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Veterinary Medicines and Product Data Management

Scientific discussion

COMFORTIS (EMEA/V/C/002233)

This module reflects the initial scientific discussion for the approval of Comfortis (as published in August 2011). For information on changes after this date please refer to the document "Changes since initial authorisation of medicine".

1. Summary of the dossier

The product was eligible for the Centralised procedure under Article 3(2)(a) of Regulation (EC) No. 726/2004.

Comfortis chewable tablets for dogs contain spinosad and are available in five different strengths, 270 mg, 425 mg, 665 mg, 1040 mg and 1620 mg. The route of administration is oral, and the target species is dogs. The (chewable) tablets are presented in packs of 6 or 36 tablets. The product is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis)* in dogs. Comfortis can also be used as part of a dermatological treatment strategy for the control of Flea Allergy Dermatitis (FAD).

2. Quality assessment

Composition

Spinosad is a fermentation product produced by a species of Actinomycetes, *Saccharopolyspora spinosa* and is a mixture of spinosyns A and D. The spinosyns represent a novel class of insecticides and were discovered in 1988. Structurally these compounds are macrolides (non-antibacterial) and contain a unique tetracyclic ring system to which two different sugars are attached.

Comfortis tablets are presented in five different strengths (270 mg, 425 mg, 665 mg, 1040 mg and 1620 mg spinosad) with an active substance content of 53.33%w/w. The active substance, spinosad (common name), is a mixture of spinosyn A and spinosyn D. Approximately 90% of spinosad is comprised of spinosyns A and D. Of that 90%, the ratio of spinosyn A to A+D is 0.85

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when calculated as spinosyn A/(spinosyn A+D). The potency of spinosad is based on factors A and D and was justified.

The active substance is declared differently in the active substance and in the product, respectively. In the active substance, the specification distinguishes between spinosyn factors exhibiting greater than 90% activity excluding spinosyn A + D, and between spinosyn factors with less than 90% activity. In the product on the other hand, only spinosyn factors A and D are specified, and all other spinosyn factors are regarded as related substances. As a consequence, during manufacture of the product, the amount of spinosad added is adjusted based on the spinosyn A + D content only.

Well established excipients are used in the manufacture of Comfortis tablets, pharmaceutical grade microcrystalline cellulose and hydroxypropyl cellulose (serving as a diluent and as a binder), croscarmellose sodium (disintegrant), colloidal silicon dioxide (glidant) and magnesium stearate (lubricant). Artificial powdered beef flavour (gamma irradiated) is included to achieve palatability of the tablets. This flavouring agent has been used previously in several authorised veterinary medicinal products.

Container

The finished product is presented in blister packs (six tablets per blister strip) inside a folding cardboard carton. The blister foil (clear PCTFE/PE/PVC laminate) is sealed with a PVC based heat seal coating (lacquered) aluminium foil (the product contact surface is PVC).

Development pharmaceutics

The choice of the excipients has been conclusively justified. It has been shown that the active substance is completely released within 30 minutes. This complies with the provisions of the European Pharmacopoeia (Ph. Eur.) 2.9.3 and Ph. Eur. 5.17.1 monographs (a single-point acceptance criterion is sufficient).

Method of manufacture

The manufacturing process is fully described and comprises wet granulation, followed by drying, then blending and compression of tablets. It is regarded as a standard manufacturing process. A comprehensive description of the manufacturing method and equipment utilised is provided in the documentation.

Control of starting materials

Active substance

Spinosad is a fermentation product produced by a species of Actinomycetes, *Saccharopolyspora spinosa* and is a mixture of spinosyns A and D. Spinosad (pharmaceutical grade) is not listed in a Pharmacopoeia.

Production of the active substance is described and well controlled. The proposed specification for the active substance is appropriate to control its quality. Information about primary reference material is available. The validations of the analytical methods used for control of the active

substance are considered satisfactory and in compliance with relevant VICH guidelines. The studies performed confirm that the analytical methods used are stability indicating. Appropriate pilot and production scale batch data have been provided.

The proposed re-test period of 24 months has been justified by stability data.

Excipients

Cellulose microcrystalline (Ph. Eur.)
Hydroxypropylcellulose (Ph. Eur.)
Croscarmellose Sodium (Ph. Eur.)
Silica, colloidal anhydrous (Ph. Eur.)
Magnesium stearate (Ph. Eur.)
Artificial powdered beef flavour (gamma irradiated) contains pig liver powder (in house specification)

All the excipients conform with the respective monograph of the European Pharmacopoeia except the flavouring agent, for which a comprehensive in house specification is applied.

Packaging materials

Appropriate specifications are provided for the primary packaging materials (blister pack components).

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

There is no TSE risk associated with the use of the Artificial Powdered Beef Flavour. Additionally, none of the other materials used in the manufacture of Comfortis tablets carries any TSE risk. Magnesium stearate is of vegetable origin. A declaration has been submitted that no starting materials are used as defined in the "Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01-Rev.2)".

Control tests during production

All the in-process controls are defined and have been justified.

Control tests on the finished product

Adequate specifications and routine tests have been described to ensure the appropriate and consistent quality of the finished product.

The following methods employed comply with the Ph. Eur.:

- Uniformity of Dosage Units
- Dissolution (performed in 0.1N HCL (0.02% Tween 80) at 75 rpm and 100 rpm (USP apparatus 2 (paddles))
- Microbial Limit Tests (the limits are in accordance with Ph. Eur. 5.1.4)

- Moisture content

The identity of spinosad is confirmed by two independent tests, IR and (spinosyn factors A + D assay) HPLC retention time.

The content of spinosyn factors A + D and of related substances is determined by a reversed phase (RP) HPLC method with isocratic elution and UV detection at 250 nm. The method is demonstrated to be stability indicating.

Batch results of pilot scale batches, which comply with the specification, have been presented.

Stability

The stability data presented satisfactorily demonstrate the claimed stability of 36 months for the tablets packaged in the proposed blister packs. No information on the photostability of the finished product has been provided, but an appropriate recommendation to keep the blister in the outer cartoon is included in the SPC and on the outer carton. Since none of the tablet strengths possess a score line, no in-use stability data have been provided for half tablets.

Overall conclusions on quality

The data provided on the active ingredient are satisfactory. Comprehensive information on the manufacture and characteristics of the active ingredient, spinosad, is provided, and routine tests and specifications are considered sufficient to assure the appropriate and consistent quality of the active substance. The proposed re-test period of 24 months is acceptable.

The excipients used are commonly employed in such tablet formulations and are acceptable, as are the packaging materials. All excipients contained in the tablets, except the Artificial Powdered Beef Flavour (containing pig liver powder), are of pharmacopoeial grade quality. The microbiological and virological safety of the Artificial Powdered Beef Flavour have been substantiated. There are no concerns in relation to TSE with any of the ingredients of the product.

The rationale for the choice of the formulation is acceptable.

An acceptable specification has been developed for release of the tablets. Control methods have been validated and the specification is considered to be relevant for a product of this type.

The stability data provided reflect the current VICH guidance. A shelf-life of 3 years was proposed, and the stability data on the finished product provided so far are acceptable as primary stability information. Data demonstrate stability of the product under both long term and accelerated storage conditions. The SPC and product information include the appropriate information.

3. Safety assessment

Pharmacodynamics

Please refer to part 4.

Pharmacokinetics

The pharmacokinetic profiles of spinosyns A and D, the main constituents of spinosad, have been characterised in a number of GLP compliant, well designed laboratory studies in rats.

Absorption

Following a single oral dose of 10 mg/kg or 100 mg/kg bw, or repeat oral doses of 10 mg/kg bw for 14 days (gavage), spinosyns A and D proved to be rapidly and extensively absorbed (bioavailability ~ 66%), with maximum plasma levels achieved at approximately 2-6 hours post-dose.

(Plasma/Tissue) Distribution

The parent molecules are broadly distributed in a variety of tissues. Amounts of less than 1% of the administered dose at 168 hours (7 days) post-dose suggest that accumulation is unlikely.

Metabolism

Spinosyns A and D are extensively metabolised. In a radiolabelled study using spinosyn A, conjugation with glutathione was identified as the major route of metabolism and biliary excretion was identified as the major route of elimination of ^{14}C-spinosyn A. The major metabolite of spinosyn A identified in the bile was the glutathione conjugate. Other metabolites identified were the glutathione conjugate of N-demethylated spinosyn A and a glutathione conjugate of O-demethylated spinosyn A.

Cysteine conjugates of spinosad, possibly resulting from further metabolism of the glutathione conjugates by gut flora, have also been identified. A similar metabolite profile was identified in another radiolabelled study using ^{14}C-spinosyn D. The main metabolites identified in the faeces were the O- and N-demethylated spinosyn D, and glutathione conjugates of the demethylated spinosyn D.

Excretion

The principle route of excretion is in the faeces, with more than 80% of the administered dose, of which the majority (~70%) was recovered in faeces within the first 24 hours post-dose. Faecal elimination appears to be biphasic with an initial elimination half-life of ~6 hours and a terminal half-life of ~30 hours. The test compound was also excreted to a lesser extent in the urine. As in the faeces, the elimination in urine appeared to occur in a biphasic manner.

In conclusion, the provided data demonstrate that following oral administration, ^{14}C-spinosyns are absorbed, extensively metabolised and rapidly eliminated in the faeces. There are no major differences in the ADME profiles of the two major factors of spinosad (that is, spinosyns A and D). No differences in the pharmacokinetic behaviour in relation to gender were identified. It can be concluded that the rats in the toxicity studies were exposed to significant systemic concentrations of spinosad.

Toxicological studies

Single dose toxicity

Most of the submitted acute toxicity studies were GLP compliant and in accordance with the

appropriate OECD Guidelines. It has been shown that spinosad has low acute toxicity following oral, intraperitoneal, and inhalation exposure. No systemic effects were observed in rabbits after dermal application up to a dose of 5000 mg/kg.

Repeat dose toxicity

Three oral subchronic toxicity studies were conducted. One study was carried out in mice (10/sex/dose) at dietary dose levels of 0, 0.005, 0.015, 0.045, or 0.12% spinosad for 13 weeks. After 6 weeks of treatment, a few mice (both sexes) fed 0.12% spinosad had died, and all other animals from this dose group were sacrificed on day 44 due to inanition and cachexia. Decreases in body weights and anaemia were observed at doses of 0.045% and 0.12%. The most prominent histopathological lesions were dose-dependent vacuolation of lymphoid, histiocytic, and epithelial cells of numerous tissues and organs which ultrastructurally corresponded with lamellar inclusion bodies. These findings were diagnosed as phospholipidosis induced by cationic lipophilic drugs. A NOAEL of 0.005% equivalent to 6 mg/kg bw/day for males and 8 mg/kg bw/day for females was identified in this study. At the next higher dose (0.015%), stomach lesions, bone marrow necrosis, and changes in organ weights were observed.

Similar effects were observed in the subchronic toxicity study in rats administered spinosad in their diet for 3 months at dose levels of 0, 0.05, 0.1, 0.2, or 0.4%. In the highest dose group 50% of the animals died or were killed moribund after 5 to 6 weeks of treatment. All other animals in this dose group were killed on day 44 of the study. Decreases in body weights were observed at 0.2 and 0.4% spinosad, and anaemia observed at 0.2% spinosad. Vacuolation was observed at all doses in nearly the same tissues and organs as in mice, except that no lesions were found in the tongue and ovaries and in contrast to the mice, the rats' caecae, testes, prostates, and thyroids were additionally affected. A NOEL was not identified in this study. The LOAEL was 0.05%, which is equivalent to 34 mg/kg bw/day.

A second subchronic toxicity study was performed in rats. Dietary levels of spinosad were 0, 0.003, 0.006, 0.012, and 0.06% administered for 13 weeks. Recovery groups fed 0 or 0.06% spinosad in the 13-week period were subsequently given non-medicated feed for four weeks in order to investigate the reversal of spinosad-induced effects. The only treatment related effect was vacuolation in the thyroid of rats of both sexes given 0.06% spinosad. After the 4 week recovery period, the incidence and severity of vacuolation was reduced compared to those at 13 weeks indicating potential reversibility. The NOEL was 0.012%, which is equivalent to 8.6 mg/kg bw/day for males and 10.4 mg/kg bw/day for females.

A 28-day dietary toxicity study was performed in rats with spinosad factors A and factor D at dietary levels of 0.1% and 0.3% each. Decreases in body weights, anaemia, and vacuolar changes in organs and tissues were induced by 0.1 and 0.3% spinosad, 0.3% factor A and/or 0.3% factor D. The results indicated that similar effects were induced by spinosad or its main components factors A and D. Spinosad and factor A revealed to be equipotent at a dose level of 0.3%, while factor D was less toxic.

In both mice and rats the most prominent effect of spinosad was dose-dependent cytoplasmic vacuolation ultrastructurally characterised by lamellar inclusion bodies in numerous organs and tissues. This condition is diagnosed as phospholipidosis induced by intracellular accumulation of cationic amphiphilic/lipophilic drugs (CAD) and subsequent formation of indigestible complexes with phospholipids as well as the inhibition of lysosomal phospholipases. A large variability has been shown among species or strains with respect to the tissue specificity and severity of the condition induced by a particular CAD.

As evident from the target animal safety studies, spinosad also induces phospholipidosis in the dog after repeated dosing and/or at higher dose levels than the recommended dosing schedule. (This is further discussed in the 'Target animal tolerance' subsection of section 4 of this report.)

Two dermal repeat-dose toxicity studies have been performed in rabbits. In the first study, rabbits received 15 applications during the 21 day interval at doses of 0, 100, 500, or 1000 mg/kg for six hours daily. In the second study rabbits were treated for 21 days at dose levels of 0 or 1000 mg/kg for six hours daily. The test material was applied on the shaved backs under a gauze patch or cheese cloth covered with non-absorbent cotton. No dermal effects and no systemic toxicity were observed in these studies. It was concluded that spinosad was not irritating to the skin of rabbits.

Tolerance in the target species

Relevant studies are covered in the 'Target animal tolerance' subsection of section 4 of this report.

Reproductive toxicity

A two-generation reproductive study was conducted in rats in order to assess the potential parental, reproductive, and neonatal toxicity of spinosad following dietary exposure. Sprague-Dawley rats, approximately 6 weeks of age at the beginning of the study, were given diets that provided 0, 3, 10 or 100 mg spinosad/kg body weight daily, 7 days/week, for two generations. After ten weeks of dietary exposure the first parental generation (P1) was mated twice to produce F1a and F1b litters. After weaning, groups of male and female pups were randomly selected to become the second parental generation (P2). After dietary exposure of approximately 12 weeks, the P2 adults were mated to produce the F2 litters. Adult females received the appropriate dietary concentration of spinosad throughout gestation, lactation and until all litters were weaned. Males received test diets after the mating periods until they were necropsied.

Signs of parental and developmental toxicity became evident at 100 mg/kg bw/day by decreased food consumption (P1 animals only), decreased body weights, increased incidence of dystocia, increased incidence of vaginal bleeding and female mortality during lactation, decreased gestation survival (F2 litters only), decreased pup body weights, decreased neonatal survival (F2 litters only). Histopathological analysis revealed cytoplasmic vacuolation in numerous organs and tissues at this dose level. No effects on fertility were observed.

Neonatal toxicity, which was induced in the highest dose group, was probably maternally mediated. The NOAEL for parental and reproductive/developmental toxicity was 10 mg/kg bw/day.

Developmental toxicity studies were performed in Sprague-Dawley rats and New Zealand White rabbits.

Pregnant rats were treated orally via gavage from days 6 to 15 of gestation with doses of 0, 10, 50, 150, 200, 250, or 300 mg/kg bw daily. Dose levels of 150 mg/kg bw/day and above resulted in maternal toxicity as evidenced by decreases in body weight and food consumption. Embryonal/ foetal parameters were not affected at any dose level. The NOEL for maternal toxicity was 50 mg/kg bw/day and the embryonal/foetal NOEL was 300 mg/kg bw/day.

In the second rat study, daily doses of 0, 10, 50, or 200 mg/kg bw were administered. On day 21 of gestation all animals were euthanised and necropsied. The uteri were examined for implantations and resorptions, and the ovaries examined for corpora lutea. The foetuses were removed, weighed, and examined for external, visceral, and skeletal alterations. Maternal toxicity was induced by oral doses of 200 mg spinosad/kg bw/day as evidenced by decreases in body

weight on day 12 and body weight gains on days 6 to 9, 9 to12 and 6 to 16. No treatment related effects were observed on reproductive or embryonal/foetal parameters at any dose level tested. Microphthalmia was diagnosed in one foetus from a dam given 50 mg/kg/day and in two foetuses from dams given 200 mg/kg bw/day. These malformations were not considered treatment related because the incidence was within the range of historical controls. The NOEL for maternal toxicity was 50 mg/kg bw/day, the NOEL for embryonal/foetal toxicity and teratogenicity was 200 mg/kg bw/day.

Pregnant rabbits were treated from days 7 to 19 of gestation with daily doses of 0, 50, 100, 200, or 400 mg/kg bw. A decreased faecal production was observed, the incidence of which was dose-dependent. Due to excessive maternal toxicity, manifested as marked decreases in food consumption, all animals given 100, 200, and 400 mg/kg bw/day were euthanised on day 13 of the study. Haemolysed blood was present in the stomach of dams of the 50 mg/kg bw group. No adverse effects on embryonal/foetal or reproductive parameters were noted in this dose group.

A maternal NOEL could not be identified in this study. The maternal LOAEL was 50 mg/kg bw/day. The NOAEL for embryonal or foetal parameters was 50 mg/kg bw/day.

In the second rabbit study, daily doses of 0, 2.5, 10, or 50 mg/kg bw/day were administered on days 7-19 of gestation. Animals were euthanised on day 28 of gestation and a limited necropsy was conducted. Ovaries were examined for corpora lutea, and uteri were examined for implantations and resorptions. Foetuses were removed from the uterus and weighed, sexed, and examined for external, visceral and skeletal alterations.

Adverse effects were observed in the highest dose group only and consisted of decreased faecal output associated with body weight loss and decreased feed consumption. Two females aborted due to severe inanition. No embryo- or foetotoxicity and no teratogenic effects were observed at any dose level.

The maternal NOAEL was 10 mg/kg bw/day and the NOAEL for embryonal/foetal toxicity and teratogenicity was 50 mg/kg bw/day.

Conclusions on reproductive toxicity including developmental toxicity:

The rat and rabbit studies provided no evidence for adverse effects of spinosad on male or female reproductive functions at dose levels below parental toxic levels. At maternally toxic doses decreased litter size, decreased neonatal survival and decreased pup body weights were observed in the multi-generation study in rats. No developmental effects occurred at maternally toxic levels. The NOAELs for parental and reproductive/developmental toxicity were 10 mg/kg bw/day.

The lowest NOAEL for maternal toxicity determined in the developmental studies was 10 mg/kg bw/day in rabbits and the NOEL for embryonal/foetal toxicity and teratogenicity was 50 mg/kg bw/day both in rats and rabbits.

It is noted that these NOAELs are close to the therapeutic dose range which is recommended for dogs (apart from the different dosing regimes, i.e., single dosing in dogs once every four weeks, daily dosing in laboratory animals). Furthermore, in line with the OECD guidelines, the protocols did not include histopathological examinations. Therefore, the NOAEL for microscopic effects (such as phospholipidosis) may have been lower if they had been examined.

Reproductive toxicity in the target animal species is covered in the 'Target animal tolerance' subsection of section 4 of this report.

Mutagenicity / genotoxicity

A full battery of *in vitro* and *in vivo* tests has been carried out. All assays were conducted up to the designated limit doses or up to doses demonstrating induced cytotoxicity. Treatment with positive control chemicals induced clear positive results in the presence and absence of metabolic activation, an S9 fraction prepared from the livers of rats. All studies were clearly negative indicating that spinosad has no genotoxic potential.

Spinosad neither induced point mutations in bacteria, nor gene mutations, nor chromosomal aberrations, nor unscheduled DNA synthesis in mammalian cells *in vitro*. Spinosad was also found to be negative in an *in vivo* micronucleus bone marrow test. Based on these results, spinosad is considered to be non genotoxic.

Carcinogenicity

An 18-month oral toxicity and carcinogenicity study in mice was carried out using doses of 0, 0.0025, 0.008, or 0.036% spinosad in the diet. Females receiving high doses were killed on day 455 of the study, due to marked signs of toxicity indicative of exceeding the maximum tolerated dose level. A supplementary study was therefore carried out in which both males and females were dosed with 0, 0.008 or 0.024% spinosad in the diet. The main effects seen in both studies included decreased body weight, thickened glandular mucosa, hyperplasia, and inflammation of the stomach, and degeneration, inflammation and/or vacuolation in several tissues. The incidence of treatment related histopathological changes increased with time. The incidence of tumours in both males and females was not increased relative to controls in any of the tested dose groups. The overall NOAEL of both chronic toxicity studies in mice was found to be 11.4 mg/kg bw/day. The highest dose levels tested were 50.9 mg/kg bw/day and 41.5 mg/kg bw/day for males and females respectively.

A combined chronic toxicity/neurotoxicity/oncogenicity study was conducted in rats. The rats were dosed with 0, 0.005, 0.02, 0.05, or 0.10% spinosad in the diet for 12 or 24 months. The highest dose level was found to exceed the maximum tolerated dose level. Treatment related effects found in the 0.02% group comprised of vacuolation of the thyroid glands only. Other treatment related effects, in the higher dosed animals included accumulation of reticuloendothelial cells in the mesenteric lymph nodes and inflammation of the lung. Except for the effects in the thyroid gland, all treatment related findings did not progress or were resolved over time. The NOAEL after 24 months was set at 0.005%, which is equal to a dose of 2.4 mg/kg bw/day. There were no increases in tumour incidence noted in any of the tested dose-groups compared to controls. The highest dose levels tested were 49.4 mg/kg bw/day and 62.8 mg/kg bw/day for males and females respectively.

Studies of other effects

Acute and chronic neurotoxicity studies were performed in accordance with GLP requirements. The NOAEL for acute neurotoxicity was 2000 mg/kg bw. For general acute toxicity, a NOAEL of 200 mg/kg bw was established due to a transient decrease in body weight at 630 mg/kg bw. In a 13 week neurotoxicity study, a NOEL for neurotoxicity was correctly set at 0.06% in the diet (42.7 mg/kg bw/day for males and 52.1 mg/kg bw/day for females). In a 1 year study, the neurotoxic NOEL was greater than 0.1% in the diet, which represented doses of 50.7 mg/kg bw/day for male rats and 63.8 mg/kg bw/day for female rats. A NOAEL for general toxicity was set at 0.02% (9.8 mg/kg bw/day for males and 12.4 mg/kg bw/day for females).

It can be concluded, that spinosad did not show any acute, subchronic and chronic neurotoxic potential.

User safety

A user safety assessment on the active substance, and an assessment on the formulation in accordance with the Guideline on User Safety (EMEA/CVMP/543/03-FINAL), have been provided.

Several studies were submitted assessing the skin and eye irritating, and skin sensitising potentials of spinosad. All studies were performed to GLP requirements. It is evident that spinosad is not irritating to the skin of rabbits, or sensitising to the skin of guinea pigs, but it is a reversible, slight to moderate eye irritant in rabbits. No studies with the formulation were submitted.

In respect to the intended once monthly use of this product, exposure for the user could be considered as acute dermally and acute orally following the accidental ingestion of one tablet (1620 mg) by children. The oral exposure is estimated to be 162 mg/kg bw. It can be agreed that dermal contact with spinosad is not considered to induce adverse effects locally or systemically via dermal absorption/hand to mouth contact in humans. No signs of systemic toxicity were observed in rabbits following dermal exposure of 5000 mg/kg bw. All excipients are commonly used in human pharmaceutical preparations and are listed in the Excipients Handbook. They are all considered non-toxic with regards to oral exposure and do not increase the absorption of spinosad. Although some of the components (hydroxypropyl cellulose, croscarmellose sodium, magnesium stearate) have a slight skin irritating potential, it was concluded that the extremely low exposure to potentially irritating excipients represents no risk of irritation for the product. However, appropriate advice to wash hands after use of the product is included in the SPC (and other product information). This warning phrase can be considered satisfactory to prevent accidental hand-to-eye contact with spinosad. There is no health concern for the adult user, including pregnant and nursing women, when the advice for correct use of the product is followed.

Possible health risks for children after acute oral exposure have also been discussed. Although the systemic availability of the product may be limited, emesis is an adverse effect commonly seen in dogs already at therapeutic doses and should be regarded as a possible acute adverse effect for the user, especially for children, after accidental ingestion of the product. A worst case scenario showed that the calculated dose is clearly above the effect dose in dogs without applying any uncertainty factor. Furthermore, although the product might be vomited following accidental ingestion, some absorption could occur. Therefore, systemic effects after accidental ingestion cannot be excluded. The possible ingested dose is similar to the relevant no-effect level for general acute toxicity at 200 mg/kg observed in an acute neurotoxicity study in rats. The CVMP consider this NOEL to be the most relevant for calculation of margins of safety for the user. When applying at least an uncertainty factor of 10 for extrapolation from rat to human, this results in no margin of safety. Therefore, in addition to the child resistant packaging, appropriate warning phrases are included in the product literature (as detailed in the next paragraph).

A study investigating the child resistance of the packaging was performed by an accredited laboratory for testing child-resistant packaging according to the Consumer Product Safety Commission's (CPSC) protocols using 50 children (of 42-51 months of age). The results indicated that the package is child-resistant according to the standards for poison prevention packaging according to current USA C.F.R. Title 16, Part 1700. The potential health risks after accidental ingestion of misplaced tablets is minimised by addition of the following warnings in the SPC (and other product information): "Children should not come into contact with the veterinary medicinal

product. Accidental ingestion may cause adverse reactions." Child-resistant packaging and all the proposed warnings are considered to satisfactorily minimise the risk for children.

Environmental risk assessment (ERA)

An ERA assessment in line with the VICH guideline GL6 was provided. Comfortis tablets are for the treatment and prevention of flea infestations in dogs, using single monthly oral doses of 45-70 mg/kg bw spinosad. In dogs, spinosad proved to be extensively metabolised and slowly excreted, primarily in the faeces, and to a lesser content in the urine. Comfortis tablets are for use in non-food animals and will be administered to individual animals only. Therefore the ERA assessment stops in phase I (according to VICH GL6).

Spinosad is not expected to pose a risk to the environment when used in dogs at monthly intervals during the flea season.

Overall conclusions on the safety documentation

Following oral administration to rats, ^{14}C-spinosyns are absorbed, extensively metabolised and rapidly eliminated in the faeces. There are no major differences in the ADME[1] profiles of the two major factors of spinosad, spinosyns A and D. No differences in the pharmacokinetic behaviour in relation to gender could be identified. It can be concluded that the rats in the toxicity studies were exposed to significant systemic concentrations of spinosad.

Spinosad has a low acute toxicity following oral, intraperitoneal, and inhalational exposure. No systemic effects after dermal application were observed in rabbits up to a dose of 5000 mg/kg.

The most prominent effect of spinosad in repeat dose toxicity studies in mice and rats is dose dependent cytoplasmic vacuolation in numerous organs and tissues. This condition is diagnosed as phospholipidosis induced by intracellular accumulation of cationic amphiphilic/lipophilic drugs (CAD). A large variability has been shown among species or strains with respect to the tissue/organ specificity and severity of the condition induced by a particular CAD.

Spinosad induces phospholipidosis in the target species, dogs, at doses higher than the recommended dosing schedule. (See Part 4.)

No dermal effects and no systemic toxicity were observed in two dermal repeat dose toxicity studies in rabbits. It was concluded that spinosad was not irritating to the skin of rabbits.

The studies on reprotoxicity performed in rats and rabbits provided no evidence for adverse effects of spinosad on male or female reproductive functions at dose levels below parental toxic levels. At maternal toxic doses, decreased litter size, decreased neonatal survival and decreased pup body weights were observed in the multi-generation study in rats. No developmental effects occurred at maternal toxic levels. The NOAELs for parental and reproductive/developmental toxicity were 10 mg/kg bw/day. The lowest NOAEL for maternal toxicity determined in the developmental studies was 10 mg/kg bw/day in rabbits and the NOAEL for embryonal/foetal toxicity and teratogenicity was 50 mg/kg bw/day both in rats and rabbits. These NOELs are close to the therapeutic dose range which is recommended for dogs (although there are differences in dosing regimes, that is, single dosing in dogs once every four weeks, daily dosing in laboratory animals).

[1] ADME: Absorption, Distribution, Metabolism, Excretion

Reproductive toxicity in the target animal species is covered in the 'Target animal tolerance' subsection of section 4 of this report.

Spinosad is not considered to be genotoxic, nor carcinogenic and did not show any acute, subchronic and chronic neurotoxic potential. Spinosad was not irritating to the skin of rabbits and not sensitising to the skin of guinea pigs. It caused reversible, slight to mild irritation in the eyes of rabbits.

In respect to spinosad, there is no health concern for the adult user, including pregnant and nursing women, when administering the product in accordance with the SPC and product information which include appropriate warnings and advice for users. The child-resistant nature of the packaging and the relevant warnings in the SPC and product information are considered satisfactorily to minimise the risks for children.

Spinosad is not expected to pose a risk to the environment when used in dogs at monthly intervals during the flea season.

4. Efficacy assessment

Pharmacokinetics

The pharmacokinetic properties of the two major constituents of spinosad, spinosyns A and D, have been evaluated in a number of non-GLP/GLP compliant laboratory studies in Beagle dogs following single or repeat administration of spinosad at different dose levels. The dose levels tested encompass the proposed therapeutic dose range of 45 to 70 mg/kg bw. Plasma concentrations were determined by LC-MS/MS. Different oral formulations were used and the final tablet formulation intended for the EU market was only used in one study (S12 study on dose linearity: 270 mg tablets). However, *in vitro* dissolution tests using the clinical trial formulations and the intermediate tablet strengths of the 425, 665 and 1040 mg intended for marketing produce comparable dissolution profiles revealing validity of the study results to all tablet strengths.

Absorption

Spinosyns A and D are rapidly absorbed after oral administration and extensively distributed (volume of distribution > 35 l/kg). Bioavailability is approximately 70%. Maximum plasma concentrations are achieved 2-4 hours post-dose and are in the range of 1917 to 6136 ng/ml (spinosyn A) and 340 to 1016 ng/ml (spinosyn D) depending on the dose (27-81 mg/kg bw).

Plasma/tissue distribution

As in the rat, the exposure (C_{max} and AUC) to spinosyns A and D in dogs proved to be consistently in a ratio comparable to that determined in the test formulation. Approximately 90% of spinosad is comprised of spinosyns A and D. Of that 90%, the ratio of spinosyn A to A+D is 0.85 when calculated as spinosyn A/(spinosyn A + D).

The exposure to spinosyn A and D (C_{max}, AUC) increased linearly with dose following repeated oral administration of spinosad 270 mg tablets (final formulation) at doses of 27, 54, and 81 mg/kg bw at 30 day intervals for 3 months. The mean elimination half life of spinosyn A ranged from 127.5 to 162.6 hours for the tested doses and monthly administrations. No accumulation was observed after three doses of 81 mg/kg bw were given, each 30 days apart.

It has been shown that the bioavailability of spinosad is increased when administered to dogs in the fed state. Advice is therefore given in the SPC and other product information that "The veterinary medicinal product should be administered with food or immediately after feeding. The duration of efficacy may be reduced if the dose is administered on an empty stomach."

An ADME study using radiolabelled ^{14}C-spinosad in dogs showed that the parent compound accumulated in perirenal fat, skin/ fat and the liver, but the data indicate that this accumulation had markedly decreased 21 days after administration of the dose.

Metabolism

As in the rat, spinosyns are extensively metabolised in dogs. As with rats, glutathione conjugation and demethylation were defined as the primary pathway of metabolism in dogs. The principle metabolites identified in the bile, faeces and in urine were demethylated spinosyns A and D, as well as glutathione conjugates of spinosyns A and D.

Excretion

The primary route of excretion is via the faeces, with approximately 35% and 50-55% of the administered dose excreted in the faeces 7 and 21 days after dose administration respectively. A further 16-19% of the administered dose is excreted via the urine.

It has been shown that when spinosad is administered to pregnant bitches (90 mg/kg bw on day 28 of gestation and again at one day prior to parturition), spinosyns A and D are excreted in substantial amounts in the colostrum/milk. Appropriate information is therefore included in section 4.7 of the SPC (and similarly in the package leaflet) "Spinosad is excreted in the colostrum and milk of lactating bitches and the safety of this for suckling puppies has not been sufficiently established. Therefore, during pregnancy and lactation, the product should only be used according to the benefit/risk assessment by the responsible veterinarian."

Pharmacodynamics

Spinosad possesses a broad spectrum of insecticidal activity. The mode of action has been studied in the standard insect model, the American cockroach, and in further studies on other insect species, and is characterised by involuntary muscle contractions, prostration with tremors, paralysis and death of the insect. These effects are primarily caused by a widespread neuronal excitation of the central nervous system, namely a persistent activation of nicotinic acetylcholine receptors and prolongation of acetylcholine responses. The underlying mechanism is different from other nicotinic agents such as neonicotinides (imidacloprid, nitenpyram), fipronil, milbemycin or avermectins (e.g., selamectin). Additionally, spinosad was shown to affect GABA neuronal receptors of insects which may contribute to its insecticidal activity. Spinosad is metabolised in insects to a limited extent and hardly penetrates through the insect cuticle. Therefore, spinosad exerts its insecticidal activity orally rather than by contact.

In mammalian *in vitro* assays spinosad and its major constituent, spinosyn A, proved to have no *in vitro* affinity to several important receptors of the mammalian central nervous system. No data demonstrating lack of affinity for the mammalian form of the nicotinic acetylcholine receptors have been presented. However, this is considered acceptable since data indicate a highly selective insecticidal activity of spinosad, and spinosad proved to be of low toxicity to mammalian species compared to older and more current insecticides. Acute, subchronic and chronic neurotoxicological studies in rats did not give evidence of neurotoxic effects.

The adulticidal activity of spinosad and/or individual spinosyn factors was evaluated in three *in vitro* assays on cat fleas, *Ctenocephalides felis*, using an artificial membrane feeding system. The signs of toxicity were similar to those reported and described in other insect species.

Like avermectins, spinosad is a substrate for P-glycoprotein (PgP) (canine MDR-1) which has been linked to neurotoxicity in Collies and related breeds. Target animal safety studies in avermectin-sensitive Collies revealed that spinosad alone, at oral doses up to 300 mg/kg bw (4.3X the maximum recommended dose), or in combination with milbemycin oxime at doses corresponding up to 5X the recommended dose, did not induce neurotoxicity. Nevertheless, a risk of neurotoxic effects when given with off-label high dose ivermectin remains, as evident from information gained from post-marketing surveillance reports in the US, indicating adverse effects in dogs following concomitant use of off label high dose ivermectin with spinosad. Appropriate information and advice is therefore included in the SPC and product literature accordingly.

Development of resistance

The spinosyns are a novel group of insecticides which primarily target nicotinic acetylcholinergic receptors (nAChRs). The binding site of neonicotinoid insecticides like imidacloprid or nitenpyram is different from the spinosad group molecules, revealing distinctly different modes of action of both insecticidal groups. Resistance in fleas has not been reported yet. No warning relating to resistance is therefore currently deemed necessary.

Target animal tolerance

Target animal safety studies

A large set of studies of spinosad in dogs was presented, including the following subchronic and chronic toxicity studies and overdose/margin of safety studies:

- One 28 day study with daily doses up to 120 mg/kg bw (1.7X the max. label dose),
- One 91 day study with daily doses up to 45 mg/kg bw (lower label dosing limit),
- One 12 month study with low daily doses (up to 8 mg/kg bw),
- One overdose study with treatment with 90-100 mg/kg bw on 10 consecutive days,
- One study using the approximate label dose (45-90 mg/kg bw) and overdose (treatment extended to 3 and 5 consecutive days) with monthly intervals in puppies, starting at 6 weeks of age, over 6 months.

There are no studies on acute overdose but overdosing (elevated spinosad plasma levels) was reached by consecutive dosing.

Emesis is identified as the limiting factor for safe dosing if the product is used at the label interval (monthly). Emesis occurs in a certain proportion of dogs which were dosed at the upper end of the label and above.

Young animals appear to be more sensitive to emesis than older ones. In the 6 month puppy study, 42% of treatment-naive 6 week old puppies vomited within the first hour following administration of a spinosad containing tablet, and 60% of these dogs vomited again when re-

dosed. The product is therefore contraindicated for use in young dogs. The chosen age restriction (>14 weeks) is based on data from the 6 month puppy study and confirmed by data from the clinical field studies. There may be a certain adaptive mechanism to spinosad. At the age of 14 weeks the puppies had already been treated twice before and it is unclear if, and to what extent, an adaptive mechanism influenced the rate of adverse events in these dogs.

In special studies on emesis, dogs were treated with 180 mg/kg bw orally or a pharmacokinetic equivalent directly intraduodenally, or per i.v. infusion. After oral administration, absorption of spinosad starts at about 20 minutes post dosing and takes place to a high extent within the first hour post application. These studies reveal that emesis is not primarily gastrically but probably gastrointestinally mediated. Accordingly, vomitus usually occurs after the substance has reached the small intestine, i.e., after 30 mins to approximately 2 hours post administration. Although the mechanism triggering emesis after treatment with spinosad has not been fully identified, data indicate a gastrointestinal trigger rather than a systemic effect.

At 2.5X times the maximum recommend single dose (overdose), the incidence of emesis increases and about 86% of the dogs vomit. Most dogs vomit more than once, some several times.

Cell vacuolation/phospholipidosis, which is a typical histopathological and most probably reversible finding in toxicity studies in rats and mice, was also found in dogs from a daily dose of 10 mg/kg bw/day onwards for 91 days. Histological findings of phospholipidosis were also observed in puppies treated with 3X (75% of the dogs) and 5X (100% of dogs) the maximum recommended dose at monthly intervals for 6 months. Unlike in the rat studies, the study design in dogs did not allow for demonstrating reversibility of the observed changes. The applicant demonstrated that phospholipidosis was unlikely to occur at the recommended dosing schedule using the bench mark dose (BMD) approach.

In addition, despite the finding of cell vacuolation in lymphoid tissues in the 3X group, these dogs did not show any abnormality that could be related to this change. Cell vacuolation is therefore considered to be a treatment-related response that results in a change in morphology, but not in an impairment of any body function. It would therefore, by definition of the OECD (OECD series on testing and assessment No. 32, 2001), not count as an adverse effect.

Other observed events included mild and transient elevations of alanine amino transferase at doses of up to 100 mg/kg bw/day for 10 days.

One dog reproductive toxicity study was presented. The study comprises of three equal sized groups of dogs (control, label dose, 3X overdose) which were treated at monthly intervals over about 6 months, covering the period of about 2 months pre-mating until weaning. The only maternotoxic effect observed was emesis in about half of the treated bitches, in each of the dose groups, within 1 hr post dosing. The study revealed a few early pregnancy losses in treated bitches, but final conclusions could not be drawn due to the small sample size.

Spinosad is excreted with the milk in substantial amounts, and the sensitivity to spinosad increases in younger animals. Due to the small group size in this study, and flaws in the data presentation, it is not possible to establish the safety of spinosad for suckling puppies of treated dams.

The SPC (sections 4.7 and 5.2) and other product information contain appropriate warnings that the safety of the product for pregnant and lactating bitches and weaning puppies has not been sufficiently established. The product should therefore only be used according to the benefit/risk assessment by the responsible veterinarian.

Target animal safety in clinical field studies

Three clinical field studies were evaluated for adverse events: two studies conducted in Europe (dose range 45-70 mg/kg bw), and one study conducted in the USA (dose range 30-90 mg/kg bw). Emesis proved to be the most frequently occurring adverse effect related to treatment in all studies.

In order to establish a dose-response curve for the parameter emesis rate (= incidence of vomiting), a meta-analysis of the three clinical field studies was conducted. Using dose as a continuous variable it was demonstrated that the emesis rate increased more than linearly with increasing dose over the whole range from 30 – 90 mg/kg bw. However, when comparing emesis rates in dose categories of 12.5 mg/kg bw step increases, there was no significant difference between the lower and the upper half of the recommended dose range within the categories or between the upper (57.5 - 70 mg/kg bw) and the next higher category (70 - 82.5 mg/kg bw). When pooling the data within the recommended dose range there was a statistically significant difference between the entire dose range (45 - 70 mg/kg bw) and at doses above the maximum recommended dose (70 - 82.5 mg/kg bw).

The frequency of emesis on the day of, or the day after, administration of the label dose 45 - 70 mg/kg bw was 5.6% after the first treatment and decreased after the second and third treatments to 4.2 and 3.6% respectively, identifying this adverse event as "common" (more than 1 but less than 10 animals in 100 animals). The emesis rate observed after the first and second treatments was higher (8%) in dogs dosed at the upper end of the dose band. If the maximum recommended dose was exceeded (70-95 mg/kg bw) the emesis rate after the first treatment increased considerably to 16.7%, becoming a very common adverse effect.

The incidence of emesis (at the label dose) was judged to be acceptable from a clinical point of view since the data demonstrated that emesis following treatment with spinosad was usually mild and a single episode, and which did not require veterinary intervention. Appropriate risk mitigation information on this adverse event is therefore included in sections 4.5, 4.6 and 4.10 of the SPC (and other product information) in order to comprehensively inform and advise both the prescribing veterinarian and the dog owner.

In addition, other adverse events for which treatment relation cannot be ruled out are diarrhoea, anorexia/decreased appetite and lethargy, which are also known from the preclinical studies.

In the studies performed in Europe, two dogs with a history of epilepsy had seizures while on spinosad treatment. Although this could be incidental, a related precaution on the use in such animals has been included in the SPC.

Adverse effects other than emesis are satisfactorily addressed in section 4.6 of the SPC (and other product literature).

Other serious adverse events occurring during the field studies were not considered to be treatment related.

The age restriction to dogs of >14 weeks derives from the preclinical margin of safety study (6 month puppy study) in conjunction with an analysis of the data derived from the clinical field studies. In the preclinical margin of safety study, reduced weight gain was observed in puppies <14 weeks. In a meta-analysis of all field studies, the vomiting frequency was somewhat higher in the lowest age group (6-10 weeks), while there was no detectable correlation between weight change and occurrence of one of the clinical signs emesis, diarrhoea or anorexia.

Dose determination / justification

The dose determination studies were conducted, either at independent companies or parasitological units from universities, as blinded laboratory studies using a randomised complete block design in which groups of treated animals were compared with untreated controls. GCP-principles were assured. A pre-study flea infestation was employed to ascertain the inherent ability of individual dogs to retain a solid flea population of >50 fleas/dog. Infestations obtained were used to rank the dogs for a randomised allocation to each study group.

Four exploratory dose determination studies were performed with various investigational formulations containing spinosad at varying potencies (10-100 mg/kg bw). Encouraging knockdown and residual activity against fleas was observed. Some acaricidal activity was also found (50 mg/kg bw orally) but a claim for this was not envisaged. No nematocidal activity was detected.

In a GCP-conforming dose determination study performed in the USA, groups of dogs were dosed with technical spinosad in gelatin capsules on study days 0, 30 and 60 at point dosages of 15, 20, 30 and 40 mg/kg respectively. Results suggest that a dosage of at least 40 mg/kg is necessary to achieve the threshold of ≥95% efficacy (arithmetic means) during the 4 weeks persistent period claimed against fleas (D30: 100%, D60: 100%, D91: 91.4%). The comparator active substance (imidacloprid) at its recommended dose showed consistent activity of 100% in the study period.

Two GCP-conforming dose determination studies conducted in Europe with dose rates of 30 or 40 mg/kg bw and covering periods of 1 and 3 months (i.e., two treatments, each given one month apart) however failed to demonstrate persistent pulicidal activity (>95% efficacy using arithmetic means) for four weeks after each spinosad treatment.

In order to explain the apparent discrepancy in residual efficacy between the American and the European dose confirmation studies, further investigations were initiated to ascertain a potential impact of feeding, flea strain or dog breeding on the performance of spinosad tablets.

Three GCP-conforming placebo controlled studies conducted in the USA were performed to evaluate the potential impact of feeding (time and whether dry or wet (canned) food) on efficacy. Equal sized groups of fasted dogs were treated orally with spinosad technical compound in gelatine capsules at a single dose (first study) or two consecutive doses one month apart (2^{nd} and 3^{rd} study) of 30 mg/kg bw.

The C_{max} and $AUC_{(0-t)}$ of spinosyns A and D were found to be significantly higher ($P<0.05$) for the fed groups than for the fasted group. There was no relevant difference in the absorption of spinosad either when the dogs were fed wet food or dry food. Correspondingly, spinosad revealed longer persistent pulicidal activity when administered to fed dogs compared to fasted animals. Appropriate advice and information is therefore included in the SPC and product literature that the product should be administered to dogs either with food or immediately after feeding, and that the duration of efficacy may be reduced administered on an empty stomach.

A GCP-conforming study conducted in Europe was undertaken to ascertain the potential impact of flea strains from a different origin (Italy, Ireland, USA, and Germany) on the persistent efficacy of spinosad after single dose of 30mg/kg bw. No significant impact of any flea strain on persistent efficacy could be demonstrated. However, overall, the results revealed that higher doses are necessary to reach the anticipated 4 weeks persistent efficacy period.

One study was designed to compare Beagles with other dog breeds, since the initial American dose determination study used mixed breed dogs whereas the European trials were carried out with pure bred Beagles. The retrospective analysis of plasma spinosad concentrations from dogs used in study showed no difference at one month post-treatment between blood levels in Beagles and other breeds. Again, the results revealed that higher doses than 30 mg/kg bw are necessary to reach the anticipated 4 weeks persistent efficacy period.

Since the European dose determination studies, conducted at 30mg/kg bw, did not comply with the 95% threshold in fleas, the applicant chose to increase the minimum dose to 45 mg/kg bw. In a further European non-GCP dose determination study, spinosad at a single dose of 45 mg/kg bw showed encouraging efficacy on day 30 post treatment (94%).

Dose confirmation

Seven GCP-conforming dose confirmation studies, using a dose range of 30-40 mg/kg bw which does not correspond to the proposed dose range of 45-70 mg/kg bw intended for the European market, were reported. The results showed that a minimum dose of 30 mg/kg bw is not sufficient to exhibit a consistent 4 week persistent efficacy period. However, a therapeutic activity of almost 100% is observable at 24 to 48 hours after treatment.

In order to confirm the persistent efficacy of 4 weeks, two dose confirmation studies have been performed with an oral dose of 45 mg/kg bw spinosad which resulted in efficacies of >98% up to day 23, while on day 30 somewhat lower efficacies of 85.6% and 91.6% were calculated, and 50% dogs with non-zero flea counts (up to 51 fleas per animal) were observed in the spinosad treated groups.

The knock down and speed of kill effectiveness after oral treatment with spinosad at 30-60 mg/kg bw was investigated in a GCP compliant controlled laboratory study in dogs experimentally infested with cat fleas. The results confirm the speed of killing fleas starting 30 minutes post-dose (36%) and reaching 100% of dead or moribund fleas within 4 hours post-dose. Section 5.1 of the SPC includes this information.

<u>Home environment studies:</u>

Four GCP compliant Simulated Home Environmental (SHE) studies were presented performed at independent laboratories using a blinded negative control randomised block design. Three studies conducted in the USA investigated the prevention of flea infestations (experimental flea infestations after commencement of treatment), while an Irish study investigated control of flea infestations (experimental flea infestation prior to first treatment).

In two of the studies conducted in the USA no robust flea life cycle could be established in the pen environment as evident from low flea counts on untreated dogs in the control pens. Of the two studies where a robust environmental challenge could be established, one (USA study) investigated the potential of spinosad to prevent infestations. In that study, monthly treatments with spinosad at oral doses of 30-87 mg/kg bw proved to be effective in preventing flea infestations with efficacy rates of 98-100% on all observation days. It should however be noted that the last artificial flea infestation was performed 14 days after the first treatment. The only challenge at present thereafter (the second and third dosing) and used in the efficacy calculation are survivors and/or hatched eggs from the fleas introduced early in the study. Thus, the study design is less than optimal and "mimics" a situation where the dog is confined to its home environment/isolated and treated for several months after a flea infestation has been observed. The other (Irish) study

investigated the potential of monthly treatments for controlling an established flea problem. After monthly treatments at oral doses of 30-60 mg/kg bw, efficacy rates were 91.02-66.90% after the first dose, 96.47-98.59% after the second dose, and 99.95-99.21% after the third dose. It is considered that the efficacy rates in the first 4 weeks after commencement of treatment (91.0, 80.3, 68.8 and 66.9% for day 7, 14, 21 and 28 post dosing) reflect not only the declining residual efficacy, but also the high persistent infestation pressure induced in the environment. Therefore with appropriate control measures, such as treatment of the home environment with suitable insecticides, etc, in the case of heavy infestations and/or at commencement of treatment should be recommended. Appropriate advice is included in the product literature (see section 4.4 of the SPC).

Field trials

Treatment and prevention of flea infestation:

Data from four comprehensive field trials were provided. These studies covered a dose range of 30-90 mg/kg bw. Two were conducted in Europe and one each in the USA/Canada and Australia respectively. The first study comprised a single spinosad administration while all other field studies were run over 90 days using regular monthly treatments (treatment days 0, 30, 60). The European and North American studies followed detailed GCP-protocols using a randomised block design. Except for the Australian study, a positive control treated with selamectin spot on was run in parallel. Dogs included had an infestation of at least 10 fleas. The Australian trial was smaller, with no control group included. Efficacy values were calculated by comparing pre- and post-treatment flea counts per dog. All dogs in multi-pet homes were treated with the same product (i.e., either spinosad or selamectin, as appropriate). Cats in participating households were treated with selamectin.

The European field trials were originally run with a dose range of 30-90 mg/kg bw but were completely re-evaluated by rejecting all data from dogs that had received less than the minimum dose (45 mg/kg bw) or more than the maximum recommended dose (70 mg/kg bw).

One study summarises the data sub-set encompassing dogs in the European one month field study receiving the refined dose range of 45-70 mg spinosad/kg bw. This includes information from 93 households in France, Germany, Netherlands and the UK. 93 selamectin treated dogs are included for comparison. During the study the short term residual activity of Comfortis was high, with 94% on day 14 and on day 30, 88% (arithmetic means; geometric means >95%). Approximately 50% of the spinosad treated dogs were completely free of fleas compared to one third of the selamectin treated dogs on day 14 and day 30. Statistically, spinosad proved to be non-inferior to the comparator product (selamectin). In fact, the spinosad efficacy values at each time point were significantly higher than the corresponding values for selamectin.

Palatability was demonstrated with a frequency of approximately 50% free choice when administered on top of food, in the hand, on the floor or in a bowl. Emesis occurred in 9.1% of the spinosad treated dogs compared to 2.4% in the selamectin treated dogs. The difference was significant (p=0.01).

The 3 month study (45-70 mg/kg bw) was conducted in France, but included dogs from Mediterranean and Atlantic climatic zones. For the spinosad group, zero flea values rose from 39.5% at day 14 to 85% at day 90 compared to 28.6% and 67.1% for the selamectin group respectively. Again, spinosad proved to be non-inferior to the comparator product (selamectin), and percentage efficacies in the spinosad group were significantly higher at each time point compared to selamectin. The reduction in flea count (arithmetic means) increased from day 14

(95.7%) to day 90 (98.5%) in the spinosad group compared to 80.2% (day 14) to 95.1% (day 90) in the selamectin group. Palatability was judged to be fair, with ~ 80% of the dogs consuming the tablet voluntarily (~ 35-40%) or in-food (~ 40-45%).

By means of a meta-analysis of three clinical field studies (the two conducted in Europe and one conducted in the USA/Canada), it could be shown that the flea infestation rate decreased (statistically significant, (p=0.0043) with increasing doses of spinosad, when dose was used as a continuous variable. Otherwise, by using categorical dose groups with 12.5 mg/kg bw step increases from 30 to 90 mg/kg bw there is no significant difference between each of the fixed categories.

In order to elucidate a possible relationship between the age or weight of a dog and the persistent efficacy, additional meta-analyses were performed by using the repeated measures linear mixed model. With respect to the weight of the animals two categories (<9 kg; >9 kg) were chosen without specific consideration of the body conditions (normal, obese, fat). No significant effect of the weight category was found (p= 0.67). The same analysis was repeated with respect to growing dogs (<1 year old) compared to adults (>1 year old). Again, there was no significant effect of the age category (p=0.44). By examination of the difference on each day, a significant difference was calculated at day 30 where the percentage reduction was 95.4% (arithmetic mean: 87.3%) in growing dogs compared to 97.7% (arithmetic mean 96.6%). Considering however that no such difference exists on day 14, day 60, day 90 (percent reduction always ≥99%) the relevance of a singular significant incidence is deemed meaningless.

Flea allergic dermatitis (FAD):

The impact of spinosad treatments on FAD were recorded during the two North American and Australian field trials.

In the three month field trial conducted in the USA/Canada, a number of dogs treated with either spinosad or selamectin dogs exhibited FAD (papules, erythema, alopecia, scaling and dermatitis/ pyodermatitis). Clinical FAD signs were scored using a standardised system (0-3). Dogs were included in the final analysis if they had received all three monthly doses (30 – 60 mg/kg bw) and had been examined by a 'treatment-blinded' veterinarian on the first and last visits. A dog was classified as having improved if its FAD score decreased by at least one level from visit 1 to visit 5. Improvements of FAD signs were observed in 86 to 100% of the spinosad treated dogs compared to 57 to 100% in the positive controls. The hallmark sign "pruritus" was improved by 96% in the spinosad group compared to 57% in the selamectin group. In this study at least one episode of emesis occurred in 11.2% of the spinosad treated dogs compared to 6.4% in the selamectin treated dogs. There was also a higher number with decreased appetite (6.7% vs. 2.1%), lethargy (5.8% vs. 3.6%) and diarrhoea (3.3% vs. 2%).

A multi-centre three month field study in Australia covering two different regions was performed with a small number of households (n=37) involved. One third showed clinical signs of FAD. The scoring system was the same as in the North American study but the values for each clinical sign were added to an overall score (max = 15 points). There was significant reduction of 91% in the total lesion score after three months. At this time 82.6% of the animals showed no signs of FAD pruritus. The results provide supporting evidence that monthly spinosad treatments do have marked beneficial effects on signs of FAD. Occasional vomit or a small amount of diarrhoea was noted in the report.

The results support the use of spinosad as a part of a treatment strategy for the control of FAD.

Other studies

No other studies were presented.

Overall conclusions on efficacy

The proposed dosage regimen of Comfortis (spinosad) is 45-70 mg/kg bw. Treatment can be repeated at 4-weekly intervals, if needed. A number of EU dose determination/dose confirmation studies, performed according to current EU guidelines, confirmed the efficacy of spinosad at a dose of 45 mg/kg bw in the treatment and prevention of flea infestations *(Ct. felis).* Efficacy rates above the threshold of 95% were maintained for 3 weeks (98-99%) but were on average slightly below the threshold of 95% on day 30 post dose (arithmetic means: 85.6 to 91.6%) and 50% of dogs had zero fleas. The data justify a claim of residual insecticidal activity for up to four weeks after single oral treatment at a minimum dose of 45 mg spinosad/kg bw, which is supported by the field efficacy studies (statistically, spinosad proved to be non-inferior to the comparator product (selamectin). In fact, the spinosad efficacy values at each time point were significantly higher than the corresponding values for selamectin. Appropriate information is included in the SPC and other product information.

Comparative studies on fed/fasted dogs indicate that spinosad chewable tablets should be given with a meal to increase the systemic availability, and appropriate advice is included to this effect.

The therapeutic activity of spinosad against an existing flea infestation (treatment claim) is clearly demonstrated at 24 to 48 hours after treatment in several studies.

The knockdown and speed of kill effectiveness was investigated in a laboratory study on dogs. Effectiveness (100%) within 4 hours post-dose could be demonstrated

Data were provided from four Simulated Home Environmental (SHE) studies. Suitable advice regarding appropriate control measures, such as treatment of the home environment with suitable insecticides in case of heavy flea infestations, is included the SPC.

Two field studies conducted in Europe were run originally at a dose range of 30-90 mg/kg bw but were re-evaluated by rejecting all data from dogs that had received less or more than the claimed dose range of 45 -70 mg/kg bw.

In a one-month multi-centre field study conducted in Europe, spinosad proved to be non-inferior to the comparator product (selamectin). (The spinosad efficacy values at each time-point were significantly higher than the corresponding values for selamectin.) Palatability of the product was demonstrated.

The second European field study was performed over a treatment period of 3 months. Spinosad proved to be non-inferior to the comparator product (selamectin) again. (The spinosad efficacy values at each time were significantly higher than the corresponding values for selamectin.) Palatability was judged to be fair.

Meta-analysis of the pivotal clinical field studies showed that the flea infestation rate significantly decreased with increasing doses of spinosad, when dose was used as a continuous variable. Otherwise by using categorical dose groups, there was no significant difference between each of the fixed categories.

No relationship between the age or weight of a dog and the persistent efficacy could be established based on additional meta-analyses of the field data.

From the results of clinical field studies performed in the USA and in Australia, it can be concluded that spinosad can be used a part of a treatment strategy for the control of FAD. Advice is included in the SPC and product literature recommending additional control measures such as the use of suitable insecticides in the home environment and vacuum cleaning, and also the treatment of other pet animals living in the same household, in order to ensure efficacy. If fleas reappear in the fourth week, the treatment interval can be safely shortened by up to 3 days.

5. Benefit Risk Assessment

Introduction

The application is for Comfortis flavoured chewable tablets for dogs. The active substance is spinosad, a compound not previously authorised in veterinary medicine in the European Community. The application is supported by a full dossier.

Benefit assessment

Direct therapeutic benefit

Comfortis chewable tablets are proposed for the treatment and prevention of flea infestations (*Ctenocephalides felis*) in dogs at a single oral dose of 45-70 mg/kg bw which may be repeated every month. Fleas do have a considerable nuisance value because of irritating bites causing distress in dogs. Hypersensitive animals may develop signs of flea allergy dermatitis. Fleas transmit a number of pathogens such as *Dipylidium caninum* and *Bartonella henselae*. Therefore, the treatment and prevention of flea infestations is considered beneficial to animal health.

Well conducted controlled clinical studies demonstrated that Comfortis is effective in the treatment of flea infestations in dogs with a persisting efficacy rate in the European laboratory studies of >95% for 3 weeks and 85.6 to 94% for 4 weeks after dosing. The data do justify a preventive effect of spinosad as a result of its residual insecticidal activity of up to 4 weeks.

Spinosad shows a rapid killing effect which provides fast relief for dogs harbouring fleas. Moreover, the rapid kill of fleas after a blood meal prevents the fleas subsequently laying eggs.

In the field studies, at the recommended dose, spinosad proved to be non-inferior to the reference product in the treatment and prevention of flea infestations in dogs.

There is clear evidence from the data that the frequency and severity of Flea Allergy Dermatitis (FAD) is significantly decreased in dogs treated with Comfortis. This is an indirect beneficial effect and therefore, the claimed use as part of a treatment strategy for the control of FAD is justified.

Additional benefits

Comfortis tablets are flavoured, palatable, and are easily taken by most dogs.

The mode of action of spinosad is different from other chemical classes used for flea control in veterinary medicine such as neonicotinoides, fiproles, milbemycins, and avermectins and no cross-resistance is expected at present. The low egg drop after treatment prevents to a certain extent the spread of resistance genes into the next population of fleas.

The indirect benefits refer particularly to children living in close contact with treated dogs, since there is no or little opportunity to transfer spinosad to human skin. The active substance, in contrast to spot ons or insecticidal sprays, will not be washed off during rain, swimming or shampooing, and does not undergo UV degradation. There is also negligible opportunity for a direct transfer of spinosad to household items or (as possible after external treatment with marketed spot ons) to expose the environment via bathing or swimming.

Humans will also benefit from the successful treatment of their dogs (as fleas also bite humans).

Risk assessment

Preclinical and clinical data reveal the most prominent adverse reaction to be emesis, which occurs within 0.5 to 2 hours post-dose, is dose-related, and is probably caused by a local effect on the small intestines. The incidence of emesis observed after the first and second treatments was higher in dogs dosed at the upper end of the dose band, and was shown to be a very common adverse event if the maximum recommended dose was exceeded. The incidence of emesis following treatment with spinosad at the label dose was judged to be acceptable from a clinical point of view as the data demonstrated that the emesis was usually mild and a single episode, and did not require veterinary intervention.

Other adverse events that are possibly treatment related include other gastrointestinal disorders (diarrhoea, soft faeces), and some cases of anorexia/loss of appetite, lethargy and seizures. These adverse events are all adequately addressed in the product literature.

Following overdosage, cell vacuolation/phospholipidosis was also found in dogs, in a dose dependant manner. Unlike the rat studies, the dog study design did not allow for demonstrating reversibility of the observed changes, however, it was demonstrated (using the bench mark approach) that phospholipidosis was unlikely to occur at the recommended dosing schedule. Cell vacuolation was considered to be a treatment related response that results in a change in morphology, but not in any known impairment of any body function, and the Committee concluded that there was no need for a margin of safety for this effect, and agreed that the SPC and package leaflet included adequate information to alert veterinarians to the possibility of phospholipidosis.

After overdosing, and/or dosing in short intervals over a prolonged period of time, other observed adverse events included slight alanine amino transferase elevations, or impaired growth (possibly secondary to emesis). Impaired growth was also reported in puppies <14 weeks old receiving 45-90 mg/kg bw spinosad, therefore the product should only be used in dogs over 14 weeks of age. This threshold was supported by data derived from the clinical field studies.

Spinosad was shown to be a substrate of P-glycoprotein, but was shown not to induce neurotoxicity in avermectin-sensitive Collies after its concurrent administration with milbemycin oxime. However, since adverse effects have been reported (from the US) after the use of spinosad in combination with high (off-label) doses of ivermectin, appropriate information is included in the product literature accordingly (SPC section 4.8).

The study of spinosad on the reprotoxicity in dogs revealed a few early pregnancy losses in treated bitches. Although the relation to treatment remained unclear, a recommendation that Comfortis should be used with caution in pregnant and lactating bitches is included in the SPC and product literature. Spinosyns A and D, the main constituents of spinosad, are preferentially excreted in colostrum/ milk of treated nursing bitches, but the available data were not sufficient to confirm the safety of this for suckling puppies so appropriate information was included in the SPC and product literature accordingly.

The Committee concluded there is no health concern for adults, including pregnant and nursing women, administering this product (to dogs) in accordance with the SPC and product literature. The child-resistant packaging in conjunction with the warnings and advice in the SPC and product literature are considered satisfactorily to minimise risks for children.

When used as recommended spinosad is not expected to pose a risk to the environment.

Risk management or mitigation measures

In order to ensure the safe use in dogs and to minimise potential risks for the user appropriate instructions for the use of the product are included in the product literature. The child-resistant packaging is an additional risk mitigation measure.

Evaluation of the benefit risk balance

Overall, the benefit risk balance is considered positive for Comfortis 270 mg, 425 mg, 665 mg, 1040 mg, and 1620 mg chewable tablets for dogs.

Spinosad has been shown to be effective in the treatment and prevention of flea infestations (*Ctenocephalides felis*) in dogs for up to four weeks. The preventative effect against re-infestations is the result of the adulticidal activity and the reduction in egg production and persists for up to four weeks after a single administration of the product. Comfortis can be used as part of the treatment of flea allergic dermatitis. To ensure efficacy, adequate supplementary measures are included in the product literature.

The (initial) systemic exposure is considered very high in comparison to the concentrations needed for efficacy. However, the appropriateness of the proposed EU dosage regimen of 45-70 mg/kg bw has been adequately demonstrated with regard to efficacy and target animal safety. The treatment related risks are adequately described in the product literature.

Conclusion on benefit risk balance

The information provided in the dossier and in response to points raised was sufficient to confirm an overall positive benefit-risk balance for this veterinary medicinal product.

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for Comfortis 270 mg, 425 mg, 665 mg, 1040 mg, and 1620 mg chewable tablets for dogs were considered to be in accordance with the requirements of Directive 2001/82/EC as amended and the benefit-risk balance was favourable.