.

The NOEL in mice was determined as 33.7 mg/kg feed (equivalent to 6.9 mg/kg/day). In rats, a NOEL was determined as 50 mg moxidectin/kg feed (3.9 mg moxidectin/kg/day) following 13 weeks treatment. A NOEL of 10 mg moxidectin/kg feed (equivalent to 0.29 - 0.30 mg/kg/day) was determined following the 13 week treatment in dogs.

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3.3. Tolerance in the target species of animal

See Part IV of the assessment report.

3.4. Reproductive toxicity, including teratogenicity

3.4.1 Studies on the effects on reproduction

Imidacloprid

The Applicant provided one GLP-compliant multiple generation reproduction study in rats receiving – 100, 250 or 700 mg imidacloprid/kg feed. Imidacloprid did not induce any effects on reproductive parameters at inclusion levels in the diet of up to 700 mg imidacloprid/kg feed (56 mg/kg). The only adverse effects recorded during the study were reduced food consumption and body weight, and induction of liver enzymes. These are the same adverse effects as recorded in the repeat dose studies in rats. The NOEL derived from this study was 250 mg imidacloprid/kg feed (equivalent to approximately 20 mg/kg/day) for technical grade imidacloprid administered daily in the diet to rats.

Moxidectin

The Applicant provided one GLP-compliant two-generation (two litters) reproduction study in rats receiving 1, 2, 5 or 10 mg moxidectin/kg feed. Moxidectin at an inclusion level of 10 mg/kg feed (0.8 mg/kg) in the diet, elicited a reduction in mean body weights (F1 males) and reduced pup survival indices in two litters. There were no adverse effects observed at inclusion levels of 2 mg/kg feed (0.16 mg/kg) and 5 mg/kg feed (0.4 mg/kg). The NOEL of moxidectin derived from this two-generation reproduction study was considered to be 5 mg/kg feed (approximately 0.4 mg/kg/day).

3.4.2 Embryotoxicity/foetotoxicity, including teratogenicity

Imidacloprid

Imidacloprid was evaluated for embryotoxicity/teratogenicity in rats and rabbits up to dose levels that produced some maternal toxicity.

In rats, doses of 30 and 100 mg/kg/day elicited reduced food consumption and reduced body weight gains in the dams. At 100 mg/kg/day, slight effects on skeletal development in the foetuses were observed, suggestive of a retardation of maturity. There were no indications of embryotoxicity or teratogenicity. The maternal NOEL was 10 mg/kg/day and the foetal NOEL at 30 mg/kg/day.

In the rabbit study, a similar pattern was observed, 24 and 72 mg/kg/day elicited reduced food consumption and reduced body weight gain in the does. A dose of 72 mg/kg/day imidacloprid resulted in delay in maturation of the skeleton. There were no indications of embryotoxicity or teratogenicity at any of the dose levels of imidacloprid in this study. A maternal NOEL of 8 mg/kg/day and a foetal NOEL of 24 mg/kg/day are derived from this study.

Moxidectin

Moxidectin was evaluated for embryotoxicity/teratogenicity in rats and rabbits up to dose levels that produced maternal toxicity.

In the rat study, doses of 10 and 12 mg/kg/day elicited overt signs of toxicity similar to those in the repeat dose studies. There were significant reductions in food consumption, body weights and body weight gains at these dose levels. There were no adverse effects observed at dose levels of 2.5 and 5 mg/kg/day. Effects on the

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foetuses were observed with dose levels of 10 and 12 mg/kg/day, in the form of increases in the numbers of foetuses with alterations. The levels of alterations were not statistically significant and were a result of incomplete ossification of the palate and ribs. These alterations were considered due to retardation of maturation in the foetuses, a result of maternal toxicity. The maternal NOEL of was 5 mg/kg/day and the foetal NOEL was 5 mg/kg/day.

In the rabbit study, doses of 5 and 10 mg/kg/day resulted in a significant increase in does with abnormal faeces, reduced food consumption and reduced body weight gain. At the top dose of 10 mg/kg/day, there were no adverse effects on foetal development. Moxidectin was not embryotoxic or teratogenic at doses up to 10 mg/kg/day in the rabbit. A maternal NOEL of 1 mg/kg/day and a foetal NOEL of 10 mg/kg/day were determined.

Based on the results of all the reproductive studies, neither imidacloprid nor moxidectin are considered to have an adverse effect on reproductive parameters or on the foetus.

3.5. Mutagenicity

Imidacloprid

Various reports were provided investigating the mutagenicity of imidacloprid with negative results except for assays of clastogenic potential. In a human lymphocyte study, imidacloprid exhibited a positive response in the absence of S-9 and an equivocal response in the presence of S-9. At a dose of 50 µg/ml, imidacloprid was negative in the study, with or without metabolic activation. In an *in vitro* study utilising Chinese hamster ovary cells, imidacloprid was shown to possess weak clastogenic activity in the absence of metabolic activation. No chromosome effects were observed in this study in the presence of metabolic activation.

In another *in vitro* study in CHO cells, imidacloprid did not induce chromosome aberrations either in the absence or the presence of metabolic activation. As the results are equivocal and only occurred in one study at high doses, imidacloprid is at worst a weak clastogen. The levels of imidacloprid that induce chromosome aberrations are significantly above those likely to be encountered when using Advocate.
The data are considered satisfactory and there is not expected to be a mutagenic hazard at the levels of exposure consistent with use of the Advocate product.

Moxidectin

Moxidectin was evaluated for mutagenic potential in one bacterial assay and three mammalian assays. The results in all four test systems were negative and moxidectin is therefore considered to be non-mutagenic.

3.6. Carcinogenicity

Imidacloprid

Two GLP-compliant two year feeding studies were provided to evaluate carcinogenicity of imidacloprid in rats and mice.

Imidacloprid was incorporated into feed at levels up to 2000 mg/kg feed in mice for periods of up to 2 years. The main adverse effect was reduced food consumption and reduction of body weight at 1000 and 2000 mg/kg feed in a dose-related pattern. The dose of 2000 mg/kg feed produced minor changes in haematology and mineralisation of the thalamus. There were no indications of a carcinogenic potential of imidacloprid in mice. The NOEL was determined as 330 mg/kg feed, equivalent to 65.6 mg/kg/day for males and 103.6 mg/kg/day for females.

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At the only inclusion level of 1800 mg imidacloprid/kg feed in the rat, the adverse reactions were the same as for the repeat dose toxicity. A NOEL of 100 mg imidacloprid/kg feed (5.7 mg/kg) was derived for males and 300 mg imidacloprid/kg feed (24.9 mg/kg) for females. There was no evidence for a carcinogenic potential of imidacloprid in rats.

On the basis of the studies provided, the CVMP concluded that imidacloprid does not possess a carcinogenic potential.

Moxidectin

Two GLP-compliant two year feeding studies were provided to evaluate carcinogenicity of moxidectin in rats and mice.

Moxidectin was incorporated into feed at levels up to 120 mg/kg feed in the study in mice. However, after 8 weeks, the top dose level was reduced to 100 mg/kg feed following mortality, weight reduction and hypersensitivity in females. The amended top dose of 100 mg/kg feed (4.5 mg/kg/day) was well tolerated and no further adverse effects were observed in this group. The NOEL was determined as 100 mg moxidectin/kg feed for both sexes. There were no indications of a carcinogenic potential of imidacloprid in the mouse at the inclusion levels investigated in the study.

In the rat study, the highest inclusion level of 60 mg moxidectin/kg feed exceeded the maximum tolerated dose and at the end of week 8, the top dose was reduced to 50 mg/kg feed. This dose elicited increased mortality in females during the last 13 weeks of the study. The NOEL determined from this study was 30 mg moxidectin/kg feed (equivalent to 4.5 mg/kg/day). There were no indications of a carcinogenicity potential of moxidectin in the rat under the conditions of this study.

On the basis of the studies provided, the CVMP concluded that moxidectin does not possess a carcinogenic potential.

III.A.4. STUDIES OF OTHER EFFECTS

4.1. Special studies

4.1.1 Skin and eye irritation

Imidacloprid

A GLP-compliant study was provided investigating the irritant/corrosive potential of imidacloprid on the rabbit skin. Imidacloprid was classified as “not irritant to the skin”.

In another GLP-compliant study for occular irritation in rabbits, imidacloprid was classified as “not irritant to the eye”.

Moxidectin

Two GLP-compliant studies were provided investigating the irritant/corrosive potential of moxidectin on the rabbit skin, the results demonstrate that moxidectin is classified as “not irritating to the skin”.

In another GLP-compliant study in albino rabbits for occular irritation, moxidectin was classified as “slightly irritant to the rabbit eye”. The SPC therefore contains appropriate warnings (sections 5.5 and 5.12) to avoid contact with the eyes.

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4.1.2 Skin sensitisation

Imidacloprid

A GLP-compliant maximisation test according to the method of Magnusson and Kligman was carried out to evaluate the potential of imidacloprid to induce skin sensitisation in guinea pigs. Intradermal induction was performed with 1% imidacloprid in a Cremophor 2% suspension in physiological saline 3 weeks after the first administration, animals were challenged with 3% and 25% imidacloprid in Cremophor 2% suspension in physiological saline. The CVMP concluded that imidacloprid is not classified as a sensitiser in the guinea pig.

Moxidectin

A GLP-compliant dermal sensitisation test according to the method of Buehler was carried out in guinea pigs. 0.4 g moxidectin was applied for six hours to the shaved flank of a guinea pig three times a week for three weeks (a total of 9 applications). Two weeks later, animals were challenged with the same concentration of the product for 6 hours., moxidectin did not induce skin sensitisation in the guinea pig. Although moxidectin was only evaluated in the much less sensitive Buehler method, the CVMP concluded that moxidectin was not classified as a sensitiser in the guinea pig.

4.1.3 Inhalation toxicity

Imidacloprid

The Applicant has provided the results of two GLP-compliant studies investigating the inhalation toxicity of imidacloprid in rats following single and repeat exposures. Acute exposure of rats to dusts resulted in no mortalities at exposure levels up to 5323 mg/m^3 and a NOEL of 1220 mg/m^3, and with aerosols an LC_{50} >69 mg/m^3 and a NOEL >69 mg/m^3. Repeat exposures to dusts at levels up to 191.2 mg/m^3 did not cause any mortalities, giving an LC_{50} of >191.2 mg/m^3 and a NOEL of 5.5 mg/m^3. The main clinical signs observed are recorded in the individual study assessments. There was no evidence of specific organ damage or local injury to the respiratory tract. It can be concluded that imidacloprid possesses a slight acute inhalation toxicity.

Moxidectin

A GLP-compliant study was carried out to investigate the acute inhalation toxicity of moxidectin in the rat at exposure levels up to 9.29 mg/l. The LC_{50} derived from this study was 2.87 mg/l for females and 3.63-4.06 mg/l for males. It was concluded that moxidectin is slightly toxic by inhalation exposure in the rat. However, since the product formulation does not provide for inhalation exposure to the animal owner, the results of this inhalation toxicity study are not considered relevant to operator safety.

4.1.4 Neurotoxicity

Imidacloprid

The Applicant provided studies on the oral neurotoxicity of imidacloprid in rats treated with a single oral dose of 50-350 mg/kg/day or repeated doses of up to 213 mg/kg. The single dose studies showed a dose-related increase in evidence of toxicity, with minimal effects (decreased activity in female rats) from a dose of 42 mg/kg/day and severe toxicity, including lethality at a high dose of 307 mg/kg. All clinical signs and neurobehavioral effects are ascribed to acute cholinergic toxicity, with complete recovery at sub-lethal doses within 7 days. Evidence of a

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minimal decrement in activity that persisted to day 14 in males receiving the high dose of 307 mg/kg/day is attributed to extreme acute toxicity and not to neurotoxicity per se.

In the repeated dose study, neurobehavioral changes were only evident in males receiving the top dose of up to 3027 mg imidacloprid/kg feed (i.e. 196 mg/kg). The NOELs derived from these studies were for males 42 mg/kg (single dose) and 9.3 mg/kg/day (repeated dose) and for females 20 mg/kg (single dose) and 10.5 mg/kg/day (repeated dose). However, it was noted that these figures were much higher than those for systemic NOELs.

Moxidectin

No studies have been performed with moxidectin. This was considered acceptable when taking into account the mechanism of action of moxidectin.

4.1.5 Antidote studies

Imidacloprid

Three studies were performed to investigate possible antidotes to imidacloprid poisoning. The results of one study in mice indicates that propranolol hydrochloride, furosemide and dimorpholamine reduced the toxicity of imidacloprid to a slight degree, that is by decreasing lethality, slightly reduced symptoms and prolonged duration before death.

Treatment with activated charcoal reduced mydriasis and the level of imidacloprid in the blood. The LD50 values of imidacloprid were 350 mg/kg in the absence of activated charcoal and 673 mg/kg following treatment with activated charcoal. The studies on potential antidotes to imidacloprid toxicity indicate that the most effective was three times administration of activated charcoal. However, it is not clear what impact activated charcoal would have in the event of accidental poisoning with the final product.

Moxidectin

No effective antidote to moxidectin poisoning in mammals was identified in the data submitted as part of the dossier.

4.2. Observations in humans

Imidacloprid

A GLP-compliant *in vitro* study was carried out to evaluate the penetration of imidacloprid through rat and human skin. The study confirmed that penetration of imidacloprid through human skin is much lower than through rat skin. This provides assurance that the results obtained with acute dermal toxicity studies in rats provides a margin of safety in humans although the proposed formulation for the final product is more complex than the vehicle used in this *in vitro* study.

Moxidectin

There is no data to support use of moxidectin in humans.

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4.3. Microbiological studies (studies on human gut flora and organisms used in food processing)

Imidacloprid

No data were provided with respect to the microbiological effects of imidacloprid. However, the Applicant claims that to the best of its knowledge, imidacloprid does not possess antimicrobial activity.

Moxidectin

Moxidectin was assessed for antimicrobial activity against a range of bacteria and compared with its parent compound nemadectin. Neither compound was shown to possess significant antimicrobial activity.

4.4. Studies on metabolites, impurities, other substances and formulation

4.4.1. Acute toxicity studies with the final product

A number of acute toxicity studies, reflecting potential routes of exposure of the operator (oral and dermal), were conducted in rats using the dog formulation. The canine formulation was selected due to the higher level of moxidectin, 2.5% in the canine formulation compared to 1% in the feline formulation. The inhalation toxicity of the final formulation was not investigated since this was considered an unlikely route of exposure to the operator with respect to the mode of application and the nature of the formulation.

The acute dermal toxicity was investigated in the rat and NOEL's of more than 2000 mg/kg/day and more than 4000 mg/kg/day (males) and 400 mg/kg/day (females), were derived from these studies. Following oral administration of the formulation, LD_{50} was calculated as 1000-1500 mg/kg and the NOEL for female rats as 200 mg/kg.

4.4.2. Local irritant effects and sensitisation of the formulation

A GLP-compliant skin primary irritation study with a spot-on formulation (10% imidacloprid and 2.5% moxidectin) in rabbits indicated that the final product is non-irritating to the rabbit skin. However, a maximisation study in guinea-pigs demonstrated that the formulation is a skin sensitiser. Although the results did not involve reactions in 100% of the animals in the test group, the product must be regarded as possessing the potential to induce sensitisation and would certainly be expected to elicit reactions in individuals pre-sensitised to the components of the formulation.

Another study in rabbits demonstrated that the final product is irritating to the rabbit eye inducing corneal opacity, iritis, conjunctival redness and conjunctival chemosis up to several days after the treatment.

4.4.3. Toxicity of metabolites, by-products and formulation ingredients

Various studies were provided on the toxicity of several metabolites of imidacloprid and moxidectin as well as a comparison of the toxicity of toxicological and commercial sources of imidacloprid and moxidectin. This allows comparison of the samples used for toxicity testing and the commercial material included in the final formulation. It was accepted that the results obtained in the toxicity tests are also valid for the commercially produced material.

III.A.5. USER SAFETY

The main toxicity of the formulation is with respect to eye irritancy and skin sensitisation. The Applicant has provided a thorough assessment of the potential exposure of humans (animal owner, operator) to the final

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product and identified dermal exposure as the principle route for the operator and accidental ingestion (by a child) as the other potential route of exposure. Due to the low vapour pressure of the product and the method of application, inhalation exposure is considered to be minimal.

On request of the CVMP, the Applicant also provided a comprehensive report of all reported human adverse reactions in all major markets for "Advantage for dogs and cats", which was used as reference product. Advantage is presented in the same pipettes as those proposed for Advocate and therefore provides an indication of the likely exposure routes for operators using Advocate. The report indicates that the most common routes of exposure are via the skin and eyes. There are no reports of ingestion of the product.

The user safety warnings included in the SPC and product literature were considered to adequately address the potential routes of exposure of the operator to the product:
"Avoid contact with skin, eyes or mouth. Do not eat, drink or smoke during application. Wash hands thoroughly after use. In case of accidental spillage onto skin, wash off immediately with soap and water. In very rare cases individual skin reactions (for example, allergies, irritation, tingling) may occur after using this product. People with a known hypersensitivity to either imidacloprid or moxidectin should administer the product with caution. If the product accidentally gets into eyes, they should be thoroughly flushed with water. If skin or eye symptoms persist, or the product is accidentally swallowed, seek medical attention and show the package insert to the physician."

The CVMP agreed that the addition of ferrets as a new target species (to the Advocate for small cat product) should not have a negative impact on the original user safety assessment, and that all relevant precautions for persons administering the product were already included in the SPC (section 4.5).

5.1 Direct contact with the product (skin, eye)

Transfer of liquid product should not occur once the main excipient has evaporated. However, it was demonstrated that even 24 hours after spot-on treatment, the active ingredients imidacloprid and moxidectin could be removed from the fur by stroking the dog. However, the CVMP concluded that there are sufficient safety margins for dermal exposure from treated animals, particularly when the solvent has evaporated from the animals fur. Even in the theoretical and most unfavourable case of close physical contact between a child and a recently treated animal, it was concluded that children are not put at risk.
The main toxicity of the formulation is with respect to eye irritancy and skin sensitisation. This has been taken into account by adding appropriate user safety warning in the product literature and the SPC.

5.2 Accidental ingestion

The Applicant has provided a user risk assessment specifically addressing the ingestion of the Advocate for dogs formulation (with the higher level of moxidectin) by a child weighing 10 kg. A 10 kg child would be exposed to twice the NOEL and a 20 kg child to the NOEL. However, the Applicant demonstrated that the container (pipette) is child-resistant. Furthermore, the CVMP considered that the residual product remaining in the pipette following treatment of an animal represents a small percentage of the original fill and is extremely difficult to extrude. The bitter taste of the product would mitigate against a child ingesting a significant quantity of the product were they to gain access to an open pipette. Also, the absence of any reports of accidental ingestion of similar products in identical pipettes provides reassurance as to the safety in use of the packaging.
The CVMP therefore concluded that the risk of accidental ingestion had been sufficiently addressed by the Applicant.

III.A.6. Ecotoxicity

Advocate contains 2 active ingredients, imidacloprid as the dermal ectoparasiticide and moxidectin as the systemic endoparasiticide. Since dogs as each individual will be dosed with more active ingredient than cats and

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they are more likely to swim in watercourses than cats, a Phase I and II assessment was provided on the use of Advocate in dogs.

Routes of entry to the environment were considered to be excretion for moxidectin, and run-off into watercourses for both active ingredients. The excretion route was not considered to be significant.

The PEC assessment for contamination of a watercourse was on the basis of a single treatment and assuming that the entire dose from the maximum size of pipette enters the watercourse. Watercourses of 100 m^3 were considered with concentrations of 4 µg/l imidacloprid and 1 µg/l moxidectin.

Data have been provided from the human safety assessment demonstrating that 24 hours post-treatment imidacloprid and moxidectin could still be removed from the fur of treated animals. Furthermore, data on losses from dogs' fur based on water immersion were provided similar to those obtained from stroking treated dogs.

Aquatic toxicity data were provided for both active ingredients. The PNEC for imidacloprid was 850 µg/l. The PNEC for moxidectin was 0.3 ng/l. The PEC/PNEC ratio was considered acceptable for imidacloprid. Taking into account that only at 84 h post treatment the PEC/PNEC ratio was less than 1 (0.33 - 0.67), the CVMP concluded that dogs should be prevented from swimming for 4 days.

The following wording therefore was included in section 5.5 of the SPC:
*"*Advocate should not enter surface waters as it has harmful effects on aquatic organisms: moxidectin is highly toxic to aquatic organisms. Dogs should not be allowed to swim in surface waters for 4 days after treatment."

Also section 4.3. (Environmental properties) mentions: "See section 5.5."

The CVMP noted that the addition of ferrets as a new target species would not result in any change to the approved dose, dose regimen or the treatment category (individual dogs, cats, and ferrets). Ferrets are accepted as a minor species and treatment of them will therefore be sporadic compared to cats and dogs. The Committee also recognised that ferrets are mainly kept indoors, that their access to swimming in surface water would be limited, and that the SPC (section 6.6) already included the following precautionary sentence: "*Advocate should not be allowed to enter surface water as it has harmful effects on aquatic organisms.*" The Committee concluded that use of the product in ferrets would not impact on the general assumptions in the environmental risk assessment previously made for this product.

III.B. RESIDUE DOCUMENTATION

As the product is only intended for administration to companion animals, there are no requirements for residue data.

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PART IV: CLINICAL ASPECTS

IV.1. PRECLINICAL STUDIES

See also Part III.

IV.1.A. Pharmacology

IV.1.A.1. Pharmacodynamics

Imidacloprid

Imidacloprid is a chloronicotinyl nitroguanidine insecticide, which binds to nicotinyl acetylcholine (nACh) receptors in the post-synaptic region of the central nervous system. Whilst the precise reasons for the highly selective action of imidacloprid on invertebrate receptors may not have been fully elucidated, extensive toxicological and safety studies have confirmed that this compound has almost no effect on vertebrates. It is claimed that, once it has bound to insect nicotinic acetylcholine receptors, imidacloprid induces slow depolarisation on cell bodies of motor neurones, which results in tetanic muscle contractions as well as destruction of the ganglia of the head and thorax leading to the death of the insect. It appears that imidacloprid acts on fleas via direct contact with the compound. It is stated that it is not necessary for the flea to bite the host animal to be killed by imidacloprid as it is absorbed through the flea's intersegmental membranes. From the work reported, it appears that this active substance becomes localised in the water-resistant lipid layer secreted by the sebaceous glands, which spreads over the entire skin and fur. If this secretion is removed from treated dogs by repeated swabbing of a clipped area with alcohol, fleas placed on the area continue to feed as normal. In contrast, fleas placed on a clipped area of a treated animal, which has not been cleaned with alcohol ceased feeding within a few minutes and died within one hour of exposure. It is claimed that imidacloprid produces no pharmacodynamic effects in the bodies of dogs or cats. Consequently, as there are no pharmacological effects, no data on this subject have been provided in the dossier.

Moxidectin

Moxidectin is a second-generation, semi-synthetic macrocyclic lactone belonging to the milbemycin family, which is closely related structurally to the avermectins. As with other related compounds, it has activity against many nematode species which parasitise animals. As with other avermectins, there is no activity against cestodes or trematodes. The mode of action of moxidectin is the same as that of ivermectin and abamectin. It causes paralysis of susceptible parasite species by altering chloride conductance into cells. The chloride ion influx lowers cell membrane resistance and causes a hyperpolarisation of the resting potential of the post-synaptic cells. This makes neurotransmission more difficult so that transmission of stimuli to muscles is prevented and a flaccid paralysis results followed by death or expulsion of the parasite from the host. It is stated that as moxidectin has no primary pharmacological effects, no data on this aspect have been provided.

The data provided on the pharmacodynamics of imidacloprid and moxidectin are considered satisfactory. Whilst imidacloprid is active only against insect species, moxidectin, being a member of the avermectin/milbemycin group, is known to have activity against ecto- as well as endoparasites. In this respect, it is noted that a spot-on formulation containing moxidectin alone used in the dose confirmation studies did reduce flea numbers, although no such activity is claimed for this component of Advocate.

A satisfactory justification for the combination product, in accordance with the CVMP Guideline on Fixed Combination Products, was provided. The principal advantage claimed for the combination is that it broadens the spectrum of activity for simultaneous treatment and/or prevention of several parasitic infections. The Applicant has acknowledged that it is not always necessary to use a combination product with broad-spectrum

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activity or to use a product throughout the year. The necessity for this depends on the local epidemiological situation and will need to be assessed by a veterinarian. The Applicant has rightly pointed out that the use of a combination product, which has been extensively tested removes the risk of incompatibility, which might arise if individual treatments are used simultaneously. It is also noted that combined treatment offers practical advantages.

The Applicant has acknowledged that no work has been conducted on the possible interaction of imidacloprid and moxidectin at the molecular level and believes that there is no interaction between imidacloprid and moxidectin during the distribution phase following topical administration. Imidacloprid largely remains on the surface of the animal and the presence of moxidectin does not appear to influence this. Studies conducted with the imidacloprid mono formulation and with the imidacloprid-moxidectin combination indicated wide inter-animal variation, but there were generally comparable concentrations of imidacloprid on the fur of treated animals at various time points following treatment. It is noted that further support for the non-interference of moxidectin on imidacloprid is provided by the results of the dose confirmation studies. In view of the very small amounts of imidacloprid absorbed systemically, it is very unlikely that it would have any influence on the metabolism or excretion of moxidectin. In addition to the different distribution of the two active substances, the type of metabolism and routes of excretion are also quite different. The Applicant considers that if any pharmacodynamic interaction exists, it is most likely to occur in insect parasites such as fleas. Dose confirmation studies in the original dossier in which the activity of moxidectin against ascarid worms was assessed in dogs and cats showed that the imidacloprid-moxidectin combination product was just as effective as the moxidectin mono formulation. It is considered that, even though specific studies at the molecular level have not been conducted, all the evidence available indicates that it is most unlikely that there is any adverse interference between imidacloprid and moxidectin.

Further information was requested from the Applicant to justify why a dose determination study for imidacloprid in the combination had not been provided taking into account the fact that moxidectin has some activity against fleas. In view of the finding in the dose confirmation study that the imidacloprid-moxidectin combination was no more effective than the imidacloprid mono formulation, the Applicant concluded that it was necessary to provide the same dose of this active substance as in the marketed imidacloprid mono product in order to achieve the same level **and** duration of efficacy. It is acknowledged that moxidectin does exhibit efficacy against fleas, but that this peaks at 7 days after dermal application of Advocate and then diminishes. However, even at 7 days, the efficacy rate of 69% is at a much poorer than what is acceptable in a flea treatment. In the practical situation, it is likely that fleas will be killed by imidacloprid on the fur of a treated animal long before they have a chance to consume a lethal dose of moxidectin from a blood meal. It is considered that the Applicant's response is satisfactory.

IV.1.A.2. Pharmacokinetics

General information

Imidacloprid

In a number of experiments it has been show that, following dermal application of an imidacloprid solution to dogs and cats, this active substance spreads widely from the site of application within 12 hours and can still be detected at various sites on the body after 4 weeks. There is very little absorption of imidacloprid into the blood stream and this was not considered to play any significant role in the distribution and activity of this agent. In dogs with imidacloprid concentrations in serum were below 0.02 mg/l during an 8 week period after topical application and never reached the lowest pulicidal level of 0.1 mg/l during this time. In cats, concentrations in blood reached a minimally effective concentration of 0.1-0.2 mg/l, but for only a short period between 2-48 hours after topical application.

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Moxidectin

As detailed in Part III, studies on the ADME characteristics of moxidectin have been conducted in rats using ^{14}C-labelled compound. Highest concentrations of drug residues were found in the fat with much lower levels in the liver, kidney and muscles. Elimination was mainly via the faeces with less than 2% being found in the urine by 7 days after treatment. The pharmacokinetic behaviour and metabolism of moxidectin was found to be comparable in all the species examined and it is assumed that this will also be the case in the dog and cat.

The pharmacokinetics of imidacloprid and moxidectin have been studied in both the dog and cat following topical application of the formulation containing 10% imidacloprid and 2.5% moxidectin. These studies involved the distribution of imidacloprid on the coat of animals (fur kinetics) and the concentrations of both active substances in the blood (serum kinetics).

DOGS

Imidacloprid

The most relevant behaviour with regard to its activity against fleas is the dispersion of the active substance over the body surface of treated dogs and its concentration in the hair. This distribution occurs quite rapidly with concentrations, which should be effective against fleas being found within one day of treatment and persisting for at least a month. There was no indication of accumulation with applications repeated at monthly intervals. The degree of systemic absorption was very low. The findings with the imidacloprid-moxidectin combination were comparable with those obtained previously with the product Advantage, which contains only imidacloprid. This indicates that the addition of moxidectin to the formulation had no significant effect on the kinetics of imidacloprid.

Moxidectin

The systemic absorption, distribution and persistence are of most relevance to efficacy. Following dermal administration of the combination product, moxidectin is extensively absorbed into the systemic circulation and evidence suggests that it is widely distributed in the body. It takes from 4-10 days for maximum concentrations in serum to be reached. It is noted that significant moxidectin concentrations in serum persisted for at least 28 days after topical application of the spot-on formulation such that Cmax and AUC values were higher following subsequent treatments administered at 30 day intervals. This caused some initial concerns as there was clearly accumulation of this active substance in the body with dosage repeated at monthly intervals as recommended in the SPC and product literature. The Applicant's opinion is that a steady state would be reached after 4-5 repeated doses and the data obtained in the cat gives some indirect support for this view. The Committee considered that the clarification was satisfactory.

CATS

Imidacloprid

The distribution across the cat’s fur occurs quite rapidly with concentrations, which should be effective against fleas being found within one day of treatment and persisting for at least a month in most cats. There was no indication of accumulation of this active substance on the fur with applications repeated at monthly intervals. The degree of systemic absorption was a little higher than in the dog, but still quite low. In fact, concentrations in serum only exceeded the probable pulicidal level in all cats at 24 hours after treatment. Concentrations were also above this level in individual cats between 8 hours and 3 days after treatment. Consequently, imidacloprid absorbed into the systemic circulation is only likely to have a very minor and short-lived effect on fleas feeding on treated cats. The findings with the imidacloprid-moxidectin combination were comparable with those

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obtained previously with the product Advantage, which contains only imidacloprid. This indicates that the addition of moxidectin to the formulation had no significant effect on the kinetics of imidacloprid.

Moxidectin

Following dermal administration of the combination product, moxidectin is extensively absorbed into the systemic circulation and evidence suggests that it will be widely distributed in the body. The time to reach maximum concentrations in the serum was only 1.3-3 days, which is shorter than in dogs. However, as in the dog, significant moxidectin concentrations in serum persisted for at least 28 days after topical application of the spot-on formulation. This led to Cmax and AUC values which were higher following subsequent treatments administered at 30 day intervals. However, the values for both of these parameters were lower after the fourth treatment than after the third, which suggests that a plateau had been reached. The findings in the study reference 15 also indicated that there was no further accumulation after treatment on day 84, i.e. the fourth treatment repeated at monthly intervals as recommended in the SPC and product literature. This finding gives further indirect support to the Applicant's opinion that a steady state would be reached in the dog after 4-5 repeated doses.

FERRETS

Since there are no data on the pharmacokinetics of moxidectin or imidacloprid in the ferret, the CVMP agreed that it would be reasonable to extrapolate the information already obtained for cats to ferrets, thereby avoiding unnecessary further studies. This was considered reasonable given that the size of the cat is similar to that of the ferret, both species are mammalian carnivores and doses of other drugs are similar for both species. This was also consistent with the minor species guideline (EMEA/CVMP/EWP/117899/2004).

IV.1.B. Tolerance in the target species of animal

DOGS

The safety of the imidacloprid 10% - moxidectin 2.5% spot-on formulation has been investigated in 8 studies in dogs. In two of these the tolerance of the combination was examined following application at the recommended dose rates to the skin of Beagle dogs and puppies using the proposed method of administration. A similar dermal tolerance study was also conducted in Collies as these are most likely to be susceptible to moxidectin intoxication. Although treatment is only indicated via topical application, two studies have been carried out, one in Beagles and the second in Collies, to assess the likely impact of accidental oral ingestion. Two studies were also conducted to examine tolerance of the formulation in heartworm positive dogs. It is noted that all the above studies were conducted to GLP standards. Finally, a field safety study, conducted to GCP, is also reported. As all the studies reported in this section were conducted in the US, it should be noted that there were small differences in the US dosing regimen due to the use of pounds (lb) rather than kilograms (kg) bodyweight when calculating dosages, which are likely to be of no practical significance.

General dermal tolerance

Study - ID 21092 was a GLP study involving Beagle dogs of 7-8 months of age, which were divided into two groups of 8 each. Animals in one group were treated topically on a single occasion with 10x the recommended dose and the other 8 received a placebo treatment (mineral oil at 10x volume). No adverse clinical signs or other significant changes were recorded in either group, which could be related to treatment. It was concluded that the imidacloprid-moxidectin formulation is safe at up to 10 times the maximum recommended dose rate.

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Dermal safety in puppies and young dogs

Study - ID 21090 was a GLP study involving Beagle puppies aged 6.5-7 weeks, which were divided into four groups. The puppies were treated topically as follows:

Placebo:	Mineral oil at 5x unit dose volume at 14 day intervals for 6 treatments
Imi/Mox:	**1x** unit dose (0.9-3x min dose rate) plus 4x unit dose volume of mineral oil at 14 day intervals for 6 treatments
Imi/Mox:	**3x** unit dose (2.7-8.5x min dose rate) plus 2x unit dose volume of mineral oil at 14 day intervals for 6 treatments
Imi/Mox:	**5x** unit dose (4.4-14.2x min dose rate) at 14 day intervals for 6 treatments

It was concluded that imidacloprid-moxidectin poses no serious safety concerns to puppies, although some might show transient vomiting, or rapid or laboured respiration at higher dose rates.

Oral safety in Beagle dogs

Study - ID 21093 was a GLP study involving adult Beagle dogs of both sexes, which were divided into two groups. Animals in one group were treated orally on a single occasion with 1x the recommended unit dose of the product and the others were dosed orally with a placebo treatment (water). 50% of the 12 treated dogs vomited within 18 hours of dosing. Most of these vomited in the first 2 hours. 50% of the dogs also had anorexia after treatment. It is highly unlikely that a dog would consume the whole of the dose of this product unless it accidentally swallowed the tube. However, even if this did happen, the effects are not likely to be serious and they would only be short-lived.

Safety studies in Collie breeds

The following two laboratory studies were conducted as it is known that some Collie dogs may show neurological toxicity to overdoses of some compounds of the macrocyclic lactone (avermectin) family. In addition, Collie and Collie type dogs taking part in a Clinical Trial in the USA were specifically observed for any such effects.

Dermal Safety in Ivermectin-Sensitive Collie

Study - ID 21094 was a GLP study involving Collie dogs aged 1-8 years, which had been shown to be sensitive to ivermectin. These were divided into three groups and treated topically as follows:

Placebo:	Mineral oil at 5x volume 3 times at 28 day intervals
Imi/Moxi:	**3x** maximum dose rate (12-20 mg/kg moxidectin) 3 times at 28 day intervals
Imi/Mox:	**5x** maximum dose rate (20-32.5 mg/kg moxidectin) 3 times at 28 day intervals

The maximum dose rate tested represents 13x the standard 2.5 mg/kg moxidectin dose. Final observations were on day 98, which was 14 days after the final treatment. No clinical abnormalities or adverse reactions were seen in any of the dogs. It was concluded that the imidacloprid-moxidectin spot-on product is safe when administered at up to 5 time the maximum recommended dose, to Collies which had tested positive for sensitivity to ivermectin.

Oral Safety in Ivermectin-Sensitive Collies

Study - ID 21095 was a GLP study involving Collie dogs aged 1-3 years, which had been shown to be sensitive to ivermectin. These were given ascending doses of the imidacloprid-moxidectin formulation orally at 14 day intervals as follows:

First dose:	0.1 mg/kg moxidectin
Second dose:	0.25 mg/kg moxidectin
Third dose:	1.0 mg/kg moxidectin

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No adverse effects were observed after the first two doses. However, after the third dose, one dog became ataxic 2 hours later and by 4-8 hours the remaining dogs exhibited severe toxicosis and were euthanased 8 hours post-treatment. It was concluded that oral dose rates of the imidacloprid-moxidectin up to 10% of that applied dermally are safe, even in ivermectin-sensitive Collies.

Clinical Safety in Collie and Collie Types of Dog

Study - ID 23085 was a controlled, blinded, field study carried out in 4 small animal hospitals in the USA in accordance with GCP. Dogs included in the study were of 46 different breeds. Approximately 60% of the dogs were treated with the test product and the remainder with a product containing the active substance selamectin. Physical and clinical evaluations were carried out by veterinarians and owners. 2% of the dogs treated with the test product and 1.5% of the dogs treated with the reference product exhibited some abnormality but, in all cases, the reactions were mild and none were considered to be connected with macrocyclic lactone toxicity. In the Collie type dogs treated with the test product, the only abnormality recorded was pruritus, which was seen in less than 10% of those dogs. However, as fleas had been identified on one of these dogs prior to treatment, the connection with the test product is uncertain.

The results of the dermal safety study demonstrated a wide safety margin for the product when used as recommended in Collies known to be sensitive to ivermectin. This was confirmed in the clinical trial involving Collie type dogs. With oral safety, there was much less margin for safety as 40% of the dose normally administered dermally produced severe neurological signs requiring euthanasia when given by mouth. Consequently, the Applicant has included a warning in the SPC and product literature that care should be taken with Collies or Collie cross-breeds to administer the product correctly and in particular to prevent oral uptake by the recipient or in-contact animals.

Safety in heartworm-positive dogs

It is noted that treatment of animals known to be infected with heartworms with macrocyclic lactones has been associated with adverse reactions. These were thought to be connected with the death of microfilaria and adult worms which resulted in emboli in the blood circulation.

Study - ID 21091 was a GLP study conducted in Beagle dogs aged 10-12 months, which were artificially infected with heartworms. This infection was carried out by surgically transplanting 8 male and 8 female heartworms into the dogs via a catheter. The dogs were allocated to one of three groups of animals and treated as follows:

Placebo: Mineral oil applied dermally at 5x volume on days 0, 14 and 28
Imi/Mox: **1x** unit dose (1.3-2.6x min dose rate) applied dermally on days 0, 14 and 28 - plus placebo at 4x volume on each occasion
Imi/Mox: **5x** unit dose (5.7-13.5x min dose rate) applied dermally on days 0, 14 and 28

Microfilariae counts were significantly lower after treatment with the imidacloprid-moxidectin formulation. The counts generally remained high in the placebo group, whilst at the same time in both the 1x and 5x groups there were more than 50% of the dogs with zero counts and the maximum number in either group was 8/ml. However, there were no significant differences in the numbers of adult heartworms collected at post mortem. The only significant clinical abnormality recorded was in a dog in the 5x group, which vomited between 2 and 3 hours after the second dose. As noted by the Clinical Expert, there was a divergent trend in LDH values in both treated groups and effects on RBC and PCV in male animals. However, it is not clear if these were related to drug treatment or the effects of treatment on the infection.

Study - ID 223037 was a GLP study conducted in dogs of mixed breeds aged 1-6 years with naturally acquired heartworm infections. The dogs were allocated to one of two groups and treated as follows:

Placebo: Mineral oil applied dermally at 5x volume on days 0, 14 and 28

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Imi/Mox: **5x** unit dose (5.4-13.6x min dose rate) applied dermally on days 0, 14 and 28

Microfilariae counts were high in both groups on day 0. The counts declined after treatment with the imidacloprid-moxidectin formulation ($p<0.1$), but also declined in the placebo group, although to a lower extent. By day 33 the counts ranged from 1-50 in the 5x group and 0-150 in the placebo group. There were, however, no significant differences in the numbers of adult heartworms collected at post mortem ($p<0.1$). The only clinical abnormalities recorded were in one dog, which had a transient mild skin reaction after each treatment and another, which vomited on day 1.

Based on the findings in these two studies, it was concluded that the treatment of heartworm positive dogs with the imidacloprid-moxidectin product is safe. However, in the interest of good clinical practice, it is recommended to test dogs for heartworm infection before treatment.

Tolerance with concomitant treatments

The Applicant has investigated the tolerance of the imidacloprid-moxidectin formulation with other concomitant treatments in 85 cases including ones from both field and laboratory studies. This included not only treatment given at the same time as the spot-on, but also any treatment given subsequently during the observation period. The other products used include 5 antiparasitics, 13 antibiotics, 3 glucocorticoids and numerous other agents used mostly on single occasions. It is reported that none of these dogs exhibited any adverse reactions to treatment with the test product and any of these other products.

Tolerance during long-term treatment

The Applicant was asked to provide data on the duration of treatment and the safety of the product with possibly continuous monthly therapy in some animals over a period up to 9 months. The Applicant has referred to data, which provide indirect support for the contention that prolonged usage of the product at monthly intervals will be safe in the target species. It was noted firstly that the same product without moxidectin (Advantage) has been used safely on a monthly basis over long periods. Secondly, it was pointed out that toxicity data have been obtained following the **daily oral** administration of both active substances over 12 month periods in dogs and a 2 year period in rats. These data were included in Part III of the original dossier and indicate good tolerance despite such regular and long-term oral administration. Thirdly, it was noted that multiples of the recommended dose were administered during the tolerance studies with the product in both dogs and cats. In both species, doses of 5 times the maximum recommended were administered at 2 week intervals on six occasions without significant side-effects. Such dosage far exceeds that which would be given by monthly treatment at the recommended dose rate for 9 months. Fourthly, the question of accumulation of the moxidectin was considered. There was no problem in the cat as it was shown that a steady state had been achieved by the fourth monthly administration. This has not been proven absolutely in the dog, but the Applicant has presented arguments, which strongly indicate that a steady state will be reached between the fourth and fifth administration. Consequently, although there are currently no data on the tolerance of Advocate in dogs and cats when dosed at monthly intervals over a 9 month period, it is considered that there are several valid reasons to believe that such treatment will be well tolerated in both species.

Reproductive safety

It is stated in section 5.6 of the SPC that, from data in Part III, laboratory studies of imidacloprid or moxidectin in rats and rabbits have not produced any evidence of teratogenic, foetotoxic or maternotoxic effects. Evidence suggests that no adverse effects are to be expected in pregnant and lactating dogs or cats, although it is acknowledged that the safety of Advocate has not been established during pregnancy and lactation. This is satisfactory.

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CATS

The safety of the imidacloprid 10% - moxidectin 1% spot-on formulation has been investigated in 5 studies in cats. In two of these the tolerance of was examined following application at the recommended dose rates to the skin of cats and kittens using the proposed method of administration. Although treatment is only indicated via topical application, a study has been carried out in cats to assess the likely impact of accidental oral ingestion. A study was also conducted to examine tolerance of the formulation in heartworm positive cats. It is noted that all the above studies were conducted to GLP standards. Finally, a field safety study, conducted to GCP, is also reported.

General dermal tolerance

Study - ID 21101 was a GLP study involving Domestic Shorthair cats of 4-5 months of age which were divided into two groups. Animals in one group were treated topically on a single occasion with 10x the recommended unit dose of the product and the others received a placebo treatment (mineral oil at 10x volume). No adverse clinical signs or significant changes in the other parameters were recorded in either group, which could be related to treatment. It was concluded that imidacloprid-moxidectin is safe at up to 10 times the maximum recommended dose rate.

Dermal safety in kittens

Study - ID 21099 was a GLP study involving Domestic Shorthair cats aged 8-9 weeks, which were divided into four groups. The kittens were treated topically as follows:

Placebo:	Mineral oil at 5x unit dose volume at 14 day intervals for 6 treatments
Imi/Mox:	**1x** unit dose (1.4-5.4x min dose rate) plus 4x unit dose volume of mineral oil at 14 day intervals for 6 treatments
Imi/Mox:	**3x** unit dose (5.0-16.4x min dose rate) plus 2x unit dose volume of mineral oil at 14 day intervals for 6 treatments
Imi/Mox:	**5x** unit dose (7.8-27.1x min dose rate) at 14 day intervals for 6 treatments

There were no significant changes related to treatment in any of the animals with respect to haematology, clinical chemistry or gross or microscopic pathology. It was concluded that the imidacloprid-moxidectin spot-on poses no serious safety concerns to kittens when applied dermally, although some might show transient mydriasis, salivation or vomiting. However, it the product is ingested orally, there may be very serious neurological adverse effects.

Oral safety in cats

Study - ID 21102 was a GLP study involving Domestic Shorthair cats aged 11-16 months, which were divided into two groups. Animals in one group were treated orally on a single occasion with 1x the recommended unit dose of the product and the others were dosed orally with a placebo treatment (water). A quarter of the treated cats salivated excessively for a time after treatment with the test product and most of the affected cats also vomited a small amount of fluid during the 24 hours after dosing. A couple of other cats also vomited a small amount of fluid likewise. There were no major differences between the test and control groups in the other parameters assessed. It was concluded that inadvertent oral ingestion of the imidacloprid-moxidectin spot-on by cats would not pose any serious safety concerns.

Clinical safety field study

Study - ID 23084 was a controlled, blinded, field study carried out in 4 small animal hospitals in the USA in accordance with GCP. Cats of a variety of different pure- and cross-breeds were included and treated with either the test product or a selamectin-containing product. Physical and clinical evaluations were carried out by

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veterinarians and owners. A few geriatric cats exhibited somnolence and lethargy following treatment with the test product. Approximately 1% of the cats also showed salivation after treatment with the test product. There were no differences between single and multiple households in the occurrence of these effects. With regard to the safety profiles, 93.8% of those treated with the test product and 100% of those treated with the selamectin product were classified post-treatment as either normal or with signs definitely not related to macrocyclic lactone toxicosis. Although approximately 14% of the cats treated with the test product exhibited some abnormalities at one or more observations, they were all were mild and none were considered as likely to be connected with macrocyclic lactone toxicity. It was concluded that the imidacloprid-moxidectin spot-on formulation was well tolerated by cats of various types when used under practice conditions.

Safety in heartworm-positive cats

It is noted that treatment of animals known to be infected with heartworms with macrocyclic lactones has been associated with adverse reactions. These were thought to be connected with the death of microfilaria and adult worms which resulted in emboli in the blood circulation. Consequently, the Applicant has commissioned a study to investigate the safety of the imidacloprid-moxidectin combination when used in cats with patent (microfilaraemic) heartworm infection.

Study - ID 21100 was a GLP study conducted in Domestic Shorthair cats aged 7-9 months, which were artificially infected with heartworms (by surgically transplanting 3 male and 3 female heartworms into each cat via a catheter). The cats were allocated to one of three groups and treated as follows:

Placebo: Mineral oil applied dermally at 5x unit dose volume on days 0, 30 and 56
Imi/Mox: **1x** unit dose (1.3-2.6x min dose rate) applied dermally on days 0, 30 and 56 - plus placebo at 4x unit dose volume on each occasion
Imi/Mox: **5x** unit dose (5.7-13.5x min dose rate) applied dermally on days 0, 30 and 56

There was a significant reduction in circulating microfilaria in all treated cats. No microfilaria were detected on study days 28, 55 and 83 in the 1x and 5x groups, whereas some cats in the placebo group still had high counts. On day 83, one cat in the placebo group had a count of 2005 microfilariae per ml of blood, one had a count of 2 and the remaining cats had zero counts. At necropsy, the mean numbers of live adult worms were 5.2, 3.6 and 3.5 in the placebo, 1x and 5x groups respectively. The numbers in the two treated groups were significantly different from the placebo group at the 0.10 level. Transient salivation was observed in one cat in the 1x group and in seven in the 5x group. One cat in the 5x group showed skin irritation at the application site after the second treatment and another in the same group also had skin irritation on one occasion in the 25 day period after the second dose. One cat in the 5x group was found dead on day 30, before the second treatment. Based on the post-mortem findings, the cause of death was considered to be heartworm infection. However, it cannot be ruled out that treatment might have had some influence on the cause of this animal's death, since the product clearly had some effect on adult forms of this parasite as the heartworm counts at the end of the study were significantly lower in both the 1x and 5x groups than in the untreated controls. Thus, the death of an adult heartworm could have been the result of treatment and this in turn could have caused the animal's death.

The Applicant concluded that the product is safe in heartworm positive cats. Nevertheless, the recommendation is to test cats for the presence of heartworm infection before treating with the imidacloprid-moxidectin spot-on product. The Committee had concerns for safety of the product in heartworm positive cats and included an additional warning:
"Treatment with Advocate may cause serious adverse effects including death in cats having adult heartworms."

Safety in pure bred cats

The Applicant has extracted data and presented on the safety of the imidacloprid-moxidectin spot-on formulation in pure bred cats from the various studies in which it had been used. It is reported that no signs of adverse effects were observed which could be related to treatment.

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Tolerance with concomitant treatments

The Applicant has investigated the tolerance of the imidacloprid-moxidectin formulation with other concomitant treatments in cats, including cats from both field and laboratory studies. This included not only treatment given at the same time as the spot-on, but also any treatment given subsequently during the observation period. It was reported that none of the cats exhibited any adverse reactions to treatment with the test product and any of these other products.

Tolerance in older and sick animals

The Applicant was asked to address the safety of the use of Advocate in the target species at the recommended therapeutic doses in older animals, in animals with hepatic and renal diseases, and in animals with neurological disorders. The Applicant noted that there are relatively few data relating to treatment in debilitated animals with Advocate. The following warning was therefore included in section 5.3 of the SPC:
"There is limited experience on the use of the product in sick and debilitated animals, thus the product should only be used based on risk-benefit assessment for these animals."

The Applicant was asked to discuss if the use of Advocate is prudent in animals with flea allergic dermatitis (FAD) and severely damaged skin since the correct application of Advocate is recommended to undamaged skin. The Applicant re-examined the clinical data to support its response. In fact, there were considerable numbers of both dogs and cats with Flea Allergic Dermatitis in the field studies. These animals generally responded well to treatment and there was no indication of any difference in tolerance between those with FAD and those without. The Applicant has also looked at other cases in which there was skin damage and concluded that there was no apparent adverse effect following treatment with Advocate. Whilst it is clearly prudent to apply the product to undamaged areas of the skin, the presence of lesions elsewhere on the body should not lead to any increased risk of intolerance. The product will only be available as a POM, thus veterinary surgeons can ensure correct application.

FERRETS

Two studies were conducted examining the tolerance of Advocate in ferrets.

Study - ID23923. Prior to the start of the main study, a small preliminary study was conducted with one male ferret and one female ferret treated with Advocate/Advantage Multi at 5x overdose for cats (i.e. 5 x 0.4 ml = 2 ml) onto the skin on the dorsal neck on a single occasion. After treatment the ferrets were observed for five days. They were seen to have matting of the hair around the treatment site on the day of treatment and no other abnormalities were observed.

The main study included a small group of ferrets (half of which were intact males and the other half were spayed females), approximately 9 months of age and weighing 0.8-1.8 kg at the time of treatment, and was a non-GCP study (no controls). The ferrets were treated with Advocate/Advantage Multi locally onto the skin on the dorsal neck 4 times once every two weeks with 2 ml (which correlates to 5x overdose for cats, i.e. 5 x 0.4 ml) resulting in dose-rates of between 10.6 - 23.8 mg/kg moxidectin and 106.7 - 238.1 mg/kg imidacloprid. Animals were examined clinically (twice daily), physically (on days -7, 15 and 46) and food and water consumption were monitored daily from day -7 to 46. Blood samples were collected on ~7 days before first treatment then on days 0, 15 (one day after 2nd treatment) and day 46.

Ferrets were observed to shake their heads following treatment and matted hair was observed at the treatment site after treatment. Other than this, all ferrets gained weight

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between days 1 and 46. There was no evidence of any trend in either haematology or biochemistry measurements. Mean haematology and biochemistry parameters were within normal ranges or matched by pre-treatment values except for elevated glucose in the female ferrets on day 46: this finding is not considered significant. None of the other changes observed in the study was considered significant or treatment related.

The Committee concluded that although this study did not fulfil the principles of GCP, they noted it was conducted satisfactorily and shows that ferrets tolerated Advocate at 4 to 5 times the recommended dosage (depending on the weight on a ferret) when repeated four times every two weeks without showing any adverse reactions. Histopathological changes in the liver and lungs were found in both the control and the treated ferrets, therefore it is not likely that the changes were related to treatment.

Study - ID27506 included farmed minks (bodyweight 1.1 - 1.6 kg) which received imidacloprid at the recommended dose volume (0.4 ml) and an equal sized group of minks (bodyweight 1.2 - 1.6 kg) which received twice the recommended dose (0.8 ml) of imidacloprid. The doses were 2.47 - 3.60 times and 5.14 - 6.84 times the minimum dose-rate for cats of 10 mg/kg, respectively. The minks were examined after treatment and up to 8 hours after treatment. No abnormalities were observed. No signs of damage to the skin or fur were seen when the pelts were examined six days after treatment.

This was only a non-GCP study in a closely related species, the Committee therefore considered that the study provides only supportive value to the target animal safety (of imidacloprid only).

Adverse reactions

Less than ten suspected adverse event reports had been received by the Applicant following the off-label treatment of ferrets with Advantage or Advocate, however the number of ferrets treated off-label over the period were unknown, so although there were only a few reports of suspected adverse events it is not possible to estimate the occurrence of such events.

In the clinical studies all treatment regimens were generally very well tolerated, and only one local reaction was observed and this was only 8 days after treatment and near the application site (transient reddening of skin). It was considered therefore the reaction was possibly due to some other cause.

Conclusion on tolerance in ferrets

The presented data support the statement in the SPC section 4.10 that: *"The product was administered to ferrets at 5 times the recommended dose, every 2 weeks for 4 treatments, and there was no evidence of adverse effects or undesirable clinical signs."*

Only the 'Advocate for Small Cats' size pipette should be used for ferrets. Suitable contraindications to help prevent the use of other pipette sizes are therefore included in both the SPCs:

- Advocate spot-on for Cats, SPC section 4.3: "*For ferrets: Do not use Advocate for Large Cats (0.8 ml) or Advocate for Dogs (any size).*"
- Advocate spot-on for Dogs, SPC section 4.3: *"For ferrets: Do not use Advocate for Dogs. Only "Advocate for Small Cats and Ferrets" (0.4 ml) must be used."*

The smallest ferret, and thus with the highest overdose in the Advocate study, tolerated 23 times the minimum dose-rate without any clinical signs (i.e. 23.8 mg/kg moxidectin and 238.1 mg/kg imidacloprid). The SPC section 4.5 states that the treatment of ferrets weighing less than 0.8 kg should be based on a risk-benefit assessment. Some small female ferrets may weigh 500 g, and thus would receive a dose-rate of 80 mg/kg imidacloprid and 8 mg/kg moxidectin, which would be anticipated to be well tolerated.

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No minimum re-treatment interval is defined in the SPC. The ferrets in the main overdose study were treated every two weeks five times and none of these ferrets showed any adverse effects. Therefore, it would be expected that fortnightly treatment at the normal dose-rate would be well tolerated.

The Committee concluded that the safety in ferrets had been demonstrated sufficiently and the SPCs included appropriate information and warnings.

IV.1.C. Resistance

Various databases, as detailed in the dossier, have been searched for any references to resistance against imidacloprid in fleas or moxidectin in nematodes or heartworm in dogs and cats. It is stated that no such references could be found. The Applicant has also referred to a published paper, which indicates that no flea strain has yet been found with a reduced sensitivity to imidacloprid. The Clinical Expert has pointed out that moxidectin cannot be considered just on its own as there is likely to be cross-resistance with other macrocyclic lactones as they all have a similar mode of action. Agents of this type, including milbemycin, have only recently been introduced for use in dogs and cats and so it is not surprising that resistance to them has not yet been identified in small animal parasites. With regard to imidacloprid, this has been in use in veterinary medicine since 1994 and no resistance has yet been identified in fleas. Although limited information has been provided in this section, it appears that there should be no problems of resistance either at present or in the immediate future with the use of the proposed imidacloprid-moxidectin products. However, as these agents become used more widely and more regularly, there must be the potential for parasites to become resistant eventually to either active substance. The efforts taken by the Applicant to monitor this situation with imidacloprid are noted and these indicate a responsible attitude to the potential problems.

The clinical expert report for the ferret application contained additional information on resistance. The Applicant had conducted a monitoring survey on an imidacloprid-only containing product for the prevention and treatment of fleas on dogs and cats, which had been marketed across the world for approximately ten years. A total of 768 flea isolates were collected over five years from the US, the UK and Germany. Of these only six field strains had over 5% survival within a larval development assay. Further analysis showed that those six strains were not significantly different to reference strains. The CVMP concluded therefore that, to date, there was no evidence for the development of resistance to imidacloprid in fleas. Furthermore the CVMP considered that use of Advocate in ferrets would only increase the use of imidacloprid very slightly over its usage in cats and dogs.

The Committee considered it unlikely that the current control measures used to prevent heartworm establishment in dogs and cats using avermectins and milbemycins would select for resistance, unless uptake within the dog and cat population (including strays) is very high. Additionally, the additional selection pressure posed by the treatment of a relatively small number of ferrets was considered unlikely to pose any substantial selection on heartworm populations.

Whilst it is possible for resistance to develop to either of the active substances, wording to address this risk is included in all the Advocate SPCs, section 4.4: "*Parasite resistance to any particular anthelmintic may develop following frequent, repeated use of an anthelmintic of that class.*" The CVMP considered it unlikely, given the size of the ferret population, that the additional claims in this application would have any impact on the likelihood of induction of resistance to imidacloprid or moxidectin.

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IV.1.D Conclusion on the Preclinical Part

Cats and dogs

The dossier contains a considerable amount of data on the pre-clinical aspects of this application and the individual sections generally cover the area well. With regard to the pharmacodynamics, it is considered that sufficient information has been provided on the actions of the two individual active substance and the details in section 4.1 of the SPC are satisfactory. The pharmacokinetic aspects have been well covered in the dossier with several relevant studies in both dogs and cats. The product is somewhat unusual in that one active substance, imidacloprid, acts almost exclusively on the surface of the animal following spot-on application, whereas the other, moxidectin, is readily absorbed in both dogs and cats when applied in this way. The information provided in section 4.2 of the SPC is considered satisfactory.

Target species tolerance is well covered in the dossier with several studies in both dogs and cats. The Applicant has considered overdosage of up to five times the maximum recommended dose rate following spot-on application in both adult dogs and cats as well as in puppies and kittens. Although the Applicant has included age limits in the warnings the CVMP still had some concerns for use of the product to animals with low body weights, thus a warning was included in the SPC and product literature. Both single and multiple dose studies have been carried out with up to six treatments being given at 14 day intervals in both species. Studies have also included Collie and Collie type dogs and pure bred cats. In addition, the effects of oral ingestion have been considered, presumably to evaluate the probable effects if a treated animal were to lick off some of the applied dose either from itself or from a treated in-contact animal. In general such oral dosage was well tolerated, except in the case of Collie dogs which are, not unexpectedly, rather more sensitive to the moxidectin component. Application of the product at the dose rate and site recommended should minimise the chances of animals ingesting the product and its bitter taste may further reduce this. Warnings and instructions covering these aspects in the SPC and product literature were considered adequate.

As one of the main indications is in the prevention of heartworm disease in dogs and cats, studies have been conducted in both species to evaluate safety in animals known to be infected with these parasites. There is naturally concern in such animals as the destruction of adult heartworms could lead to vascular embolisms with serious consequences. The combination product apparently had little or no effect on adult heartworms in dogs and no significant adverse effects were recorded. However, the product did have an effect on adult heartworms in cats and one cat died as a result of heartworm infection. There is a warning in the SPC and product literature to test both dogs and cats for heartworm infection before treatment, although the reasons for this differ between dogs and cats. In the case of dogs there is a statement that the product can be used safely in animals infected with adult heartworms, but that veterinary advice should be sought as the product has no effect on the adult parasites. In cats, the SPC at section 5.5 indicates that if adult heartworm infection is diagnosed, the infection should be treated in accordance with current scientific knowledge.

Warnings for use during pregnancy and lactation are adequate. No intolerance has been seen when using the product concomitantly with several other veterinary medicinal products.

The safety of the combination product has generally been confirmed in the various clinical studies with only minor and transient effects in a few cases, which did not require any corrective treatment.

The question of resistance has only been covered briefly in the dossier. However, there should be no problems of this type either now or in the near future, although the situation will need to be monitored in the longer term.

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Ferrets

The original application for cats and dogs contained satisfactory preclinical studies together with their evaluation. No data were provided on the pharmacokinetics of moxidectin or imidacloprid in the ferret, however the CVMP noted that the size of cats is broadly similar to that of ferrets, that both species are mammalian carnivores and also that doses of other drugs are similar for both species. The Committee accepted that it would be reasonable to extrapolate the information already obtained for the cat to the ferret, thereby avoiding further unnecessary studies and that this was consistent with the minor species guideline (EMEA/CVMP/EWP/117899/2004). Consequently only tolerance and efficacy were evaluated within this application.

Target species tolerance was investigated in two studies in ferrets. Overdosage of up to five times the maximum recommended dose rate following spot-on application in ferrets showed no adverse effects.

CVMP considered there were no changes to the environmental safety, user safety or resistance profile as a result of use of the product in ferrets and that the safety in ferrets had been demonstrated sufficiently and the SPC included appropriate information and warnings.

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IV.2. CLINICAL STUDIES

DOGS

Details are provided of 25 studies conducted in dogs to investigate the efficacy of the imidacloprid-moxidectin spot-on formulation. These included 6 dose determination studies, 18 laboratory dose confirmation studies and 2 field trials. It is stated that all of the studies were conducted with the final formulation of the product, however, this cannot be the case with the dose determination studies in which the concentrations of moxidectin varied.

CATS

Details are provided of 19 studies conducted in cats to investigate the efficacy of the imidacloprid-moxidectin spot-on formulation. These included 4 dose determination studies, 13 laboratory dose confirmation studies and 2 field trials. It is stated that all of the studies were conducted with the final formulation of the product, however, this cannot be the case with the dose determination studies in which the concentrations of moxidectin varied.

FERRETS

Details are provided of 1 study with imidacloprid and moxidectin (Advocate) and 2 studies with imidacloprid (Advantage) to investigate the efficacy in ferrets of the imidacloprid-moxidectin spot-on formulation in the prevention and treatment of flea infestations. One further study with the product was provided investigating efficacy the prevention of heartworm. All studies in ferrets were laboratory dose confirmation studies.

IV.2.1. Laboratory trials

Dose Determination Studies

DOGS

It is stated that, as imidacloprid is already approved at a dose rate of 10 mg/kg, the dose determination studies were only conducted to ascertain the optimum dose for moxidectin. From the published references, it is clear that heartworm larvae are much more susceptible to moxidectin administered orally than hookworms or roundworms. Consequently, the Applicant has considered mature *Ancylostoma caninum* and mature *Toxocara canis* as the dose-limiting species with regard to this active substance. It is also noted that, in the tests discussed later, roundworms proved to be less sensitive to moxidectin than hookworms.

On the basis of results of the 6 studies, 4 in Germany and 2 in Australia, it was decided to progress with a moxidectin dose rate of 2.5 mg/kg bodyweight - this corresponds to the formulation containing imidacloprid 10% - moxidectin 2.5% and a dose volume of 0.1 ml/kg. None of the studies conducted in Germany were in accordance with GLP. However, the first two were basically pilot studies and the in-house standards used in all of them appear adequate for the purposes of the studies. The Australian studies were conducted in accordance with GCP and are considered satisfactory. The numbers of animals used were quite small in all study groups, although more were included in the groups treated with the final selected formulation containing imidacloprid 10% and moxidectin 2.5%. Thus, there were sufficient numbers to show a significant difference ($p<0.05$) in the number of worms passed compared to the worms remaining at post-mortem with this formulation. It is clear that *T. canis* was the least sensitive of the worms tested, but a moxidectin dose rate of 2.5 mg/kg was adequate to give an efficacy rate >90%. It is agreed that the formulation selected should give a satisfactory efficacy rate against hookworms, roundworms and heartworms when administered topically to dogs at a dose volume of 0.1 ml/kg bodyweight.

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CATS

It is stated that, as imidacloprid is already approved at a dose rate of 10 mg/kg, the dose determination studies were only conducted to ascertain the optimum dose for moxidectin. From the published references, it is clear that heartworm larvae are much more susceptible to moxidectin administered orally than hookworms or roundworms. Consequently, the Applicant has considered mature *Ancylostoma tubaeforme* and mature *Toxocara cati* as the dose-limiting species with regard to this active substance. It is also noted that, in the tests described later, roundworms proved to be less sensitive to moxidectin than hookworms.

On the basis of the results of 4 studies carried out in Australia, it was decided to progress with a moxidectin dose rate of 1.0 mg/kg bodyweight - this corresponds to the formulation containing imidacloprid 10% - moxidectin 1.0% and a dose volume of 0.1 ml/kg. These studies were conducted in accordance with GCP and are considered satisfactory. The numbers of animals used were relatively small in all study groups, although they were adequate for this type of study. It is clear that *T. cati* was the least sensitive of the worms tested, but a moxidectin dose rate of 1.0 mg/kg was adequate to give an efficacy rate >98%. It is agreed that the formulation selected should give a satisfactory efficacy rate against hookworms, roundworms and heartworms when administered topically to cats at a dose volume of 0.1ml/kg bodyweight.

Dose Confirmation Studies

DOGS

As noted above, 18 laboratory dose confirmation studies have been conducted with the imidacloprid 10% - moxidectin 2.5% spot-on formulation in dogs and reference is also made in this section to the results in a field study. All of these studies were conducted in accordance with GCP in both Europe and in the USA. In these studies, the product was dispensed in the same dose volume sizes as are proposed for marketing, i.e. 0.4, 1.0, 2.5 and 4 ml, and animals were treated with these according to the weight band in which they belonged. It is recommended that the dose should be divided and applied at several spots along the back in large dogs (20 lb in the US and 25 kg in Europe). This was the system used also in the studies reported in this section.

Fleas

It is firstly noted by the Applicant that the efficacy of imidacloprid (Advantage) for the control and prevention of fleas on dogs has been established previously. With this product a single topical application of 10% (w/v) imidacloprid solution at a rate of 0.1 ml/kg provides more than 90% flea control for at least 30 days. Two studies were conducted in dogs to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against fleas. Both of these used artificial flea challenge, firstly before a single treatment and then at weekly intervals up to 5 weeks after this treatment. Thus both efficacy against existing flea infestations and the duration of efficacy could be ascertained. The Applicant has also referred to a field study conducted in France.

Flea Allergy Dermatitis (FAD)

The Applicant has referred to studies conducted with Advantage in which it was shown that this product produced a rapid improvement in the signs of allergy in animals with FAD. In these, there was almost complete remission by day 28 after a single treatment. It is argued that, as moxidectin has been shown to have no deleterious effect on the activity of imidacloprid, it can be concluded that the combination product would be as efficacious in the control of FAD in dogs. The claim proposed in the SPC and product literature is that the product can be used as part of a treatment strategy for flea allergy dermatitis. This is the standard wording approved by the CVMP for products of this type.

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Heartworm

Four studies were conducted in dogs in an attempt to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against heartworms. In all of these an artificial infection with *Dirofilaria immitis* was carried out using L_3 larvae. Details of the studies are provided. The inability to produce patent heartworm infections in most placebo control dogs means that the findings are of no value in evaluating the efficacy of the imidacloprid-moxidectin spot-on formulation in the prevention of heartworm disease in this species. It would seem possible that insufficient time was allowed between infection and necropsy for the larvae to moult and develop to the adult stage.

The Applicant was asked to further address the efficacy of prevention of heartworm infection in dogs. Three studies were carried out in the USA in accordance with GCP. The other details were as follows:

Study ID-No. 23291 involved Beagle dogs (50% male, 50% female) aged 11-12 months and housed individually in pens. All animals were infected with 50 L3 larvae of *D. immitis* removed from mosquitoes on Day -33. On Day 0, half the dogs were treated with Advocate Spot-on (10% imidacloprid and 2.5% moxidectin) in pre-filled tubes according to the US dosing schedule. The other half received treatment with a placebo, which contained excipients only. After approximately 90 minutes, half the dogs in each group were bathed and shampooed with a proprietary shampoo. This was washed off thoroughly with water after contact for 2-3 minutes. On Day 146 or 147 after infection, necropsy was carried out on all dogs and they were examined for adult heartworms. All animals in the placebo groups had large numbers of heartworms, whereas those treated with the test product had none. The geometric mean number of worms in each group was as follows:

Heartworm count (range)			
Advocate	Advocate + bathing	Placebo	Placebo + bathing
0	0	34.54 (26-42)	35.07 (26-40)

Thus Advocate completely prevented the development of heartworm in the treated dogs and this effect was not diminished when the dogs were bathed and shampooed 90 minutes after the spot-on treatment.

Study ID-No. 23324 involved Beagle dogs (50% male, 50% female) aged 7.5-8.5 months and housed individually in pens. All animals were infected with 50 L3 larvae of *D. immitis* removed from mosquitoes on Day -45. On Day 0, Group 1 was treated with Advocate (10% imidacloprid and 2.5% moxidectin) spot-on from pre-filled tubes according to the US dosing schedule. Group 2 was treated with a spot-on formulation containing only moxidectin 2.5% and Group 3 was treated with a spot-on formulation containing only imidacloprid 10% (Advantage), all at equivalent dose volumes. On Day 164 after infection, necropsy was carried out on all dogs and they were examined for adult heartworms. All animals in the Advantage-treated group had large numbers of heartworms, whereas those treated with Advocate or moxidectin alone had none. The geometric mean number of worms in each group was as follows:

Heartworm count (range)		
Advocate	Moxidectin 2.5%	Imidacloprid 10%
0	0	37.6 (33-43)

Thus both Advocate and the moxidectin 2.5% spot-on completely prevented the development of heartworm in the treated dogs, whereas Advantage (imidacloprid 10%) did not confer protection against infection.

Study ID-No. 23318 involved Beagle dogs (50% male, 50% female) aged 6.5-8 months and housed individually in runs. All animals were infected with 50 L3 larvae of *D. immitis* removed from mosquitoes on Day -34. On Day 0, animals in Groups 1-4 were treated with Advocate Spot-on (10% imidacloprid and 2.5% moxidectin) in

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pre-filled tubes according to the US dosing schedule. The dogs in Group 5 received spot-on treatment with Advantage (10% imidacloprid). Following treatment, all the dogs were subjected to bathing or shampooing as detailed below. On Day 153 after infection, necropsy was carried out on all dogs and they were examined for adult heartworms. All animals in the Advantage-treated group had large numbers of heartworms, whereas those treated with the test product had none. The geometric mean number of worms in each group was as follows:

Heartworm count (range)				
Advocate + simulated rain/swim 60 min. post-treatment	**Advocate** + simulated rain/swim 24 hr., 7, 14, 21 & 28 days post-treatment	**Advocate** + shampoo & bath 4 hr. post-treatment	**Advocate** + shampoo & bath 24 hr. post-treatment	**Advantage** (imidacloprid) + simulated rain/swim 24 hr., 7, 14, 21 & 28 days post-treatment
0	0	0	0	21.7 (11-33)

Thus Advocate completely prevented the development of heartworm infection in the treated dogs and this effect was not diminished when the dogs were exposed to simulated rain or swimming, or if they were bathed and shampooed, at various times after the spot-on treatment.

It is recorded that no side effects were observed in any of the animals treated in these three studies. Based on the results of these three studies, the Applicant has concluded that Advocate is highly effective in the prevention of heartworm infection when administered 33-45 days after infection with *D. immitis* microfilariae. Also, that this activity is not diminished when dogs are shampooed from 90 minutes after treatment, or when dogs are exposed to water at one or more intervals from 1 hour after treatment. It is also clear from the studies that it is the moxidectin component which confers this prophylactic effect and that the presence of imidacloprid in the combination product does not diminish this.

It is noted that no explanation is provided by the Applicant as to why it was possible in these three studies to produce patent heartworm infections in the control groups whereas this had not been possible in the 4 studies reported in the original dossier. It is also noted that two further studies have been reported in the responses to questions in which difficulties in establishing consistent heartworm infections were encountered. These were study ID-No. 2305 in dogs, in which only 25% of the control animals had heartworms and study ID-No. 23178 in cats, in which no heartworms were found in any animals. Nevertheless, the three new studies described above appear to be valid and it is considered that the Applicant has resolved this major deficiency in the original data. Consequently there is now sufficient justification for the proposed claim for the prevention of heartworm in dogs as stated in section 5.2 of the SPC.

Ascarids

Seven studies were conducted in dogs to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy of treatment against ascarids, *Toxocara canis* and *Toxascaris leonina*. These studies appear to have been conducted to a satisfactory standard and the reporting is good. It is evident from the findings, that the moxidectin in the combination spot-on product is very effective in the proposed dosage range against natural and artificial infections with both *T. canis* (L_4, immature adults and adults) and *T. leonina* (adults). It is also clear that the inclusion of imidacloprid in the formulation has no adverse effect on the efficacy of the moxidectin component.

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Hookworms

The Applicant has referred to 8 studies conducted in dogs to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against the hookworms *Ancylostoma caninum* and *Uncinaria stenocephala*. Reference is also made to a study described earlier which provided data on *A. caninum* in addition to that on *T. canis*. In that study, *A. caninum* was found in one placebo animal and in several dogs treated with the imidacloprid mono formulation, but none were found in the groups treated with either moxidectin alone or the imidacloprid-moxidectin combination. It was concluded, not only that the test formulation was fully effective against *A. caninum,* but also that the inclusion of imidacloprid had no deleterious effects on efficacy against this parasite. It is considered that the studies reported in this section were satisfactory. It is clear from the results obtained that the imidacloprid-moxidectin spot-on formulation is 100% effective against both natural and artificial infections with *Ancylostoma caninum* and *Uncinaria stenocephala* in dogs when administered topically using the dosage regimen recommended in the SPC and product literature. As well as being effective against adult forms of these hookworms, the product was also equally effective against L_4 larvae and immature adults. However, it was not adequately effective against L_3 larvae.

Further data was requested on the preventive efficacy against gastrointestinal nematodes. The Applicant provided the results of a recent GCP study (**ID-No. 25829**) conducted with Advocate. Beagle dogs (50% male, 50% female) of approximately 11 weeks of age and free of worms were used. They were housed singly in cages during the entire study. On Day 0 they were randomly allocated to two equal-sized groups (A or B). The animals in group A were then treated topically with the test formulation at a dose rate of 0.1 ml per kg bodyweight. Animals in group B were also treated topically at the same dose volume but with a placebo formulation containing excipients only. On Day 18 all the dogs were infected with L3 larvae of *U. stenocephala*. The worm counts on Day 39 were as follows:

Treatment Group	Geometric Mean Worm Counts				
	L3	L4	Imm. Adults	Adults	Total
A (test)	0	0	0	0	0
B (placebo)	0	0.36	10.10	16.59	28.61

It was concluded that the test formulation had 100% persistent efficacy in dogs when administered 18 days prior to an *U. stenocephala* infection. The Applicant pointed out that *U. stenocephala* is the nematode with the shortest pre-patent period (approximately 14 days), so that it could theoretically establish a patent infection in the inter-treatment interval. However, it is clear from the results of this study that the moxidectin in the formulation is still fully effective against this parasite 18 days after treatment, and its efficacy against immature stages means that it is able to prevent adult gastrointestinal nematode burdens developing. The Committee concluded that preventive efficacy has been demonstrated against *U. stenocephala* when monthly treatment interval is used.

Trichuris vulpis

Although no studies have been performed specifically to evaluate the efficacy of the imidacloprid-moxidectin spot-on formulation against *Trichuris vulpis*, some of the dogs included in both treated and placebo control groups in 6 of the dose confirmation studies already discussed were naturally infected with this parasite. Thus it is possible to acquire efficacy data for this parasite. Details of the studies that are relevant to *T. vulpis* are provided.

There were no significant differences in *T. vulpis* faecal egg counts on day 0 in any of the studies which indicates a similar level of infection in both treated and placebo groups. At necropsy, there were 814 worms in

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all the placebo control dogs and only 2 worms in one dog treated with the test combination. The efficacy rates were 100% in all cases except study reference 60 where it was just under 97%. The combined results for the 6 studies (as presented by the Applicant) can be summarised as follows:

Treatment Group	Geometric mean numbers of worms	Percentage Efficacy
Placebo	17.5	-
Imidacloprid-moxidectin	0.1	99.6
Moxidectin	0	Too few dogs to calculate
Imidacloprid	124	

According to the Clinical Expert's statistical assessment, a summary odds ratio for the five studies in which *T. vulpis* were present at post-mortem in the control or treated groups showed that there were significantly more dogs with no worms in the treated animals than in the controls. It was concluded that the imidacloprid-moxidectin spot-on formulation was highly effective against *T. vulpis* in naturally infected dogs.

The studies referred to in this section are considered satisfactory. Although not specifically intended for the evaluation of the efficacy of the test product against the dog whipworm, there were sufficient numbers of dogs naturally infected with *T. vulpis* to permit a valid evaluation. The imidacloprid-moxidectin spot-on formulation is highly effective against this parasite when used as recommended in the SPC and product literature.

CATS

As noted previously, 13 laboratory dose confirmation studies have been conducted with the imidacloprid 10% - moxidectin 1% spot-on formulation in cats and reference is also made in this section to the results in a field study. All of these studies were conducted in accordance with GCP in both Europe and in the USA. In these studies, the product was dispensed in the same two dose volume sizes as are proposed for marketing, i.e. 0.4 ml for cats $\leq$4 kg bodyweight, and 0.8 ml for cats >4 kg.

Fleas

It is firstly noted by the Applicant that the efficacy of imidacloprid (Advantage) for the control and prevention of fleas on cats has been established previously. With this product a single topical application of 10% (w/v) imidacloprid solution at a rate of 0.1 ml/kg provides more than 90% flea control for at least 30 days. Two studies were conducted in cats to confirm the dosage of the imidacloprid/ moxidectin spot-on product for efficacy against fleas. Both of these used artificial flea challenge, firstly before a single treatment and then at weekly intervals up to 5 weeks after this treatment. Thus both efficacy against existing flea infestations and the duration of efficacy could be ascertained. The Applicant has also referred to a field study conducted in France. Details of the three studies are provided.

Flea Allergy Dermatitis (FAD)

The Applicant has referred to studies conducted with Advantage in which it was shown that this product produced a rapid improvement in the signs of allergy in animals with FAD. In these, there was almost complete remission by day 28 after a single treatment. It is argued that, as moxidectin has been shown to have no deleterious effect on the activity of imidacloprid, it can be concluded that the combination product would be as efficacious in the control of FAD in cats. The claim proposed in the SPC and product literature is that the product can be used as part of a treatment strategy for flea allergy dermatitis. This is the standard wording approved by the CVMP for products of this type.

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Heartworm

Three studies were conducted in cats to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against heartworms. In all of these an artificial infection with *Dirofilaria immitis* was carried out using L_3 larvae. Details of the studies are provided. It was concluded that the test formulation was 100% effective in preventing the development of adult heartworms in cats. In addition to the above dose confirmation studies, the Applicant has also referred to the tolerance study conducted in heartworm positive cats. It is pointed out that, in this study, no microfilariae were detected on study days 28, 55 and 83 in the cats treated with 1x or 5x the recommended dose of the imidacloprid-moxidectin spot-on product, whereas placebo cats had up to 2005 microfilaria per ml of blood. The Applicant has also noted that, although cats are biologically less suitable hosts for *D. immitis*, the number of recognised cases of heartworm disease in cats is increasing. The geographic range of heartworm in cats is similar to that in dogs, but the rate of infection is lower and the life-span of adult heartworms is shorter in cats (2 years). Signs of heartworm disease in cats are rather non-specific and could be confused with other conditions.

In comparison with the situation in dogs as discussed previously, the Applicant has encountered far fewer difficulties in confirming the activity of the test formulation in the prevention of heartworm disease in cats. Nevertheless, they did have a similar problem in one study as that seen in dogs in which no heartworms were found in either the treated or control animals. Fortunately, the results in the other two studies were clear-cut and easy to interpret. Whilst adult heartworms were not found in all the placebo treated control cats, the majority were successfully infected and the lack of heartworms in any of the cats treated with the test formulation confirmed conclusively that the product was effective.

The studies reported in this section appear to have been well conducted and are sufficiently well reported. Two of the studies gave a clear result with the efficacy of the test formulation in preventing the development of heartworm infection being demonstrated conclusively. Also, it is evident that the efficacy of the moxidectin component in producing this effect was not compromised by the inclusion of imidacloprid in the formulation. In conclusion, it is considered that the imidacloprid-moxidectin spot-on product will prevent heartworm disease in cats when used routinely at monthly intervals as recommended in the SPC and product literature in heartworm endemic areas.

Whilst the designs used in the heartworm studies did not permit efficacy against L3 to be studied specifically, there seems no real doubt from all the evidence available in published papers and work carried out with Advocate, that moxidectin is highly effective against all larval stages of *D. immitis*. It is considered that the Applicant is correct in the belief that monthly administration of Advocate from one month before the mosquito season will kill L3 and L4 larval stages of the parasite before they develop to L5's and begin systemic migration. Thus there should be no risk of aberrant larval migration in animals treated with Advocate as recommended in the SPC and product literature.

Ascarids

Five studies were conducted in cats to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against *Toxocara cati*. Details of the studies are provided. In all cases the differences between treated and control groups were statistically significant. The test formulation achieved a high efficacy rate against artificially induced infections with L_4 and immature adult forms of *T. cati*. These studies appear to have been conducted to a satisfactory standard and the reporting is good. It is evident from the findings, that the moxidectin in the combination spot-on product is very effective in the proposed dosage range against natural and artificial infections with *T. cati* (L_4, immature adult and adult forms). It is also clear that the inclusion of imidacloprid in the formulation has no adverse effect on the efficacy of the moxidectin component.

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Hookworms

The Applicant has referred to 5 studies conducted in cats to confirm the dosage of the imidacloprid-moxidectin spot-on product for efficacy against the hookworm *Ancylostoma tubaeforme*. The first four of these were laboratory studies and the last is one of the field trials. Details of the studies are provided. The Applicant has also referred to the field study conducted in Germany and France, which is discussed in the next section of the assessment report as this provides additional information on the activity of the test product against natural infections with *Ancylostomatidae*. As will be seen, the test product produced a 99.64% reduction in faecal egg count in the cats infected with hookworms. The Clinical Expert has also noted that further evidence for the efficacy of the test formulation against *A. tubaeforme* in cats is provided in 2 studies, which were summarised in the previous section above. Both of these studies were conducted in naturally infected cats and, in addition to *T. cati*, a number of animals carried infections with *A. tubaeforme*. In one study, worms of this species were found in almost half of the placebo group cats, but in none of those treated with the test product. In the second study, almost half of the placebo controls carried this parasite whereas none were found in the cats treated with the imidacloprid-moxidectin spot-on.

The studies reported in this section were satisfactory and well reported. It was unfortunate that there were inadequate infection levels in the first two studies to provide a valid estimate of efficacy, although it is noted that no worms were found in the cats treated with the test product. On the other hand, there was good evidence of efficacy against all developmental stages of *A. tubaeforme* from L_3 to adult in the other two studies, which used artificial infections. In addition, the results from the field trial confirm that the imidacloprid/ moxidectin spot-on formulation produced >99% reduction in faecal egg counts in cats naturally infected with *Ancylostomatidae* when used as recommended in the SPC and product literature. The evidence from the earlier dose determination studies in naturally infected cats provides further corroboration of efficacy against *A. tubaeforme*.

FERRETS

Fleas

One study was performed with the product (Advocate, which contains imidacloprid and moxidectin) and a further two studies with imidacloprid only (Advantage) to examine the efficacy against flea infestation in ferrets.

Summary of studies conducted to examine the efficacy of Advocate or Advantage against fleas in ferrets.

Study Reference	Dose regimen	Comparator in the study
ID25828	Single treatment with 1 pipette containing 0.4 ml Advocate	Untreated control ferrets (same number)
ID24985	Single treatment with 10 mg/kg imidacloprid only (Advantage)	Untreated control ferrets (same number)
ID30771	Single treatment with 1 pipette containing 0.4 ml imidacloprid 10% only (Advantage)	Untreated control ferrets (same number)

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Study - ID25828: a GCP, randomised study was conducted in the EU (bodyweight of male ferrets 1.3 - 2.0 kg, female 0.8 - 1.3 kg). Adult ferrets (10 - 24 months of age; 50% each gender) were randomly allocated to two equally sized groups (treatment group/untreated control group), on the basis of bodyweights and day -6 flea counts (pre-treatment infestation with fleas on day -7). The ferrets were all subsequently infested with 50 unfed adult cat (*Ctenocephalides felis)* fleas each on days -1, 7, 14, 21 and 28. Treatment of the ferrets in the test group was conducted on day 0, when one pipette containing 0.4 ml of Advocate was applied to the skin at the base of the skull to all animals in group 1. The dose rate of imidacloprid ranged from 20.0 to 30.8 mg/kg for males and from 30.8 to 50 mg/kg for females (CVMP calculation). Flea counts were performed on day 1 and days 8, 15, 23 and 30, approximately 24 (up to two weeks after treatment) or 48 hours (from two weeks onwards) after infestation on each occasion (the ferrets were sedated for the flea combing). The table below shows the group geometric means and the efficacy at each time point. Efficacy remained greater than 90% at all assessment points up to and including day 30. On day 30 the individual flea counts ranged from 0 (2 animals) to 24 (one animal) fleas. The range in the control group at the same time point was 16 – 48. Sufficient efficacy (i.e. at least 95%) was shown up to and including 23 days (97.6%) after treatment. No adverse events or significant abnormal findings were observed during the study.

Geometric group mean flea counts and percentage efficacy following treatment of ferrets with 0.4 ml Advocate on a single occasion.

Study Day	Geometric group mean flea counts		% Efficacy
	Control Group	Treated Group	
1	23.6	0	100
8	32.1	0	100
15	29.7	0	100
23	20.6	0.5	97.6
30	29.4	2.7	90.8

The CVMP concluded that this GCP study had been conducted largely in line with the CVMP guideline EMEA/CVMP/005/2000-Rev. 2 and that acceptable efficacy has been demonstrated up to study day 23.

Although the Committee noted there were some deviations from the CVMP guideline they concluded that they were all justified.

CVMP agreed that imidacloprid is effective against fleas but noted that the duration of effect is difficult to ascertain. This study can be considered as a pivotal study for the flea efficacy and duration of efficacy since Advocate was used in the treatment and the product was applied to the skin at the base of the skull. The duration of efficacy in ferrets was clearly shorter than what was demonstrated in dogs and cats even though the dose-rate of imidacloprid in ferrets was higher. Although the application was made within the framework of the Minor Species Guideline it is not considered sound to accept a lower level of efficacy in the ferrets. However, in order to address the risk of a shorter protection period, particularly in heavy flea infestations, a new sentence is included in the "Dosage schedule for Ferrets" part of the SPC (section 4.9) as follows: *"Under heavy flea pressure it may be necessary to repeat administration after 2 weeks"*.

Study - ID24985: in this study, conducted to Good Clinical Practice (GCP), the ferrets were divided into 2 groups, then infested with 60 unfed adult fleas each on day -1 and then weekly on days 7, 14, 21 and 28. Half of the ferrets (males weighing 1.5-1.7 kg, females weighing 0.9-1.0 kg) were treated with the imidacloprid (only) containing product Advantage at a dose-rate of 10 mg imidacloprid /kg, the minimum dose rate recommended for cats (one ferret was underdosed). The other half of the ferrets acted as untreated controls (males weighing 1.5-1.7 kg, females weighing 0.8-1.0 kg). Efficacy at approximately eight hours post-treatment was 95.3%, with

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100% by one day post-treatment. Thereafter it diminished to 92.9% on day 8. One suspected adverse event was detected but no other significant abnormal findings were observed.

The Committee noted this study has been conducted with a product (Advantage) which contains only imidacloprid (0.1ml / kg bodyweight). The results of this study can be only supportive. It is notable that the duration of efficacy is only approximately one week.

Study – ID30771: this efficacy study in ferrets was conducted in the USA. The ferrets (intact males and neutered females) were divided into two groups according to their bodyweight and sex (equal numbers of ferrets in each group). Each of the ferrets was then infested with 50 adult fleas on each of days 6, 13, 20 and 27. The ferrets in the treatment group were treated with one 0.4 ml pipette of the imidacloprid (only) containing product Advantage on day 0 onto the skin at the base of the skull. Depending on the bodyweight of a ferret the imidacloprid dose rate ranged from 36.4 to 47.05 mg/kg for males (bodyweight range 0.85 - 1.1 kg) and 57.1 to 66.6 mg/kg for females (bodyweight of smaller females 0.6 - 0.7 kg). The other half of the ferrets served as controls. Flea comb counts were conducted on days 7, 14, 21 and 28.

It was noted that although this study was conducted with a product (Advantage) containing the same amount of imidacloprid (0.4 ml/dose) as the recommended dose of Advocate for ferrets, the results of the study could be considered as supportive. It was notable that the duration of efficacy was approximately three weeks and also that the efficacy was not as high as mentioned in the CVMP guideline (at least 95%). Efficacy was over 90% up to and including day 21 (93%), thereafter efficacy diminished to 70.6% by day 28.

The bodyweight of ferrets included in this study varied from 0.8 – 2.0 kg. Whilst the efficacy in heavier ferrets was not in question, the duration of efficacy was not considered conclusive in animals over 2 kg so the CVMP therefore considered that it was necessary to include a warning in the SPC section 4.4 that *"The product's efficacy has not been tested in ferrets weighing over 2 kg and therefore the duration of effect might be shorter in these animals."*

Conclusions regarding efficacy in ferrets against fleas:

No field study has been conducted in ferrets. CVMP noted that data submitted in the original (dog and cat) application included field studies which supported the efficacy of the product against fleas under field conditions. The Committee agreed that efficacy against fleas had been evaluated in ferrets in the dose confirmation studies and that adequate target animal safety data are available, therefore CVMP agreed that in this case, the absence of field studies in ferrets had been satisfactorily justified. Furthermore, this approach is in line with the CVMP guideline on efficacy and target animal safety requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004).

According to the efficacy studies provided by the Applicant and conducted with both Advocate and Advantage the full efficacy of 95% and above is reached shortly after the treatment and reflected by the first flea counting on study day 1. The efficacy after approximately 24 hours was 100% in all three studies.

The duration of efficacy varies in all three studies. Only one single efficacy study with ferrets was conducted with combined imidacloprid and moxidectin (Advocate) and the Committee's conclusions relied strongly on this. In this most relevant study the efficacy was still >95% up to study day 23 and fell then below 90% on study day 30. The efficacy should be at least 95% for adult fleas at each counting according to the CVMP guideline for flea and tick control in dogs and cats (EMEA/CVMP/005/2000-Rev.2). All assessments of flea kill have been made 24 or 48 hours after challenge according to CVMP guideline EMEA/CVMP/005/2000-Rev.2. The flea counting showed however that the presence of fleas was clearly below 50% on the control animals especially at study

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days 1 and 23. Although this is a deviation from the CVMP guideline the CVMP agreed that this concern had been adequately addressed.

The Committee concluded that the main dose confirmation GCP efficacy study supports the claim for ferrets for treatment and prevention of flea infestation (*Ctenocephalides felis*) and the duration of efficacy of 3 weeks only was demonstrated for the prevention of flea infestations in ferrets (one week shorter than the duration of efficacy in cats and dogs). The studies also indicated that intervals of four weeks between treatments may be insufficient in cases of a very heavy flea challenge. The tolerance study indicated that treatments at fortnightly intervals are well tolerated, so in the case of a very heavy challenge, treatment may be safely repeated at intervals of two weeks. Therefore the following sentences in the SPC (section 4.9) were included for the ferret presentation: *"One treatment prevents further flea infestation for 3 weeks. Under heavy flea pressure it may be necessary to repeat administration after 2 weeks.*"

Heartworm

One study has been conducted to examine efficacy against heartworm in ferrets and is summarised below. Although no field study was performed this is consistent with the CVMP guideline on worm control in dogs and cats where the default requirement for field studies is cancelled due to the potential pathogenicity of infection in the dog and cat. The same argument would apply to ferrets.

Summary of study conducted to examine the efficacy of Advocate against heartworm in ferrets

Reference	Weight of treated ferrets	Dose regimen	Comparator in the study
Study ID289843	bodyweight on day -1 = 1.2 - 2.1 kg	Single treatment with 1 pipette containing 0.4 ml Advocate	Untreated control ferrets (same number)

Study - ID289843 – To evaluate the efficacy of Advocate in preventing establishment of adult heartworms (*Dirofilaria immitis)* a blinded, randomised, controlled GCP study was conducted in the USA. On study day -30 a group of 7.5 month old male ferrets were artificially infected subcutaneously with 25 third stage *D. immitis* larvae. On day 0 all the ferrets in the treated group received 0.4 ml Advocate as a spot-on onto the skin at the base of the skull, thus the dose-rate ranged from 1.90 mg/kg to 3.33 mg/kg moxidectin depending on the weight of a ferret. At day 124, heartworms were recovered and counted. The geometric mean number of heartworms in the treated group was 0.0 and in the control group 17.2 (range 13 – 23), thus the treatment was 100% effective in preventing the establishment of heartworms. No adverse events occurred during the study.

Although it is desirable to have studies conducted in Europe, CVMP noted that this US study was reliably performed under GCP control. There was no field study for heartworm, but this is consistent with the CVMP guideline on worm control in dogs and cats where the default requirement for field studies is cancelled due to the potential pathogenicity of infection in the dog and cat. The same argument would apply to ferrets.

In the SPC section 4.9 a dosage schedule for ferrets has been added. In addition, for heartworm prevention similar statements as for cats have been given:

> *"Ferrets in areas endemic for heartworm, or those which have travelled to endemic areas, may be infected with adult heartworms. Therefore prior to treatment with Advocate, the advice provided in section 4.5 should be considered.*
>
> *For prevention of heartworm disease, the product must be applied at regular monthly intervals during the time of year when mosquitoes (the intermediate hosts which carry and transmit heartworm larvae) are present. The product may be administered throughout the year or at least 1 month before the first*

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expected exposure to mosquitoes. Treatment should continue at regular monthly intervals until one month after the last exposure to mosquitoes.
In non-endemic areas there should be no risk of ferrets having heartworm. Therefore they can be treated without special precautions."

The CVMP agreed that the study adequately demonstrated the efficacy of the product for prevention of heartworm disease in ferrets.

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IV.2.2. Field trials

Two field trials have been conducted in dogs and two in cats to study the efficacy of the imidacloprid-moxidectin spot-on formulation against natural parasitic infections in animals kept in their normal home environment. In each species, one study was designed to investigate efficacy against fleas and the other investigated efficacy against intestinal nematodes. In the latter two studies, animals were also examined for fleas so that an estimation could be made of the incidence of concurrent nematode and flea infestations.

DOGS

Details of the two clinical trials in dogs are provided.

ID-No. 24774 was a GCP field trial in France. The dogs were initially recruited from 13 veterinary practices. These dogs were of a wide variety of types, ages and weights. Just over half the dogs were kept in the home and the remainder in outdoor pens/kennels. The dogs were included in the study provided they satisfied the enrolment criteria, which included an existing flea burden of at least 3 fleas. Following a flea count and physical examination on day 0, the animals were treated according to a randomisation table with either the test product or the positive control. The method of application and the dose rates used were those proposed in the SPC and product literature for the test product and those approved for the positive control. Following treatment, the animals were examined again on study days 1, 14, 28 and 35. The study was blinded with equal numbers of dogs treated with the test formulation as with the positive control product. The population characteristics of both groups were analysed statistically and it was found that there were no significant differences between them. At every examination time point, the animals were combed and the numbers of live and dead fleas counted manually. The percentage of dogs completely free of fleas at each time point in the two groups were as follows:

Treatment	Dogs free of fleas (%)			
	Day 1	Day 14	Day 28	Day 35
Test product	59.5	77.4	77.4	60.7
Advantage	51.2	71.4	76.2	66.7

In both groups, there were significant reductions in flea counts compared to pre-treatment counts, but no significant differences between the two groups in mean live flea count over the study period. By day 28 after a single treatment with either product, the reduction in geometric mean flea counts was 96.3% in the test group and 95.8% in the group treated with Advantage. The difference was not statistically significant.

With regard to the safety, it was noted that there were no general adverse events, which could be related to treatment with either product. Also, there was no significant difference between the two groups with respect to reported local adverse effects. In the dogs treated with the test formulation, 9 local reactions were recorded. All but one of these were on days 0 or 1. Slight pruritus occurred in 5 dogs and local slight greasy fur was recorded in 2 dogs on day 1 only. One dog showed slight erythema and pruritus 10 minutes after treatment, but this had resolved by day 1. Another dog had moderate erythema as well as slight scabs and greasy fur on day 1, but was normal by day 14. None of these reactions required any corrective treatment and all resolved spontaneously.

It was concluded that both the imidacloprid-moxidectin spot-on formulation and the positive control product, Advantage, were similarly highly effective against fleas on dogs.

This study was well designed and well conducted and the standard of reporting is high. The lack of an untreated control group is noted, but as it could be deemed unethical not to treat infected animals, this is considered acceptable. In any case, the pre-treatment counts could be considered as valid controls for each animal. The

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choice of the positive control product was appropriate. The test sites were suitable and the numbers of dogs included were sufficient to provide statistically valid results. As noted by the Clinical Expert, this type of study is a severe test of a flea control product, as fleas can re-infest a host animal at any stage before subsequent flea counts and the product takes a certain length of time to exert its effect. Thus, even a flea picked up by the dog in the veterinarian's waiting room prior to examination would be counted. Consequently, it is probably not surprising that only around three quarters of the animals treated with either product were completely free of fleas at the 28 day examination. Despite this, the percentage reduction in the numbers of fleas at this time point was >95% for both the test and control products. It is, therefore, agreed that the imidacloprid-moxidectin spot-on formulation provided a good level of flea control for at least 28 days following a single application which was comparable with that achieved by a well-established product applied in the same way.

ID-No. 24507 was a GCP field trial in France and Germany. The CRO co-ordinating the study utilised 7 veterinary practices in two regions of Germany and 5 veterinary practices in two regions of France. The basis for inclusion of dogs in the study was that they were positive for ascarid and/or hookworm infection as assessed by means of faecal egg counts. This was carried out between 1 and 7 days before treatment. Following this testing, the recruited dogs included pure-bred animals (the majority) and cross breeds. The ages of the animals ranged from 8 weeks to 13 years and weighed from 1.9-46 kg. All were housed in private homes.

Prior to treatment (day 0), a physical examination was carried out and this included an assessment for fleas. The dogs were then allocated to one of the two treatments using a site specific randomisation sheet. This was designed to allocate twice as many dogs to the test treatment as to the positive control. The method of application and the dose rates used for the test imidacloprid-moxidectin spot-on formulation were those proposed in the SPC and product literature. The positive control product is a tablet formulation and this was administered orally at the approved recommended dose rate. Following inclusion in the study, the animals were clinically observed again between 8 and 13 days after treatment when another faecal sample was taken for faecal egg count examination. The study was blinded as the person conducting the faecal egg counts was unaware which treatment had been used. Approximately two-thirds of the dogs were treated with the test formulation and one-third with the positive control product. Analysis of variance showed that there was no significant difference between the two groups with regard to pre-treatment faecal egg counts. Efficacy was assessed by calculating the reduction in the geometric mean faecal egg counts between the first sample taken pre-treatment and the second sample taken 8-13 days after treatment. The pre- and post- treatment faecal egg counts were highly significantly different with both the test and positive control products ($p<0.0001$). The detailed results were as follows:

Treatment (Day 0)	**Geom. Mean Faecal Egg Count**			
	T. canis		***Ancylostomatidae***	
	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.
Test product	462.9	5.5	462.4	0.4
Positive control	413.9	1.4	263.3	0.2

Treatment (Day 0)	**Reduction (%)**	
	T. canis	***Ancylostomatidae***
Test product	98.81	99.92
Positive control	99.66	99.91

Of all the dogs included in the study, 16.8% had concurrent flea and nematode infestations. It is reported that no adverse events or mortality were seen during the study. It was concluded that the imidacloprid-moxidectin spot-on formulation was safe and highly effective in the treatment of natural infestations due to *Toxocara canis* and *Ancylostomatidae* in dogs.

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This study was well designed and well conducted and the standard of reporting is high. The choice of the positive control product was complicated by the fact that there was no comparable anthelmintic product, which could be applied as a spot-on. However, the use of Drontal Plus is considered appropriate as it is effective against the nematodes of relevance in this study and, although blinding was not possible during treatment, the fact that the person performing the faecal egg counts did not know which treatment had been given did provide a satisfactory degree of blinding. The test sites were suitable and the numbers of dogs included were sufficient to provide statistically valid results. It is clear from the results that the imidacloprid-moxidectin spot-on formulation was safe to use and that it provided a high level of efficacy against *T. canis* and *Ancylostomatidae* when used in dogs as recommended in the SPC and product literature.

Effects of Water Contact/Shampooing after Treatment of Dogs

The Applicant has referred to two studies, one is study which has been considered above under efficacy against heartworms and the other is the French field study considered in the previous section. Unfortunately, although the heartworm study had been designed to evaluate the effects of wetting in a very thorough manner, no conclusions could be drawn from it as the infection rate in the placebo control animals was so low.

From the results of the field study in which efficacy against fleas was studied, the Applicant has analysed the data for two sub-groups of dogs. One group consisted of dogs, which had had no contact with water, and dogs with at least one contact with water during the study. Dogs in the latter group had either had a bath or been shampooed. None of the dogs in the study had contact with water within two hours of treatment. Equal numbers of dogs from the imidacloprid-moxidectin and Advantage groups had contact with water during the study. The effect of wetting on flea numbers and efficacy can be summarised as follows:

	Imidacloprid-moxidectin Group		Advantage Group	
	Wetted	All dogs	Wetted	All dogs
Flea count day 1	13.18	10.54	13.15	12.86
Flea count day 28	2.44	1.36	2.33	1.50
% efficacy	88.2	96.3	89.1	95.8

The mean flea counts on day 35 were not significantly different for the dogs, which had had contact with water. In fact, almost 80% of these dogs had repeated contacts with water over two or more examination time point intervals. Thus, it is argued that the activity of both products was severely challenged with regard to its 'waterproofness'.

The Committee requested further efficacy data against heartworms and the Applicant provided three new studies, which have been described earlier in this report. The effect of wetting and shampooing were studied in two studies. The efficacy of moxidectin against heartworm infection was not diminished when the dogs were bathed and shampooed 90 minutes after the spot-on treatment or if the dogs were exposed to simulated rain or swimming, or if they were bathed and shampooed, at various times after the spot-on treatment. With regard to the activity of imidacloprid, it is clear that repeated exposure to water does reduce efficacy against fleas to some extent. However, the product remains generally effective in this respect and if treatment was repeated at monthly intervals, the practical significance might not be too great. This relative resistance to wetting might be expected from the distribution of imidacloprid in the fatty secretions of the skin and hair coat. However, it is not clear to what extent this might apply to the moxidectin component. The latter is, of course, absorbed systemically following topical application, but maximum blood levels occur only after 4.6 to 9.3 days. In the SPC and product literature it is stated that treated animals should not be allowed to bathe in water courses for 4 days after treatment. However, this warning is given principally because of ecotoxicity concerns rather than possible effects on efficacy. It is evident that there is some reduction in efficacy against fleas following wetting. Whilst, this might not be adversely affected if the wetting occurred more than 10 days after application of the product, it

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could be affected if a dog, for example, went swimming or was bathed between 2 hours and 10 days after treatment. The SPC has been amended at section 5.10 to read '*Brief contact of the animal with water on one or two occasions between monthly treatments is unlikely to significantly reduce the efficacy of the product. However, frequent shampooing or immersion of the animal in water after treatment may reduce the efficacy of the product.*'

CATS

Details of the two clinical trials in cats are provided.

ID-No. 24767 was a GCP field trial in France. Cats were initially recruited from 12 veterinary practices and were mostly of the European domestic type with lower numbers of Persians and Siamese. The ages of the animals ranged from 0.2-17.5 years and they weighed from 1-7.3 kg. Most of the cats were kept in the home and only a small number in pens. The cats were included in the study provided they satisfied the enrolment criteria, which included an existing flea burden of at least 3 fleas.

Following a flea count and physical examination on day 0, the animals were treated according to a randomisation table with either the test product or the positive control. The method of application and the dose rates used were those proposed in the SPC and product literature for the test product and those approved for the positive control. Following treatment, the animals were examined again on study days 1, 14, 28 and 35. The study was blinded and equal numbers of cats were treated with the test formulation and with the positive control product, although the flea count statistical analysis was not possible on 1 cat in group 2 (positive control). The population characteristics of both groups were analysed statistically and it was found that there were no significant differences between them.

At every examination time point, the animals were combed and the numbers of live and dead fleas counted manually. On day 0, the mean flea count was 8.8 (range 3-31) in the test group and 10.0 (range 3-32) in the positive control group. The percentage of cats completely free of fleas at each time point in the two groups were as follows:

Treatment	**Cats free of fleas (%)**			
	Day 1	**Day 14**	**Day 28**	**Day 35**
Test product	59.7	76.4	81.7	61.1
Advantage	59.2	84.5	87.3	56.3

In both groups, there were significant reductions in flea counts compared to pre-treatment counts, but no significant differences between the two groups in mean live flea count over the study period. By day 28 after a single treatment with either product, the geometric mean flea counts were 1.23 in the test group and 1.18 in the group treated with Advantage. The corresponding reduction rates were 96.9% and 97.7% respectively. The difference was not statistically significant. It was note that the skin condition in both groups showed a significant improvement over the course of the study.

With regard to the safety, it was noted that there were no serious adverse events, which could be related to treatment with either product. Details of the non-serious adverse effects are summarised. In the cats treated with the test formulation, 7% exhibited pruritus, 2% erythema, 1% greasy fur, 1% salivation and 1% vomiting (once, and 1 hour after treatment). All but one of these were on days 0 or 1 and almost all were classed as slight. None of these reactions required any corrective treatment and all resolved spontaneously. The findings for the positive control product were basically similar and there were no statistical differences between groups in this respect.

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It was concluded that both the imidacloprid-moxidectin spot-on formulation and the positive control product, Advantage, were safe and similarly highly effective against fleas on cats.

The choice of the positive control product was appropriate as it contained only imidacloprid and was applied in an identical fashion and at the same dose rate to the test product, thus full blinding of the participants was possible. As noted by the Clinical Expert, this type of study is a severe test of a flea control product, as fleas can re-infest a host animal at any stage before subsequent flea counts and the product takes a certain length of time to exert its effect. Thus, even a flea picked up by a cat in the veterinarian's waiting room prior to examination would be counted. Consequently, it is probably not surprising that only around 82-87% of the animals treated with either product were completely free of fleas at the 28 day examination. Despite this, the percentage reduction in the numbers of fleas at this time point was around 97% for both the test and control products. It is, therefore, agreed that the imidacloprid-moxidectin spot-on formulation provided a good level of flea control for at least 28 days following a single application which was comparable with that achieved by a well-established product applied in the same way.

ID-No. 24506 was a GCP field trial in France and Germany. The CRO co-ordinating the study utilised 13 veterinary practices in three regions of Germany and 8 veterinary practices in two regions of France. The basis for inclusion of cats in the study was that they were positive for ascarid and/or hookworm infection as assessed by means of faecal egg counts. This was carried out between 1 and 7 days before treatment. Following this testing, the recruited cats comprised 96% cross-bred animals and 4% pure-breds. The ages of the animals ranged from 8 weeks to 15 years and weighed from 0.5-6.2 kg. All were housed in private homes.

The cats were allocated to one of the two treatments using a site specific randomisation sheet. This was designed to allocate approximately twice as many cats to the test treatment as to the positive control. The method of application and the dose rates used for the test imidacloprid-moxidectin spot-on formulation were those proposed in the SPC and product literature. The positive control product is a tablet formulation containing praziquantel and pyrantel embonate and this was administered orally at the approved recommended dose rate. Following inclusion in the study, the animals were clinically observed again between 7 and 13 days after treatment when another faecal sample was taken for faecal egg count examination. The study was blinded as the person conducting the faecal egg counts was unaware which treatment had been used. 65% of the cats were treated with the test formulation and the remaining 35% with the positive control product. Analysis of variance showed that there was no significant difference between the two groups with regard to pre-treatment faecal egg counts.

Efficacy was assessed by calculating the reduction in the geometric mean faecal egg counts between the first sample taken pre-treatment and the second sample taken 7-13 days after treatment. The pre- and post- treatment faecal egg counts were highly significantly different with both the test and positive control products ($p<0.0001$). The detailed results were as follows:

Treatment (Day 0)	**Geom. Mean Faecal Egg Count**			
	T. cati		***Ancylostomatidae***	
	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.
Test product	1054.8	0.1	259.9	0.9
Positive control	1149.8	0.4	264.6	0.0

Treatment (Day 0)	**Reduction (%)**	
	T. cati	***Ancylostomatidae***
Test product	99.99	99.64
Positive control	99.96	100

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3% of the cats were found with *Toxascaris leonina* infestation pre-treatment. The majority of these were treated with the imidacloprid-moxidectin spot-on and only one with the control product. Both products proved to be 100% effective against this parasite as judged by faecal egg count reduction. Of the total number of cats included in the study, 26.95% had concurrent flea and nematode infestations.

One suspected adverse drug reaction report was completed during the study. This was in a cat treated with the reference product, which showed salivation after the tablet had been given. However, it recovered shortly afterwards without any treatment being necessary. No reactions were reported for the test product.

It was concluded that the imidacloprid-moxidectin spot-on formulation was safe and highly effective in the treatment of natural infestations due to *Toxocara cati* and *Ancylostomatidae* in cats.

The choice of the positive control product was complicated by the fact that there was no comparable anthelmintic product, which could be applied as a spot-on. However, the use of control product is considered appropriate as it is effective against the nematodes of relevance in this study and, although blinding was not possible during treatment, the fact that the person performing the faecal egg counts did not know which treatment had been given did provide a satisfactory degree of blinding. The test sites were suitable and the numbers of cats included were sufficient to provide statistically valid results. It is clear from the results that the imidacloprid-moxidectin spot-on formulation was safe to use and that it provided a high level of efficacy against *T. cati* and *Ancylostomatidae* when used in cats as recommended in the SPC and product literature.

FERRETS

Data submitted in the original (dog and cat) application included field studies which supported the efficacy of the product under field conditions against fleas and heartworm. Although no field studies were conducted in ferrets, efficacy was evaluated for the claimed indications in this minor species in dose confirmation studies and adequate target animal safety data are available, therefore CVMP agreed that in this case, the absence of field studies in ferrets had been satisfactorily justified for the indications claimed. This approach is in line with the CVMP guideline on efficacy and target animal safety requirements for veterinary medicinal products intended for minor uses or minor species (EMEA/CVMP/EWP/117899/2004).

IV.2.3. Conclusion on the Clinical Part

Data from a considerable number of studies have been presented. The Applicant has adequately summarised the data and the Clinical Expert Report generally offers a good level of critique. Apart from four early pilot dose determination studies in dogs, all other studies were conducted in accordance with GLP or GCP. Standard parasitological methods were used in most cases and the relevant VICH and CVMP guidelines followed. Most studies were also well blinded to avoid bias in the results. The data have generally been subjected to appropriate statistical analysis to establish the significance of the findings. Also, geometric means have been used in almost all cases in calculating efficacy rates. In general the animals used in the studies could be considered as representative of the target population and there was no indication of any difference in efficacy or safety between short or long haired animals.

In the case of the dose determination studies, no data are provided with respect to the imidacloprid component as the optimum dosage of this active substance is known from previous studies with Advantage and the Applicant's experience with this product, which has essentially the same formulation, but without the moxidectin. This is considered acceptable. It is noted that the dose rate for imidacloprid is the same for both dogs and cats. Also, as the Applicant wished to use the same concentration of imidacloprid in the new product, this fixed the dose volume. Thus, the dose determination studies involving the moxidectin component required the testing of formulations containing different concentrations of this active substance. In both cats and dogs, it is considered

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that the Applicant has correctly selected the least sensitive of the parasites for which activity is claimed in deciding on the optimum moxidectin dosage. Based on the results obtained in these studies, it is considered that the dose rates selected for moxidectin are appropriate for the dog and cat respectively. As these dose rates are different for the two host species, it was necessary to provide different formulations for the dog and cat. Thus, the dog formulation contains 10% imidacloprid and 2.5% moxidectin, whereas the cat product contains 10% imidacloprid and 1.0% moxidectin.

The dose confirmation studies were carried out logically, investigating the efficacy of the combination product against each of the parasites for which efficacy is claimed. In all cases, the test product was tested in comparison with a placebo spot-on formulation, which contained only inactive ingredients. It is also noted that, in many cases, the combination product was tested in comparison with the appropriate 'mono' substance formulation. This made it possible to detect any adverse influence between the two active substances with respect to efficacy. From the results obtained, it is clear that neither active substance has any such effect.

The studies on efficacy against fleas were well performed and reported. They are considered satisfactory to confirm that the combination imidacloprid-moxidectin spot-on product is effective in the treatment and prevention of flea infestations in dogs and cats when used as recommended and at the proposed dose rate as detailed in the SPC and product literature. It is interesting, but not surprising, that the moxidectin component also had a limited effect on fleas. However, it is also clear that moxidectin had no adverse effects on efficacy against fleas and that the combination product was as effective as the authorised product, Advantage, in this respect. The proposed claim in respect of Flea Allergy Dermatitis is also considered acceptable in view of the comparable efficacy of the test product with Advantage.

The dose confirmation studies provided convincing evidence that the combination product will offer satisfactory efficacy against all the species of parasite for which activity is claimed. However, an experimental confirmation of the preventive effect has only been demonstrated against *Uncinaria stenocephala*. The prevention of heartworm claim (L3 and L4 larvae) has been sufficiently demonstrated in both dogs and cats.

The field trials were of a high standard and were well reported. They were conducted in a range of European geographical sites. The animals included were of diverse types typical of the dogs and cats, which would be treated with the product. In all cases, the positive control products used were appropriate and the lack of any untreated controls is deemed acceptable as non-treatment of infested animals could be considered unethical. The dog and cat formulations were dispensed in the various pipette sizes as intended for the product and they were used according to the recommendations and at the dose rates proposed in the SPC and product literature. Thus, the appropriate unit dose volumes were used according to the bodyweight ranges of the animals. The product was used topically in all cases with the spot-on formulation being applied between the shoulder blades in dogs and on the neck of cats at the base of the skull.

With regard to field efficacy against flea infestations, both the degree of activity and duration of effect of the imidacloprid-moxidectin spot-on formulation was very similar to that of the control product Advantage in dogs and cats. The data are, therefore, considered satisfactory to support the claims made for Advocate in this respect. It is also noted that with Advantage, there is advice in the SPC and product literature that re-infestation from the emergence of new fleas in the environment may continue to occur for six weeks or longer after treatment. There is also a recommendation to use a suitable environmental treatment to deal with adult fleas and their developing stages in the animal's surroundings. It would seem logical on this matter to give the same information and advice for this product as that approved for Advantage and the product literature has been updated accordingly.

With regard to the prevention and treatment of flea infestations in both species, it is claimed that larval flea stages in the pet's surroundings are killed by contact with an Advocate-treated pet. The Applicant was asked to substantiate this claim. The main support in this section is a comprehensive review of the flea situation in small animals and the evidence provided by 14 papers relating to the larvicidal effects of Advantage. These data are

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applicable to Advocate. The researchers investigated the transfer of larvicidal effects of this spot-on formulation from the treated animal's hair coat to polyester fleece blankets. The results indicated that even after only a few hours contact between the animal and this material, the latter exhibited a marked larvicidal effect, which persisted for several weeks. In the published paper by Dryden *et al*, the objective was to compare the ability of imidacloprid alone (spot-on) with lufenuron (oral) and pyrethrin spray in combination to control fleas in households with pets. The study was conducted in Florida and involved dogs and cats from 34 different households. The results clearly illustrate the efficacy of imidacloprid alone in reducing the flea population in the animal's environment after a period of time, even without any other concomitant treatment of the environment. Consequently, it is considered that the Applicant has now justified the claim that larval flea stages in the pet's surroundings are killed by contact with an Advocate-treated pet.

The field efficacy of the test product against the principal ascarid and hookworm species of nematodes in dogs and cats was very similar to that achieved with the respective praziquantel and pyrantel embonate tablet formulations in these host species. The data are, therefore, considered satisfactory to support a claim against these nematodes.

Although the efficacy of a monthly interval for the treatment of fleas has been justified the Applicant was asked to support such a treatment interval for roundworms, hook worms and whipworms. Although it is standard veterinary practice to routinely treat adult dogs and cats only every 3 or 6 months, the Applicant has challenged such long intervals based on the opinions of two eminent parasitologists. It is accepted by the Applicant that the decision whether or not to treat at monthly intervals must be left with the veterinarian who can take into account the likelihood of worm infestation. The Applicant has proposed that monthly treatment with Advocate would provide a satisfactory level of prevention for all the nematode parasites for which it is indicated. This is supported by the results of a recent GCP study conducted with Advocate at the Hanover School of Veterinary Medicine. The full report on this study is presented. Although the frequency of anthelmintic treatment recommended for Advocate is greater than that currently used for other such products, it is considered that the Applicant has made a logical and valid argument to support this regimen. In practice, it will be the veterinarian who decides if this product, with its monthly treatment schedule, is appropriate for any particular animal.

With regard to roundworm, hookworms and whipworms, it is claimed that Advocate is effective in the treatment and prevention of infestations. However, only efficacy against established infestations has been investigated and no preventative effect has been demonstrated. It is accepted that, for parasites with a pre-patent period longer than 4 weeks, monthly treatment should prevent the establishment of adult worms in the gut. However, this may not be the case with hookworms, which can have a shorter pre-patent period. In view of this, it is considered that the product should only be indicated for the treatment of infestation caused by nematode parasites.

Efficacy against larval and immature stages of *Toxocara canis* in dogs has been confirmed, but efficacy against *Toxascaris leonina* has only been shown for the adult form of this parasite.

The product was initially authorised only for use in dogs and cats, but an application was made later for ferrets. Although data was limited to target species tolerance and dose confirmation studies and there were no field studies performed in ferrets, the CVMP concluded that the data provided adequately demonstrated the efficacy of the product for the treatment and prevention of flea infestations (*Ctenocephalides felis*) and the prevention of heartworm disease (L3 and L4 larvae of *Dirofilaria immitis*) in ferrets.

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V. OVERALL CONCLUSIONS AND BENEFIT/RISK ASSESSMENT

Advocate spot-on is a fixed-combination product containing two active substances. Use of the fixed combination has been adequately justified; it broadens the spectrum of treatment of parasitic infections in animals suffering from, or at risk from, mixed parasitic infections. The use of a single product also reduces the risk of using incompatible products.

The method of manufacture of both the cat/ferret and dog products (which differ in the ratio of the active substances in the solution) is well defined and controlled. The specifications are considered suitable for products of this type and batch analyses data demonstrates production to a consistent quality. The immediate packaging comprises single-dose pipettes in a range of sizes in order to accommodate wide ranges of body-weights in the target species. The outer packaging comprises blister packs. This is acceptable for this product. The proposed shelf-life of 2 years when stored below 30°C is supported by the stability data presented. Overall the quality of the product has been demonstrated.

In mutagenicity tests, imidacloprid was considered a weak clastogen. However, it was accepted that the concentration inducing chromosome aberrations would be significantly above the dose likely to be used.

Moxidectin has been classified as slightly irritating to the eye. Also, a maximisation study in guinea-pigs demonstrated that the final product is a skin sensitiser. Although the results did not involve reactions in 100% of the animals, the product possesses the potential to induce sensitisation and is expected to elicit reactions in individuals pre-sensitised to the components of the formulation. An appropriate warning has therefore been included in the SPC and product literature of the product.

Imidacloprid is considered to be of low to moderate toxicity following oral administration, while moxidectin is considered to be moderately toxic. Dermal exposure has been identified as the principle route for the operator and accidental ingestion as the other potential route of exposure. Due to the low vapour pressure of the product and the method of application, inhalation exposure is considered to be minimal. The assessment of oral exposure has been limited to mishandling the product by an adult or ingestion of the contents of a pipette by a child. Although the pipette is classed as “child resistant”, additional warning has been included in the SPC and product literature.

In view of the high aquatic toxicity of moxidectin a warning has been included in the SPC and the product literature indicating that “Advocate should not enter surface waters as it has harmful effects on aquatic organisms: moxidectin is highly toxic to aquatic organisms.” Also, taking into account that only at 84 h post treatment the PEC/PNEC ratio was acceptable, the CVMP concluded that dogs should be prevented from swimming for 4 days by adding an appropriate recommendation into the SPC and product literature.

The pharmacokinetic aspects have been well covered with several relevant studies in both dogs and cats. The product is somewhat unusual in that one active substance, imidacloprid, acts almost exclusively on the skin and hair/fur of the animal following spot-on application, whereas the other, moxidectin, is readily absorbed in both dogs and cats when applied in this way. Although no pharmacokinetic studies were conducted in ferrets adequate justification for this was provided.

Target species tolerance is well covered and overdosage of up to five times the maximum recommended dose rate has been considered following spot-on application in both healthy adult dogs, cats and ferrets as well as in puppies and kittens. Both single and multiple dose studies have been carried out with up to six treatments being given at 14 day intervals in both dogs and cats. Oral dosage was well tolerated, except in the case of Collie dogs which are, not unexpectedly, rather more sensitive to the moxidectin component. There is a warning in the SPC and product literature to test both dogs and cats for heartworm infection before treatment, although the reasons

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for this differ between dogs and cats. In the case of dogs there is a statement that the product can be used safely in animals infected with adult heartworms, but that veterinary advice should be sought as the product has no effect on the adult parasites. In cats, the SPC at section 5.5 indicates that if adult heartworm infection is diagnosed, the infection should be treated in accordance with current scientific knowledge. Warnings for use during pregnancy and lactation are adequate.

The studies on efficacy against fleas confirm that the combination imidacloprid-moxidectin spot-on product is effective in the treatment and prevention of flea infestations in dogs, cats and ferrets when used as recommended. The proposed claim in respect of Flea Allergy Dermatitis in dogs is also considered acceptable.

The prevention of heartworm claim (L3 and L4 larvae) has been sufficiently demonstrated in dogs, cats and ferrets.

Efficacy against established roundworm, hookworm and whipworm infestations has been shown. No preventative effect has been demonstrated, except against *Uncinaria stenocephala*. It is accepted that, for parasites with a pre-patent period longer than 4 weeks, monthly treatment should prevent the establishment of adult worms in the gut. However, this may not be the case with hookworms, which can have a shorter pre-patent period. In view of this, it is considered that the product should only be indicated for the treatment of infestation caused by nematode parasites. Efficacy against larval and immature stages of *Toxocara canis* in dogs has been confirmed, but efficacy against *Toxascaris leonina* has only been shown for the adult form of this parasite.

Based on the CVMP review of data on quality, safety and efficacy, the CVMP considered by consensus that the benefit/risk profile of Advocate spot-on solutions was favourable in the treatment of cats, dogs and ferrets suffering from, or at risk from, mixed parasitic infections.

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