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Veterinary Medicines and Product Data Management

Scientific discussion

This module reflects the initial scientific discussion for the approval of Activyl (as published in February 2011). For information on changes after this date please refer to module 8.

1. Summary of the dossier

Activyl, a spot-on solution contains indoxacarb as the active substance and is presented in containers of one or four pipettes for single-use. It is indicated for the treatment and prevention of flea infestation. The target species are dogs and cats. The applicant for this veterinary medicinal product is Intervet International B.V., The Netherlands.

The active substance of Activyl is indoxacarb an ectoparasiticide for topical use, ATCvet QP53AX27, which after bioactivation by insects' enzymes interferes with the nervous system of the parasites and causes paralysis and death.

The benefits of Activyl are its effectiveness in the treatment and prevention of flea infestations in dogs and cats. The most common side effect is hypersalivation if the animal licks the application site.

The approved indication is: *For dogs and cats:*

Treatment and prevention of flea infestation (Ctenocephalides felis)

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

Developing stages of fleas in the pet's immediate surroundings are killed following contact with Activyl treated pets.

The GMP status of the dosage form manufacturing, assembly and release sites is satisfactory.

The pharmacovigilance system in place complies with the requirements in the guideline on monitoring of compliance with pharmacovigilance regulatory obligations and pharmacovigilance inspections for veterinary medicinal products in Volume 9 of the Rules governing medicinal products in the EU.

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2. Quality assessment

Composition

Activyl contains indoxacarb 195 mg/ml in a non-aqueous solution of triacetin, ethyl acetoacetate and isopropyl alcohol and is packed in single-dose spot-on applicators of different sizes for both dogs and cats.

Container

Activyl is packed in single-dose spot-on pipettes composed of a polypropylene/cyclic-olefin-co-polymer/polypropylene blister foil and an aluminium/polypropylene co-extruded lidstock which are then secondary packed in aluminium sachets. The unit dose pipettes are presented as 0.51 ml, 0.77 ml, 1.54 ml, 3.08 ml, and 4.62 ml for very small dogs, small dogs, medium dogs, large dogs and very large dogs respectively. Two sizes of pipettes 0.51 ml and 1.03 ml are authorised for small cats and large cats respectively.

Development Pharmaceutics

The choice of the formulation is based on the solubility of indoxacarb in several tested solvents and evaluation of appearance on the animal in an iterative process of appearance evaluations. Preservatives are not required as the product is for single-use. An early formulation has been replaced by the final formulation to achieve less 'run-off' of the product from the skin of the animals. Clinical and non-clinical studies have been performed with both formulations. A clear overview of the products that were used in the submitted clinical and non-clinical studies has been provided. As preliminary data indicated an increase in water content upon exposure to higher relative humidity environments, an aluminium sachet was added as additional packaging.

Method of manufacture

Indoxacarb is dissolved in the mixture of ethyl acetoacetate, triacetin and isopropyl alcohol. As a flammability/safety precaution, the tank is blanketed with nitrogen. The bulk solution is filtered in-line in the filling process. The process is straightforward and considered as a standard process. The fill weight is based on the assay and the previously determined residual volume that will remain in the containers after expression of the dose. Validation of pilot-scale batches indicates that the manufacturing process yields a robust reproducible product, yet the fill weight will be confirmed in the validation with production scale batches, together with mixing speeds and times, purge volumes, filling speed, and hold times.

Control of starting materials

Active substance

Indoxacarb is a new active substance, not described in the European Pharmacopoeia, USP or JP. The active substance is the S-enantiomer. The active substance master file (ASMF) system is used for this application. The synthesis has been described in sufficient detail. Adequate information on the synthesis and control of the starting materials has been provided. The process is evaluated based on representative batches. The applicant has its own control methods. The control tests and specifications are suitable to ensure a product of consistent quality is produced. A re-test period of 24 months, when stored not above 30°C, is accepted based on submitted 12–month normal and 6-month accelerated stability results.

Excipients

The excipients triacetin and isopropyl alcohol are controlled according to the requirements of the Ph.Eur. For ethyl acetoacetate reference is made to the tests and requirements in the Food Chemical Codex (FCC). These references are appropriate.

Satisfactory certificates of analysis are provided for each of the excipients.

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

Declarations on the absence of TSE material have been provided by the active substance manufacturer and the drug product manufacturer. These declarations are appropriate.

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

Control tests during production

Not applicable.

Control tests on the finished product

Specifications have been set for appearance, identification by HPLC and HPTLC, concentration of the active substance and related substances, water content, and uniformity of dosage units. The average delivered mass is specified and determined by calculation from the concentration of the active substance and the average delivered volume per spot-on applicator. The release requirement of 95.0 – 105.0% is in line with the CVMP Guideline on the quality aspects of single-dose veterinary spot-on products (EMEA/CVMP/QWP/544461/2007 - effective 28 February 2010). The wider shelf-life requirement of 90.0 – 105.0% will be re-evaluated when additional stability data are available. The other specifications are acceptable and in line with relevant VICH Guidelines. The methods have been described in sufficient detail. Results of batch analysis of pilot-scale batches confirm compliance to the proposed specifications.

Stability

The results of the submitted stability data (18 months normal and intermediate and 12 months accelerated) justify the proposed shelf life of 24 months without specific storage condition. In the on-going stability studies the weight of individual containers should be evaluated over the storage times. The stability studies are being conducted with the product packed in a slightly different sachet and by a slightly different process. Suitability of the packaging will be confirmed in stability studies with production scale batches.

OVERALL CONCLUSION ON QUALITY

The dossier provides an appropriately detailed description of the active substance and the chosen formulation, and demonstrates that production of the active substance and the product leads to a consistent quality. The analytical methods are well described, and data of their validation confirm their suitability. Stability studies have been performed according to relevant VICH guidelines. The primary stability studies are on-going. The stability studies done on the active substance allow a re-test period

of 2 years, if not stored above 30°C for the active substance indoxacarb and a shelf-life of 2 years, stored in the original packaging in order to protect from moisture for the drug product.

Overall the documentation on quality relating to both indoxacarb, the active substance and the final product is satisfactory and provides reassurance that product of consistent quality will be produced.

3. Safety assessment

Pharmacokinetics

Pharmacokinetics in non-target animals

Absorption

In rats, absorption of radiolabelled indoxacarb occurred readily following oral gavage at low doses but it was saturated at higher doses (approximately 8 to 14% at 150 mg/kg BW). Absorption reached a plateau between 5 and 27 hours after treatment with elimination half-lives of 35 to 188 hours in plasma, depending on the radiolabel given and the sex of the animal. The half-lives were shorter in males than in females.

Peak time was similar for female and male rats given a dose of 5 mg/kg BW, while female rats had higher peak time when given a dose of 150 mg/kg BW.

Distribution

In rats given radiolabelled indoxacarb by oral gavage, only low levels of residues were found in tissues, because the dose was almost completely excreted. Females retained more residues in tissues than males. The major portion of the radioactivity in tissues was associated with fat. In fat, the main metabolite was IN-JT333 (more than 85 to 100% of the extracted residue).

Metabolism

Metabolism in insects

In the insect, the rate of metabolic conversion of indoxacarb to the active (and more toxic) metabolite IN-JT333 was high (at least 90%) and rapid (within 4 hours) after ingestion of indoxacarb in susceptible insects. Formation of IN-JT333 (i.e., "bioactivation") is the major metabolic pathway of indoxacarb in insects.

Metabolism in rats

Less than 10% of an orally administered dose is transformed into IN-JT333, independent of the enantiomer ratio. Indoxacarb was converted into mainly nontoxic metabolites.

Parent indoxacarb in bile was found to represent less than 1% of the dose, indicating that the liver is a significant site for the metabolism of indoxacarb.

A difference in the level of metabolites retained was observed between male and female rats. In order to identify the metabolic pathway and to confirm these differences, an *in vitro* study in rat hepatocytes was carried out. The difference in metabolism between male and female rats was confirmed by the *in vitro* experiment, indicating a greater production of metabolite IN-JT333 by female rats. This pattern is consistent with the profile of metabolites excreted in the faeces following oral gavage dosing. These factors are likely responsible for the greater sensitivity of female rats for (high) exposure to indoxacarb, observed in the toxicity studies.

Excretion

From different studies it appeared that rats given radiolabelled indoxacarb at an oral dose of 5 mg/kg BW excreted approximately 35-50% of the dose in urine and approximately 25-45% in faeces. At a dose of 150 mg/kg BW, excretion of radioactivity was in the range of 10-20% of the dose in urine and 65-80% of the dose in faeces. The major fraction of the radiolabel was excreted in the excreta within 72 to 96 hours after treatment. The mean percentage of the dose recovered by 7 days after treatment was above 90%.

Pharmacokinetics in dogs and cats

The pharmacokinetics of indoxacarb was studied in the dog and the cat, both after oral and topical administration, in a total of 3 studies. As for the rat, less than 10% of an orally administered dose is transformed into IN-JT333.

Topical application of radiolabelled indoxacarb to dogs and cats revealed concentrations in hair and skin at the treatment site higher than on the rest of the body areas at one week after application, while the concentration in hair was always much higher than in skin. In general, the concentrations in hair and skin in the regions outside the treatment site were fairly similar for each animal indicating the material had spread evenly. Although the dose level for cats was 1.7-fold that of dogs, the respective mean concentrations in hair and skin outside of the treatment site for dogs and cats were comparable. This observation, along with the accompanying observation that a higher percentage of dosed radioactivity was excreted in cats, might be explained by oral ingestion of the product via grooming behaviour in cats. However, oral absorption in cats by grooming cannot easily be distinguished from higher dermal absorption with the presented data.

Dermal absorption of indoxacarb from the final formulation during 28 days was at least 19% and 34% in dogs and cats, respectively.

Oral absorption of indoxacarb administered in a polyethylene glycol formulation in dogs and cats after 0.32 mg/kg BW treatment was approximately 85% and 82%, respectively.

The absorbed indoxacarb was extensively metabolised by the liver to a variety of small metabolites eliminated in urine and faeces. Indoxacarb metabolism in cats and dogs was similar.

With respect to the toxic metabolite the following can be concluded:

- The active metabolite IN-JT333 accounted for more than 6% of the radioactivity given to dogs by topical administration, and was only found in the skin and the plasma. In cats treated topically, this metabolite accounted for more than 1.8% of the radioactivity, and was found only in skin, plasma and faeces.
- The recovery of the radioactive dose was low but consistent, ranging from 45 to 46% for dogs (exclusive of carcass) and 30 to 41% for cats (exclusive of carcass). Recovery was determined in the carcass of one cat and it gave only 6% additional recovery.
- It was concluded that, in mammals, indoxacarb is extensively metabolised/degraded by the liver via routes including cytochrome P450-mediated attack of the indanone and oxadiazine rings, while N-decarbomethoxyllation is a relatively minor pathway.
- The rapid metabolic degradation is a key factor responsible for the high animal and human safety of indoxacarb. In addition, mammals are much less efficient in their ability to convert indoxacarb to the active and toxic metabolite IN-JT333.

Toxicology

To support the toxicology evaluation of this product, published literature references to studies on single dose toxicity, repeat dose toxicity and reproductive toxicity were provide as detailed below in tabular format. Only those studies were presented which were relevant for the assessment of the possible human health hazards associated with the use of the veterinary medicinal product and were performed with racemic (50:50) mixture of enantiomers in dogs (as target animal for Activyl and as model animal used in human toxicology), conducted with purified S-enantiomer in rats (as Activyl contains purified S-enantiomer) and run with IN-JT333 in rats (as IN-JT333 is the pharmacologically active metabolite of indoxacarb).

All the other studies performed during the development of indoxacarb for crop protection use are summarised in the open literature and are not included in the present application.

Single dose toxicity

Study type	**Animal species**	**Route and dose**	**Conclusion**
acute dermal toxicity indoxacarb	male + female rat	skin; 5000 mg/kg BW	LD_{50} > 5000 mg/kg BW
acute oral toxicity indoxacarb purified S-enantiomer	male + female rat	gastric intubation males: 400, 640, 1000,1953 mg/kg; females: 123, 192, 300, 400 mg/kg. BW	oral LD_{50} male 843 mg/kg BW female 179 mg/kg BW
acute oral toxicity Activyl	female rat	oral gavage; final formulation at 175, 550, 2000 mg/kg BW	clinical signs at 2000 mg/kg BW
acute dermal toxicity Activyl	male + female rat	skin; final formulation at 2000 mg/kg BW	dermal LD_{50} > 2000 mg/kg BW
acute oral toxicity metabolite IN-JT333	male + female rat	oral; 10, 30, 50, 100, 200 mg/kg BW	oral LD_{50} male 52 mg/kg BW female 39 mg/kg BW
acute single dose neural toxicity study 75:25 ratio indoxacarb	male + female rat	oral male: 0, 25,100, 200 mg/kg BW female: 0, 12.5, 50, 100 mg/kg BW	NOEL systemic male: 100 mg/kg BW female: 12.5 mg/kg BW neurotoxic: male: 100 mg/kg BW female: 50 mg/kg BW

Repeat dose toxicity

study type	**animal species**	**route and dose**	**conclusion**
90 days oral safety 50:50 indoxacarb	male + female dog	oral; 40, 80, 160 and 640 ppm in the diet, daily	NOEL male 80 ppm(i.e., 2 mg/kg BW) female 160 ppm (i.e., 5 mg/ kg BW)
1 year oral safety 50:50 indoxacarb	male + female dog	oral; 40, 80, 640, 1280 ppm in the diet, daily	NOEL 40 ppm (i.e., 1.1 mg/kg BW for males & 1.3 mg/kg BW for females)

90 day oral toxicity indoxacarb (S-enantiomer)	male + female rat	oral; male: 0, 8, 20, 50, 100, 200 ppm female: 0, 3, 8, 20, 50, 100 ppm in the diet, daily	NOEL male: 50 ppm (i.e., 3.2 mg/g BW) female: 20 ppm (i.e., 1.7 mg/g BW)
14 day oral toxicity metabolite IN-JT333	male + female rat	oral; 0, 2, 10, 40, 100 ppm in the diet, daily	NOEL 10 ppm (i.e., 0.88 mg/kg BW for males & 0.87 mg/kg BW for females)
28-day dermal exposure 75:25 indoxacarb	male + female rat	skin; 50, 500, 1000, or 2000 mg/kg BW/day	NOEL male: 1000 mg/kg BW/day female: 50 mg/kg BW/day
18 month oncogenicity feeding study 50:50 indoxacarb	male + female mice	oral; 0, 20, 100, 200 ppm in the diet daily	NOEL 20 ppm (i.e., 2.6 mg/kg BW for males & 4 mg/kg BW for females)
2-year oncogenicity feeding study	male + female rat	oral; male: 0, 20, 40, 60, 125, 250 ppm female: 0, 10, 20, 40, 60, 125 ppm in the diet, daily	NOEL male: 60 ppm (i.e., 2.4 mg/kg BW/day) female: 40 ppm (i.e., 2.1 mg/kg BW/day)
90-day oral neurotoxicity; 75:25 ratio indoxacarb	male + female rat	oral; male: 0, 10, 100, 200 ppm female: 0, 10, 50, 100 ppm in the diet, daily	NOEL systemic: 10 ppm (i.e., 0.6 mg/kg BW for males & 0.7 mg/kg BW for females) NOEL neurotoxicity male: 200 ppm (i.e., 11.9 mg/kg BW/day) female: 100 ppm (i.e., 6.1 mg/kg BW/day)

Reproductive toxicity

study type	**animal species**	**route and dose**	**conclusion**
peri- and postnatal toxicity study; indoxacarb	pregnant rat	oral; 0 (Vehicle), 1.0, 1.5, 2.5, 4.0 mg/kg BW/day D6-D24	NOEL maternal rat and pups: 1.5 mg/kg BW/day
2-generation reproduction/fertility study	rat	Oral 20, 60, or 100 ppm in the diet, daily	NOAEL parental and pup development: 20 ppm (i.e. 1.3 mg/kg BW/day) No effect on fertility: 100 ppm (i.e. 6.7 mg/kg BW/day)
maternal and developmental toxicity	rat	oral; 0, 0.5, 1.0, 2.0, 3.5 mg/kg BW; daily	NOEL 2 mg/kg BW/day
maternal and developmental toxicity	rabbit	Oral 0, 250, 500, 1000 mg/kg BW/day	NOEL 500 mg/kg BW/day
oral developmental	female rat	oral;	NOAEL maternal:

neurotoxicity study; indoxacarb	+ offspring	0, 0.5, 1, 1.5 and 3.0 mg/kg BW/day	1.5 mg/kg BW/day. NOAEL developmental F1 male: 1.5 mg/kg BW female: 3 mg/kg BW
reproductive safety study from pre mating to end of lactation	cat + offspring	topical; 0 and 75 mg/kg (= 3x treatment dose) at 28-day intervals	increased number early pregnancy terminations; decreased litter size

Mutagenicity / genotoxicity

Twelve studies concerned the possible mutagenic/genotoxic and carcinogenic effects of indoxacarb. No such effect was observed and indoxacarb was considered devoid of mutagenic/genotoxic effects.

Carcinogenicity

Based on the results of the genotoxicity studies and the two carcinogenicity studies (rats and mice), indoxacarb can be considered as not carcinogenic.

Studies of other effects

Dermal and ocular irritation

Dermal toxicity of the final formulation of indoxacarb is low, with an LD_{50} of > 2000 mg/kg for the male and female rat. Activyl was not considered to be a skin irritating contact sensitiser in studies in the rabbit and guinea pigs respectively. The skin irritation study was conducted in rabbits and the skin sensitisation study was conducted in guinea pigs. The final formulation is moderately irritating when administered to rabbits' eyes.

Neurotoxic studies

Three studies in rats (acute, repeated dose, developmental toxicity) were conducted with a special focus on possible neurotoxic effects, in view of the specific pharmacological properties of indoxacarb.

The study results indicate that systemic toxicity is more relevant than a specific neurotoxic effect. In fact no specific neurological effects have been observed.

Given the relatively high susceptibility of the rat for toxic effects of indoxacarb, the occurrence of neurotoxic effects in other animal species is not expected.

Haemolytic effects

The specific toxic effect of indoxacarb is mild regenerative red cell haemolysis, which is thought to be the result of oxidative damage to red blood cells mediated by a metabolite, an N-hydroxylarylamine. A number of studies were conducted to assess the comparative haemolytic potential of this metabolite.

As mechanistic studies have demonstrated that oxidation of glutathione in red blood cells (RBCs) following *in vitro* exposure to N-hydroxyarylamines serves as a useful surrogate for their *in vivo* haemolytic potential, these effects were investigated after exposure of RBCs from rats, dogs and humans to this metabolite.

Study results indicate that:

- Exposure of RBCs from rats, dogs and humans to this metabolite produces dose-dependent oxidation of glutathione, confirming the haemolytic potential of this N-hydroxylarylamine metabolite.
- The rank order of species sensitivity to *in vitro* oxidation of erythrocyte glutathione by this metabolite is rat >> dog > human. Specifically, human erythrocytes are up to 2 to 3-fold less sensitive than dog erythrocytes and up to 4 to 5-fold less sensitive than rat erythrocytes to this metabolite -induced oxidation of glutathione.
- The oxidative effects of dapsone hydroxylamine, the haemolytic metabolite of the antimycobacterial substance dapsone, are more severe than those of this metabolite in dog and human erythrocytes; in the rat, responses were similar for dapsone hydroxylamine and this metabolite.

In conclusion, species differences in the oxidative response to this metabolite exposure *in vitro* indicate that humans are likely to be less sensitive than either rats or dogs to the *in vivo* haemolytic action of this compound.

User safety

A user safety risk assessment has been provided in accordance with the CVMP Guideline on user safety for pharmaceutical veterinary products (EMEA/CVMP/543/03-FINAL).

Exposure assessment
Users (professional and non-professional) may become exposed to Activyl (including its (in)active ingredients) via the following routes:

- Dermal exposure: - accidental spillage of the product on the skin during treatment.
  - stroking the treated animal.
  - opening the product accidentally and access to the used pipette (children)
- Oral exposure: - hand-to-mouth contact (after stroking)
  - opening the product accidentally and access to the used pipette (children)
- Ocular exposure: - hand-to-eye contact (during treatment or after stroking).

Hazard identification and characterisation
For the assessment of risks after exposure to Activyl (see scenarios above), the following toxicity data of indoxacarb or the final formulation of Activyl are considered of relevance:

- Dermal exposure
  1) A 28-day repeated-dose dermal toxicity study in rats receiving indoxacarb at doses of 0, 50, 500, 1000 or 2000 mg/kg BW for 6 hr/day revealed a NOAEL for systemic toxicity (reduced body weight, reduced body weight gain, food consumption and food efficiency) of 50 mg/kg BW in female rats.
  2) The active ingredient indoxacarb (both as a purified S-enantiomer and as a mixture of enantiomers) is classified as a skin sensitiser (R43). Therefore Activyl may possess skin sensitising properties.
- Oral exposure:
  1. Systemic NOAEL in the acute oral neurotoxicity study in rats: 12.5 mg/kg BW

2. In a 90-day repeated-dose dietary toxicity study, rats were given indoxacarb at doses of 0, 0.56, 1.4, 3.2, 6.6 or 14 mg/kg BW/day (males) or 0, 0.25, 0.68, 1.7, 4.1 or 8.5 mg/kg BW/day (females). The NOAEL was 1.7 mg/kg BW/day, based on reduced body weight gain and haemolytic effects at higher doses in female rats.

- Ocular exposure:

  Activyl (final formulation) was tested on the eyes of rabbits. Iritis and/or discharge were observed until day 8 post-treatment. On day 15 post-treatment, eye effects were no longer observed. Based on this study, Activyl was found to be moderately irritating to the eyes of rabbits.

Risk characterisation

Comparing exposure estimates with the relevant NOAELs, the Margins of Exposure (MOEs) were calculated. Although the MOEs for all exposure scenarios considered were greater than one, some were low such that it cannot be ruled out that adverse health effects may occur after dermal exposure (via accidental spillage) and oral exposure (via hand-to-mouth contact when opening the product or getting access to the used pipette) in adults and children. However, appropriate risk mitigation measures were proposed to prevent and/or reduce exposure.

Risk management and risk communication

The following warnings as included in the product information will sufficiently mitigate the risks for the user:

- *Do not eat, drink or smoke while handling the veterinary medicinal product*
- *Wash hands immediately after use*
- *The foil pouch is child-resistant. Keep the product in the foil pouch until use, in order to prevent children from getting access to the product*
- *Keep the used pipette out of reach and sight of children*
- *The solvents, hence the veterinary medicinal product is highly flammable. Keep away from heat, sparks, open flame or other sources of ignition.*
- *As the veterinary medicinal product may cause moderate eye irritation, avoid contact with eyes. If this occurs, rinse slowly and gently with water.*

Environmental safety

A phase I assessment revealed that no Phase II risk assessment is necessary, because the product is only used in non-food producing animals (dogs and cats). Nevertheless, because the product has insecticidal properties, the following precaution will be necessary:

Activyl should not be allowed to enter surface waters as indoxacarb may have harmful effects on aquatic organisms.

Overall conclusions on safety

Due to its use for agricultural purposes, the safety of indoxacarb has been studied thoroughly. However, these studies served another purpose, i.e. user and consumer safety rather than safety in the target species. Consequently, differences in study design, implementation and reporting as well as formulations used were observed. Although the information is valid as such, extrapolation to safety-aspects of indoxacarb in target animal species required careful consideration of data.

Based on the no observed effect levels (NOAEL) generated with the racemic (50:50) mixture of enantiomers, the purified S-enantiomer and the 75:25 ratio of enantiomers in rats (3 months studies), the toxicity of all three materials is quantitatively and qualitatively similar. Adverse effects seen in

subchronic feeding studies in mice and dogs with the racemic (50:50) mixture of enantiomers are qualitatively very similar to those seen in rats, moreover mice appeared less sensitive than rats.

Adverse events observed in the *in vivo* repeat dose toxicology studies in rats and dogs were mild haematological effects and effects on the body weight. This toxicological effect of indoxacarb is mild regenerative red cell haemolysis, which is thought to be the result of oxidative damage to red cells mediated by the putative metabolite N-hydroxyarylamine. This metabolite contains the structure of the hydroxylated metabolites of aniline, a known substance causing haemolysis (or methaemoglobinaemia). Slight increases in methaemoglobin as well could be seen in rats in subchronic oral toxicity studies with the purified S-enantiomer. Methaemoglobin results from oxidation of haemoglobin iron from the ferrous to the ferric state.

Reproduction toxicity was only studied in laboratory animals and not in the target species.

In view of the data on reproduction toxicity in rats, it is concluded that effects on reproduction in the target species are unlikely to occur. However, given the observations made in the cat (increased number early pregnancy terminations; decreased litter size) when treated prior to mating, then through mating, gestation and lactation, the product is not recommended for use in breeding, pregnant and lactating animals.

Possible risks for the user are mitigated by appropriate warnings and precautions which are included in the product information.

4. Efficacy assessment

Pharmacodynamics

Indoxacarb is a synthetic insecticide belonging to the oxadiazine class of insecticides (oxadiazine analogue of the pyrazoline ring system). Its original use was for the control of pest insects on a wide variety of plants, including vegetables, fruit trees, cotton, and row crops. Because of its use in agriculture, extensive information is available on the toxicity profile of this molecule, as well as its safety for mammals and the environment.

Indoxacarb has not been authorised previously for use as a veterinary medicinal product in the European Union (EU).

Indoxacarb is a chiral molecule. The S-enantiomer (>99% S-isomer; DPX-KN128) is the insecticidal active substance; the R-enantiomer has no such activity, but both enantiomers are similarly effective with respect to the toxic effects.

For crop protection indoxacarb was used as a racemic (50:50) mixture of enantiomers (DPX-JW062). Processes were subsequently improved allowing the development of a 75:25 ratio of enantiomers (mixture containing 75% S-, and 25 % R-isomer; DPX-MP062). Finally it was possible to produce the purified S-enantiomer (>99% S-isomer; DPX-KN128). Safety studies for agricultural purposes have been carried out with various ratios of isomers. The final veterinary product only contains the purified S-enantiomer.

In order to be effective indoxacarb has to be metabolised. In the insect indoxacarb is converted to a more toxic substance IN-JT333. Indoxacarb requires bioactivation by insects before it exerts its insecticidal effect.

Bioactivation in insects follows enzymatic cleavage via N-decarbomethoxyllation by insect-esterases.

Higher animals, including mammals, primarily degrade indoxacarb to inactive metabolites via alternate routes (cytochrome P450-mediated attack of the indanone and oxadiazine rings).

Oral uptake by insects is the major route of bioactivation and pharmacodynamic effect compared to contact uptake. The insecticidal active metabolite of indoxacarb (IN-JT333/DCJW) acts primarily as a strong voltage dependent sodium channels blocker in the insect. Cessation of feeding occurs very rapidly after contact of the insect with indoxacarb, followed by the cessation of egg laying (oviposition), paralysis and death. Indoxacarb has both ovilarvicidal and adulticidal activity.

Submitted information on the pharmacodynamics concerns published literature. No original study data were submitted.

The activity of indoxacarb against arthropods was tested *in vitro* in four insect and two tick species. Specific Activity Assays were conducted to evaluate the anti-ectoparasiticide activity of indoxacarb concerning the feeding activity (medicated diet) on adult *Ctenocephalides felis* (cat flea) and on adult and nymphs of *Ornithodoros moubata* (chicken tick), contact activity (impregnated paper) on adult *Ctenocephalides felis*, contact activity (immersion) on *Boophilus microplus larvae* (cattle tick) and contact/feeding activity (medicated diet) on *Lucilia cuprina* (Blow fly) and *Aedes aegypti* (mosquito), including larvae first through third instar larvae and insect growth regulation (IGR) test for both species.

These tests confirm that indoxacarb has no acaricidal activity, but is toxic to insects only. The major route is oral uptake. Fleas removed from dogs treated with Activyl at the 24 hours comb counts, unlike fleas collected from placebo-treated animals, died within 24 hours of being in an incubator.

Impact of resistance development to efficacy

Indoxacarb is a novel active substance in veterinary medicine. No resistance data were submitted. All strains used in (pre-) clinical studies appeared to be susceptible.

Up to now only one active substance from the pyrazoline family has been used in veterinary medicine. Resistance against indoxacarb has not been reported, a conclusion that is mainly based on experience with this substance in agriculture.

Target animal safety

The selected treatment doses of 15 mg/kg BW and 25 mg/kg BW as the minimal recommended treatment doses in dogs and cats, respectively were based on dose-determination studies.

Three overdose studies using the recommended route of administration were carried out in each species, starting at a minimum age of 8 weeks and with treatment duration of up to 6 months. Treatment intervals of 2 and 4 weeks were applied. In all cases, topical administration of 3x and 5x overdoses were well tolerated, without systemic adverse effects. Kittens experienced overdose treatments as being unpleasant, as shown by the shaking of the head. Kittens also showed transient scratching at the application site, greasy application site (clumped hair) and white residue at the application site in all of the treatment groups (1x, 3x and 5x); some squinting in the higher dose groups (3x and 5x); and some salivation in the high dose group (5x). A number of these findings were considered to be volume related as signs were also observed in the placebo-treated group, receiving a volume equal to that of the 5x-group.

Oral safety studies were carried out with a dose of 0.1 rising to 0.7x the recommended treatment dose and followed by 1 x recommended treatment dose in both species. Adult cats showed transient and/or intermittent excessive salivation, licking of the face, shaking the head, and squinting after oral application of the formulation at doses of 0.1-0.7x the treatment dose.

No treatment related adverse effects were observed in the dog. No signs of local irritation were observed in both species.

It is noted that the studies involved healthy animals without flea infestations. It is known that FAD can lead to an increased permeability of the skin, due to inflammation. Consequently the absorption of indoxacarb may increase in these cases, however in view of the pharmacokinetic and toxicity studies it is not likely that this will result in adverse effects.

Dose determination, justification, and confirmation

Dog

Four studies were carried out to establish an effective treatment dose in the dog. Studies involved Beagle dogs only.

From a dose-range of 7.5 -15 mg/kg BW a dose of 15 mg/kg BW was selected as the optimal treatment dose. This dose was also used to study the ovicidal and larvicidal effects.

A dose of 15 mg/kg BW (volume 0.077 ml/kg; concentration 195 mg indoxacarb/ml) did result in an adequate level of flea control up to 4 weeks after treatment (>95% based on arithmetic means). Efficacy was comparable to that of a reference product.

Most flea counts in the earlier studies were carried out 24 hours after (re)infestation. Later on, other data indicated that the onset of flea killing was relatively slow although cessation of feeding and paralysis occurs very rapidly after contact of the insect with indoxacarb, and counts in the later studies were based on a 48-hours interval which provides a better indication for efficacy.

The same dose was effective to prevent hatching of flea eggs for a period of 5 weeks.

For the field study the dose of 15 mg/kg BW was converted to a dosage as a fixed volume per body weight range.

Cat

Twelve studies were carried out in the cat. Using the same formulation as for the dog, a range of 10-17.5 mg/kg BW was used for dose-finding. However, the observed reduction in flea counts were considered insufficient and the range was increased to 20-31.25 mg/kg bodyweight. A dose of 25 mg/kg bw appeared the optimal treatment dose, with a persistency for up to 4 weeks.

The same dose prevented the further development of flea larvae in the immediate environment of treated cats for a period of up to 5 weeks.

For the field study the dose of 25 mg/kg BW was converted to a dosage as a fixed volume per bodyweight range.

Field trials

To confirm the efficacy and safety of indoxacarb under field conditions, five multicentre field studies were conducted in dogs and cats naturally infested with fleas. Four of them included dogs only or cats only and one study included both dogs and cats.

Four studies were carried out in Australia using a development formulation, with households being included rather than cats and dogs. Efficacy was adequate in reducing flea burdens and signs of FAD, but the reports indicate a difference with EU-environments and climatic conditions.

One study was carried out in the EU (Spain, France and Germany) with the final formulation. The study involved numerous individual veterinary practices. A total of 110 dogs and 97 cats were included in the Indoxacarb group. 101 dogs and 85 cats were kept as per protocol efficacy analysis. A total of 97 dogs and 100 cats were included in the control group, treated with an authorised reference product containing fipronil. 89 dogs and 85 cats were kept as per protocol efficacy analysis.

Animals were treated every 4 weeks with a total of 3 treatments. Counting of fleas, flea allergy dermatitis assessment, observations on local reaction at the application site and general attitude were carried out on Day 0 and every two weeks on Days (+2) 14, 28, 42, 56, 70 and 84.

Results in dogs

In the dog average flea counts at D0 were 11.8 for the indoxacarb-group and 15.6 for the reference product group. Counts ranged from 0 to 191.

Overall percent reduction in flea counts (geometric mean / arithmetic mean) in dogs were:

Study day	Visit	Indoxacarb	Reference product (fipronil)
D0	V1	0	0
D14 (±2)	V2	97.7 / 97.4	94.3 / 93.0
D28 (±2)	V3	97.6 / 96.0	93.1 / 90.5
D42 (±2)	V4	99.7 / 99.6	97.6 / 97.2
D56 (±2)	V5	99.0 / 98.7	94.1 / 90.5
D70 (±2)	V6	99.7 / 99.7	97.7 / 95.1
D84 (±2)	V7	99.8 / 99.6	96.9 / 95.4

Results in cats

In the cat average flea counts at D0 were 10.1 for the indoxacarb-group and 10.9 for the reference product group. Counts ranged from 0 to 46.

Overall percent reduction in flea counts (geometric mean / arithmetic mean) in cats were:

Study day	Visit	Indoxacarb	Reference product (fipronil)
D0	V1	0	0
D14 (±2)	V2	95.6 / 93.8	92.4 / 85.9
D28 (±2)	V3	95.8 / 94.6	90.3 / 83.3
D42 (±2)	V4	98.4 / 97.0	95.8 / 93.5
D56 (±2)	V5	97.0 / 95.7	94.4 / 86.8
D70 (±2)	V6	99.6 / 99.4	96.2 / 93.5
D84 (±2)	V7	99.4 / 98.9	97.1 / 94.4

Flea Allergy Dermatitis (FAD)

In both groups the percentage of dogs and cats with flea allergy dermatitis decreased markedly over time. In the Indoxacarb treated group, dogs and cats were considered cured from signs of FAD from the sixth visit in dogs and the fourth visit in cats. At the end of the observation period (V7), one dog and one cat still had signs of FAD in the fipronil group. The number of dogs and cats with flea allergy dermatitis were not different between the two treatment groups at each of the seven visits.

Adverse effects
In less than 10% of dogs scratching was observed, and very few dogs showed signs of hair loss and itching and one dog showed a cutaneous lesion. In less than 5% of cats adverse effects were observed, consisting of excitation, anorexia, vomiting an excessive salivation. A local reaction (hair loss) was observed in another cat. Signs resolved with or without therapy.

The results from the field studies demonstrate the efficacy of indoxacarb in controlling flea infestations in dogs and cats. As was expected on the basis of the dose-finding studies, efficacy increases in time and treatments need to be repeated before an adequate level of control can be achieved. The level of efficacy was similar to that of the reference product.

Based on the findings of the EU-study, it is concluded that safety and efficacy (adulticidal and inhibition of development of larvae to adult stages) of the final indoxacarb formulation has been demonstrated for the dog and the cat, when used as proposed.

Other studies
One study was carried out to assess the effect of water immersion and shampooing on the efficacy of indoxacarb in the dog. In another study, the effect of exposure to sunlight was assessed.

Shampooing, water immersion or sunlight did not result in a reduced efficacy, when flea counts were made after one week.

Overall conclusion on efficacy

Indoxacarb is a novel substance in the EU and intended for the control of flea infestations in the dog and the cat. After being converted into its toxic metabolite IN-JT333 by the insect, it has a high and specific receptor affinity, rapidly leading to inactivation of the insect and death.

In the insect, the rate of metabolic conversion of indoxacarb to the active metabolite IN-JT333 is high (at least 90%) and formation of IN-JT333 (i.e., "bioactivation") is the major metabolic pathway of indoxacarb in insects.

Mammals are much less efficient in their ability to convert indoxacarb to IN-JT333. In mammals, the metabolic pathway of indoxacarb differs from that of in insect leading to conversion of indoxacarb to mainly nontoxic metabolites (e.g., IN-KG433).

After topical administration indoxacarb becomes systemically available, but levels remain far below those needed for toxic effects. A specific haemolytic effect has been demonstrated, but levels needed to elicit this effect are not reached in the target animal when the product is used as recommended.

Tolerance studies were carried out in the target species as a spot on treatment at the recommended dose as well as 3x and 5x overdoses. The minimum age was 8 weeks for both species. No adverse reactions were observed.

Applied as a spot-on formulation, adequate levels of flea control are achieved for a dose of 15 mg/kg BW in the dog and of 25 mg/kg BW in the cat. Optimal efficacy is achieved after 48 hours and maintained for up to 4 weeks in the dog and the cat.

Treatment results in a parricidal effect for up to 5 weeks in both animal species.

Adequate efficacy was demonstrated under EU-field conditions.

5. Benefit risk assessment

Introduction

Activyl is a spot-on solution for the treatment and prevention of fleas in dogs and cats, and can be used as part of a treatment strategy for flea allergy dermatitis. The product contains one active substance, indoxacarb, which has not been previously authorised as a veterinary medicine in the EU. Indoxacarb is an ectoparasiticide belonging to the oxadiazine chemical family, and has a history of use in plant protection.

Benefit assessment

Direct therapeutic benefit

Clinical studies have demonstrated adequate efficacy of Activyl under EU-field conditions. The mode of action is characterised by the bioactivation of indoxacarb into a metabolite that subsequently blocks the sodium channels in the fleas. This toxic metabolite is produced in high amounts in insects but in low amounts in mammals.

Risk assessment

Indoxacarb exerts its effects by blocking the sodium channels in the fleas, but shows no overt neurotoxic signs in mammals because the responsible metabolite is formed in quite low amounts in mammals. However, other metabolites of indoxacarb are capable to cause some adverse effects at high doses. In particular an N-hydroxylarylamine metabolite is considered to be responsible for red blood cell haemolysis.

Nevertheless, indoxacarb in its final formulation is well tolerated in dogs and cats, even at dermal doses ten times the recommended dose.

The safety for the user has been adequately addressed. Although all margins of exposure were greater than one, some were too low to exclude any toxic effects.

Risk management or mitigation measures

No specific risk management or mitigation measures seem to be needed for the safety of the target animals.

To protect children from exposure to the content of a pipette, the applicant provided a child-resistant packaging. Other proposed warnings were considered adequate to mitigate any other possible risks arising from user exposure to the product.

Evaluation of the benefit risk balance

It can be concluded that the product appears to be effective and safe for target animals and for the users. Some outstanding issues need to be addressed by the applicant post-authorisation; however these are related to the quality part and will not impact on the benefit risk balance.

Conclusion on benefit risk balance

The benefit risk balance is considered to be positive.

Conclusion

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EC as amended.