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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Daxocox (EMEA/V/C/005354/0000)

INN: enflicoxib

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Ecuphar NV submitted on 31 January 2020 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Daxocox, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

On 17 February 2021, the CVMP adopted an opinion and CVMP assessment report.

On 20 April 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Daxocox.

The eligibility to the centralised procedure was agreed upon by the CVMP on 20 June 2019 as Daxocox contains a new active substance (enflicoxib) which is not yet authorised as a veterinary medicinal product in the Union.

The applicant applied for the following indication: for the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease) in dogs.

The active substance of Daxocox is enflicoxib, a non-steroidal anti-inflammatory drug (NSAID) belonging to the coxib class, which acts by selective inhibition of the enzyme cyclo-oxygenase 2. The target species is dogs.

Daxocox tablets contain 15, 30, 45, 70 or 100 mg enflicoxib and are presented in packs containing 4, 10, 12, 20, 24, 50 or 100 tablets.

The rapporteur appointed is Rory Breathnach and the co-rapporteur is Cristina Muñoz Madero.

The dossier has been submitted in line with the requirements for submissions under Article 31 of Regulation (EC) No 726/2004.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated June 2018) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Batch release of the dosage form takes place in the Netherlands at Lelypharma B.V., Lelystad. A GMP certificate has been provided for this site based on an inspection conducted in June 2020.

A QP declaration for the active substance manufacturing sites was provided from the Qualified Person (QP) at the EU batch release site. The declaration is issued on foot of an audit of the site in January 2019.

Overall conclusions on administrative particulars

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

The GMP status of the active substance has been satisfactorily established and is in line with legal requirements. A valid GMP certificate for the site of manufacture and batch release of the finished product has been provided.

Part 2 - Quality

Composition

The finished product is presented as brown, round, convex tablets containing the active substance enflicoxib in 5 tablet strengths: 15 mg, 30 mg, 45 mg, 70 mg and 100 mg. The other ingredients are silicified microcrystalline cellulose, mannitol, sodium laurilsulfate, crospovidone, dried flavour, pigment blend, copovidone, sodium stearyl fumarate and talc.

Containers

The tablets are packaged in an aluminium/aluminium blister within an outer carton. The packaging material complies with the relevant EU requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Development pharmaceutics

The tablets used in the clinical trials consisted of a range of 3 divisible tablets, whereas the final formulations consist of 5 non-divisible tablets. The tablets used for the stability studies and for product registration were manufactured according to the same manufacturing process (blending and direct compression) used in the manufacture of the clinical trials formulation. However, there is a minor difference in the formulation between the clinical batches and the final formulation in that the flavouring agent was changed from 'beef liver roasted flavour' to 'dried flavour' due to the discontinuation of the beef liver roasted flavour. The two flavours have similar characteristics and are present at the same concentration in the two formulations. In addition, active substance used in the proposed formulation is manufactured at the proposed active substance manufacturing site with an optimised route of synthesis, whereas that used in the clinical trial batches was manufactured at a different active substance manufacturing site by a previous route of synthesis. Comparative batch analytical data has been provided for the batch of the active substance used in the clinical trial batches and three batches of the active with the optimised process used for the proposed formulation and the results comply with the updated active substance specification, and are comparable between batches

and between sites. Comparative pXRD data is provided on the same four batches, demonstrating that all have the same polymorphic form. Comparative specific optical rotation data has been provided for the same four batches indicating that both routes of synthesis produce the same racemic mixture. In addition, justification of the choice of a racemate instead of a single enantiomer has been provided. Comparative particle size data has been provided for the batch of the active substance produced used in the clinical trial batches and three batches of the active with the optimized process used for the proposed formulation and the results are comparable between batches and between sites. In addition, confirmation has been provided that the particle size of the batches of of mannitol used in the clinical trial batches also complied with the proposed particle size limits for this excipient.

Direct compression technology was selected for the formulation due to its simplicity. Derivation of the formulation is logical and well described in the dossier and the formulation components are commonly used in this dosage form. The function of each excipient is clearly detailed and their selection was based on screening studies based on the rheological characteristics and ability to enhance the solubility of the active substance.

Investigation of the dissolution test is described. Comparative dissolution testing was performed for the 120 mg tablets used in the clinical trials versus batches of the 15 mg, 30 mg, 45 mg, 70 mg and 100 mg proposed product strengths at pH 1.2, 4.5 and 7.5. The similarity factor f2, as per the 'Guideline on the conduct of bioequivalence studies for veterinary medicinal products' EMA/CVMP/016/2000-Rev.3, was also calculated for each of the profiles, and were within 50 – 100, suggesting that the dissolution profiles are similar. It was confirmed that the conditions followed for the evaluation of the similarity factor are in line with the requirements of the 'Guideline on the conduct of bioequivalence studies for veterinary medicinal products' EMA/CVMP/016/2000-Rev.3-corr. Information has been provided on the discriminatory nature of the dissolution method, including detail on the manufacture of a 'bad batch' in which changes were made to the proportion of excipients and its resultant dissolution profile. The proposed limit for the finished product dissolution test was amended in line with the recommendations of the 'Reflection paper on the dissolution specification for generic oral immediate release products with systemic action' EMA/CHMP/CVMP/QWP/226031/2017, enhancing the discriminatory nature of the dissolution method.

Method of manufacture

The manufacturing process is a standard direct compression process. A is mixed, sieved and mixed again. The remaining sieved ingredients are then added to the premix blend and mixed. The final bulk blend is then sieved and compressed into tablets. The tablets are packed into blisters, packed into cartons, and a final inspection is carried out. In-process controls are adequate for this type of manufacturing process and pharmaceutical form. Process validation data has been provided for three full scale finished product batches and the data provided demonstrates that all critical parameters are within acceptable limits, and that a quality product is consistently produced, and validation data has also been provided to support the proposed 4 week bulk hold time for the tablets.

Control of starting materials

Active substance

The active substance enflicoxib is not monographed in the Ph. Eur. and data on the active substance is provided according to the Active Substance Master File (ASMF) procedure. The active substance specification includes tests for appearance, identity, assay, impurities, residual solvents, water content,

heavy metals, and residue on ignition and particle size distribution. Acceptable batch analysis data has been provided. The primary reference standards used by the finished product manufacturer for the testing of the active substance are as per ASMF section 3.2.S.5.

Excipients

The excipients of the formulation are controlled in accordance with their respective Ph. Eur. monographs with the exception of silicified microcrystalline cellulose, dried flavour and pigment blend. Silicified microcrystalline cellulose is not monographed in the Ph. Eur. and is therefore controlled in line with its USP/NF monograph. In addition, it has been confirmed that the individual components of this excipient, microcrystalline cellulose and colloidal anhydrous silica, comply with their Ph. Eur. monographs. The excipients dried flavour and pigment blend are controlled in line with in-house specifications. The compliance of the excipients with the requirements of Ph. Eur. 5.1.4 for non-sterile substances for pharmaceutical use is detailed. A satisfactory specification is provided for the dried flavour. A satisfactory specification is provided for the pigment blend along with a declaration of compliance with Regulation EC No 231/2012 laying down specifications for food additives.

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

Valid TSE declarations from the manufacturers of the active substance and excipients have been provided.

Control tests on the finished product

The finished product release specification controls relevant parameters for the dosage form. Parameters on the specification are: appearance, uniformity of dosage units, identification of the active substance and of the pigment, assay, related substances, dissolution, average tablet weight and microbiological testing. The validation of the analytical methods is in accordance with the VICH guideline GL2 'Validation of analytical procedures: Methodology'. Batch analysis data is provided for three full scale process validation batches of each strength. The data demonstrates compliance with the proposed specifications and are comparable between batches. Information is provided on the reference standards used in the testing of the finished products.

Stability

The proposed specification for shelf life is the same as that for release with the following exception: uniformity of dosage units.

A stability study was conducted with three full scale process validation batches each of the lowest (15 mg), median (45 mg) and highest (100 mg) strengths of tablets. As the tablets are compressed from a common blend, a partial bracketing approach in accordance with VICH guideline GL45 was used. The batches were packaged in Alu/Alu blisters, however the lidding foil used in the stability studies did not include the paper and polyethylene layers that are present in the proposed packaging, and so the blister used in the stability studies is considered worst-case in comparison to the proposed commercial packaging, which is acceptable.

Samples were stored at 25ºC/60% RH, 30ºC/65% RH and 40ºC/75% RH according to VICH guideline GL3 and stability data is available to 18 months. The study is scheduled to continue up to 60 months. All results are in compliance with the currently proposed specification. Although there have been some

trends noted in assay, there is no overall trending apparent with respect to storage conditions. The provided stability data supports the proposed shelf-life of 30 months with no special temperature storage conditions. No photostability studies have been provided, which is accepted as the blister packaging will provide adequate protection from light, and so, the storage condition of "Store in the original package in order to protect from light" is included in the relevant sections of the SPC and product literature.

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the finished product is satisfactory.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics.

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical aspects relevant to the performance of the product have been investigated. Information relating to the discriminatory nature of the dissolution test has been provided, along with amended limits for dissolution to ensure batches continue to display dissolution properties similar to that of clinical trial batches.

The manufacturing process is a standard one. A detailed description of the manufacturing process has been provided along with relevant in-process controls. Process validation data has been provided for three full scale finished product batches and the data provided demonstrates that all critical parameters are within acceptable limits, and that a quality product is consistently produced.

The active substance enflicoxib is not monographed in the Ph. Eur. and data on the active substance is provided in the form of an Active Substance Master File. The proposed applicant's active substance specifications are considered to be acceptable. Acceptable batch analysis data has been provided.

Acceptable specifications have been provided for the excipients. Acceptable information has been provided for the container-closure systems. Valid TSE declarations from the manufacturers of the active substance and excipients have been provided.

The finished product release specification controls relevant parameters for the dosage form.

Stability data has been provided for the active substance and finished product. The provided stability data supports the proposed shelf-life of 30 months with no special temperature storage conditions but including a labelling requirement for photo-sensitivity.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. Based on the review of the data on quality, the manufacture and control of the product are considered to be acceptable.

Part 3 – Safety

The active substance of Daxocox is enflicoxib, a new active substance not authorised for a veterinary medicinal product in the EU before. Firocoxib, mavacoxib, robenacoxib and cimicoxib are other compounds from the coxib class that are currently licensed in Europe for the management of degenerative joint disease in dogs. The majority of these products are administered on a daily basis. However, mavacoxib has been authorised within the EU with an extended between-treatment-interval

(initially 14 days followed by monthly re-treatments thereafter). Consequently, the proposal to have a longer re-treatment interval (weekly) than 24 hours is not novel for Daxocox.

Enflicoxib (E-6087) is a drug substance described as belonging to the anti-inflammatory and anti-rheumatic group of products known as 'coxibs' and therefore belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs).

The product is intended for oral administration to dogs for the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease).

The product is intended to be administered before or with the dog's meal using an initial dose of 8 mg enflicoxib per kg bodyweight (bw), followed by repeated doses of 4 mg enflicoxib per kg bw every 7 days.

The product is available in five tablet strengths: 15, 30, 45, 70 and 100 mg tablets for dogs.

A full safety file in accordance with Article 12(3)(j) has been provided.

Enflicoxib is also referred to as E-6087 in the application dossier and both names are used interchangeably in this assessment report.

Safety documentation

Enflicoxib (acronym: E-6087) is a new coxib that contains one stereogenic centre at the C5 atom of the 4,5-dihydro-1H-pyrazole moiety. It is a 50:50 racemic mixture with a more active enantiomer, (*S*)-(-) enflicoxib (acronym: E-6232), and a less active enantiomer, (*R*)-(+) enflicoxib (acronym: E-6231).

Given that the active moiety has a stereogenic centre, the CVMP 'Note for guidance: Investigation of chiral active substances' (EMEA/CVMP/128/95-FINAL) is considered to have relevance for this application.

Pharmacodynamics

The enzyme cyclooxygenase (COX) has two well-defined isoforms, COX-1 and COX-2. The enzyme cyclooxygenase 1 (COX-1) catalyses the transformation of arachidonic acid into prostaglandin G_2. This substance is then peroxidised, in a reaction catalysed by the same enzyme, yielding prostaglandin H_2, which is then further transformed to biologically active prostaglandins (e.g. prostaglandin E_2) and other eicosanoids by various other enzymes. The synthesis of prostaglandins, which are important mediators of inflammation (swelling, rash, etc.) and pain, is known to be inhibited by NSAIDs.

In vitro inhibition of COX enzymes

In order to characterise the pharmacodynamics of the active moiety, a number of *in vitro* isolated enzyme studies were conducted to demonstrate the inhibition of COX enzymes by enflicoxib. In these studies, purified enzymes were obtained from bovine seminal vesicles (COX-1) and sheep placenta (COX-2).

An *in vitro* study was performed to determine the activity (50% inhibitory concentrations [IC_{50}]) of enflicoxib on the enzymatic reactions of COX-1 and COX-2 when compared with other anti-inflammatory agents (celecoxib, naproxen, NS-398 and rofecoxib) whose effect is attributed to the specific inhibition of the COX-2 enzyme. Results of this experiment suggest that enflicoxib displays COX-inhibition with a greater selectivity for the COX-2 than the COX-1 isoenzyme.

In another *in vitro* study, enflicoxib and each of the enantiomers (E-6231 and E-6232) were tested simultaneously to compare their potency and COX-2 selectivity. Results suggest that enflicoxib is approximately 114 times more active in inhibiting COX-2 than COX-1.

E-6231 (*R*-enantiomer) did not inhibit COX-1 (at 500 µM) and only exhibited 30% inhibition of COX-2 (at 100 µM). The IC_{50} of E-6232 (*S*-enantiomer) was 0.53 µM at which concentration it exhibited 84% inhibition of COX-2 but was inactive in inhibiting COX-1. Therefore, enflicoxib and its more potent *S*-enantiomer (E-6232) can be considered selective COX-2 inhibitors with the S-enantiomer being responsible for the main pharmacological effect.

It was reported that E-6132 is the main active metabolite of enflicoxib, so an *in vitro* study was conducted to quantify the inhibitory potency of E-6132 in an isolated COX-1 and COX-2 enzyme assay. The IC_{50} of E-6132 in the COX-1 assay was 133 µM, while, in the COX-2 assay, the IC_{50} was 0.5 µM. This suggests that E-6132 is about 266 times more potent in inhibiting COX-2 than COX-1, with a similar IC_{50} value to E-6232 (*S*-enantiomer) as obtained in the previously mentioned study.

Two *in vitro* studies using whole blood from humans and dogs were conducted to demonstrate the inhibition of COX enzymes by enflicoxib.

Human blood samples were taken from 4 healthy male volunteers. The effects on COX-1 activity were evaluated with coagulated blood and the effects on COX-2 activity were evaluated using heparinised whole blood samples in the presence of lipopolysacharride (LPS) stimulation. The results indicate that both enflicoxib and the *S*-enantiomer (E-6232) show similar inhibitory effects against COX-2 (as measured by inhibition of thromboxane B2 formation [TxB_2]). However, the *R*-enantiomer (E-6231) did not exhibit any anti-COX-2 activity. This is consistent with the findings of the previously reported studies above. Similarly, both enflicoxib and the *S*-enantiomer (E-6232) showed inhibitory effects against COX-1 (as measured by inhibition of prostaglandin E2 formation [PGE_2]). However, the *R*-enantiomer (E-6231) did not exhibit any anti-COX-1 activity.

Enflicoxib was more active towards COX-2 than the E-6232 enantiomer, but with less potency than E-6232 as E-6087 was 43 fold more active towards COX-2, whereas E-6232 was only 2.6 fold more active towards COX-2. Although these results were obtained using human blood (as opposed to canine blood), given that the arachidonic cascade pathway is understood to be the same in both species, the findings are considered supportive for the target species.

In a similar manner, inhibitory activity of enflicoxib (racemic mixture), E-6232 (*S*-enantiomer), E-6231 (*R*-enantiomer) and E-6132 (main metabolite) against COX isoenzymes (COX-1 and COX-2) in dog whole blood were compared in a GLP-compliant study using standard enzyme immunoassay (EIA) methods. Comparison of mean IC_{50} values demonstrated that the order of greatest potency for inhibitory activity against COX-2 was as follows: E-6132 > E-6232 > enflicoxib (E-6087) > E-6231, confirming the findings from previous studies.

Following on from the above *in vitro* studies, a number of *in vivo* studies were performed to investigate inhibition of COX enzymes.

***In vivo* inhibition of COX enzymes**

Three non-GLP-compliant *in vivo* studies using rat carrageenan exudate models were conducted to demonstrate the inhibition of COX enzymes by enflicoxib.

The results of these studies indicate that the parent compound enflicoxib, its *S*-enantiomer (E-6232) and its main metabolite (E-6132) principally inhibit PGE_2 in inflammatory exudate and therefore, based on the hypothesis that this represents COX-2 inhibition, can be considered to represent COX-2 selectivity and that, based on ED_{50} values, the metabolite appears to have more potent COX-2 inhibitory properties than its parent enflicoxib. Results also indicate that enflicoxib and its enantiomers E-6231 and

E-6232 demonstrate no inhibition of PGE_2 production in the gastric mucosa and therefore theoretically showed no inhibition of the COX-1 isoenzyme in the gastric mucosa. However, the metabolite E-6132 did show some inhibitory activity on PGE_2 production in the gastric mucosa, with a mean inhibition of 55.9% at a 40 mg/kg bw dose. Whilst these studies were not carried out in the target species, it can be accepted that, overall, they support results observed *in vitro* concerning the selectivity of enflicoxib, its *S*-enantiomer E-6232 and its main metabolite E-6132 as COX-2 inhibitors.

The inhibitory activity of enflicoxib compared to celecoxib and aspirin given by the intraperitoneal route was tested using the 6-day air pouch model where the injection of sterile air in the scapular region of mice followed by an injection of LPS or zymosan into the pouch triggers an inflammatory reaction. The ED_{50} of enflicoxib on inflammatory exudate PGE_2 levels was 0.4 mg/kg bw and mean inhibition at doses of 0.039, 0.156, 0.625, 2.5 and 10 mg/kg bw was 25.3, 50.2, 53.8, 58.3, and 79.5%, respectively, suggesting a dose-related effect.

***In vivo* anti-inflammatory activity**

In addition to investigation of inhibitory activity against COX isoenzymes, the anti-inflammatory effect was investigated in two *in vivo* studies.

In one study, the activity of enflicoxib was investigated after induction of inflammatory oedema in rats using carrageenan injected into the paw. The inhibitory activity of enflicoxib was compared with celecoxib administered by the oral route at various doses (0.63, 2.5, 10 and 40 mg/kg bw) and a control group receiving arabic gum only. A plethysmograph was used to measure the volume of the paw. The ED_{50} of enflicoxib had not been reached at either 4 or 5 hours therefore, calculations were made using the ED_{25}. The ED_{25} for enflicoxib at 4 hours was 6.23 mg/kg bw and at 5 hours was 2.48 mg/kg bw. The mean inhibitory activity of enflicoxib at 4 hours for doses of 0.625, 2.5, 10 and 40 mg/kg bw was 11.9, 19.4, 27.9 and 26.9%, respectively. The inhibitory activity levels at 5 hours at doses of 0.625, 2.5 and 10 mg/kg bw were 10.8, 24.9 and 39.6%, respectively, suggesting increasing inhibition with time. It can be accepted that this study suggests an anti-inflammatory effect of enflicoxib in the rat model used.

Another study investigated the anti-inflammatory activity of enflicoxib on the induction of chronic arthritis in rats using *Mycobacterium butyricum* given orally. Enflicoxib was administered by the oral route at various doses (0.039, 0.156, 0.625, 2.5, 10 and 40 mg/kg bw per day) and a control group receiving arabic gum only. A plethysmograph was used to measure the volume of the paw and was compared to a paw that had not been injected. The ED_{50} for enflicoxib was 0.50 mg/kg bw/day. A significant dose-related activity in the inhibition of arthritis for enflicoxib at doses of 0.039, 0.156, 0.625, 2.5, 10 mg/kg bw/day were observed. A significant difference in results for doses of 0.625, 2.5, 10 and 40 mg/kg/day were observed in comparison to the control group. Maximum inhibition of arthritis of 84.4% was observed in rats administered 10 mg/kg bw. It is noted that rats administered the highest dose of 40 mg/kg bw were not observed to have a greater inhibition of arthritis than those administered 10 mg/kg bw.

***In vivo* analgesic activity**

The inhibitory activity of enflicoxib was investigated following oral administration against the hyperalgesia induced by thermal stimulus in the paw of a rat inflamed by carrageenan. 405 rats were distributed into several groups and each animal received an injection of sterile saline in the left rear paw and carrageenan in the right rear paw. The control group received only arabic gum, while the test group were administered enflicoxib at varying doses (range 0.01–10 mg/kg bw) by the oral route two hours after injection into the paw with carrageenan. The results of this study suggest that E-6087 has an anti-hyperalgesia and consequently an analgesic effect when administered to rats, which was observed to be

dose-dependent. The ED_{50} for E-6087 was 0.21 mg/kg bw. E-6087 demonstrated a significantly greater activity than celecoxib.

In a similar experimental model, the anti-hyperalgesic activity of enflicoxib was compared to its enantiomers E-6231 and E-6232. The ED_{50} for enflicoxib was 0.48 mg/kg bw, 25.8 mg/kg bw for E-6231 (*R*-enantiomer) and 0.19 mg/kg bw for E-6232 (*S*-enantiomer). The minimum dose at which enflicoxib showed a significant difference when compared to the control was 0.31 mg/kg bw. It can be accepted that the results of this study suggest that the *S*-enantiomer is likely to have the greatest anti-inflammatory effect when compared to the parent moiety and the *R*-enantiomer and that the laboratory model indicates that this anti-inflammatory effect includes an anti-hyperalgesia (analgesic) effect.

Conclusions on pharmacodynamics

It can be accepted that the applicant has investigated the pharmacodynamic effects of the parent moiety (E-6087) and both enantiomers (*S*-enantiomer E-6232 and *R*-enantiomer E-6231) in accordance with the CVMP 'Note for guidance: Investigation of chiral active substances' (EMEA/CVMP/128/95-FINAL).

Whilst most of the studies appear to be non-GLP-compliant, the following can be accepted:

- Enflicoxib appears to selectively inhibit the inducible form of cyclooxygenase, i.e. COX-2, both *in vitro* and *in vivo*;
- Isoform-specific assays *in vitro* and *in vivo* show that enflicoxib and its *S*-enantiomer (E-6232) as well as active metabolite E-6132 appear to selectively inhibit the COX-2 isoform;
- The *R*-enantiomer (E-6231) does not appear to have significant inhibitory effect against any COX isoform *in vitro* or *in vivo*;
- E-6087, E-6232 and E-6132 were shown to have anti-hyperalgesic and anti-inflammatory properties in experimental models of inflammation and pain in rats;

Based upon the data provided, it can be accepted that the active substance enflicoxib, its *S*-enantiomer E-6232 and its metabolite E-6132 appear to be selective inhibitors of the COX-2 enzyme.

Data has been provided to characterise (both *in vitro* and *in vivo*) the pharmacodynamic properties of the active moiety (E-6087) and both of its enantiomers. The *S*-enantiomer (E-6232) appears to be significantly more active than the *R*-enantiomer (E-6231) and the metabolite E-6132 appears to have greater potency than either E-6087 or E-6232.

The *in vivo* studies conducted in the rat are considered supportive of the proposed anti-inflammatory and anti-analgesic activity of E-6087 under the experimental conditions used.

It can be accepted that the studies provided suggest that there is limited inhibitory activity by the parent compound E-6087 and its enantiomers E-6231 and E-6232 against COX-1. The impact on the activity of the COX-1 enzyme in renal *papillae* was only considered in one *in vivo* study and considered only the parent compound E-6087. Given that NSAIDs are known to have possible adverse effects on the gastrointestinal and renal systems, the product will be expressly contraindicated for use in animals with impaired renal function and in animals suffering from gastrointestinal disorders, protein or blood-losing enteropathy or haemorrhagic disorders.

Pharmacokinetics

A number of studies investigating the pharmacokinetics of the product have been provided, assessing the pharmacokinetics of the parent moiety as well as its enantiomers and metabolites. Those relevant

to assess the safety of enflicoxib are discussed below and those studies relevant for the proposed posology are discussed in part 4 of this report.

Absorption

^{14}C-labelled ADME study

The absorption, distribution, metabolism and excretion of enflicoxib was studied in 16 male Wistar rats after single administration of 2.5 mg/kg bw intravenously and 5 mg/kg bw by the oral route using ^{14}C-labelled compound. Plasma levels, urinary and faecal excretion, the metabolic profiles of urinary and faecal excretion and parameters defining the kinetics of a single dose of enflicoxib and its metabolites in rats were determined under fasting conditions.

Following intravenous (i.v.) administration of 2.5 mg/kg bw, a double-peak phenomenon was observed which could be related to the entero-hepatic cycle of the formed metabolites. The total radioactivity plasma levels showed a slow elimination, with a half-life ($t_{1/2}$) of 51 h for the i.v. route and $t_{1/2}$ of 144 h for the oral route. Urinary excretion was minimal, accounting for 5–6% of the dosed radioactivity by both routes. Radioactivity was excreted gradually, with a recovery of 75 and 83% after intravenous and oral administration, respectively, at the eleventh day of collection. The metabolic profile of urinary excretion showed about eleven metabolites, with the metabolite 'M8' being the main one. The parent drug was practically not detected and the metabolite profile was identical following both administration routes. Glucuronide or sulphate conjugates were not observed. Faecal excretion was found to be the main excretion route and the main metabolite (M8) represented 16–20% of the administered dose. The remaining metabolites, (including E-6132) showed percentages between 3 and 6%. Enflicoxib excretion was 29% and 4% following oral and i.v. administration, respectively. The metabolic profile of faecal radioactivity showed the existence of three metabolites (M8, M7 and M9 [or E-6132]) and the parent drug following i.v. administration as well as a fourth metabolite (M4) in addition to the parent drug following oral administration. M8 was the main metabolite in both routes, representing 16–20% of the administered dose at 72 hours. The dose-normalised oral:intravenous AUC ratio was used to determine an absorption index value of 101%, suggesting complete absorption following oral administration.

Based on the findings from this study conducted in rats, it is accepted that almost complete absorption of enflicoxib is to be expected following oral administration, that urinary excretion is expected to be limited (maximum of 6%) and that the faecal route is the main route of excretion.

According to the applicant, the long-lasting pharmacodynamic activity of enflicoxib is believed to be due to its metabolite M9 (E-6132). However, two additional main phase I metabolites of enflicoxib have been described in the dog, namely M7 and M8. A study was therefore performed to determine the persistence of M7 and M8 in the plasma of dogs. Dogs were dosed orally with enflicoxib at 8 mg/kg bw under fed conditions and samples were analysed by LC-MS-MS using a qualified bioanalytical method. M7 was quantifiable in the plasma of all animals from 4 h up to at least 72 h after dosing. M8 was quantifiable from 0.5 h up to at least 240 h. T_{max} (time to reach the peak plasma concentration C_{max}) ranged from 4 to 48 hours for M7 and from 12 to 24 hours for M8, respectively. The elimination half-lives were similar for M7 and M8, i.e. 50.0 and 42.7 h, respectively. The $t_{1/2}$ values for M7 and M8 were determined to be approximately 9-fold lower than that for metabolite M9 (E-6132; $t_{1/2}$ = 430 h) determined in another study, indicating that M7 and M8 do not contribute to the prolonged pharmacologic activity of enflicoxib. Based upon these results, together with the data from the pharmacodynamic studies, the applicant concluded that E-6132 is the most representative metabolite to be characterised in the pivotal pharmacokinetic and PK/PD studies in dogs. The applicant also investigated the inhibitory potential of M8 on COX-1/COX-2 *ex vivo* in dog blood in comparison to the

inhibition induced by enflicoxib and E-6132. Based on the results provided, it was concluded that M8 does not inhibit the COX-1 or COX-2 isoenzymes. Results indicate that E-6132 is the metabolite responsible for pharmacological effect (as opposed to M8).

Plasma/tissue distribution

A study was performed to determine the extent of protein binding of E-6087 and the *S*-enantiomer (E-6232) to human plasma protein by ultracentrifugation technique at a concentration of 200 ng/ml using HPLC chromatographic techniques. The results showed that E-6087 and E-6232 are highly bound to human plasma proteins (97.5% and 96.5%, respectively).

A separate GLP-compliant study was conducted to investigate the extent of protein binding by E-6087 and the *S*-enantiomer (E-6232) to canine plasma protein using rapid equilibrium dialysis (RED) followed by mass spectrophotometry. The unbound fraction of enflicoxib and E-6132 was 1.5% and 0.5% in dog plasma, respectively, demonstrating that the parent compound and its metabolite E-6132 (M9) exhibit a high protein-binding affinity in canine plasma. Given these findings, it may be expected that this may have implications for potential competition/interactions with other protein-bound active substances.

A further study was performed to explore the *in vitro* blood-to-plasma partitioning of enflicoxib and its metabolite (E-6132) in dogs and rats, as enhanced uptake of E-6132 into erythrocytes could be one possible explanation for the longer *in vivo* half-life observed for this metabolite when compared to enflicoxib. Whole blood samples were taken from 3 untreated female Beagle dogs and 15 untreated male Wistar rats. LC-MS analysis was used to calculate the blood-plasma concentration ratio (Cb/Cp). The red blood cell (RBC) to plasma ratio ($K_{RBC/PL}$) was also calculated from these values. The assays were performed at three increasing blood concentrations (1, 5 and 15 µg/ml). The parent compound demonstrated a species difference in the blood cell distribution between the rat and dog. In dogs, enflicoxib was mainly distributed to the plasma compartment at high blood concentrations (Cb/Cp of 0.66 at 15 and 5 µg/ml), while it was equally distributed between red blood cells and plasma at the lower blood concentration of 1 µg/ml (Cb/Cp: 1.07).

Interestingly, in dogs, E-6132 showed dose-dependent distribution favourable to the cell compartment with the Cb/Cp increasing from 0.95 to 1.40 and 2.49 at 15, 5 and 1 µg/ml blood concentrations, respectively. It can be concluded that enflicoxib and its metabolite E-6132 are highly bound to plasma proteins, with the parent compound favouring the plasma compartment at higher concentrations, whereas E-6132 favours the cellular compartment in a dose-dependent manner.

Although the applicant has not investigated tissue distribution of enflicoxib, an additional study was conducted to investigate the extent of distribution of enflicoxib and its two metabolites E-6132 and M8 in synovial fluid of dogs when administered intravenously. Based upon the findings from this study, it was accepted that distribution of E-6132 from plasma to synovial fluid is limited when measured up to 35 days after i.v. administration of enflicoxib.

Metabolism

A preliminary study was conducted to evaluate the *in vitro* metabolic profile of enflicoxib in rat and human liver microsomes. Sample analysis was performed using high performance liquid chromatography (HPLC) and three metabolites were found (M7, M8 and M9 [E-6132]). Metabolic profiles were similar in both species, with the metabolite with the highest absorption in both species having a similar retention time and UV absorption spectrum, suggesting it could be the same metabolite. Enflicoxib and E-6132 had different UV absorption spectra making it possible to study them

separately. Biotransformation rates were slightly higher in rats when compared to humans, at 385 and 188 pmol/min/mg protein, respectively. Based on the findings from this study, it can be accepted that all metabolites present in humans were also found in the rat, supporting the selection of this species for toxicological evaluation, as humans are not expected to be exposed to different metabolites than those produced in rats.

The *in vitro* metabolism of ^{14}C-labelled enflicoxib was studied in human, rat, dog and mouse hepatic microsomes. Three metabolites were formed, two with a pyrazole structure and another one with pyrazoline structure. One of the two pyrazole metabolites corresponds to E-6132. The other two metabolites are the hydroxylated pyrazole (M7) and the hydroxylated pyrazoline (M8) molecules. The biotransformation rate (expressed in pmol/min/mg protein and, with reference to the concentration of cytochromes P450 [CYP], in pmol/min/nmol CYP) of enflicoxib was 477.4 pmol/min/nmol CYP in humans, 163.7 in rats and 52.1 in dogs. The parent compound is principally metabolised to M8, consistent with previous study findings. It is noted in this study that the biotransformation rate of enflicoxib was higher in humans than in rats (447 vs 163 pmol/min/mg protein), unlike findings seen in another study. Of interest is the fact that the metabolite E-6132 (considered by the applicant to be the metabolite principally responsible for pharmacodynamic effect) was not detected in the presence of dog microsomes during this study but was detected in the presence of human and rat microsomes. The applicant suggests that this was as a result of the low sensitivity of the analytical method.

Another study was conducted to evaluate the *in vitro* metabolism of the *R*-enantiomer (E-6231) and the *S*-enantiomer (E-6232) by the action of enzymes present in mice, rat and human hepatic microsomes as well as in human intestinal microsomes. The metabolism was also studied in the presence of recombinant human microsomes expressing different P450 cytochromes (CYP) and flavin-containing monooxygenases. ^{14}C-labelled substance was used at a concentration of 10 µM. Consistent with the findings for the parent compound, the metabolic profile of both enantiomers showed three predominant metabolites, i.e. M7, M8 and M9 (E-6132), with M8 being the most predominant in all cases. Phase I metabolism of E-6232 in humans was mainly catalysed by CYP3A4. Enzymes involved in the transformation of E-6231 in humans were CYP2C19 and CYP3A4.

A study was conducted to explore the potential secondary metabolism of enflicoxib metabolites *in vitro*. The parent compound, its metabolite E-6132 and the main metabolite M8 were incubated separately with cryopreserved hepatocytes and liver microsomes from dog and rat and analysed by LC-MS. Nine metabolites of enflicoxib were detected in the study. M8 was transformed to metabolite M7, suggesting that M7 is a secondary metabolite of enflicoxib formed by reduction of the pyrazoline moiety of M8. E-6132 was demonstrated not to be the source of neither M8 nor M7.

While M8 has been identified as a hydroxyl pyrazoline derivative of enflicoxib, the chemical structure of M7 remained to be fully elucidated. Consequently, a study was conducted to generate M7 *in vitro* and identify its chemical structure. Enflicoxib was incubated with human recombinant CYP3A4 in order to generate sufficient M7 for chemical identification purposes using LC-MS techniques. Approximately 60% of the parent compound was metabolised into its 3 main phase I metabolites: E-6132 (pyrazole derivative of E-6087), M8 (hydroxylated E-6087) and M7. Results suggest that the M7 metabolite is a secondary metabolite of enflicoxib, produced from M8 by reduction of its pyrazoline moiety.

A study was conducted to explore the potential intestinal metabolism of enflicoxib *in vitro* in the dog and rat. Enflicoxib was incubated separately with intestinal microsomes from dog and rat in the presence of NADPH cofactor. Three metabolites were detected, matching with the exact masses of E-6132, M8 and M7, suggesting a first-pass effect of enflicoxib is possible in dogs and rats after oral administration. However, the rate of enflicoxib metabolism *in vitro* was determined to be very low in both rats and dogs.

Following incubation of enflicoxib with cryopreserved hepatocytes and liver microsomes in dogs, three

main metabolites were detected: E-6132 (pyrazole derivative of E-6087), M8 (hydroxylated E-6087) and M7 (hydroxylated pyrazole derivative of E-6087). Based on results of PK analyses, the applicant considered that both M7 and M8 achieve lower blood levels and last for a shorter length of time than E-6132. Therefore, E-6132 is considered by the applicant to be the main active metabolite responsible for the long-lasting activity of the product. Consequently, E-6132 is the metabolite that has been characterised in the pivotal PK/PD studies in dogs.

The metabolite E-6132 exhibits a very low clearance, much lower than the parent compound and M7 or M8. As it is detected in blood for a comparatively longer period than the other metabolites (M7 and M8) after administration of a single dose of enflicoxib, the applicant proposed that this permits the repeated administration of the product at once weekly intervals, which is considered beneficial for treatments with prolonged treatment periods.

Excretion

Enflicoxib predominantly undergoes biliary excretion, with faecal excretion found to be the main excretion route and the main metabolite (M8) representing 16–20% of the administered dose. The remaining metabolites (including E-6132) showed percentages between 3 and 6%. Enflicoxib excretion was 29% and 4% following oral and i.v. administration, respectively.

In vivo pharmacokinetic studies in mice and rats

A study was conducted to determine the pharmacokinetic parameters of enflicoxib and its metabolite E-6132 in male and female mice after single oral administration of enflicoxib at a dose of 2000 mg/kg bw. Plasma levels of enflicoxib were higher than those observed for E-6132. After oral administration of E-6087, t_{max} was 4 h for male and 6 h for female rats. Metabolite formation was very slow for both sexes with a t_{max} of 24 hours.

In another study, the pharmacokinetics of enflicoxib in male and female Wistar rats after single-dose administration of 1, 5 or 25 mg/kg bw by the oral route and 1 mg/kg bw by the intravenous route was investigated. 60 rats of each sex were administered 1 mg/kg bw i.v., 57 rats of each sex were administered 1 mg/kg bw and 5 mg/kg bw orally, 15 rats of each sex were administered 25 mg/kg bw orally and 15 males were administered 1 mg/kg bw orally in the presence of food. After intravenous administration, $t_{1/2}$ was 24.5 h in males and 33.5 h in females. Total plasma clearance was lower in females (0.13 l/h/kg) than in males (0.22 l/h/kg). Following oral administration, the peak plasma concentration (C_{max}) was reached between 2–6 h after administration in both sexes. Bioavailability was moderate in females (47.4–65%) and good in males (62.3–99.2%). There were no clear sex-related differences for C_{max} and AUC. Food decreased bioavailability by approximately 22%, but $t_{1/2}$, T_{max} and C_{max} remained unaffected. Elimination after oral administration was faster in males ($t_{1/2}$ = 15.3–19.2 h) than in females ($t_{1/2}$ = 17.7–28.0 h).

A study was conducted to determine the plasma levels and the pharmacokinetic parameters of metabolite E-6132 after oral administration of enflicoxib to male and female rats at the dose of 5 mg/kg bw under fasting conditions. A C_{max} of E-6132 was reached 24 h after administration. Substance elimination was faster in males than in females with a $t_{1/2}$ of 30.7 h in males and 122.4 h in females. The concentrations of E-6132 were 10 times lower than those of enflicoxib between 0.25 to 3 h post administration and 4–10 times lower within 4 to 8 hours. Based on the findings of this study, it would appear that lower concentrations of E-6132 are observed when compared with the parent enflicoxib.

Having studied the PK profile of the parent moiety and the metabolite E-6132 in rodents, the applicant provided the results of a study investigating the PK profile of the two enantiomers (E-6232 [*S*-

enantiomer] and E-6231 [*R*-enantiomer]) and the possible interconversion of the two enantiomers after separate oral administration of 5 mg/kg bw dose of each substance to rats. Higher levels of the *R*-enantiomer E-6231 in plasma were detected than for the *S*-enantiomer E-6232. No *in vivo* interconversion between enantiomers was observed. An elimination half-life of 18.3 h for E-6231 was determined and it was concluded that the pharmacokinetics of E-6231 are similar to those of the parent compound enflicoxib.

In vivo pharmacokinetic studies in humans

Results of three studies conducted in humans were provided.

Results of a study to determine the safety, tolerability and pharmacokinetics of six increasing single doses of enflicoxib in healthy human volunteers were provided. Forty subjects were exposed to different enflicoxib doses ranging from 1 to 300 mg administered orally as capsules. Both C_{max} and $AUC_{0\text{-}48h}$ in plasma were only found to be dose-proportional in the lower dose range (5–25 mg). Dose increases from 25 to 100 mg resulted in only minor increases in AUC and C_{max}, although this finding may have been affected by poor solubility of the active substance.

Results of another study were provided in which the bioavailability of two enflicoxib capsule formulations (capsules with active compound, capsules with active compound and excipients) were compared with an alcoholic solution in healthy volunteers administered a single 100 mg oral dose. The relative bioavailability of capsules of active ingredient only and capsules with excipients averaged 84% and 81%, respectively, suggesting high bioavailability after oral intake in humans.

In the third study, enflicoxib was administered at a single dose of 100 mg by the oral route in an alcohol formulation to 6 male volunteers. Blood samples were collected for up to 168 h post administration. C_{max} for enflicoxib and E-6132 was 556 and 338.7 ng/ml, respectively. $AUC_{0\text{-}t}$ for enflicoxib and E-6132 was 16,915 and 43,182 ng*h/ml, respectively. T_{max} for enflicoxib and E-6132 was 1.3 and 128 h, respectively. $T_{1/2}$ for enflicoxib was 34.2 hours, but no $t_{1/2}$ could be determined for E-6132. Based on the findings from this study, it would appear that the metabolite E-6132 has a much longer elimination half-life when compared with the parent moiety. This is consistent with the findings from previous studies conducted in mice.

In vivo pharmacokinetic studies in dogs

A study was conducted to evaluate the bioavailability of enflicoxib in 6 male and female Beagle dogs after oral (5 mg/kg bw) and i.v. (1 mg/kg bw) administration on a single occasion. Enflicoxib (using a particle size of 45 µm) was administered to each animal by oral and intravenous routes with an interval of 2 weeks. Blood samples were collected up to 120 hours. The analytical determination was performed by HPLC with fluorescence detection. The bioavailability was moderate (30–53%). After oral administration of the substance, additional peaks were observed which could be related to enterohepatic circulation. Following intravenous administration, the distribution volume (V_{ss}) adjusted for bodyweight, was 29.53 ± 4.02 l in males and 34.15 ± 6.98 l in females. Mean residence times (MRT) and elimination half-lives ($t_{1/2}$) following oral administration were 35.17 ± 9.35 h and 25.83 + 6.93 h, respectively. Following i.v. administration, an MRT of 27.45 ± 7.11 h, an elimination half-life of 24.50 ± 6.87 h and a total plasma clearance (CL) of 0.10 ± 0.03 l/h/kg were obtained. Elimination was observed to be similar regardless of the administration route.

Another study was conducted to characterise the plasma kinetics of micronised enflicoxib after oral administration in dogs and to study the influence of a reduction in particle size. Results indicated similar peak plasma levels (C_{max}) between males and females. Similar $AUC_{0\text{-}\infty}$ values were also obtained for males and females. Bioavailability was 69.9% for males and 81.1% in females. Elimination and MRT were not influenced by the smaller particle size. Based on the findings from this study, it can be accepted that increased absolute bioavailability arises from use of a smaller particle size.

A pilot study was conducted to evaluate the pharmacokinetics of enflicoxib and its metabolite E-6132 in 8 Beagle dogs following a single oral administration of enflicoxib capsules at 1 mg/kg bw and 4 mg/kg bw on a single occasion under fasted conditions. For enflicoxib, t_{max} was 6 h for the 1 mg/kg bw dose, and 18 h for the 4 mg/kg bw dose. For E-6132, t_{max} was 132 h (5.5 days) for both dose rates. Overall mean C_{max} values for enflicoxib were 384.5 ng/ml for the 1 mg/kg bw dose and 872.1 ng/ml for the 4 mg/kg bw dose. Systemic exposure (as measured by $AUC_{0-\infty}$) was 14923.8 ng/h/ml and 47047.5 ng/h/ml for 1 mg/kg bw and 4 mg/kg bw, respectively. Overall mean C_{max} values for E-6132 were 165.8 ng/ml for the 1 mg/kg bw dose and 577.0 ng/ml for the 4 mg/kg dose, respectively. AUC_{0-t} was 40,783.7 ng/h/ml and 132,329.1 ng/h/ml for the 1 and 4 mg/kg bw doses, respectively. $T_{1/2}$ values for enflicoxib for the 1 and 4 mg/kg bw doses were 31.0 and 29.3 h, respectively. $T_{1/2}$ values for E-6132 could not be determined. Based on the results of this study, it can be accepted that a dose-related effect in the target species was observed in respect of PK parameters when comparing the 1 and 4 mg/kg bw dose rates. It can be accepted that the pharmacokinetics of the metabolite E-6132 in the dog is consistent with that reported previously in the laboratory animal studies in terms of a long elimination half-life.

A GLP-compliant study was conducted to determine the absolute bioavailability and the effect of feeding on bioavailability of enflicoxib and its metabolite E-6132 under fed or fasted conditions in 30 healthy Beagle dogs. Twenty (10 fed and 10 fasted) healthy Beagle dogs (5 males and 15 females) were administered enflicoxib orally at a dose of 8 mg/kg bw. Ten healthy Beagle dogs (5 males and 5 females) were administered a dose of 2 mg/kg bw intravenously. Results indicated that enflicoxib was rapidly transformed to the active metabolite E-6132. T_{max} for enflicoxib after oral administration was higher in fed conditions (2 h) than in fasted conditions (4 h). The elimination half-life ($t_{1/2}$) of enflicoxib was 12 h after i.v. administration and 18–20 h after oral administration and was not influenced by feeding. However, the metabolite E-6132 showed much longer elimination phases, with a $t_{1/2}$ of 185, 406 and 354 h for intravenous, oral fed and oral fasted conditions, respectively. Food increased the rate and extent of absorption and the relative bioavailability was determined to be 143.3% for enflicoxib. Of particular note from this study is the finding that the elimination half-life of the metabolite E-6132 has been determined to be long (353 h in fasted dogs and 406 h in fed dogs).

Taking into account the elimination half-life stated and the outcome of PK/PD modelling, a warning will be included in section 4.4 of SPC not to administer other non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids concurrently or within 2 weeks of the last administration of this veterinary medicinal product.

Two pilot efficacy studies were conducted.

In one study, osteoarticular pain and inflammation was experimentally induced using a reversible arthritis model by means of an intra-articular injection of urate crystals in the femorotibial joint of the leg of dogs (9 male dogs, 3 for each group) at day 0 (group 1) or 2 (group 2) and repeated at days 7, 14 and 21 in both groups. Dogs were treated orally at days 0 and 12 with 4 mg/kg bw of enflicoxib by means of a capsule. Plasma concentrations of enflicoxib and its active metabolite E-6132 were evaluated before treatment and on days 7, 14 and 21 (group 1) and on days 2, 7, 14 and 21 (group 2). After repeated dose administration of 4 mg/kg bw of enflicoxib at days 0 and 12, at the last measured time point (21 days), mean plasma levels of E-6132 were 5–10 times higher than those obtained for the parent compound, confirming a slower elimination half-life for the metabolite in this experimental model as observed in previous studies in healthy dogs. Although the proposed loading dose (8 mg/kg bw) was used in this study, the retreatment interval is longer (12 days) than proposed (7 days).

In the second study, using the same experimental model to induce osteoarticular pain and inflammation as the previous study, thirteen male Beagle dogs were randomly allocated to four groups.

Animals in groups A and B were administered a dose of 4 mg/kg bw of enflicoxib in one capsule on days 0, 1, 7, 14 and 21. Animals in group C were administered a loading dose of 8 mg/kg bw of enflicoxib as two capsules on day 0 and then 4 mg/kg in one capsule on days 7, 14 and 21. One animal was included in group D as an untreated control. Animals were fasted in this study. Blood samples were collected from group C on days 9, 21, 28, 35, 42, 49 and 56. Results indicated that exposure to metabolite E-6132 was higher than for the parent compound. Following repeated oral administration of a loading dose of 8 mg/kg bw of enflicoxib to dogs on day 0, followed by doses of 4 mg/kg bw on days 7, 14 and 21 (group C), mean plasma levels of the parent compound were above the limit of quantification (5 ng/ml) up to day 35, i.e. 14 days after the last dose administered. Animals were continuously exposed to its metabolite (E-6132) during the whole sampling schedule up to day 56, which represents 35 days after the last dose administered.

Toxicokinetic studies

The applicant also provided in this section PK information from three repeat dose toxicology studies providing information on toxicokinetics (2 studies in rats and 1 study in dogs). Only the PK information from these studies is summarised below as the toxicity aspect of these studies are discussed in more detail in the toxicology and target animal safety sections of this document.

The first study in rats was a 4-week repeat-dose study. Enflicoxib was administered daily at doses of 12.5, 25, 61 and 150 mg/kg bw/day for four weeks and measured in plasma (days 1 and 28 of treatment) by HPLC with fluorescence detection in conjunction with an on-line solid-phase extraction system. The pharmacokinetic profile of enflicoxib was characterised by lower plasma levels and a shorter elimination half-life ($t_{1/2}$) on the last day of treatment when compared with the first day of treatment. Dose-related kinetics were observed on day 1 in C_{max} and AUC_{0-24}, but not at day 28. The linearity observed on day 1 was obtained at all tested doses except the highest one. T_{max} appears to have been modified by continuous administration of enflicoxib and was shorter on the last day of treatment than on the first day (6–12 h vs 2–6 h for days 1 and 28, respectively). No accumulation of enflicoxib after repeated administration in rats was observed.

In the second study in rats, plasma levels of both enantiomers (E-6231 and E-6232) were determined in rats after daily oral administration of 10, 25 and 70 mg/kg bw/day enflicoxib for 14 days. Samples were analysed by an on-line solid-phase extraction system connected to a HPLC with fluorescence detection. The enantiomers showed different pharmacokinetic behaviour. As for the parent moiety, no cumulative effects were seen. Continuous administration over 14 days resulted in an induction effect, as expressed by a decrease in C_{max} and AUC_{0-t}. This metabolic induction is more pronounced in the case of E-6231.

Enflicoxib was administered to male and female dogs once daily for 4 weeks at doses of 1, 4 and 15 mg/kg/day using gelatin capsules. Three dogs of each sex were administered 1 and 4 mg/kg bw whereas 5 dogs of each sex were administered 15 mg/kg bw. Blood samples were collected on study days 1 and 28 at baseline, 1, 2, 4, 6, 8, 10, 12, 16 and 24 h. Repeat daily dose administration was characterised by higher plasma levels on day 28 when compared to day 1, suggesting substance accumulation in dogs. The findings from this study differ to that from the previously reported study in rats given that accumulation of enflicoxib was observed. An accumulative factor was reported within the range of 2.1 to 3.8 and appears to be independent of the dose administered. This finding has significance in terms of dose and frequency of dose administration and also has direct relevance for the potential for accumulation of the metabolite E-6132.

Toxicological studies

A number of single and repeated dose toxicological studies with enflicoxib were carried out in mice,

rats and dogs.

Single dose toxicity

In a GLP-compliant single dose study in mice, a group of 5 male and 5 female mice were administered an oral dose of 2000 mg/kg bw of enflicoxib and monitored for 15 days. As one death was observed at day 4, this dose was considered lethal in mice.

In a GLP-compliant single dose study in mice, two groups of 5 male and 5 female mice were administered a dose of 500 or 750 mg/kg bw of enflicoxib by the intraperitoneal route and monitored for 15 days until necropsy. At 750 mg/kg, death of two females occurred on days 8 and 13. At 500 mg/kg bw, 2 females showed abnormal gait, hunched back and piloerection. The gastrointestinal tract was identified as the target organ, as duodenal perforation and stomach ulcers were reported at necropsy.

In a GLP-compliant single dose study in rats, a group of 5 male and 5 female rats were administered an oral dose of 2000 mg/kg bw of E-6087 and monitored for 15 days. As one death was observed at day 4, this dose was considered lethal in rats. Necropsy revealed an extremely dilated stomach and ulcers in the pyloric region, a perforated ulcer in the duodenum, dilated intestines, pale liver and multiple fibrous adhesions in the peritoneal cavity.

In a GLP-compliant single dose study in rats, two groups of 5 male and 5 female rats were administered a dose of 500 or 750 mg/kg bw of E-6087 by the intraperitoneal route and monitored for 15 days until necropsy. At 750 mg/kg bw, death of one male occurred at day 6. At necropsy, duodenal perforation at the pyloric zone, mucopurulent peritonitis, reddish renal *medulla* and *papillae* and adhesions in the peritoneal cavity were observed. Decreased spontaneous activity, hunched back, piloerection, ataxia, ptosis and constipation were observed in all females and most males at this dose. Healed ulcers at the duodenal pyloric zone were observed on necropsy of one male and one female.

These GLP-compliant studies in mice and rats were performed prior to the publishing of the updated OECD test guideline 420 in 2001. However, toxicological effects following single dose administration were evaluated in mice and rats following standard methods.

Whilst a range of doses (500, 750 or 2000 mg/kg bw) administered either by the intraperitoneal or oral route were investigated, it would appear that sighting studies (normally investigating doses of 5, 50, 300 and 2000 mg/kg bw) have not been performed prior to the selection of these dose rates.

It is noted that the applicant has not determined an acute toxicological reference value below which toxicity does not occur. As only a limited range of exposures have been investigated (500–2000 mg/kg bw), only limited conclusions may be consequently drawn.

Clinical signs were observed in mice receiving an intraperitoneal 500 mg/kg bw dose and a 1000 mg/kg bw oral dose. Clinical signs were observed in rats receiving an intraperitoneal 500 mg/kg bw dose and a 2000 mg/kg bw oral dose. However, studies in which doses less than 2000 mg/kg bw were administered orally were not performed in rats.

Repeat dose toxicity

Repeated dose oral toxicity studies with enflicoxib were conducted in rats and dogs. The 4-week toxicity studies in rats and dogs were preceded by preliminary shorter-term studies, in order to establish suitable dose levels for administration.

In a GLP-compliant study, four groups of Sprague-Dawley rats (5 male and 5 female per group)

received enflicoxib by oral gavage at 60, 200, 600 and 900 mg/kg bw/day for 14 days. Lethal effects were observed at doses of 200 mg/kg bw/day. No adverse effects were reported in rats administered 60 mg/kg bw.

In a GLP-compliant 4-week study in Sprague Dawley rats, enflicoxib was administered by oral gavage to four groups (36 male and 36 female each) at dose levels of 12.5, 25, 61 and 150 mg/kg bw/day. Deaths were observed in 4 males and 15 females at 150 mg/kg bw/day and in 2 females at 61 mg/kg bw/day. The applicant concluded that the NOAEL is 12.5 mg/kg bw/day.

In a GLP-compliant study, doses of 0, 10, 25 or 70 mg/kg bw/day of both enantiomers (E-6231 or E-6232) were administered orally to seven groups of 10 rats for 14 days. It was concluded that no significant differences were found in the toxicological profile of the substances E-6231 and E-6232 at the tested doses. Although the applicant concluded that there were no significant differences between enantiomers, no NOAEL was derived, as skin lesions (considered to have resulted from scratching) were observed in animals administered the lowest dose rate (10 mg/kg bw).

As pharmacokinetic studies have demonstrated that the metabolite E-6132 is formed from enflicoxib, it can be accepted that in studying the administration of enflicoxib and its enantiomers, the toxicology of E-6132 will also have been studied.

Two repeat-dose toxicity studies were conducted in the target species dogs. A preliminary dose range-finding toxicity study was performed in Beagle dogs over fourteen days and in a second study, enflicoxib was administered orally to Beagle dogs for 28 days.

In the first GLP-compliant study, doses of 60, 30 or 20 mg/kg bw were administered orally in gelatine capsules to 6 fasted Beagle dogs (one male and one female in each treatment group). 60 mg/kg bw (group 1; n = 2) and 30 mg/kg bw (group 2; n = 2) doses were administered once daily for 14 days while the 20 mg/kg bw (group 3; n = 2) dose was administered once daily for 28 days. Mortality was observed only in the 60 mg/kg bw/day group (one male). A decrease in the erythrocyte count and in the haemoglobin and haematocrit values were recorded in all groups. Gastrointestinal effects (blood in faeces and vomiting) were observed in all three groups. Histopathology revealed duodenal ulcers in the deceased male treated with 60 mg/kg bw/day (perforated), in the two animals administered 30 mg/kg bw/day and in the female administered 20 mg/kg bw/day. Renal pelvises were congested in the female treated with 60 mg/kg bw/day and in the male at 30 mg/kg bw/day. No NOAEL could be determined in this study as toxicological effects were reported at all dose levels.

In the second GLP-compliant study, 32 dogs were divided randomly into 4 treatment groups. The control (group 1; n = 10) received empty gelatine capsules. Doses of 1 mg/kg bw (group 2; n = 6), 4 mg/kg bw (group 3; n = 6) and 15 mg/kg bw (group 4; n = 10) were administered once daily for 28 days. Two males and females from the control group and the 15 mg/kg bw group underwent a 2-week recovery period to assess reversibility of events observed during the treatment period. Although the product was administered daily, it is noted that fatalities were observed in the 15 mg/kg bw/day dose group, blood in faeces was observed in all dose groups, duodenal ulceration, decreases in erythrocyte count and haematocrit was observed in both the 15 mg/kg bw/day and the 4 mg/kg bw/day dose groups and decreased total protein and albumin levels and albumin/globulin ratio appears to have been observed in all three groups. Given the observation of adverse effects at the lowest dose administered in this study (1 mg/kg bw/day), this was considered as the LOAEL rather than NOAEL.

A NOAEL of 12,5 mg/kg/day has been derived for laboratory animals (rats) from the study data provided. Two sub-acute repeat-dose toxicity studies were conducted in the target species dogs. It was not possible to derive a NOAEL from either of these studies given the occurrence of adverse events at the lowest doses studied (1 mg/kg bw/day), which is considered more representative of a LOAEL than a NOAEL. Based on these findings, it is concluded that enflicoxib has a relatively narrow therapeutic

margin in the target species when administered at the recommended treatment dose but at a higher frequency (once daily) than proposed (once weekly).

Tolerance in the target species of animal

The tolerance in the target animal is described under part 4.

Reproductive toxicity

Study of the effect on reproduction

No guideline-compliant reproductive toxicity studies have been conducted.

Whilst the applicant has described some of the studies provided as reproductive toxicity studies, these are considered more typical of developmental toxicity studies. In the absence of studies specifically investigating reproductive toxicity, the applicant has proposed that the product is not to be used in pregnant or lactating animals.

However, according to VICH GL 43, reproductive safety studies are required for systemically absorbed APIs intended for use in breeding animals and if reproductive safety studies have not been conducted in the target species, labelling should reflect this and state that safety has not been determined in breeding, pregnant or lactating animals or their offspring.

In light of the above, the product is contraindicated for use in animals intended for breeding purposes.

Study of developmental toxicity

In one toxicity study, enflicoxib was administered by the oral route (gavage) to four groups of 6 pregnant female Sprague-Dawley rats at dose levels of 40, 60, 80 and 100 mg/kg bw/day from day 6 up to and including day 15 of gestation. The females were sacrificed on day 20 of gestation and their uterine content was examined. Maternal toxicity (death) was recorded at dose levels of 60 mg/kg bw/day and above. All deaths were associated with the presence of perforated ulcers in the pyloric region of the gastrointestinal tract.

In a developmental study in rabbits, enflicoxib was administered by oral gavage to four groups of 6–8 pregnant female rabbits at doses of 10, 15, 25 and 35 mg/kg bw/day, from day 6 to 18 of gestation inclusive. Rabbits were sacrificed on day 29 of gestation and their uterine content was examined. Deaths were recorded in all enflicoxib-treated groups, except in those receiving 25 mg/kg bw/day. Most deaths were associated to the presence of gastrointestinal lesions. An increase in pre- and post-implantation losses, a lower percentage of live foetuses, a higher number of foetal deaths and an increased frequency of resorptions occurred at doses of 10 and 25 mg/kg bw/day. It was concluded that enflicoxib provoked embryotoxicity and that the decrease in live foetuses was directly related to the administered dose.

The applicant has proposed that, as the safety of enflicoxib has not been established during pregnancy and lactation, the product should be contraindicated for use in pregnant or lactating animals. This is considered appropriate. In light of the above findings, the SPC indicates that laboratory studies in rats and rabbits have shown evidence of foetotoxicity at maternotoxic doses.

Genotoxicity

The genotoxic potential of enflicoxib was assessed by means of two bacterial reverse mutation assays (Ames test), one human lymphocyte chromosome aberration assay and one mouse bone marrow micronucleus tests. In addition, a third Ames test was conducted using the metabolite E-6132.

In a GLP-compliant Ames test conducted in accordance with OECD GLs 471 and 472, *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 as well as *Escherichia coli* strain WP2 uvrA pKM101 were incubated with enflicoxib in the presence or absence of metabolic activation (S9 mix). Results indicate that enflicoxib does not induce mutations in either *S. typhimurium* or *E. coli* with or without metabolic activation. It is accepted that enflicoxib is non-mutagenic in this test.

In a second GLP-compliant Ames test conducted in accordance with OECD GL 471, *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 as well as *Escherichia coli* strain WP2 uvrA pKM101 were tested in the presence or absence of S9 mix. Revertant count values obtained in both positive and negative controls were within the laboratory historical control range values in all five strains used in this experiment. Results indicate that enflicoxib does not induce bacterial mutations in either *S. typhimurium* or *E. coli* with or without metabolic activation. It is accepted that enflicoxib is non-mutagenic in this test.

In a GLP-compliant chromosomal aberration test conducted in accordance with OECD GL 473 and using human peripheral lymphocytes, clastogenic activity of enflicoxib in the presence and absence of S9 mix was investigated. In the presence of metabolic activation, the highest concentration 100 µg/ml was toxic. A concentration of 66.7 µg/ml decreased the mitotic index down to 61% of the control value and was selected as the highest concentration for metaphase analysis. All cultures treated with enflicoxib showed a similar frequency of cells with chromosome aberrations when compared to the vehicle control. It was concluded that enflicoxib showed no evidence of clastogenic activity in this test.

In a GLP-compliant mammalian erythrocyte micronucleus test conducted in accordance with OECD 474, doses of 500, 1000 or 2000 mg/kg bw were given orally by gavage once a day for two consecutive days. The frequency of micro-nucleated polychromatic erythrocytes in the groups treated with enflicoxib was similar to the negative control group at all tested doses and at all sampling times, with no significant variations. It was concluded that enflicoxib does not induce the formation of micronuclei in polychromatic erythrocytes of mouse bone marrow when administered orally at doses up to 2000 mg/kg bw, and therefore showed no signs of genotoxic potential in this test.

In another GLP-compliant Ames test conducted in accordance with OECD GL 471, *S. typhimurium* strains TA1535, TA1537, TA98 and TA100, and *E. coli* strain WP2 uvrA pKM101 were incubated with the metabolite E-6132 in the presence or absence of metabolic activation. Revertant count values obtained in both positive and negative controls were within the laboratory historical control range values in all five strains used in this experiment. Results indicate that E-6132 does not induce bacterial mutations in either *S. typhimurium* or *E. coli* with or without metabolic activation. It is accepted that E-6132 is non-mutagenic in this test.

It can be accepted that the standard battery of *in vitro* and *in vivo* tests have been conducted on the compound enflicoxib, i.e. bacterial reverse mutation tests, a chromosomal aberration test and a mammalian erythrocyte micronucleus test. Results from these studies indicate that enflicoxib is not a genotoxic substance under the conditions of these tests.

Although the product is not intended for administration to food-producing animals, the applicant has also investigated genotoxicity of the metabolite E-6132 in an Ames test. The results indicate that the metabolite E-6132 is not a genotoxic substance under the condition of this test.

Carcinogenicity

No carcinogenicity data have been provided. This was justified by the applicant on the grounds of lack of genotoxic potential, the lack of structural alerts and the lack of findings relevant to neoplastic lesions in repeat dose toxicity studies.

Whilst it is acknowledged that no chronic repeat-dose toxicity study data has been provided, given the absence of any indication of mutagenicity or genotoxicity from the battery of tests conducted, it can be accepted that there appears to be no indication (results from those studies) or findings from the repeat-dose toxicity studies that might signal a potential for carcinogenicity of enflicoxib.

Consequently, the applicant's justification for the absence of carcinogenicity data can be accepted.

Studies of other effects

A series of studies was conducted in laboratory animals to investigate possible effects of enflicoxib on the central and autonomic nervous system, the cardiovascular system, the respiratory system, diuretic activity, anti-convulsant activity, cholinergic, histaminic and serotoninergic activity, basal temperature and gastro-intestinal motility. Enflicoxib was not observed to elicit significant effects on any of the systems studied at the doses administered.

In a GLP-compliant study conducted in accordance with OECD GL 439, the potential dermal irritation of a Daxocox formulation was evaluated in an *in vitro* skin irritation assay using the reconstructed dermal epidermis skin model EpiSkin. Another GLP-compliant study in accordance with OECD GL 429 was conducted to investigate the skin-sensitising potential of the candidate product. The test item used in these studies was identical to the final formulation. Based on the results of these studies, the product can be considered as non-irritant and it can be accepted that the product was not shown to be a skin sensitiser.

A GLP-compliant study in accordance with OECD GL 491 was conducted to investigate potential for ocular irritancy of the candidate product. The test item in this study was not identical to the final formulation (different flavouring agent). Instead of following OECD GL 405, the applicant used a step-down/bottom-up approach (STE-OECD 491). Based upon the findings from this study, the applicant suggests that the formulation was safe at 0.05% but that no prediction on ocular irritancy could be made. Given this conclusion, it is considered that the STE method used was not fully suitable for the intended purpose as it is unknown at what concentration between 0.05% and 5% the cell viability would remain above 70%. The applicant subsequently conducted a study in accordance with OECD GL 405 to investigate the potential for acute ocular irritation/corrosion of the candidate formulation. Based upon the findings from this study, it can be accepted that the product was shown not to be an ocular irritant or corrosive.

Excipients

The excipients included in the candidate formulation are generally recognised excipients used in either veterinary or human medicinal products. Silicified microcrystalline cellulose (refined wood pulp) is used to improve the binding, disintegrating, compactation and liquidity capability of the tablets as well as a filler. Mannitol (hexahydric alcohol mannose) is used as diluent. Sodium lauryl sulphate is used as an anionic surfactant, emulsifying agent, lubricant and wetting agent for tablets. Crospovidone and copovidone is used as disintegrant, dissolution enhancer, suspending agent and tablet binder. Iron oxides (inorganic) are used as colorant and UV absorber. Sodium stearyl fumarate is used as lubricant. Talc (inorganic) is used as anticaking agent, glidant, tablet diluent and lubricant.

However, no information on the flavouring 'dried flavour' is available. The safety of the excipients for the user is considered further in the user safety assessment.

User safety

Two studies in human volunteers were performed and are mentioned under the pharmacokinetics section above.

The applicant has presented a user safety risk assessment which has been generally conducted in accordance with CVMP guideline EMEA/CVMP/543/03-Rev.1. This is considered acceptable.

Two types of users were identified, professional (e.g. veterinary surgeons and veterinary medical assistants) and non-professionals (e.g. owners and children).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of dermal and oral exposure. However, ocular exposure following hand-to-eye contact cannot be excluded.

It is considered likely that adverse events will not occur as a result of dermal contact with these tablets, as sensitisation and irritation studies have confirmed that the product does not cause these effects in the tests conducted.

With regard to accidental oral exposure, the applicant has considered that ingestion of the largest (100 mg) tablets by a small child (10 kg bw) should be used as a worst-case scenario.

According to previous study findings, all metabolites observed in rats were also observed in humans (including E-6132) and therefore it can be accepted that the toxicological profile of E-6132 has been considered.

Based on the information provided, the CVMP considered the data available from a phase I human trial following single administration to be the most suitable for establishing a toxicological reference value (using a LOAEL of 1.67 mg/kg and applying an appropriate uncertainty factor). Using that information, it was concluded that an unacceptable risk exists following oral ingestion of the product in both children and adults (assuming a child weighing 10 kg ingests a 100 mg tablet or a 60 kg adult ingesting a 100 mg tablet both of which exceed the TRV of 1.67 mg/kg, even before accounting for individual variability). However, noting the nature of the proposed risk mitigation measures (child-proof packaging certified in accordance with ISO [EN] 14375 is proposed, product packaging recommends storage out of the sight and reach of children) and the fact that the product is non-divisible, thereby avoiding the need to store part-used tablets between administrations, the CVMP concluded that the risk mitigation measures proposed, with slight modifications, were adequate to mitigate against the risk identified. Notwithstanding the above, the applicant has highlighted the potential risk in children accidentally ingesting a tablet by including a warning in the SPC indicating that prolonged pharmacological effects, e.g. gastrointestinal disorders, may be observed.

Regarding pregnant women, the calculations show that there is no margin of exposure in case of (although improbable) accidental ingestion and findings reported in the preliminary teratogenicity studies submitted, revealed treatment-related effects on foetuses and pregnant females, both in rats and rabbits and as such the applicant has included a warning in the SPC for this specific sub-group of users.

Environmental risk assessment

A phase I environmental risk assessment (ERA) was provided according to the relevant CVMP/VICH guidelines.

Daxocox tablets are intended for the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease) in dogs. The product will therefore only be administered to individual dogs.

Phase I:

Given the nature of the active substance (NSAID) and that the product is only intended for administration to non-food animals, the environmental risk assessment may end in phase I.

Conclusions on the environmental risk assessment

An ERA was provided according to relevant CVMP/VICH guidelines. Based on the data provided, the ERA can stop at phase I, as none of the phase I criteria are met. Daxocox is not expected to pose a risk for the environment when used according to the SPC.

It can be concluded that the product will not present an unacceptable risk for the environment when handled, administered, stored and disposed of in accordance with the recommendations proposed for inclusion in the SPC.

Overall conclusions on the safety documentation

The applicant has provided the results of studies including some using ^{14}C-radiolabelled material in order to investigate the ADME of enflicoxib in rats, mice, humans and dogs.

Absorption: In laboratory animal studies, almost complete absorption of enflicoxib was reported in rats. However, feeding was noted to reduce bioavailability of enflicoxib in rats by approximately 22%.

In one study conducted in dogs the particle size of enflicoxib was demonstrated to have an impact on bioavailability, with a smaller micronised particle size increasing plasma concentration and bioavailability.

In a pilot study in fasted Beagle dogs, a dose-related effect in the target species was observed with respect to PK parameters of enflicoxib, suggesting a slow rate of absorption from the gastrointestinal tract as well as a very slow rate of metabolite (E-6132) formation. In a study on the effect of feeding, enflicoxib was better absorbed with food, with maximum concentrations reached more rapidly under fed conditions than under fasted conditions, and relative bioavailability increasing by approximately 43%.

Distribution: One *in vitro* study indicated that enflicoxib and the metabolite E-6132 have a high binding affinity for dog plasma proteins. Another *in vitro* study demonstrated that, in dogs, enflicoxib at high concentrations is mainly distributed to the blood plasma compartment and that metabolite E-6132 demonstrated concnetration-dependent distribution favouring the red blood cell compartment.

Given the high protein binding affinity, it may be expected that this may have implications for potential competition/interactions with other protein-bound active substances.

Another *in vivo* study in dogs suggests that enflicoxib has a low liver extraction ratio and that the clearance is independent of hepatic blood flow.

In a further study, it was determined that distribution of E-6132 from plasma to synovial fluid is limited.

Metabolism: The applicant has provided a series of *in vitro* studies investigating the metabolic transformation of enflicoxib. The applicant has focused on three main metabolites of enflicoxib in particular, namely pyrazole hydroxylated (M7), pyrazoline hydroxylated (M8) and pyrazole E-6132 (M9).

According to the applicant, the long-lasting pharmacodynamic activity is believed to be due to the E-6132 metabolite. Results of study of M7 and M8 pharmacokinetics in fed dogs confirmed that half-life values for these metabolites were approximately 9-fold lower than for E-6132. However, in one *in vivo* study investigating the metabolism of E-6087 in hepatic microsomes in dogs, the main metabolite was M8 and metabolite E-6132 was not observed.

Given that E-6087 is a racemic mixture, an *in vitro* study on the metabolism of its enantiomers E-6231 and E-6232 was provided, which again demonstrated the formation of the three main metabolites M7, M8 and E-6132. Another *in vitro* study demonstrated that E-6132 was not the source of M8 or M7. However, the study demonstrated that M7 was likely formed by the reduction of M8, which was confirmed by another study. Results from an additional study suggest that M7 and M8 do not contribute to the prolonged pharmacologic activity of E-6087 when compared with E-6132.

In another study, it was found that M8 does not inhibit the COX-1 or COX-2 enzyme and that E-6132 is the metabolite responsible for pharmacological effect.

One study demonstrated that all metabolites present in humans were also found in the rat, supporting the selection of this species for toxicological evaluation, as humans are not expected to be exposed to different metabolites than those produced in rats.

Excretion: In one *in vivo* study, the excretion pattern in rats indicated biliary excretion as the main route and minimal urinary excretion, with the majority of enflicoxib and its metabolites being excreted in faeces. The main metabolite excreted through faeces was M8, whereas M9 (E-6132) accounted for between 3 and 6% of the administered dose.

In a pilot study in fasted Beagle dogs, a dose-related effect in the target species was observed with respect to PK parameters.

In another study, the elimination half-life of metabolite E-6132 was determined to be long (353 h in fasted dogs and 406 h in fed dogs).

The pharmacokinetic studies provided are generally of a good standard and overall the pharmacokinetics are generally well described. One study was provided using dogs in a fed state, which showed a significant increase in bioavailability.

Whilst ADME studies were performed in rats using radiolabelling, no such study was performed in the target species, i.e. dogs.

Single dose toxicity: GLP-compliant studies in mice and rats were performed.

It is noted that the applicant has not determined a toxicological reference value below which toxicity does not occur. As only a limited range of exposures have been investigated (500–2000 mg/kg bw), only limited conclusions may be consequently drawn.

Clinical signs were observed in mice receiving an intraperitoneal 500 mg/kg bw dose and a 1000 mg/kg bw oral dose.

Clinical signs were observed in rats receiving an intraperitoneal 500 mg/kg bw dose and a 2000 mg/kg bw oral dose. However, doses less than 2000 mg/kg bw were not administered orally to rats.

Repeat dose toxicity: Repeat-dose toxicity studies have been conducted in rats and dogs.

The main clinical signs observed in repeat dose studies in rats were neurological including ataxia, ptosis, piloerection and hunched back. Necropsy demonstrated the gastrointestinal system to be significantly affected with ulcers and perforation around the pyloric zone being seen. However, it is noted that renal and liver changes were also observed. The applicant concluded that the NOAEL is 12.5 mg/kg bw/day.

In another study, wounds and scales on the snout and/or scapular zone of several female rats receiving the lowest dose (10 mg/kg bw/day) of E-6232 were observed, which were considered attributable to increased scratching activity in these animals. Consequently, it is considered that a NOAEL has not been derived for laboratory animals (rats) from the study data provided.

Two sub-acute repeat-dose toxicity studies were conducted in the target species dogs. Again, it is not considered possible to derive a NOAEL from either of these studies, given the occurrence of adverse events at the lowest doses studied (1 mg/kg bw/day) which is considered more representative of a LOAEL than a NOAEL.

It is considered that the results of repeat-dose toxicity studies indicate that this compound is considered to have a relatively narrow therapeutic margin in the target species when administered at the recommended treatment dose but at a higher frequency (once daily) than proposed (once weekly).

Reproductive (including developmental) toxicity: Results of one study reported the finding of perforated ulcers in the pyloric region of the gastrointestinal tract, confirming previous findings that the gastrointestinal tract is one of the target organ systems for this active substance in terms of toxicity. Another study reported mortality (considered to be treatment-related) and a higher number of foetal deaths and reabsorptions at the lowest dose rate (10 mg/kg/day). The applicant's proposal to indicate in the SPC that the safety of this veterinary product has not been established during pregnancy and lactation and to contraindicate use in pregnant or lactating animals is considered appropriate. In addition, the SPC indicates that laboratory studies in rats and rabbits have shown evidence of foetotoxicity at maternotoxic doses.

No guideline-compliant studies on reproductive toxicity were provided. In accordance with VICH GL 43, the absence of any reproductive toxicity study data is considered to preclude the use of the product in animals intended for breeding purposes. Consequently, in the absence of such data, the SPC specifically contraindicates use of the product in breeding animals.

Genotoxicity and carcinogenicity: The standard battery of *in vitro* and *in vivo* genotoxicity tests have been conducted on the compound enflicoxib. Results from these studies indicate that enflicoxib is not a genotoxic substance under the conditions tested.

Although the product is not intended for administration to food-producing animals, the applicant has also investigated genotoxicity of the metabolite E-6132 in a bacterial reverse mutation assay. The results indicate that the metabolite E-6132 is not a genotoxic substance under the condition tested.

The findings from these studies can be accepted, that is, there is no indication of genotoxic potential for the active substance in enflicoxib.

No carcinogenicity data have been provided. This was justified by the applicant based on the lack of genotoxic potential, the lack of structural alerts, and the lack of findings relevant to neoplastic lesions in repeat dose toxicity studies. This can be accepted.

Based on the results of studies investigating the potential for dermal and ocular irritancy and skin sensitisation, the candidate product can be considered as non-irritant to the skin, not to harbour skin-sensitising potential and not to be an ocular irritant or corrosive.

User safety: The applicant has presented a user safety risk assessment which has been generally conducted in accordance with CVMP guideline EMEA/CVMP/543/03-Rev.1. Whilst a number of NOAELs in animals have been mentioned by the applicant, none are considered by the CVMP to have been adequately justified and many are considered to be either irrelevant for the exposure scenario or to represent LOAELs as opposed to NOAELs.

According to previous study findings, all metabolites observed in rats were also observed in humans (including E-6132) and therefore it can be accepted that the toxicological profile of E-6132 has been considered.

Using a toxicological reference value (TRV) derived from a phase I human trial, it was concluded that a potentially unacceptable risk for the user arises following oral ingestion of the product. However, the CVMP concluded that the proposed risk mitigation measures were adequate to mitigate against the risk identified.

Regarding pregnant women, the calculations show that there is no margin of exposure in case of (although improbable) accidental ingestion, and findings reported in the preliminary teratogenicity studies submitted revealed treatment-related effects on foetuses and pregnant females, both in rats and rabbits. Therefore, a warning has been included for this specific sub-group of users.

Environmental safety: A phase I environmental risk assessment (ERA) was provided according to relevant CVMP/VICH guidelines. Given the nature of the active substance (NSAID) and that the product is only intended for administration to non-food animals, the environmental risk assessment may end in phase I.

It can be concluded that the product will not present an unacceptable risk for the environment when handled, administered, stored and disposed of in accordance with the recommendations proposed for inclusion in the SPC.

Part 4 – Efficacy

Pharmacodynamics

Please refer to Part 3.

Pharmacokinetics

Please refer to Part 3.

Dose justification / Dose determination / Dose finding studies

The applicant has presented a rationale for selection of the dose and the weekly administration schedule of enflicoxib (Daxocox) for the treatment of pain and inflammation in dogs suffering from osteoarthritis (degenerative joint disease).

Reference is made to several studies that have previously been discussed in the pharmacokinetics and pharmacodynamics sections of this report (see Part 3).

In a four-week toxicity study in dogs, signs of toxicity were seen at 15 mg/kg bw/day. At 4 mg/kg bw/day, only occasional presence of occult blood in faeces and a reversible decrease of erythrocyte count, proteins and haematocrit was observed. The dose of 1 mg/kg bw/day was identified as the no-observed adverse effect level (NOAEL). Considering these results, doses above 4 mg/kg bw were discarded for further development.

In this toxicity study the toxicokinetics of enflicoxib in Beagle dogs after daily oral administration at 1, 4 and 15 mg/kg bw/day, for four weeks was characterised. Enflicoxib was quantified in plasma samples but the levels of the active metabolite E-6132 were not determined. Therefore, a pharmacokinetic

study to define the plasma levels of both enflicoxib and E-6132 after the oral administration of enflicoxib was considered necessary to be able to establish any PK/PD relationship.

A single dose PK study was performed following single oral administration of enflicoxib at dose of 1 and 4 mg/kg bw, as a tentative range of safe and efficacious doses. Results showed that parent and metabolite concentrations at 1 mg/kg bw did not reach the COX-2 IC_{50}, previously defined in isolated enzyme test at any time. However, at 4 mg/kg bw, average levels of the metabolite were observed to be above its IC_{50} for 14 days.

Therefore, doses of 1 mg/kg bw or lower were not considered further for clinical development, as the plasma levels achieved were not considered adequate for efficacy.

Considering these results, a dose of 4 mg/kg bw was used in two pilot efficacy studies using an acute arthritis induction experimental model.

In the first study which was not GLP-compliant, efficacy of enflicoxib was investigated in an acute arthritis model after oral dosing. Osteo-articular pain and inflammation were experimentally induced using a reversible arthritis model in which lameness (pain and inflammation indicator) and pain induced by stifle joint palpation were scored.

Nine male Beagle dogs, 23 - 36 months old, weight range 8.96 – 11.66 kg were randomly allocated to one of the three treatment groups. A urate crystal injection was performed in the stifle (femorotibial) joint of each dog to induce an experimental acute arthritis on Day 0 (Groups 1 and 3), and Day 2 (Group 2) and was repeated on Days 7, 14 and 21 in all animals. Animals in Groups 1 and 2 were administered a single, oral dose of enflicoxib at 4 mg/kg bw in one capsule 2 hours before (Group 1) or 2 days before (Group 2) intra-articular injection and 2 days before the third intra-articular injection (i.e. 12 days later). Animals in Group 3 were administered mavacoxib.

The anti-inflammatory and analgesic efficacy of the treatments were assessed by investigating two clinical signs of arthritis: visual lameness (whilst standing and walking) and pain during (stifle) palpation. Their intensity was determined using a scoring system on each day of intra-articular urate crystal injection just before injection and 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 9 h and 12 h later. The combined visual lameness score, determined as the sum of the standing and walking lameness scores, was also considered. Only 3 animals per treatment group were included in this study and therefore no statistical analysis was performed on the results and only descriptive analysis has been provided. Further, no untreated control group was included. Lameness and pain scores were assessed by study investigators, not veterinary surgeons. It is noted that no objective measure of lameness was used (e.g. force plate analysis) and instead a visual assessment of lameness/gait was used. Whilst the applicant suggests that intensity and duration of pain seemed to be lower than pain observed on the day of first administration in animals treated with enflicoxib and similar to animals treated with mavacoxib, given the small sample size, the absence of a negative control, the method of assessment used and the absence of any statistical analyses, it is considered that little can be concluded from this study in terms of the adequacy of the proposed posology (either dose rate or re-treatment interval).

In the second study, efficacy of enflicoxib was again investigated in an acute arthritis model after oral dosing. Two treatment designs which differed by the loading dose were tested in this study. In the first one, the loading dose consisted in enflicoxib administration at 4 mg/kg bw repeated 24 h later (Day 0 and Day 1). In the second one, the loading dose consisted of a single enflicoxib administration of 8 mg/kg bw (Day 0). Then, the two treatment designs were the same, with enflicoxib administrations of 4 mg/kg bw on Day 7, 14 and 21. The intra-articular injection of urate crystals was performed on three occasions for each animal as follows: Group A: on Day 2, Day 9 and Day 23; Group B: on Day 4, Day 14 (before treatment) and Day 28; Group C: on Day 0 (2 hours after treatment), Day 9 and Day 21 (before treatment); Group D: on Day 0, Day 14 and Day 28. Again, the anti-inflammatory and

analgesic effect of the treatments was assessed by investigating two clinical signs of arthritis: visual lameness (whilst standing and walking) and pain during (stifle) palpation using the same scoring system.

Only 4 animals were included in each treatment group in this study and therefore no statistical analysis was performed on the results and only descriptive analysis has been provided. Unlike the previous study, an untreated control group was included, however, this consisted of only one animal. This study was not blinded and lameness and pain scores were assessed by study investigators, not veterinary surgeons. No objective measure of lameness was used (e.g. force plate analysis) and instead a visual assessment of lameness/gait was used. Given that pain and lameness scoring are subjective, there is a possibility of treatment bias given the absence of blinding. Even using a loading dose of 8 mg/kg bw, it would appear that there was little difference between the treated and control animals in intensity and duration of lameness and pain induced 2 hours after the loading dose. Whilst the investigators have suggested that the duration and intensity of pain as well as pain during palpation was lower in treated animals than control animals when compared with the previous study, it would appear that these findings were only at certain time points and were not necessarily consistently shown at all time points. For example, no pain was reported in 2 out of 4 animals at Day 23 in Groups A & B (4 mg/kg bw loading dose) whereas no pain was reported at Day 21 in only 1 out of 4 dogs in Group C (8 mg/kg bw loading dose).

In light of the above and given the small sample size, the absence of any statistical analyses and the absence of blinding, it is considered that little can be concluded from this study in terms of the adequacy of the proposed posology (either dose rate or re-treatment interval).

The applicant concluded that the results of these two pilot studies indicate that weekly dosing is needed and that dose loading is required like for other long-acting NSAIDs. Rather than administering two doses close together, the applicant considered a single administration of a double dose preferable.

In order to further support the proposed dosing schedule, PK/PD modelling has been utilised.

PK-PD modelling

In support of the proposed dose, the applicant presented a series of PK/PD model simulations analysing data from several studies.

The selection of the optimal dosing schedule for enflicoxib was further explored in three steps:

1) Development of a population PK model of parent compound (enflicoxib) and its active metabolite (E-6132) including all available PK/PD data from PK, TK and the pilot efficacy studies,

2) Establish the "Therapeutic Window" (to define the minimum effective concentration and maximum well-tolerated level), and

3) Define the efficacy/safety profile at different dose schemes, in order to select the optimal oral posology for enflicoxib.

A population PK model was developed using NONMEM using the first order conditional estimation method with interaction. A first version was developed with the trials performed in Beagle dogs.

The model was established using a two-compartment model for parent compound with first-order elimination and absorption (with lag time) along with a three-compartment model for metabolite with first order elimination. This model assumed a linear PK behaviour of enflicoxib and E-6132 over an oral single dose and weekly multiple maintenance doses in a range from 1 to 8 mg/kg bw.

The three datasets related to:

a) Toxicity study in Beagle dogs with continuous administration for 28 days and a 14-day recovery period. Dogs were administered enflicoxib in capsules at dose levels of 1, 4 and 15 mg/kg bw/day during a four-week period. A total of 228 samples with enflicoxib levels above the limit of quantification from 12 Beagle dogs were included in this dataset. The E-6132 exposure was not measured in this study.

b) Pharmacokinetics study of enflicoxib following single oral administration to Beagle dogs at the doses of 1 and 4 mg/kg bw. A total of 341 samples for enflicoxib and 341 samples for E-6132 from 26 Beagle dogs were included in this dataset.

c) Pilot efficacy study: assessment of two oral administrations (days 0 and 12) of enflicoxib following an acute arthritis induction in dogs & Pilot efficacy study: efficacy assessment of an oral administration of enflicoxib following an acute arthritis induction in dogs using two multiple dosing schedules in Beagle dogs: i) 4 mg/kg bw daily for two days followed by three weekly doses of 4 mg/kg bw , and ii) Loading dose of 8 mg/kg bw followed by three weekly doses of 4 mg/kg bw. A total of 18 enflicoxib-treated animals, that have at least one pair of E-6132 - lameness score at the same day, were included in the PD dataset, supplying a total of 71 pairs of PD observations.

The efficacy and safety profiles of different weekly oral dosing schedules of enflicoxib were explored using simulation techniques implemented in the NONMEM program and several Monte Carlo simulations were performed in order to reflect the expected range of variability in concentration values after administration of different dosing schedules using the final model parameter estimates. Since enflicoxib is being developed for the chronic treatment of arthritis in dogs, 1000 simulations of enflicoxib and E-6132 levels were performed after multiple oral weekly administration of enflicoxib to attain the PK steady state at different dosing schedules:

- Loading dose of 8 mg/kg bw followed by maintenance dose of 4 mg/kg bw weekly.
- Loading dose of two daily doses of 4 mg/kg bw followed by maintenance dose of 4 mg/kg bw weekly.
- Loading dose of 10 mg/kg bw followed by maintenance dose of 5 mg/kg bw weekly.

The 90% prediction intervals, the typical population PK profiles, the Minimum Effective Concentration (MEC) for metabolite and the Minimum Toxic Concentration (MTC) for parent and metabolite were displayed in one plot, in order to visually evaluate the viability of each dosing schedule.

Results indicated an average MTC for enflicoxib on day 28 after 4 mg/kg bw/day of 2216 ng/mL. MTC for E-6132 metabolite was determined to be 1808 ng/mL. The MEC for E-6132 is 536 ng/mL and levels higher than 704 ng/mL do not seem to provide an improvement of efficacy.

It is noted that the applicant has indicated that the PK model predicted a metabolite accumulation ratio of 31 following daily administration and E-6132 maximum levels on day 28 of 1808 and 7231 ng/mL for 1 and 4 mg/kg bw/day, respectively and it was not feasible to confirm that the PK model predicts adequately the metabolite accumulation after daily doses of enflicoxib.

Based upon the predicted modelling, the applicant has concluded that a loading dose of 8 mg/kg bw is preferable to that of 4 mg/kg bw on two consecutive days from an efficacy perspective. However, the justification for improved efficacy of the 8 mg/kg bw loading dose appears to be based upon the findings from the two pilot efficacy studies which, as previously highlighted, are considered to provide little reassurances in terms of the adequacy of the proposed posology (either dose rate or re-treatment interval).

Indeed, it would appear from the predictions from the PK modelling that both loading dose schedules appear to be similar in terms of the percentage of animals predicted to have plasma E-6132 concentrations above the MEC and below the MTC.

Further information on the proposed dose and treatment interval has been provided in additional PK/PD modelling which is further discussed below.

Dose confirmation studies

A GCP-compliant dose confirmation study (DA/184/C) was conducted to evaluate the efficacy of two target doses of 2 mg/kg bw and 4 mg/kg bw enflicoxib administered orally once weekly in the treatment of natural canine osteoarthritis (OA).

The study was a blinded, randomised, controlled, parallel-group, multi-centre field trial with a negative and a positive control, to evaluate the efficacy and to determine the effective dose of enflicoxib in dogs with natural OA presented as veterinary patients. In addition, the safety of the compound was evaluated throughout the study. The study included 242 client-owned dogs from 28 veterinary practices in France and Spain; 236 dogs were included in the ITT dataset (115 female and 121 male). Mean age was 9.35 years old with 178 purebreds and 58 mixed breeds. Mean bodyweight was 28.5 kg (range 5.0 – 65.0 kg). The study included dogs ≥12 months showing clinical signs of OA for at least 3 weeks prior to enrolment. Confirmation of OA was by radiographic evidence of presence of OA and a minimum clinical score (CSS) ≥6. If more than one joint was affected by OA, the most severely affected joint was assessed for efficacy. The study included 4 treatment groups: enflicoxib at maintenance dose of 4 mg/kg bw (T1) and 2 mg/kg bw (T2), placebo (T3), and mavacoxib (T4 - administered as per the label dose). An initial loading dose of enflicoxib (4 mg/kg bw and 8 mg/kg bw, respectively) was administered on Day 0 followed by weekly oral doses of 2 mg/kg bw and 4 mg/kg bw enflicoxib, respectively until Day 35. Placebo was administered at the same weekly schedule and mavacoxib was administered on Days 0 and 14 and placebo on Days 7, 21, 28 and 35. Doses were administered with food or immediately before the main meal.

Clinical assessment of pain and lameness including for posture, lameness at walk, lameness at trot and pain at palpation/manipulation were performed by the investigator using numerical rating scales to calculate the CSS (Clinical Sum Score). In addition, during each clinical assessment on Days 0 (prior to treatment), 7, 14, 21, 28, 35 and 42 the investigator interviewed the owner (Days 21 and 35 via telephone interview) and recorded the animal owner's assessment using the CBPI (Canine Brief Pain Inventory) score. The CBPI allowed to quantify the severity (pain severity score) and the impact of chronic pain (pain interference score) by assessing a number of criteria including description of the pain, level of activity, enjoyment of life, ability to rise and ability to walk, run and climb up.

Blood samples were collected on Day 0 prior to treatment and haematology and clinical chemistry parameters were analysed. In addition, blood samples were collected on Day 28 for the population pharmacokinetics.

The primary efficacy outcome parameter was CSS assessed by the investigators according to treatment and time. Secondary outcome parameter was CBPI measured by the animals' owner and the CBPI score was compared between treatments and within treatments (versus baseline). Two subsets of the CBPI score, the Pain Severity Score (PSS) and the Pain Interference Score (PIS) were calculated and compared between treatments and within treatments (versus baseline).

Nevertheless, the primary efficacy endpoint selected only reflects an overall response, whilst the individual response cannot be shown. Whilst some animals could improve notably, in other dogs this improvement could be modest or may not be even noted. This information is important in order to

identify possible differences in the efficacy of the veterinary medicinal product regarding the severity of the pathology, age or any other factors. Therefore, the applicant was requested to propose a suitable recovery threshold scientifically supported and to recalculate the efficacy of the different treatment groups based on the individual improvement of dogs. The applicant reconsidered the adequacy of the threshold for demonstrating efficacy in terms of clinical relevance and subsequently conducted supplementary statistical analyses on two additional data populations, in which the thresholds selected are in line with those supported by peer-reviewed published literature. Based on the results provided, it can be accepted that these supplementary analyses of the pivotal outcome parameter (CSS<6) provide additional support for the selection of a weekly maintenance dose of 4 mg/kg as opposed to the lower dose of 2 mg/kg and that using the revised threshold for determining efficacy, it can be accepted that administration of enflicoxib resulted in a clinically relevant reduction in CSS and CBPI (PSS & PIS) scores.Out of the 242 dogs enrolled in the study, 221 completed the study on Day 42. Efficacy analysis was performed both on the Intention to Treat (ITT) and Per Protocol (PP) populations. It is noted that the candidate formulation administered in this study is not the same as that intended to be marketed; however, the only difference between the formulation used in this study and the final formulation is a flavouring excipient.

Although a positive control group was included, a non-inferiority approach has not been appropriately used in this study in terms of its design or analysis. Based upon the results of this study, it would appear that a more rapid reduction in CSS values was obtained in the dogs administered a maintenance dose of 4 mg/kg bw when compared with those administered 2 mg/kg bw. A statistically significant difference compared to untreated animals was observed from 14 days onwards at 4 mg/kg bw and from 28 days onwards at 2 mg/kg bw. Mavacoxib-treated dogs were observed to have a statistically significant reduction in CSS values from 14 days (similar to enflicoxib at a dose of 4 mg/kg bw).

Although a statistically significant reduction in mean CSS values has been reported, it is noted that the magnitude of the difference is limited. For example, a reduction in mean CSS from 9.02 to 4.0 (difference of 5.02) was reported in the 4 mg/kg bw group, representing an absolute difference of 2.75 when compared to the placebo group. Concerning the more subjective secondary parameter CBPI score as measured by owners, it is noted that a more rapid reduction in CBPI score was obtained in the dogs administered a maintenance dose of 4 mg/kg bw when compared with those administered 2 mg/kg bw. A statistically significant difference compared to untreated animals was observed from 28 days onwards at 4 mg/kg bw and from 35 days onwards at 2 mg/kg bw. Mavacoxib-treated dogs were observed to have a statistically significant reduction in CBPI score from 35 days (similar to enflicoxib at a dose of 2 mg/kg bw).

It can be accepted that overall, the findings from this study suggest a more favourable outcome in terms of CSS and CBPI assessment when the product is administered at a maintenance dose of 4 mg/kg bw compared to 2 mg/kg bw.

At the end of the study, owners considered their dogs to have been responders (improvement in CBPI score at end of study compared to baseline) in 76.2% of animals in the negative control (placebo) group, suggesting a high placebo effect in the assessment by owners. Furthermore, no statistically significant difference between treated and untreated animals was reported for CBPI. This raises concerns in terms of the clinical relevance of the statistically significant p-values reported for the mean CSS values (primary efficacy parameter).

Given that the primary efficacy parameter was based upon mean CSS values assessed with reference to posture, lameness at walk, lameness at trot and pain at palpation/manipulation as performed by the study investigator, it can be accepted that this parameter is appropriate for the assessment of pain. However, it is noted that the proposed indication is for the treatment of both pain and inflammation.

Consequently, in the absence of any specific parameters designed to assess inflammation (for example inflammatory markers, physical characteristics (e.g. heat, swelling, redness) or radiographic/ultrasonographic examinations), it is unclear as to how CSS values have specifically assessed effect of the product on inflammation.

That said, evidence of an anti-inflammatory effect of enflicoxib under laboratory conditions has been provided and which included assessment of inflammation elicited by injection of urate crystal in the stifle joint of dogs and in experimental models (rat carrageenan exudate, inhibition of carrageenan-induced oedema in rats, mouse air pouch, *Mycobacterium butyricum* induced arthritis model in rats).

Further, the CVMP has previously concluded that the connection between the analgesic and anti-inflammatory effect for NSAIDs is accepted and has acknowledged that these effects can hardly be separated and evaluated apart from each other. On that basis, and given the parameters assessed under field conditions (CSS and CBPI), then assuming a claim for treatment of pain is adequately supported, then a claim for treatment of inflammation can also be accepted based upon the data provided.Concerning safety, it is noted that a total of 112 adverse events were reported (37 in dogs administered 4 mg/kg bw, 19 in dogs administered 2 mg/kg bw, 33 in dogs receiving placebo treatment and 23 in dogs administered mavacoxib). It can be accepted that the majority of adverse events were related to gastrointestinal disorders such as diarrhoea/soft faeces, nausea or vomiting.

The nature and frequency of co-morbidities in animals included in the dose confirmation study is somewhat limited and more typically represented local as opposed to systemic disorders. Animals with compromised renal or hepatic function and/or gastrointestinal disorders were not included in the study. Given that use of NSAIDs is normally contraindicated in such animals, their exclusion can be accepted. The SPC expressly contraindicates use of the product in animals with impaired renal or hepatic function, animals suffering from gastrointestinal disorders, protein or blood losing enteropathy or haemorrhagic disorders or animals with cardiac insufficiency.

Plasma levels of enflicoxib and its metabolite E-6132 were measured from plasma samples taken on Day 28.

There was a large variability in concentrations of both enflicoxib and E-6132 when administered at either 8 mg/kg bw loading dose followed by 4 mg/kg bw or 4 mg/kg bw loading dose followed by 2 mg/kg bw.

Concentrations of E-6132 when administered at 8 mg/kg bw loading dose followed by 4 mg/kg bw. At Day 28, the lowest concentration of E-6132 was 480.76 ng/mL, which is below the average MEC value (536 ng/mL) predicted from PK modelling, whereas the highest concentration was 2940.12 ng/mL, which exceeds the average MTC predicted (1808 ng/mL). At Day 42, the lowest concentration was 273.01 ng/mL, which again is below the predicted average MEC value and highest concentration was 3377.04 ng/mL, which even further exceeds the average MTC value. Mean was 1910.07 ng/mL with SD 693.56, again which exceeds the predicted MTC.

Concentrations of E-6132 when administered at 4 mg/kg bw loading dose followed by 2 mg/kg bw. At Day 28, the lowest concentration was 344.51 ng/mL, which is below the predicted average MEC, whereas the highest concentration was 1786.58 ng/mL. At Day 42, the lowest concentration was 339.41 ng/mL, which again is below the predicted average MEC and the highest concentration was 2049.46 ng/mL, which exceeds the predicted average MTC.

The safety of the proposed dosing schedule was demonstrated in the TAS study and justification for the maintenance dose of 4 mg/kg bw was justified based on additional PK/PD modelling and the improved efficacy supported by supplementary statistical analyses of the pivotal dose confirmation study.

Additional PK-PD modelling

The applicant has conducted further PK modelling using the PK data derived from the above study as the previous population PK model was established in Beagle dogs (mean weight of approximately 10 kg) without the inclusion of total body weight as a covariate.

The PK dataset that was used to establish the new popPK model included a total of 341 samples for enflicoxib and 341 samples for E-6132 from 26 Beagle dogs, using the same PK dataset as that used in previous study PK model but including allometric exponents in all volumes and clearances (exponents of 1 and 0.75, respectively) to permit extrapolation to other body weights.

Using the new PK modelling approach, the MTC for enflicoxib was upwardly revised from 2216 ng/mL to 6723 ng/mL. This has been justified on the grounds that no relevant toxicological effects were observed at 4 mg/kg bw/day and was established estimating the upper confidence interval (95% CI) of the maximum enflicoxib level predicted by the second PK model on Day 28 after daily oral administration of E-6087 at 1 mg/kg/day (NOAEL). Following the same criteria, the MTC for E-6132 was upwardly revised from 1808 ng/mL to 4258 ng/mL. This has been justified on the grounds of a NOAEL of 1 mg/kg bw/day, and the MEC for E-6132 was downwardly revised from 536 ng/mL to 411 ng/mL.Model simulation predicts that, after oral weekly administration of enflicoxib, 87% of E-6132 PK steady state was achieved after 6 weekly administrations in dosing schedules that incorporates loading dose as an initial dose. The 90 and 95% of steady-state for E-6132 was attained after 7 and 10 weekly administrations, respectively.

Whilst it is noted that the PK data for enflicoxib and E-6132 derived from study animals in dose confirmation field study have been used to 'improve' the PK/PD modelling previously conducted, a number of concerns are considered to arise in terms of the relevance and clinical significance of the findings from that study and consequently, the relevance of the revised predictions from the PK/PD modelling.

Concerning the need for a loading dose, the applicant states that the results of PK/PD modelling indicate that a loading dose of 8 mg/kg is needed to ensure a relatively short onset of effect and that if only administering weekly doses of 4 mg/kg (i.e. without a loading dose), effect would not be expected until after a second dose. Further, the applicant suggests that 90% of steady-state concentrations of E-6132 would not be achieved until 7 weeks without a loading dose, but 5 weeks with a loading dose of 8 mg/kg. Results from the dose confirmation study do tend to support this prediction.

Concerning the maintenance dose, the applicant indicates that the results of the clinical studies have been reanalysed and the outcome of this reanalysis indicates that a maintenance dose of 2 mg/kg is not as effective as a maintenance dose of 4 mg/kg.

Concerning the re-treatment interval, the applicant has conducted additional pharmacokinetic simulations to compare dosing intervals of 7 days and 14 days. The output from this modelling suggests that 0.1%, 1.7% and 5.3% of treated dogs would not achieve minimum effective concentrations (MEC) values of 411, 536 and 700 ng E-6132/mL, respectively when administered 4 mg/kg once weekly, whereas 15.3%, 26.5% and 41.9% of treated dogs would not achieve minimum effective concentrations (MEC) values of 411, 536 and 700 ng E-6132/mL, respectively when administered 4 mg/kg once fortnightly. Based on this additional modelling, the applicant concludes that a weekly re-treatment interval is required to ensure adequate effectiveness.

In conclusion, whilst the predictions from the revised PK/PD modelling are noted, no conclusion in respect of safety or efficacy of the proposed dosing schedule is considered possible and it was considered that the suitability of the proposed dosing schedule would need to be confirmed under clinical field study conditions.

Target animal tolerance

The applicant has provided the results of a single GLP-compliant target animal tolerance study (VX05LK).

Thirty-two healthy Beagle dogs aged 8-10 months old were included and randomly allocated to 4 treatment groups. Group 1 (1X) received enflicoxib at the therapeutic dose (one loading dose of 8 mg/kg bw followed by weekly maintenance doses of 4 mg/kg bw). Group 2 (control) received a placebo, Group 3 (3X) received enflicoxib at three times the therapeutic dose (one loading dose of 24 mg/kg bw followed by weekly maintenance doses of 12 mg/kg bw) and Group 4 (5X) received enflicoxib at five times the therapeutic dose (one loading dose of 40 mg/kg bw followed by weekly maintenance doses of 20 mg/kg bw). Enflicoxib was orally administered to animals for 7 months (Groups 1 and 3, with Group 2 administered a placebo), however it is noted that the animals in 5xRTD group were sacrificed after 13 weeks on the grounds that it is unlikely that overdose in the field would continue for such an extended duration; however, such justification is not considered to be consistent with the VICH GL43.

It can be accepted that a sufficiently comprehensive range of safety parameters was assessed in this study and which included physical examination, body weight, food consumption, ophthalmic examination, electrocardiography, blood pressure and pulse rate, endoscopy of the oesophageal and gastric mucosa, blood mucosal bleeding time. Blood samples for haematology and blood chemistry were taken pre-treatment and on weeks 4, 8 and 13, 17, 21, 25 and 30. Samples for urinalysis and faecal occult blood were also collected at pre-treatment and throughout the study. Blood samples for toxicokinetics were taken from all animals pre-treatment and on weeks 1, 13 and 31 at 6, 24, 48, 72, 96, 120, 144 and 168 hours post-dose. Animals in Group 4 (5X) were sacrificed following 13 weeks of treatment and animals in Groups 1, 2 and 3 were sacrificed following the 7 months of treatment. All animals were subject to necropsy including macroscopic and microscopic organ examination. Bone marrow smears were prepared immediately following death and organs weighed. Interim results (after 13 weeks) and final results (after 7 months of treatment) indicate that there were no test item-related effects on body weight, food consumption, ophthalmology, electrocardiography, blood pressure, haematology or urinalysis parameters, faecal occult blood, endoscopy, buccal mucosal bleeding time, organ weight, macroscopic or microscopic examination.

Given the proposed indication (treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease)), it is considered that the study animals are not fully reflective of the intended target population (older animals with osteoarthritis or degenerative joint disease). Consequently, systemic tolerance to the product in older animals that are likely to have comorbidities is unlikely to have been adequately characterised in this study.

Concerning the pharmacokinetic data generated, not unexpectedly, higher C_{max} and AUC values for enflicoxib were observed following the initial dose compared to the subsequent 50% lower doses. However, it is noted that for the metabolite E-6132, both C_{max} and AUC were generally higher after 13 and 31 weeks of treatment compared to after one week of treatment. For example, at the recommended posology, AUC values for E-6132 increased from 196500 in week 1 to 307300 and 304300 after 13 and 31 weeks of treatment in males and from 207600 in week 1 to 244900 and 259600 in females after 13 and 31 weeks of treatment, respectively, suggesting some accumulation of this metabolite. The applicant has estimated an accumulation ratio for E-6132 of 3.1 in males and 2.2 in females after 31 weeks at the recommended posology, confirming that accumulation is taking place relative to week 1. Of significance is the observation that the terminal rate constants and corresponding terminal half-lives (t½) of E-6132 during week 1, 13 and 31 could not be estimated

There was no statistically significant difference between mean AUC_{168} values at weeks 13 and 31. In

light of the above, it can be accepted that steady-state pharmacokinetics appears to have been reached within the follow-up period of the TAS study in animals administered 4 mg/kg once weekly and that further accumulation of E-6132 is not expected at this dose.

No adverse effects were reported in this study.

However, it is noted that elevated blood urea concentrations were reported in both sexes at all dose levels from week 4 onwards and that a dose-response effect was observed. However, there was no increase in the magnitude of this difference with control over the course of the study. Cholesterol values were also high in week 13 in both sexes and at all dose levels when compared to both pre-treatment and control. However, from week 25 this finding was only observed in male animals. There was no clinically relevant correlation between the observed increased blood urea concentrations, plasma protein fractions, cholesterol values and red blood cell indices. Nonetheless, given that an elevation of urea and cholesterol was reported at the recommended treatment dose, this finding has been adequately reflected in the proposed SPC.

Two field studies were conducted and the applicant has presented a summary of the tolerance findings from both studies. Based on the information provided, it would appear that when compared with another NSAID (mavacoxib) and placebo, enflicoxib was reported to have the highest incidence of adverse reactions cemost of which appear to have been associated with the gastrointestinal tract (emesis, diarrhoea, soft faeces or gastroenteritis) and most were reported as being mild. However, it is noted that more severe reactions such as haemorrhagic diarrhoea and fatality have been reported for enflicoxib in these studies.

The SPC expressly contraindicates use of the product in animals with impaired renal or hepatic function, animals suffering from gastrointestinal disorders, protein or blood losing enteropathy or haemorrhagic disorders or animals with cardiac insufficiency.

Clinical field trials

Clinical studies

A GCP-compliant field study was conducted to investigate the efficacy and safety of a target dose of 4 mg/kg bw enflicoxib administered orally once weekly in the treatment of natural canine osteoarthritis (OA). The study was a blinded, randomised, controlled, parallel-group, multi-centre field trial with a negative and a positive control, to evaluate the efficacy of enflicoxib in dogs with natural osteoarthritis presented as veterinary patients. 180 client-owned dogs were enrolled from 20 veterinary practices in France and Spain; 82 were female and 98 male dogs with mean age 9.30 years old of which 135 were purebreds and 45 were mixed breeds. Mean bodyweight was 27.29 kg (range 5.0 – 63.0 kg). Dogs were ≥12 months showing clinical signs of OA for at least 3 weeks prior to enrolment, which was confirmed by radiographic evidence of presence of OA and a minimum clinical score (CSS) ≥6. If more than one joint was affected by OA, the most severely affected joint was assessed for efficacy.

The study included 3 treatment groups: enflicoxib at maintenance dose 4 mg/kg bw (T1, n=78), placebo (T2, n=22), and mavacoxib (T3, n=80) administered at the label dose. An initial oral dose of enflicoxib at 8 mg/kg bw was administered on Day 0, followed by weekly oral doses of 4 mg/kg bw until Day 35, inclusive. Placebo (CP, T2, negative control) was administered orally (as tablets) once weekly on Days 0 to 35 inclusive. Mavacoxib (positive control) was administered according to the manufacturer's product label on Days 0 and 14. On Days 7, 21, 28 and 35 animals allocated to this treatment group (group T3) received placebo tablets. Doses were administered with food or immediately before the main meal.

Clinical assessment of pain and lameness including posture (0-3), lameness at walk (0-6), lameness at trot (0-6) and pain at palpation/manipulation (0-3) were performed by the investigator using numerical rating scales (total score 0-18) to calculate the CSS (Clinical Sum Score). In addition, during each clinical assessment on Days 0 (prior to treatment), 7, 14, 21, 28, 35 and 42, the investigator interviewed the owner (Days 21 and 35 via telephone interview) and recorded the animal owner's assessment using the CBPI. The CBPI allowed to quantify the severity (pain severity score) and the impact of chronic pain (pain interference score) by assessing a number of criteria including description of the pain, level of activity, enjoyment of life, ability to rise and ability to walk, run and climb up. Blood samples were collected on Day 0 prior to treatment and haematology and clinical chemistry parameters were analysed. In addition, blood samples were collected for population pharmacokinetics assessment.

The primary efficacy outcome parameter was CSS assessed by the investigators according to treatment and time. Secondary outcome parameter was CBPI measured by the animals' owner and the CBPI score was compared between treatments and within treatments (versus baseline). Two subsets of the CBPI score, the Pain Severity Score (PSS) and the Pain Interference Score (PIS) were calculated and compared between treatments and within treatments (versus baseline).

Nevertheless, the primary efficacy endpoint selected only reflects an overall response, whilst the individual response cannot be shown. Whilst some animals could improve notably, in other dogs this improvement could be modest or may not be even noted. This information is important in order to identify possible differences in the efficacy of the veterinary medicinal product regarding the severity of the pathology, age or any other factors. Therefore, the applicant was requested to propose a suitable recovery threshold scientifically supported and to recalculate the efficacy of the different treatment groups based on the individual improvement of dogs.

Out of 180 dogs enrolled in the study, 174 completed the study on Day 42. Efficacy analysis was performed both on the Intention to Treat (ITT) and Per Protocol (PP) populations. It is noted that the candidate formulation administered in this study is not the same as that intended to be marketed; however, the only difference between the formulation used in this study and the final formulation is a flavouring excipient (the CVMP accepts that the difference in flavouring agent will have no influence on bioavailability).

Given that the primary efficacy parameter was based upon mean CSS values assessed with reference to posture, lameness at walk, lameness at trot and pain at palpation/manipulation as performed by the study investigator, it can be accepted that this parameter is appropriate for the assessment of pain. However, it is noted that the proposed indication is for the treatment of both pain and inflammation. Consequently, in the absence of any specific parameters designed to assess inflammation (for example inflammatory markers, physical characteristics (e.g. heat, swelling, redness) or radiographic/ultrasonographic examinations), it is somewhat unclear as to how CSS values have specifically assessed effect of the product on inflammation.

That said, evidence of an anti-inflammatory effect of enflicoxib under laboratory conditions has been provided and which included assessment of inflammation elicited by injection of urate crystal in the stifle joint of dogs and in experimental models (rat carrageenan exudate, inhibition of carrageenan-induced oedema in rats, mouse air pouch, *Mycobacterium butyricum* induced arthritis model in rats).

Further, the CVMP has previously concluded that the connection between the analgesic and anti-inflammatory effect for NSAIDs is accepted and has acknowledged that these effects can hardly be separated and evaluated apart from each other. On that basis, and given the parameters assessed under field conditions (CSS and CBPI), then assuming a claim for treatment of pain is adequately supported, then a claim for treatment of inflammation can also be accepted based upon the data provided.

It can be accepted that the study animals were sufficiently representative of the intended target population in terms of age, breed and gender.

To be enrolled, a minimum Clinical Sum Score (CSS) of ≥6 was required. Based upon the results of this study, the candidate product was demonstrated to be superior to placebo for the primary efficacy parameter (CSS) at each of the study time points. Mean CSS decreased from 9.77 at Day 0 to 3.82 at Day 42 in dogs administered enflicoxib compared to the control group where the CSS decreased from 9.59 at Day 0 to 7.86 at Day 42 (an absolute difference of 4.22 between groups) and CSS values were statistically significantly different between enflicoxib-treated dogs and placebo-treated dogs at all time points.

Within-treatment analysis suggests that enflicoxib-treated dogs had statistically significantly lower CSS values compared to baseline at all time points. Analysis of CSS global values expressed as AUC also demonstrated a statistically significantly lower AUC value compared to placebo. It is accepted that the results of this study indicate that the candidate product is superior to placebo in terms of the primary efficacy outcome parameter CSS.

Concerning the secondary outcome parameter assessed by owners (CBPI score), a statistically significant difference between enflicoxib-treated dogs and placebo-treated dogs was observed from Day 21 onwards, but not before. Interestingly, CBPI scores for dogs administered mavacoxib were not found to differ to placebo-treated dogs at any time point when using the ITT dataset. It is accepted that the results for this secondary outcome parameter measured by owners is supportive of the more objective outcome parameter CSS.

Concerning the investigation of non-inferiority between enflicoxib and mavacoxib, the candidate product was demonstrated to be non-inferior to the comparator product for the pivotal efficacy outcome parameter (CSS).

It is noted that by the end of the study, owners considered their dogs to have been responders (improvement in CBPI score at end of study compared to baseline) in 72.7% of animals in the untreated (placebo) group, suggesting a high placebo effect in the assessment by owners. Unlike in the previous study, a statistically significant difference between treated and untreated animals was reported.

Concerning safety, it is noted that a total of 52 adverse events were reported (28 in dogs administered E-6087, 23 in dogs administered mavacoxib and 1 in the placebo group). It can be accepted that the majority of adverse events related to gastrointestinal disorders such as diarrhoea/soft faeces, nausea or vomiting. However, of the 7 AEs considered serious or life-threatening, 3 occurred in enflicoxib-treated animals and were associated with emesis & dehydration, perforated gastric ulcer and apathy/anorexia/hypothermia.

The nature and frequency of co-morbidities in animals included in the field study is somewhat limited and more typically represented local as opposed to systemic disorders. Animals with compromised renal or hepatic function and/or gastrointestinal disorders were not included in the study. Given that use of NSAIDs is normally contraindicated in such animals, their exclusion can be accepted.

Given the concerns raised by the CVMP in respect of the clinical relevance of the difference in CSS scores and the approach used to demonstrating efficacy, the applicant was requested to consider a more suitable and clinically supported threshold for determining efficacy and to recalculate efficacy of the treatment groups based upon individual animal (as opposed to group mean) data.

The applicant reconsidered the adequacy of the threshold for demonstrating efficacy in terms of clinical relevance and subsequently conducted supplementary statistical analyses on two additional data populations.

These additional data populations take into consideration recently published study reports in peer-reviewed journals that set out inclusion criteria and efficacy thresholds for assessing response to treatment of osteoarthritis in dogs, that is, the thresholds selected are in line with those supported by peer-reviewed published literature.

Both additional populations (PP2 & PP3) are composed of animals that meet criteria for minimum CSS score and in addition satisfy criteria in respect of the secondary parameter CBPI which is composed of two categories – pain severity score (PSS) and pain interference score (PIS). The additional restriction of the study population to animals with minimum PSS and PIS scores (≥2) provides a more clinically defined study population in terms of pain assessment than based solely on CSS scores and is therefore likely to facilitate identification of a more clinically relevant response.

For the PP2 dataset, only animals with both a CSS≥6 and baseline PSS and PIS scores ≥2 were included. A responder is defined as animals with a decrease in CSS to <6 (primary endpoint) and a decrease in PSS ≥1 and PIS of ≥2 (secondary endpoint), at Day 42.

For the PP3 dataset, only animals with both a CSS≥8 and baseline PSS and PIS scores ≥2 were included. A responder is defined as animals with a decrease in CSS to <6 (minimum of a 3-point reduction) and a decrease in PSS ≥1 and PIS of ≥2 at Day 42. This dataset in particular is considered to be representative of animals assessed as having greater pain. Further, it can be accepted that by limiting the PP3 dataset to animals with CSS≥8 results in animals requiring a reduction in CSS score of at least 3 points (CSS<6) which again is considered to provide a more severe test of clinical response.

The impact of a number of explanatory variables (age, gender, body weight, pure bred, limb and CSS/PIS/PSS scores at baseline) was investigated using backward selection of variables to determine which remained in the final multivariate regression model.

The sample size was inevitably reduced due to the revised inclusion criteria and resulted in 171 animals being included in the PP2 dataset and 132 in the PP3 dataset (compared to 180 and 175 in the original ITT and PP datasets).

For the PP2 dataset, in relation to the primary efficacy parameter (CSS), enflicoxib demonstrated a significantly higher percentage of responders (CSS<6) on day 42 than the placebo group (73.97% responders versus 28.57% responders in the placebo group) and differences versus placebo were statistically significant at all days (7, 14, 28 & 42) compared to the control group.

Concerning the secondary outcome parameter (CBPI) and responders for a reduction of PSS score by ≥1 and PIS score ≥2 on day 42, there were 90.41% responders in the enflicoxib group versus 42.86% responders in the placebo group. Moreover, differences versus placebo were statistically significant at all days (7, 14, 21, 28, 35 & 42).

For the PP3 dataset, in relation to the primary efficacy parameter (CSS), enflicoxib demonstrated a significantly higher percentage of responders on day 42 than the placebo group (69.64% responders versus 13.33% in the placebo group) and differences versus placebo were statistically significant at all days (7, 14, 28 & 42) compared to the control group.

Concerning the secondary outcome parameter (CBPI) and responders for a reduction of PSS score by ≥1 and PIS score ≥2 on day 42, there were 87.5% responders in the E-6087 group versus 40% responders in the placebo group and differences versus placebo were statistically significant at all days (7, 14, 21, 28, 35 & 42).

Whilst it is acknowledged that the supplementary analyses provided by the applicant is *ad hoc* in nature and was not pre-specified, it is accepted that the analyses have been conducted in order to address the concerns raised by the CVMP in respect of the clinical relevance of assessment of response based solely on CSS scores. In the opinion of the CVMP, the additional restrictions used in establishing

the PP2 and PP3 datasets can be accepted as providing a more severe test of clinical response and non-inferiority between enflicoxib and mavacoxib can be accepted as having been adequately demonstrated based on the pivotal efficacy outcome parameter (CSS<6 at day 42) and using a non-inferiority margin of 10%.

Based on the results provided, it can be accepted that administration of enflicoxib resulted in a clinically relevant reduction in CSS and CBPI (PSS & PIS) scores and enflicoxib was shown to be non-inferior to mavacoxib.

Other studies

Following on from the pivotal field study, the applicant has provided the results of a further analysis of the PK data originating from the two field studies in order to investigate the incidence of a prolonged half-life of enflicoxib and E-6132 in dogs.

The applicant used C_{min} values as an indirect measurement of terminal half-life is to explore the magnitude of C_{min} levels, as if a prolonged half-life is shown, an accumulation will be observed. Dogs were identified that showed unexpectedly high C_{min} values of enflicoxib, suggesting a prolonged half-life of enflicoxib. This was also done for C_{min} values of E-6132 to see if the same dogs that showed unexpected high C_{min} values of enflicoxib also showed unexpected high C_{min} values of E-6132.

Using an alternative approach, individual terminal half-lives of enflicoxib and E-6132 in dogs with OA were estimated using the maximum a-*posteriori* Bayesian method.

Whilst the findings of this analysis are noted, the validity and relevance of these findings should be interpreted with caution as the predictions differ to those derived from previous PK modelling conducted by the applicant.

For example, the applicant has concluded that steady-state plasma concentrations of the metabolite E-6132 are reached after four weekly administrations. However, such a conclusion does not match previous findings where the applicant concluded that mean concentration of E-6132 increased from 1582.92 ng/mL at Day 28 to 1910.07 ng/mL by Day 42 when administered using the proposed dosing. It is considered that the results provided from this additional PK modelling do not satisfactorily address the potential safety concerns arising from prolonged administration of enflicoxib and the associated potential for accumulation of the metabolite E-6132 in the target species and this aspect would need to be addressed in the TAS study. As for the other PK/PD modelling results presented by the applicant in this application, it is considered that whilst the findings are noted, no definitive conclusion on the adequacy or otherwise of the minimum effective concentration of E-6132 or indeed of the proposed dosing schedule is considered possible from the modelling results and the suitability of the proposed dosing schedule must be confirmed under clinical field study conditions.

The applicant has presented the results of a further PK modelling study to compare the PK profile of two different tablet formulations on the grounds that a difference exists between the tablets used in field trials and those proposed to be commercialised. The product used in those trials was a breakable tablet of three different strengths (15.6, 60 and 120 mg), allowing for a flexible combination of fractions to adjust to the different dosages tested and accurately treat the animals according to their body weights. However, the final formulation intended for commercialisation was adapted to 5 different non-divisible tablets (15, 30, 45, 70 and 100 mg).

These tablets are manufactured under the same manufacturing process (blending and direct compression) as the ones used in the efficacy field trials. The qualitative composition of the different strengths is the same except for the flavouring agent, which has been changed although, maintaining the same concentration as in the tablets used in the efficacy field trials.

The composition of the different strengths is quantitatively proportional. The ratio between the amount of each excipient and the amount of active substance is the same for all strengths.

In addition, the active substance used in the formulation of the finished product tablets is manufactured following a different (optimised) route of synthesis. The only study where the formulation intended to be marketed was used was the pivotal target animal tolerance study.

This PK modelling analysis was performed by means of visual predictive check (VPC). The PK profiles of enflicoxib and E-6132 after administration of enflicoxib tablets used in the efficacy field trials were simulated using the previously developed popPK model in dogs. Once the simulations were completed, the observed PK levels of enflicoxib and E-6132 after administration of finished product tablets of enflicoxib from the TAS study (VX05LK) were superimposed to these PK simulations, and then the distributions between simulations and observations were compared to explore similarities. The applicant states that results indicate that no noticeable pharmacokinetic differences of enflicoxib and E-6132 were observed when bridging from tablets used in efficacy field trials to finished product tablets used in the target animal tolerance study.

The applicant has used further PK modelling to compare the PK profiles of enflicoxib and E-6132 using PK data from the pivotal target animal tolerance study (VX05LK) where the formulation intended to be marketed was used and PK data from a revised PK modelling (Study EV-013/09-SN) that included PK data from the dose confirmation field study (Study DA/184/C).

Based on the results provided, the applicant concludes that there are no significant differences in modelled PK profiles between the formulations used in the field studies and the final formulation intended for marketing and which was used in the pivotal target animal tolerance study.

Whilst the findings of this analysis are noted, the validity and relevance of these findings should be interpreted with caution. Section 7.1.g of the CVMP 'Guideline on the conduct of bioequivalence studies for veterinary medicinal products' (EMA/CVMP/016/2000-Rev.3) states that studies to compare the rate and extent of absorption between two formulations or products containing identical active substances are generally not required if the product is a reformulated product by the original manufacturer that is identical to the original product except for small amounts of colouring agents, flavouring agents, preservatives or other excipients, which are recognised as having no influence on bioavailability.

The applicant has stated that the formulation only differs in respect of the flavouring agent – 'dried flavour' is to be used in the formulation to be marketed whereas 'beef liver roasted flavour' was included in the formulation used in the field trials.

In light of the aforementioned CVMP guideline requirements and given the fact that the tablets intended for marketing include a different flavouring agent, the CVMP accepted that the difference in flavouring agent will have no influence on bioavailability.

Palatability/acceptability study

In support of the palatability/acceptability of the candidate formulation, the applicant has provided the results of two studies.

In the first study report, the applicant has included information on treatment compliance and acceptance for enflicoxib tablets for dogs using data from the dose confirmation field study and the pivotal efficacy field study.

The compliance and acceptance of enflicoxib tablets was assessed when orally administration to dogs was compared to a placebo. Treatments were administered either in the clinic by the dispenser or by the owner at home. If required, administration was performed by placing the tablet in the back of the dog's mouth to ensure maximum compliance and if necessary was given in conjunction with a small amount

of food to ensure the tablets were fully swallowed. Regurgitated tablets were re-administered if possible. After day 35 the owner assessed the general level of acceptance of the treatment by the dog as either poor, satisfactory, good or excellent. The percentage of animals in each category was compared between groups using a Chi-square test. Overall, it is reported that 99.65% of animals took enflicoxib tablets without problems and no regurgitation or vomiting was observed, compared to 99.1% of the placebo group. Products were administered with food in 98.52% of animals receiving enflicoxib and in 98.02% of animals receiving placebo. According to the owner's assessment, enflicoxib tablets were readily accepted by most dogs and only 10% of the dogs showed poor acceptance to the administration of the product, with no statistically differences compared to the placebo group.

In both the dose confirmation field study and the pivotal efficacy field study, the formulation of the test product and the placebo contained the excipient 'beef liver roasted flavour' as opposed to the flavouring agent proposed for inclusion in the formulation intended to be marketed ('dried flavour'). Consequently, the information provided in respect of acceptance/palatability of the older developmental formulation used in the field studies is not considered to be directly applicable to the formulation proposed for marketing.

That said, it is reported that in 98.52% and 98.02% of dogs administered the test product enflicoxib and the placebo respectively, the product was administered with food. Consequently, it is unclear as to whether the administered tablets were accepted voluntarily (before feeding), or whether acceptance was only when administered with food or when placed at the back of the mouth.

Given the above deficiencies it is considered that the information provided from these studies is inadequate for the purposes of concluding on acceptability/palatability of the final formulation intended to be marketed.

During product development and after the conduct of both the dose confirmation field study and the pivotal efficacy field study, the applicant made a change to the flavouring agent. The 'beef liver roasted flavour' excipient used in the field studies was exchanged for the excipient special 'dried flavour'.

In order to demonstrate the acceptability of the tablets with the new flavouring, the applicant conducted a palatability study.

14 dogs (8 females and 6 males) of different breeds/cross breeds and aged in the range 3 – 14 years and in the weight range 6 to 34 kg were included in a cross-over design study. Tablets were first offered in an empty bowl to assess voluntary acceptance for one minute. In case of failure, tablets were then offered by hand for an additional minute. After this, the tablets were administered by forced administration directly in the mouth.

No claim for GLP compliance has been made for this study. There are a number of divergences from the guideline recommendations for this study. The number of animals administered each product was 14 (as opposed to 25), each animal was only administered each product on a single occasion (as opposed to twice) and the threshold used for success was 75% (as opposed to 80%).

However, it is noted that the applicant has proposed that a threshold of 75% should be sufficient to demonstrate that the new flavour in the product will not affect acceptability to dogs when administered with food given that the aim of the study was not to claim palatability of the product in the SPC and the SPC will recommend the product be administered with food. The applicant proposes that the SPC states that the product is well accepted by most dogs and should be given immediately before or with a dog's meal.

Whilst palatability has not specifically been claimed, the wording proposed for inclusion in the SPC is considered to imply voluntary acceptance by most dogs and therefore is considered to indirectly imply that the product is palatable enough to ensure voluntary uptake. Given that a specific CVMP guideline

has been drafted to provide guidance on when a claim for palatability can be accepted (Guideline on the demonstration of palatability of veterinary medicinal products, EMA/CVMP/EWP/206024/2011), the use of different wording that is similar in meaning would negate the value of such a guideline.

Consequently, as the applicant has failed to adequately demonstrate palatability of the candidate formulation in accordance with the relevant CVMP guideline, reference to the product being 'well accepted' by most dogs was omitted from the SPC.

Overall conclusion on efficacy

Dose determination:

In justifying the selection of a dose of 4 mg/kg bw as a candidate treatment dose for the proposed indication, reference has been made to a pharmacokinetic study that investigated the pharmacokinetics of enflicoxib and its metabolite E-6132 in dogs.

Two pilot efficacy studies were conducted in further support of the proposed dosing schedule, however it is considered that little can be concluded from these studies in terms of the adequacy of the proposed posology (either dose rate or re-treatment interval).

Results of PK/PD modelling were presented in support of the weekly dosing schedule. The modelling was conducted using data that originated from one toxicokinetic study, one pharmacokinetic study and two pilot efficacy studies. Concerning the re-treatment interval, the applicant has conducted additional pharmacokinetic simulations to compare dosing intervals of 7 days and 14 days. Based on this additional modelling, it can be concluded that a weekly re-treatment interval is required to ensure adequate effectiveness.

Dose confirmation:

A GCP-compliant dose confirmation study was conducted to evaluate the efficacy of two target doses of 2 mg/kg and 4 mg/kg enflicoxib administered orally once weekly in the treatment of natural canine osteoarthritis. Based upon the results of this study, it would appear that a more rapid reduction in CSS (Clinical Sum Score) values was obtained in the dogs administered a maintenance dose of 4 mg/kg when compared with those administered 2 mg/kg. Although a statistically significant reduction in mean CSS values has been reported, it is noted that the magnitude of the difference is limited.

It can be accepted that overall, the findings from this study suggest a more favourable outcome in terms of CSS and CBPI (Canine Brief Pain Inventory) assessment when the product is administered at a maintenance dose of 4 mg/kg compared to 2 mg/kg. However, by the end of the study, owners considered their dogs to have been responders (improvement in CBPI score at end of study compared to baseline) in 76.2% of animals in the untreated (placebo) group, suggesting a high placebo effect in the assessment by owners. Furthermore, no statistically significant difference between treated and untreated animals was reported in the CBPI assessment by owners.

Target animal tolerance:

The applicant has provided the results of a target animal tolerance study.

The pivotal target animal safety study included young Beagle dogs. Given the proposed indication (treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease)), it is considered that study animals are not fully reflective of the intended target population (older animals with osteoarthritis or degenerative joint disease). Consequently, systemic tolerance to the product in older animals that are likely to have comorbidities will not have been adequately characterised in this study.

No adverse effects were reported in this study. However, it is noted that elevated blood urea concentrations were reported in both sexes at all dose levels from week 4 onwards and that a dose-response effect was observed. Cholesterol values were also high in week 13 in both sexes and at all dose levels when compared to both pre-treatment and control, however, from week 25 this finding was observed in male animals only. There was no clinically relevant correlation between the observed increased blood urea concentrations, plasma protein fractions, cholesterol values and red blood cell indices. Nonetheless, given that an elevation of urea and cholesterol was reported at the recommended treatment dose, this finding has been adequately reflected in the proposed SPC.

The applicant has presented a summary of the tolerance findings from the two field studies conducted. Based on the information provided, most of the adverse reactions were associated with the gastrointestinal tract. These findings are appropriately reflected in the SPC.

Field study:

A GCP-compliant efficacy field study was conducted.

It can be accepted that the results of this study indicate that the candidate product is superior to placebo in terms of the primary efficacy outcome parameter CSS.

The results for the secondary outcome parameter (CBPI) measured by owners is supportive of the more objective outcome parameter CSS.

Concerning the investigation of non-inferiority between enflicoxib and mavacoxib, the candidate product was demonstrated to be non-inferior to the comparator product for the primary efficacy outcome parameter (CSS).

In response to concerns raised, the applicant reconsidered the adequacy of the threshold for demonstrating efficacy in terms of clinical relevance and subsequently conducted supplementary statistical analyses on two additional data populations.

Based on the results provided, it can be accepted that administration of enflicoxib resulted in a clinically relevant reduction in CSS and CBPI (PSS & PIS) scores and enflicoxib was shown to be non-inferior to mavacoxib.

Part 5 – Benefit-risk assessment

Introduction

Daxocox is a tablet containing enflicoxib, a new active substance. Enflicoxib is a non-steroidal anti-inflammatory drug (NSAID) belonging to the coxib class and acting by selective inhibition of the enzyme cyclo-oxygenase 2.

The product is intended for use in dogs for the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease). The proposed first dose of 8 mg enflicoxib/kg body weight administered orally and the maintenance dose of 4 mg enflicoxib/kg body weight repeated every 7 days have been confirmed.

The dossier has been submitted in line with the requirements for submissions under Article 31 of Regulation (EC) No 726/2004.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The benefit of Daxocox is its efficacy in the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease) in dogs.

The proposed indication was investigated in laboratory study models, two pilot efficacy studies, a field dose confirmatory study and a pivotal efficacy field trial.

The active substance is metabolised to an active metabolite and it is considered that this metabolite is responsible for the therapeutic effect reported.

Additional benefits

As Daxocox is to be administered at weekly intervals due to a long-lasting effect, a potential additional benefit of this product is the need for less frequent administration and therefore potential for improved owner compliance and animal acceptance when compared with products that require daily re-administration.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Risks for the target animal:

Administration of Daxocox in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions were gastrointestinal and include vomiting, soft faeces and/or diarrhoea. The SPC adequately reflects all tolerance findings.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment:

Daxocox is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk management or mitigation measures

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, user, and environment and to provide advice on how to prevent or reduce these risks.

User safety:

User safety risks have been identified, mainly the risks associated with exposure in children, adults and pregnant women. These risks have been addressed by the safety warnings in the SPC.

Evaluation of the benefit-risk balance

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

The product has been shown to be efficacious for the treatment of pain and inflammation associated with osteoarthritis (or degenerative joint disease) in dogs.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Daxocox is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.