SCIENTIFIC DISCUSSION

Name of the veterinary medicinal product:	Zubrin oral lyophilisates
Marketing Authorisation holder:	S-P Veterinary Schering-Plough House Shire Park Welwyn Garden City Hertfordshire AL7 1TW United Kingdom
Active substances:	Tepoxalin
International non-proprietary name:	Tepoxalin
Pharmacotherapeutic group (ATCvet code):	Anti-inflammatory and anti-rheumatic products, non-steroids (QM01A)
Therapeutic indication(s):	Reduction of inflammation and relief of pain caused by acute musculoskeletal disorders or acute exacerbation of chronic musculoskeletal disorders
Target species	Dogs
Withdrawal period:	N / a

I. INTRODUCTION

Zubrin oral lyophilisates (30 mg, 50 mg, 100 mg and 200 mg) contain the new chemical entity tepoxalin, 5 - (4 - chlorophenyl) n - hydroxy-1-(4-methoxyphenyl)-N-methyl-1H-pyrazole-propamide, molecular weight 385.85, an orally active non-steroidal anti-inflammatory drug with inhibitory effects on cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism.

The product is intended for the reduction of inflammation and relief of pain caused by acute musculoskeletal disorders or acute exacerbation of chronic musculoskeletal disorders in dogs. Treatment consists of 10 mg tepoxalin per kg bodyweight administered orally once daily. The duration of treatment is dependent on clinical response but should not exceed 4 consecutive weeks.

II. CHEMICAL, PHARMACEUTICAL AND BIOLOGICAL DOCUMENTATION

II A Composition

II A 1. Composition of the medicinal product

The composition of Zubrin oral lyophilisate is presented in table 1 below:

Ingredient	Ref.	mg/tablet	mg/tablet	mg/tablet	mg/tablet	Function
Tepoxalin	In-house	30 mg	50 mg	100 mg	200 mg	Active substance
Gelatin	Ph. Eur.					Structure and flexibility
Mannitol	Ph. Eur.					Rigidity
Purified water*	Ph. Eur.					

*Removed during manufacture by lyophilisation

II A 2. Container

The product is packaged in unit dose blisters of 10 tablets per card for all strengths. The package consists of an aluminum laminate blister pack with aluminum laminate lidding foil, contained in an outer cardboard carton. The blister is composed of five layers PVC/OPA/Aluminium/OPA/PVC with a paper foil laminate seal composed of aluminum/PET.

II A 3. Clinical trial formulations

The batches used in the clinical trials have the same composition as the product intended for marketing.

II A 4. Development pharmaceutics

The aim was to develop a product that could effectively reduce inflammation and relief of pain associated with musculoskeletal disorder in dogs. The attempt was based on development of freeze-dried tablets, which could be dissolved rapidly on the tongue and hence be readily swallowed without the need for water. The tablets are formed by freeze drying an aqueous suspension of the active ingredient in pre-formed pockets in film or laminate, which also forms part of the primary packaging.

Initial batches for formulation development were dosed into PVC/PVdC packs, 3-layer all aluminium and 5-layer aluminium packs. Five-layer aluminium material was the material of choice for this product as it is robust and sacheting of the product is not required. Initial formulation work concentrated on the formulation of strengths 50 mg (12 mm unit, 200 mg fill weight) and 200 mg (18 mm unit, 800 mg fill weight).

Gelatin and mannitol are used in the formulation to ensure fast dispersion properties while giving adequate tablet strength. Gelatin imparts the essential structure of the unit and ensures that it retains some flexibility. Mannitol is included in the formulation at a level of 2% w/w. Mannitol crystallises during the freezing process and gives the units rigidity. It also has the function of improving appearance, texture and taste. The optimisation of gelatin and mannitol were based on the measurement of the tensile strength as well as disintegration time. According to the manufacturer a tensile strength measurement of 0.15 Nmm^{-2} or greater will enable the unit to be removed from the primary package and handled normally without damage.

In conclusion, the development pharmaceutics took into consideration appearance of the product, disintegration time, tensile strength, particle size and weight and content uniformity.

II B Method of preparation

II B 1. Manufacturing formula

The manufacturing formula has been given for a typical batch of 180 kg. The quantity of active ingredient is adjusted based on the potency of the active ingredient. The actual batch quantity of active ingredient is given by the formula:
Actual batch quantity = theoretical quantity x 100 % / Potency assay (%)

II B 2. Manufacturing process and in process controls

Gelatin, mannitol and purified water are heated and mixed. Tepoxalin is added to the mixture and mixed to obtain a homogenous suspension. The absence of control of the particle size in the suspension before freezing has been adequately justified.

The suspension is transferred into pre-formed blister pockets using a dosing system. Although the same suspension is used for all strengths, the pocket size of the blister for the various strengths is different. The filled blister is then passed through a freeze tunnel (cooled by using liquid nitrogen) where the product is rapidly frozen. The frozen product is transferred into a refrigerated storage cabinet where it is stored prior to freeze-drying. Frozen product is freeze-dried and sealed.

In-process controls include fill weight (beginning, middle and end) of the filling operation, and samples were inspected for physical defects and weight after freeze drying. At the beginning and during the sealing operation, a sufficient number of sealed blisters are removed and visually inspected.

II B 3. Validation of the manufacturing process

Efforts have been made to develop the manufacturing process of the finished product. These concern manufacturing of six pilot batches of each strength 200 mg and 50 mg (18 - 50 kg), one batch of strength 100 mg (18 kg) and three batches of strength 30 mg (18 kg). The manufacturing process of the batches is the same as the proposed commercial process but differs in some parameters (i.e. freeze tunnel temperature and freeze drying cycle etc). All the batches were manufactured at DDS Scherer, United Kingdom, the proposed commercial manufacturer.

The process development takes into consideration mixing of ingredients, dosing, packaging, freeze-tunnel set-point, drier shelf temperature and cycle time, sealing temperature, sealing pressure, content and weight uniformity (start, middle and end), disintegration time (start, middle and end). In addition the last three batches of strengths 50, 100 and 200 mg have been tested in compliance with specifications. Following a request by the Committee, the Applicant has provided complementary information, which clarified satisfactorily the different process parameters. The Applicant has also confirmed that process validation data for full scale batches will be submitted when available.

II C Control of starting materials

The active substance is not described in a pharmacopoeia.

II C 1. Active ingredient not described in a Pharmacopoeia

Nomenclature and description of the active substance have been presented (see II C 1.2.1).

II C 1.1 Specifications and routine tests

The proposed specifications for the active substance are consistent with those expected for an active pharmaceutical ingredient.

The Applicant's headquarters are located in the United States. Therefore the United States Pharmacopoeia (USP) methods have been preferred. However, in the quality requirements for the active substance (heavy metals, melting point and microbial control), European Pharmacopoeia (Ph.Eur.) methods will be used when developing future specifications.

Identity is established by infrared absorption (IR) and verified by High Performance Liquid Chromatography (HPLC). Process-related substances and potency of the active substance are determined by HPLC method. In addition to HPLC method a Thin-layer-chromatography (TLC) method is also used for control of impurities. Residual solvent is controlled by gas chromatographic method. Water content is determined by Karl Fischer method.

The HPLC method used for determination of potency and impurities are validated regarding specificity, linearity, precision, limit of quantification (LOQ), limit of detection (LOD), ruggedness, solution stability and system suitability. The TLC method used for determination of impurities are validated regarding specificity, linearity, precision, LOQ, LOD, solution stability and system suitability. The Gas-Chromatography (GC) method is validated regarding specificity, linearity, precision, limit of detection and limit of quantification, ruggedness, solution stability and system suitability. One batch of reference standard has been manufactured. A specification with the analytical methods has also been presented for the reference standard. The standard was examined by mass spectrometry, nuclear magnetic resonance (NMR) and combustion analysis.

The quality control of the active substance and the validation of the analytical procedures are considered to be acceptable.

II C 1.2 Scientific data

II C 1.2.1 Nomenclature and description

Nomenclature and description of the active substance have been adequately presented.

International Nonproprietary Name (INN):	Tepoxalin
International Union of Pure and Applied Chemistry (IUPAC):	1*H*-Pyrazole-3-propanamide, 5-(4-chlorophenyl)-*N*-hydroxy-1-(4-methoxy-phenyl)-*N*-methyl
Chemical name:	5-(*p*-Chlorophenyl)-1-(*p*-methoxyphenyl)-*N*-methylpyrazole-3-propiono-hydroxamic acid
CAS no.:	103475-41-8
Description:	White crystalline powder
Molecular formula:	$C_{20}H_{20}ClN_3O_3$
Molecular weight.	385.85

The proposed structure of tepoxalin is supported by route of synthesis, elemental analysis, ^{1}H- and ^{13}C-NMR, mass-spectrometry (MS) and X-ray.

Appearance:	White crystalline powder, free from foreign matter or other visual contamination.
Solubility:	Soluble in about 47 parts of acetone, in about 35 parts of ethanol and in about 3.5 parts of chloroform. Practically insoluble in water.
pH value:	pH ca 6.0 (1% suspension in water)
Melting point:	125°C to 130°C

The efforts made to determine the solubility and polymorphism of the active substance have been discussed. Solubility has been assessed with different solvents and at different pHs. The recrystallisation from ethyl acetate does not promote the formation of polymorphs. The respective thermograms were identical to the reference standard.

II C 1.2.2 Manufacture and in process controls

The active substance was originally manufactured at Schering-Plough (Avondale) Company, Rathdrum, Co. Wicklow, Ireland; however, the manufacturing site of the active substance changed in June 2001 to SEAC Chemie Fine in France. The manufacturing process and the proposed specification as well as test methods are the same as those already approved. Certificates of analysis have also been submitted for three batches of the drug substance manufactured at the SEAC site and two batch analysis data presented from the French manufacturing site demonstrate that manufacture of the drug substance is reproducible and consistently meets the current specification of the drug substance.

II C 1.2.3 Development Chemistry

Originally, Johnson & Johnson (J&J) in USA developed the active substance. However, the manufacturing process was first transferred to Schering-Plough Avondale (SPA) in the UK. The manufacturing process used at SPA was the same as the process developed and used by J&J. The synthesis of tepoxalin is a 3-stage process. The route of synthesis is sufficiently described, and the major phases in the synthesis of tepoxalin are controlled during the reaction. In June 2001, the manufacturing site of the active substance changed to SEAC Chemie Fine, France.

II C 1.2.4 Impurities

Eleven potential synthetic and degradation impurities have been proposed by the Applicant. They have been characterised by Liquid Chromatography - Mass Spectometry (LC-MS). Authentic samples were then synthesised to confirm the structure of the impurity. A list and a brief description of these impurities have been presented. In practice, only 4 impurities may be detected in tepoxalin.

The Applicant has committed to provide revised limits for related impurities for the active substance tepoxalin (except the limit for individual unknown impurities) after additional manufacturing experience at the manufacturing site of the active substance has been gained. These data were provided and the CVMP concluded that the follow up measures had been fulfilled for the 50 mg, 100 mg and 200 mg presentations. However, during the review of the data, it was proposed to tighten some of the impurity limits in the active substance specification and the finished product specification. Therefore, the Marketing Authorisation Holder provided two appropriate Type I variations tightening the impurity limits in the active substance specification (total unknown impurities, total impurities detected with HPLC and total impurities detected with TLC) and the finished product specification (release and shelf-life).

II C 1.2.5 Batch analysis

Results of batch analysis have been presented. Batch data from 5 batches are provided. J&J supplied the Applicant with three batches to support registration and “clinical studies”. These batches were manufactured at two different sites and ranged in size from 13 - 48 kg. The two remaining batches were manufactured by SPA located in Ireland. The size of the batches produced at SPA were 24.0 kg and 22.7 kg, respectively. These two were tested and compared against the three batches supplied by J&J.

The results showed that the drug substances are equivalent in either of the manufacturing site. Furthermore, the results confirm the capability to produce a consistent quality of tepoxalin at the proposed site (SPA). All limits set are justified except the limits for impurities and microbial contamination.

All toxicological and clinical studies were performed with Johnson and Johnson batches.

II C 1.2.6 Potential risk of Bovine Spongiform Encephalopathy (BSE):

Judging from the manufacturing process for tepoxalin, there appears to be no risk of BSE or TSE transmission. In April 2001, the applicant submitted a Certificate of Suitability of the European Pharmacopoeia for Gelatin in order to comply with Commission Directive 1999/104/EC..

II C 2. Excipients

All other ingredients are of Ph. Eur grade. The Applicant has submitted the certificate of analysis for gelatin and mannitol but not for purified water.

II C 3. Packaging material

Certificates of analysis from the supplier for each film and foil number have been provided.

II D Control tests on intermediate products

Not applicable.

II E Control tests on the finished product

The finished product is manufactured at DDS Scherer, United Kingdom. The manufacturer in charge of batch release isSP Bray, Ireland.

II E 1. Specifications and routine tests for release and end of shelf-life

The specifications and routine tests have been adequately presented.

Identity is verified by HPLC and UV. Potency, identity, content uniformity and degradation products are determined by a HPLC procedure. The content of the active substance in dissolution study was determined using HPLC method. Water content is determined by Karl Fischer method.

II E 2. Validation of analytical methods

The HPLC methods are validated regarding specificity, precision, linearity, accuracy, and limit of quantitation. The analytical procedures are considered suitable for their intended purpose.

The process for the manufacturing of the finished product follows conventional pharmaceutical practices, which utilise a solution compounding step, filling into pre-formed blister pockets using a dosing system followed by freezing, lyophilisation and sealing. The process validation/optimisation was based on the 50 and 200 mg strengths and it was not adequately validated/optimised for the 30 and 100 mg strengths. The Applicant committed to provide process validation data from full-scale batches when available.

II E 3. Batch analysis

Data are presented for 3 batches of the finished product (one batch of each strength 50 mg, 100 mg and 200 mg). The batch sizes are from 55 460, 14 800, 14 020 units, respectively. All batches are manufactured at the proposed site of manufacturing. All limits set in the specifications are justified except the limit for degradation products.

The Applicant committed to revise and if appropriate tighten the limits for degradation products in the finished product when additional manufacturing experience is gained.

In quality requirements (microbial contamination and uniformity of content) of the finished products USP methods were selected. For an application in Europe, tests should be performed according to the European Pharmacopoeia. However, the microbial contamination tests in the USP and in the Ph. Eur. do not differ significantly. Furthermore, the USP method for uniformity of content is more stringent than the Ph. Eur. as the USP method takes into account the precision of the assay and values measured against the label claimed rather than the calculated average content. USP methods have therefore been accepted.

The batch size of finished product (i.e. the solution that is filled into blisters and then freeze dried) was originally 180 kg for all strengths of the oral lyophilisate; however, in July 2001 and June 2002 variations were approved modifying the size to 50 kg (30 mg dose strength), 175 kg (50 mg dose strength), 295 kg (100 mg dose strength) and 265 kg (200 mg dose strength).

II F Stability

II F 1. Stability tests on active ingredient

Four batches of tepoxalin were evaluated for stability using a range of challenge stability conditions. Two batches were supplied by J&J, another two batches were manufactured by SPA. Up to 3 months of data are available for all 4 batches stored at 25°C/60% RH, 30°C/60% RH and 40°C/75% RH. Solid photo-degradation studies were also conducted. Data are available of up to 30 days for all batches exposed to light. The parameters studied are appearance, assay, related impurities, melting range and water content. The methods used in the primary stability study are the same as those applied to control of the active ingredient. The studies revealed that tepoxalin is stable under the following conditions of storage: 40°C/75% RH/3 months, 30°C/60% RH/3 months, 25°C/60% RH/3 months and exposure to light for 30 days. When stored under these conditions, no significant changes were observed in any of the stability parameters analysed.

Supportive stability studies were conducted on three batches (range from 1 kg to 6.6 kg) at room temperature (24°C/50% RH), 37°C and 50°C. Additionally, tepoxalin was stored under photostability conditions of 1,000-foot candles for 30 days. J&J using the same synthesis process as that proposed for commercial production manufactured these batches. No further information was given for these batches. Parameters studied are appearance, assay, related impurities, loss on drying and UV. The results of these studies showed that the parameters are remained within the proposed specification when stored for 36 months at room temperature.

Further to a request from the Committee, results from on-going stability studies on the active substance have been submitted. Data from storage up to 3 months at 40°C/75%RH have also been submitted. Since the results comply with the specifications until 18 months at 25°C, a re-test period of 18 months has been accepted.

II F 2. Stability tests on the finished product

Stability studies have been performed on two pilot scale batches (18 kg) of the finished product (30 mg) and one pilot scale batch (18 kg) for each further strength (50 mg, 100 mg and 200 mg). All 5 batches are stored following the guidelines of the ICH at accelerated and long-term storage conditions (4°C and 25°C/60% RH, 30°C/60% RH and 40°C/75% RH).

Up to 3 months of data were available for all four strengths stored at above mentioned conditions and packaged in Lidding Foil Laminate. Slight differences occurred in the composition of the 100 mg Lidding Foil Laminate compared to the 30 mg, 50 mg, 200 mg presentations regarding the heat seal lacquer, PET content and calendered kraft paper. However, it was found that when the Lidding Foil

In addition, supporting stability data were available for two strengths, the 50 mg and 200 mg presentations stored up to 6 months at 40°C/75% RH and up to 12 months at 4°C and 25°C/60% RH, 30°C/60% RH. The accelerated data supports that extrapolation can be undertaken. These batches were packaged in Lidding Foil Laminate identical to that of 100 mg strength. No significant changes have been observed in any of the monitored parameters under any of storage conditions. A decrease in the disintegration time was observed when the product was stored for 3 months. The potency of the active ingredient remains within the proposed specification. Three potential degradation products have been identified at approximate relative retention times (RRT) of 0.75, 1.24 and 2.27. All of these degradation products were present in the initial samples and no significant increase was observed.

The Applicant has proposed a shelf-life of 24 months when stored below 30°C, but the original data submitted cover for strengths 50 and 200 mg for up to 12 months at 4 °C and 25°C/60% RH, 30°C/60% RH. Therefore, the Committee concluded that a shelf-life can be extrapolated to 18 months.

In a variation submitted in June 2001, stability data for up to 24 months were submitted for all four strengths (all batches) stored at 4 °C and 25 °C/60%RH, 30 °C/60%RH and up to 6 months at 40 °C/75% RH and packaged in Lidding Foil Laminate. The methods used were the same as the previous ones in the specifications for the finished product. Based on the results presented Zubrin was considered to be stable at least up to 24 months when stored at 4 °C, 25 °C /60% RH and 30 °C /60% RH and, therefore, a shelf-life of 24 months was accepted.

III. SAFETY AND RESIDUE DOCUMENTATION

III.A Safety

Zubrin oral lyophilisates (30, 50, 100 and 200 mg) contain tepoxalin as active ingredient. Tepoxalin is a non-steroidal anti-inflammatory drug inhibiting both the cyclo-oxygenase (CO) and the lipooxygenase (LO) pathways of the arachidonic acid metabolism. Only commonly used tablet excipients are included (gelatin and mannitol) in the composition of the final product.

III.A.2 Pharmacological studies

III.A.2.1 Pharmacodynamics

Inhibitory effect on cyclo-oxygenase (CO) and lipooxygenase (LO)

Tepoxalin has been shown in a number of *in vitro, ex vivo* and *in vivo* studies to possess dual cyclo-oxygenase (CO) and lipooxygenase (LO) inhibitory properties.

The IC_{50} values and estimated plasma levels associated with the ED_{50} values were in most cases within the plasma concentration range achieved with the therapeutic dose of the product. *In vitro*, tepoxalin inhibited cyclo-oxygenase-1 (COX-1) with at least a thirty-fold higher potency than for cyclo-oxygenase-2 (COX-2). The potencies of tepoxalin as LO and CO inhibitor, respectively, seemed to be in the same range. However, some *in vivo* studies showed less potency against LO than against CO.

At higher concentrations than those clinically relevant, inhibition of the production of cytokines was shown *in vitro*.

The *ex vivo* eicosanoid synthesis in whole blood was investigated in adult beagle dogs of both sexes treated orally or intravenously with 10 mg tepoxalin/kg bodyweight. Although C_{max} for the metabolite was higher after intravenous than oral administration, similar ED_{50} values (0.015 and 0.014 mg/kg,

respectively) for cyclo-oxygenase inhibition after intravenous and oral administration of tepoxalin in dogs were achieved 1 hour post treatment.

The active carboxylic acid metabolite was present in plasma at markedly higher levels than tepoxalin and is most likely responsible for the prolonged duration of tepoxalin-induced inhibition of CO (more than 24 hours). The acid metabolite seems to possess a pharmacodynamic profile similar to the parent compound. Inhibition of COX-1 and COX-2, with a higher potency for COX-1, was shown. The potential LO inhibitory activity by the metabolite has not been investigated. In an acetylcholine, phenyl-*p*-quinone and endothelin-1-induced abdominal irritant test in mice, the metabolite showed similar or better anti-nociceptive activity than tepoxalin.

Anti-inflammatory and analgesic effects

Anti-inflammatory and analgesic activities have been demonstrated in a number of test models in mice, rats and dogs. Doses employed in most cases are estimated to be associated with systemic exposure levels in the range expected at the recommended clinical dose (10 mg/kg). However, in some studies, particularly those concerning analgesic activity, higher doses were required, e.g. antigen-induced arthritis in rabbits, gait scoring in dogs and adjuvant arthritic rat flexion assay. In the hot-plate test in mice and air-induced abdominal irritant test in rats, no or very slight effects were recorded.

In *in vivo* studies, tepoxalin showed generally similar or slightly less potency than naproxen and indomethacin when compared on a dose basis.

The analgesic activity was studied in a canine arthritis model (knee joint synovitis). Arthritis was induced by intra-articular injection in a knee joint of immune complexes and sodium urate injection. Tepoxalin was given orally as a sodium salt solution immediately prior to the intra-articular challenge. The doses used were somewhat higher (0, 25, 50 and 100 mg/kg) than the recommended therapeutic doses (10 mg/kg). Inhibition of LTB_4- and PGE_2-production (LO and CO inhibition, respectively) and decreased gait score were investigated. Lameness was assessed 2 and 4 hours after challenge. Treatment effects were visible after 2 hours, where the best effect was seen after the dose 50 mg/kg. A clear dose related effect was seen after 4 hours. LTB_4 production was inhibited in a dose-related manner at both 2 and 4 hours. PGE_2 production was only marginally inhibited at 2 hours, but was inhibited by more than 50% at 4 hours. The inhibition did not appear to be dose related. The inflammatory cells in the synovia samples were harvested and stimulated with calcium ionophore A23187. PGE_2 was inhibited in a dose-related manner at both 2 and 4 hours. LTB_4 was also significantly inhibited at both time points.

The relatively high doses that were required in some of the *in vivo* studies may be due to limited absorption following oral administration. Problems with absorption and a very high inter-individual variability in plasma levels of tepoxalin have been observed in pharmacokinetic and toxicity studies. In the study report, as an alternative explanation for the limited analgesic activity, it was proposed that the exertion of analgesia might be mediated through the anti-inflammatory activity.

In vitro **inhibition of lymphocyte proliferation** in human blood mononuclear cells by tepoxalin was reversed by the addition of iron salts. Furthermore, the inhibition of the activated transcription factor nuclear factor kappa B (involved in cytokine production) was also reversed by iron. These data suggest that tepoxalin is an iron chelator, which is not unexpected for a hydroxamic acid. However, as the IC_{50} values for these effects were markedly higher than the corresponding values for CO and LO inhibition and above the therapeutically relevant concentrations, the clinical relevance of this is considered low.

Anti-asthmatic effects

The anti-asthmatic effect of tepoxalin was studied in guinea pigs (arachidonic acid-induced or antigen-induced bronchospasm) and sheep (naturally allergic). The ED_{50} of tepoxalin for inhibition of the high dose response (CO inhibition) was 0.03 mg/kg intravenously and for the low dose response (LO inhibition) 10 mg/kg intravenously The corresponding values at intraduodenal administration were

0.3 mg/kg and 200 mg/kg, respectively. In the antigen model (leukotriene-mediated bronchospasm), the ED_{50} values were 15 mg/kg intravenously and 200 mg/kg orally. Hence, oral administration of tepoxalin resulted in minimal activity against LO while its ability to inhibit CO was retained. In sheep, tepoxalin (600 mg twice daily, 4½ days) inhibited the late phase bronchospasm. The effect on the early response was minimal.

No safety pharmacology studies regarding effects on cardiovascular, respiratory, central nervous, gastrointestinal (except for the ulcerogenic effects) or renal systems have been performed.

III.A.2.2 Pharmacokinetics

In all species investigated, dose-related but less than dose-proportional increases in plasma levels of tepoxalin and its active carboxylic acid metabolite were shown. A rapid conversion to the metabolite was observed and the metabolite was found at markedly higher plasma concentrations (C_{max} and AUC) than tepoxalin. The inter-individual variability in plasma levels was very high for both tepoxalin and the metabolite.

Absorption

The plasma pharmacokinetics of tepoxalin was investigated in 10 dogs, treated orally with the final formulation (oral lyophilisate). Tepoxalin (0, 5, 10, 20 and 40 mg/kg bodyweight) was administered within one hour after feeding. The washout period between the treatments was 14 - 20 days. Blood was sampled at different intervals up to 48 hours post dosing. Tepoxalin and its acid metabolite were analysed by a validated HPLC method. T_{max} for tepoxalin was reached after 1 - 3 hours. Mean C_{max} varied from 0.59 µg/ml in the lowest dose group to 2.63 µg/ml in the highest dose group. The results indicated that the absorption of tepoxalin is dose dependent, but not dose proportional and that thus absorption is a saturable process.

Tepoxalin was rapidly converted to its acid metabolite. The concentrations of the metabolite were significantly higher than the tepoxalin concentrations. The C_{max} levels of the metabolite were 2 - 3 times higher than those of tepoxalin and the metabolite AUC was 5 - 10 times greater than the tepoxalin AUC. The tepoxalin concentrations were very low a few hours after dosing, while the metabolite level remained at a higher level and were detectable during the entire sampling period. The metabolite is an inhibitor of cyclo-oxygenase, but apparently not of lipooxygenase. The difference in pharmacokinetics probably explains why inhibition of cyclo-oxygenase is of longer duration than lipooxygenase inhibition. Considerable individual variation occurred both in the tepoxalin and the metabolite levels. Therefore, it was suggested that the ability to absorb and metabolise tepoxalin differs significantly between individual dogs.

Metabolism

Four non-fasted dogs were given a single oral dose of ^{14}C-tepoxalin. Plasma was collected 1 hour prior to dosing and 1 and 4 hours after dosing. Excreta were collected 0 - 6 hours, 6 - 12 hours, 12 – 24 hours and then daily for 7 days. Total radioactivity in plasma, urine and faeces was determined. Plasma radioactivity was 1.8 - 8.7 ppm parent equivalents at 1 hour post treatment and 1.7 - 5.9 ppm parent equivalents at 4 hours post treatment. HPLC analysis of plasma extracts showed that tepoxalin was rapidly metabolised. After both 1 and 4 hours the major residue was the acid metabolite, representing a mean of 61.0 $\pm$ 10.3% and 86.4 $\pm$ 4,9%, respectively, of total extract radioactivity. An unknown metabolite was seen in plasma at both time points (about 1% of total radioactivity). Approximately 98% of the recovered dose was found in faeces and about 1% was found in urine. More than 90% of the recovered dose was found within 72 hours post dosing. The residue percentages of tepoxalin and its acid metabolite in urine were 9.3 $\pm$ 7.3% and 15.8 $\pm$ 5.0%. The major residues in urine were unknown metabolites.

A similar metabolism study was performed in mice with 10 mg/kg of ^{14}C-tepoxalin orally. Radioactivity was measured in plasma and faeces and tepoxalin and its metabolites were analysed using HPLC. Tepoxalin was rapidly metabolised: 1 hour post treatment the parent compound was

almost completely metabolised, constituting only 1.7 ± 0.5% of plasma radioactivity. The major residue at 1 hour post treatment was the acid metabolite which constituted 68.4 ± 12.4%. Tepoxalin was eliminated both in faeces and urine. More than 85% of the radioactivity was excreted within 24 hours after dosing. The fraction recovered in faeces constituted 64.28 - 68.40% of the administered dose, and the fraction recovered in urine 26.26 - 33.36%.

The results of these studies show that the renal excretion of tepoxalin and its active acid metabolite differ between mice and dogs. The elimination pathway in the dog is almost exclusively via faeces, only about 1% was recovered in urine, while about 30% of the dose was eliminated via urine in the mouse.

Effects of fasting and dietary fat content on oral bioavailability

The oral bioavailability was investigated in a cross-over study in six beagles. Tepoxalin (10 mg/kg) was administered intravenously or orally as oral lyophilisate after a 12 hours fasting period, in association with feeding of a commercial low fat diet (15.4% fat), or in association with feeding of a high fat commercial diet (28.7% fat). The washout periods between treatments were at least 10 days.

Tepoxalin was rapidly absorbed after oral administration, T_{max} was about 2 hours. In fasted dogs, C_{max} was 0.53 + 0.20 µg/ml. The mean values were significantly higher when treatment was given in association with feeding, 1.19 + 0.29 µg/ml (high fat meal) and 1.08 + 0.37 µg/ml (low fat meal). The mean oral bioavailability was 50.3 + 29.3% in fasted dogs. Bioavailability was numerically higher in fed dogs, 119.86 + 118.68% (high fat meal) and 74.72 + 44.53% (low fat meal). $T_{1/2}$ was 0.92 + 0.18 hours after intravenous injection and 2.82 + 1.05 hours after oral treatment in the fasted state and 3.23 + 2.40 and 3.72 + 2.38 hours in fed dogs, respectively.

The plasma concentration of tepoxalin, and thus all pharmacokinetic parameters, varied widely between individual dogs and between treatments, therefore the difference between fed and fasted dogs was not statistically significant. However, since tepoxalin has very low water but high fat solubility, it is assumed that bioavailability is higher in fed than in fasted dogs. The Committee therefore recommended the use of tepoxalin in fed dogs, this has been addressed in the SPC under section 5.7 (Posology and method of administration).

Multiple dose studies

Several studies investigating the long-term use of tepoxalin were of limited use only. Dogs were treated twice daily with oral doses of tepoxalin (15 mg/kg to 300 mg/kg) for 4 weeks, 13 weeks, 26 weeks and 52 weeks, respectively. The final oral lyophilisate formulation was not used but a powdered formulation with a very poor absorption of tepoxalin. Blood was collected for analysis of tepoxalin and its metabolite on various days. The mean peak concentrations of both tepoxalin and the metabolite were dose related. The peak concentration was of similar height on all sampling days, and there were no signs of accumulation. The plasma levels of both tepoxalin and its metabolite increased less than proportionally with increases in the dose, indicating saturation of absorption. At doses above 200 mg/kg, no further increase in plasma level concentration was observed. T_{max} occurred at about 1 - 2 hours. Feeding seemed to increase the oral bioavailability as did high fat diet compared with low-fat diet. However, the variability in these values was very high. A high protein binding (more than 98%) was observed in dog plasma both for tepoxalin and its acid metabolite. The result of the studies showed that accumulation did not occur at daily doses much higher than the recommended dose, 10 mg/kg bodyweight.

III.A.3 Toxicological studies

III.A.3.2 Single dose toxicity

Single dose toxicity studies were performed in mice (up to 225 mg/kg intravenously, 400 mg/kg orally), rats (up to 120 mg/kg intravenously, 400 mg/kg orally) and rabbits (100 mg/kg orally,

80 mg/kg dermally). Lethality was observed at intravenous administration in mice (≥125 mg/kg) and rats (120 mg/kg). In oral single dose toxicity studies, deaths due to gastric ulceration occurred from the lowest dose administered in mice (50 mg/kg) and at 400 mg/kg in rats. Clinical signs of toxicity included hypoactivity, laboured breathing, hypothermia and urine staining of the fur.

III.A.3.3 Repeat dose toxicity

In repeated dose toxicity studies in mice and rats, renal, hepatic and gastro-intestinal lesions were observed. Specific studies investigating the ulcerogenic effect of tepoxalin in rats and dogs showed effects (gastric lesions) at higher doses than of other NSAIDs such as naproxen and indomethacin. At endoscopic evaluation of the gastric mucosa in dogs following rising doses for five weeks, similar lesion scores for tepoxalin (25 - 2700 mg/kg) and naproxen (1 - 30 mg/kg) were achieved (0 - 1.5 of possible 0 - 6).

Toxicity following repeated administration of tepoxalin via oral gavage was investigated in mice (3 months), rats (up to 6 months) and dogs (up to 1 year). In rodents, well-known NSAID-related effects, such as gastric lesions, severe hepatic and renal toxicity and decreased red blood cell parameters, were observed. The effects on the liver and kidney occurred already at a dosage lower than the clinically expected system exposures to tepoxalin and its acid metabolite. The difference in renal toxicity in mice and dogs can be related to the difference in renal excretion. Signs of renal toxicity did not occur in the canine safety studies in dogs treated with high doses for 1, 6 and 12 months, while signs of renal toxicity occurred in mice after high doses.

Tepoxalin has been investigated in toxicity studies lasting up to 1 year and employing higher than recommended doses in dogs. No indications of any cardiovascular, respiratory or central nervous system mediated effects were observed. Furthermore, the product is contraindicated in animals suffering from cardiac, hepatic and renal disease and where there is a history of gastrointestinal ulceration. The lack of studies specifically addressing the safety pharmacology of tepoxalin can be accepted.

III.A.3.4 Target species tolerance

Tolerance in the target species was investigated in studies of 7 days (0, 15, 75, and 750 mg/kg/day), 4-weeks (0, 20, 100 and 300 mg/kg bodyweight/day), 13-weeks (20 to 300 mg/kg/day), 26-weeks (20 to 300 mg/kg/day) and one-year (0, 10, 30 and 100 mg/kg bodyweight). The safety studies showed that tepoxalin was well tolerated. Adverse reactions occurred at doses equal to or higher than the suggested loading dose, but only after long term treatment. The *no observed adverse effect level* (NOAEL) was 300 mg/kg bodyweight in the 4 week study, 100 mg/kg bodyweight in the 13 week study and 20 mg/kg bodyweight and 30 mg/kg bodyweight, respectively, in the 26 week and 52 week studies. Thus, the length of the treatment period appeared to influence NOAEL.

The safety studies were not performed with the final oral lyophilisate formulation and absorption of tepoxalin was far from complete. Dogs treated with the formulation used in the safety studies at the NOAEL dose levels were exposed to much higher plasma concentrations than dogs given a single clinical dose. Therefore, the safety of doses higher than the clinical dose can be considered sufficiently documented.

Skin lesions, such as alopecia and erythema, occurred at slightly higher incidences in treated dogs in the toxicity studies. Alopecia was also reported in the clinical trials. Skin rash has also been reported as adverse events in humans administered tepoxalin. Accordingly the SPC includes an appropriate warning under point 5.3 (Undesirable effects).

The adverse reactions seen in the clinical studies were mainly associated with known effects of NSAIDs on the gastrointestinal tract and included vomiting, hypersalivation and diarrhoea. The overall frequency of adverse effects was similar for tepoxalin treatment and treatment with other NSAIDS such as carprofen or meloxicam.

As tepoxalin was shown to be rapidly metabolised and excreted in the faeces (99%), it was considered that dogs with mildly deficient renal function could be treated. However, a warning against the use of Zubrin in dogs with markedly decreased renal function should be included in the SPC as the functioning of the kidney is very dependent on adequate cyclo-oxygenase activity.

The safe use of Zubrin in older dogs has been demonstrated in a study involving 107 dogs with a mean age of 11 years (1 – 17 years) and a treatment period of 28 days.

III.A.3.5 Effects on reproduction

Effects on reproductive function have been studied in rats (fertility, embryofoetal development and peri-post natal development) and rabbits (embryo-foetal development). In all studies, clear signs of toxicity (liver and gastrointestinal effects) were observed in the high dose groups of both sexes and in both species (50 mg/kg bodyweight/d in rats and 30 mg/kg bodyweight/d in rabbits, respectively). There were no effects on male fertility parameters. However, reproductive organs were not examined in either males or females. Expected effects of a prostaglandin inhibitor on early pregnancy (e.g. increased early resorption, increased pre-, and post implantation losses; reduced implantation sites, reduced viable foetuses) were observed in both species. Administration during the organogenetic period resulted in embryo-foetal toxicity (e.g. reduced foetal weights, incomplete ossification of various bones or other skeletal variations). In one rat study (general reproductive function) and in the rabbit study, there were a few foetuses with microphthalmia / aphthalmia in treated groups. No other signs of malformations were observed. In the report of the rabbit study, a relationship to treatment for the observed microphthalmia could not be excluded.

There are no reproductive toxicity studies in dogs, and the relevance for the dog of the reproductive toxicity data obtained in rat and rabbit, is not known. However, it can be concluded that tepoxalin appeared to have a reproductive toxicity profile similar to other NSAIDs.

Since safety is not documented in breeding animals or in pregnant or lactating females, the use in pregnant and lactating dogs and in bitches intended for breeding is contra-indicated.

III.A.3.7 Mutagenicity

The genotoxicity of tepoxalin has been studied in a number of test systems addressing mutagenic potential in bacteria and mammalian cells, and clastogenic effects in various mammalian *in vitro* cell system as well as *in vivo*. It can be concluded that tepoxalin lacked mutagenic potential, both in the absence and presence of rat S9 (negative in Ames' test and in the mutation assay using CHO cells, HGPRT-locus).

However, tepoxalin (+/-S9 from rat) showed a potential to induce chromosomal aberrations *in vitro* in CHO cells. In contrast, data from an assay in peripheral human lymphocytes did not indicate a clastogenic effect. The mouse lymphoma assay is considered to be inconclusive due to uncertainties and shortcomings of the study. The *in vivo* micronucleus test in mice (200 mg/kg, orally) was negative. Available plasma concentration data in mice (3-month toxicity study) show approximately the expected clinical exposure to tepoxalin, but considerably higher than clinical exposure to the metabolite at an oral dose of 40 mg/kg. Since saturation of absorption of tepoxalin has been shown at higher doses in other animal species, it is not possible to estimate plasma concentrations at a dose of 200 mg/kg based on extrapolations. However, the plasma concentrations *in vivo* were certainly lower than the concentrations inducing chromosomal aberrations *in vitro* (25 - 34 μg/ml).

The investigation of genotoxicity showed no strong evidence for mutagenic potential of tepoxalin, however an induction of chromosomal aberrations *in vitro* in CHO cells was noted. To make a full evaluation of the possible genotoxicity, systemic exposure data for the *in vivo* micronucleus test in mice and comparative data on the metabolism in mice, rats and dogs would be necessary.

No additional information has been provided to further elucidate the findings in the *in vitro* clastogenicity studies. In CHO cells, tepoxalin caused increased aberration frequencies primarily in

the absence of metabolic activation at only slightly cytotoxic concentrations. In contrast, no clastogenic potential was identified in human lymphocytes exposed to clearly cytotoxic concentrations. No clastogenic effects were seen *in vivo* in the micronucleus test in mice, dosed once daily for 3 days with doses resulting in toxicity. Metabolic characterisation data confirmed that the mouse is a relevant test species. However, due to rapid metabolism, the exposure of bone marrow to the parent compound was possibly not sufficiently high.

In summary, tepoxalin was clastogenic in one *in vitro* test system but lacked effects in another *in vitro* test, thus not appearing to be a potent clastogen. This is further supported by the *in vivo* data. Further genotoxicity studies would most likely not add any information to the battery of tests already performed. The Committee therefore agreed that no specific warning was warranted in the SPC.

III.A.3.8 Carcinogenicity

No carcinogenicity studies were submitted. A two-year oral carcinogenicity study in rats was stopped in week 56 - 58 due to project termination.

III.A.4 Studies on other effects

Hepatotoxicity

In a 3-month exploratory study in rats, tepoxalin (20 and 40 mg/kg oral gavage) induced reversible higher liver weights and microscopic centrilobular hepatocellular hypertrophy. No effects were observed at 5 mg/kg. Peroxisomal enzyme activity and total cytochrome P-450 content were not affected. Similar results were reported from a 1-year rat carcinogenicity study (tepoxalin 30 mg/kg) that was stopped in week 56 - 58 due to project termination.

III.A.4.1 Local tolerance studies

Single (4.6 ml/kg, 2% solution, 80 mg/kg) or 4-week dermal administration (2, 10, 20 mg/kg/day) of tepoxalin produced mild erythema in rabbits. A similar effect was observed after application of the vehicle (propylene glycol 30% in absolute alcohol).

Tepoxalin (1 ml, 2% solution) or vehicle administered intraocularly in rabbits produced moderate ocular irritation as conjunctival redness, chemosis, discharge and corneal opacities.

In a contact sensitisation study in guinea pigs, tepoxalin (0.5 ml, 2% solution) did not induce any sensitisation reactions. The positive control, dinitrochlorobenzene, induced the expected response.

III.A.4.2 Immunotoxicity

N/a

III.A.4.3 Observation in humans

In studies in humans, single doses of 5 mg up to 800 mg or repeated doses of up to 500 mg twice daily for one month have been administered. Adverse events such as abnormal liver function tests, rash, diarrhoea and abdominal pain were reported. A potentially serious adverse event (renal calculus) and three withdrawals (due to rash and, pharyngitis and vaginal irritation) were reported following 14 – 33 days of treatment with tepoxalin (600 - 1000 mg/day). However, no serious toxicity would be expected following accidental ingestion of Zubrin oral lyophilisate in humans.

The Applicant provided an evaluation of the risk involved in case of accidental ingestion assuming a worst case scenario that a 10 kg child ingested the content of 1 blister i.e. 8 tablets (which was the number of tablets per blister at the time of the original authorisation), which would correspond to 160 mg/kg. This concentration was considered as being below the no toxic effect level of 300 mg/kg (2 x 150 mg/kg, given 5 - 6 hours apart). Data in dogs showed that absorption is saturated at doses above

200 mg/kg. Furthermore, data in humans indicated that absorption increases less than dose-dependently. The Committee agreed to add the following wording in the SPC and product information: '*In case of ingestion of a number of oral lyophilisates, the advice of a doctor should be sought immediately'*.

III.A.5 Ecotoxicity

A phase I environmental risk assessment was submitted. Given the use of tepoxalin in companion animals and its indication as an anti-inflammatory agent, it was concluded that this product does not require a Phase II assessment.

IV. PRECLINICAL AND CLINICAL DOCUMENTATION

IV.1 Pre-Clinical Documentation

IV.1.A.1 Pharmacodynamics

See III A 2.1

IV.1.A.2 Pharmacokinetics

See Safety file, III A 2.2

IV.1.B Target species tolerance (field study)

See also - Safety file, III A 3.4
A field study was conducted in Germany and France in 107 dogs of 34 different breeds. The median age was 11 years and the median weight was 31 kg. The most common diagnoses were osteoarthritis, spondylosis and hip dysplasia. Clinical symptoms were discovered at least one month prior to enrolment and the chronical nature of the disease was in most cases verified by X-ray investigation. The dogs were given an initial loading dose of 20 mg/kg and were thereafter given 10 mg/kg daily for 27 days. Clinical investigation was performed prior to treatment and on days 13 and 27. The scored parameters were: ease of ambulation/locomotion, weight bearing, pain and resistance to forced movement, general attitude and demeanour. Blood was collected for complete haematological and clinical chemistry examination on days 0, 13 and 27. There was no trend between pre- and post treatment for any of the parameters.

All clinical parameters improved significantly from day 0 to both day 13 and day 27. The overall assessment of the investigators showed that about 86% of the dogs were improved on day 13 and 87% on day 27. Ninety-seven of the 107 dogs completed the 28 day course of therapy. Of the 10 dogs that discontinued therapy, seven were related to adverse reactions and 3 were due to lack of owner's compliance. Based on the results from this study, it was concluded that tepoxalin was consistently safe in old dogs.

The most common adverse reaction was gastrointestinal upset. Eight dogs were reported as having shown both diarrhoea and vomiting. Blood did not occur in faeces or vomitus except for one case. Torsion of the spleen was diagnosed in this 8 year old dog and the dog underwent surgery but died of peritonitis caused by perforation of the duodenum. It was not clear if the splenic torsion initiated the dog's vomiting, or the vomiting initiated the splenic torsion. One 12 year old dog showed diarrhoea from day 3 to day 12. The dog was removed from the trial because of diarrhoea and was treated with dexamethasone.

In 2006, the indications and the duration of treatment with Zubrin were extended in order to allow the treatment of chronic musculoskeletal disorders. In order to support this, a new 90-day target animal safety study and a new multicentre European 56-day efficacy study were provided. Apart from gastrointestinal reactions (mainly vomiting, diarrhoea), no treatment related side effects were observed

in the treated animals. The incidence of gastrointestinal adverse reactions was similar in both groups (Zubrin and a positive control). Warnings concerning such adverse reactions are already included in the product literature. In addition, the product literature includes a warning to re-evaluate the clinical response in the dog for the need for continuation of treatment. The CVMP, therefore, concluded that no further warnings were necessary to be made in the product literature.

IV. 2 Clinical documentation

IV.2.1 Dose and duration of treatment

Dose selection

The treatment dose (10 mg tepoxalin per kg bodyweight) was not based on dose titration studies but on pharmacodynamic studies in dogs (complete inhibition of the PGF_{2a} synthesis in canine blood and significant drop in whole blood LTB_4 activity) and data derived from the use of tepoxalin in human medicine (3 - 7 mg/kg bodyweight). As the pharmacokinetics of tepoxalin is similar in humans and dogs, a similar dose was assumed for humans and for dogs.

Furthermore, preliminary clinical studies showed that the dose of 10 mg tepoxalin per kg bodyweight was as effective and safe as carprofen and no adverse reactions in dogs treated for up to 1 year had been observed.

Loading dose studies:

A loading dose of 20 mg/kg was originally suggested by the Applicant to ensure a rapid onset of the effect. Since this dose was not supported by appropriate data, on request of the Committee, further studies were provided. However, a loading dose was not supported by the results presented in the new studies. In the absence of further data on the pharmacokinetic profile, bioavailability, further clinical data and because of the apparent great inter-individual ability of dogs to absorb and metabolise tepoxalin, the Committee did not support a loading dose.

Dose confirmation study

Furthermore, a blinded field study was performed to support efficacy of the dose of 10 mg/kg. Eighty-six dogs of 31 different breeds, a median age of 7 years and median weight of 32 kg were included in a study which conducted in the USA. The most common diagnoses were osteoarthritis, hip dysplasia, spondylosis and intervertebral disc syndrome. Forty-five cases were considered to suffer from acute disease and 41 from an exacerbation of a chronic condition. The dogs were randomly allocated to three treatment groups and treated with 5.0, 7.5 or 10 mg/ kg bodyweight for 7 days.

The following parameters were scored before and after treatment: ease of ambulation/locomotion weight bearing, pain and resistance to palpation, pain and resistance to forced movement, general attitude and demeanour. The owner and the investigator made an overall evaluation.

Each parameter improved significantly between day 0 and day 6 in all groups. The dose of 10 mg tepoxalin per kg bodyweight resulted in statistically significant improvement in ambulation/locomotion and weight bearing when compared with the other dose groups. The results for the other parameters were numerically, but not statistically better in the highest dose group. The results of the owners' and the investigors' overall evaluation was that 96% of the dogs treated with 10 tepoxalin per kg were improved or vastly improved. The corresponding figures for the other dose groups were 71% and 76%.

Four cases of adverse reactions were observed: all cases occurred in the dose group 7.5 mg/kg. Diarrhoea occurred in 3 dogs of which one also showed abdominal discomfort and one dog appeared depressed and lethargic and showed a stiff gait. The therapy was discontinued and the clinical signs disappeared in each case.

The strengths applied should allow for an accurate dosage for dogs with a weight of 3 kg or more. Dogs below 6 months of age should not be treated as most of the dogs included in the clinical trials were older and the dogs used in the long-term safety studies were 7 - 8 months at start of treatment. An appropriate warning is included in the SPC and product information.

Since efficacy was only documented during 7 days treatment, the indication has been limited to treatment of acute cases (Reduction of inflammation and relief of pain caused by acute musculoskeletal disorders or acute exacerbation of chronic musculoskeletal disorders).

Field studies

Acute musculoskeletal disorders

Beside dose confirmation and target animal safety studies, efficacy of tepoxalin in dogs with inflammatory and painful musculo-skeletal disorders was demonstrated in four clinical studies of sufficient size performed in USA (3 studies) and Germany and Italy (1 study) comprising 639 dogs (357 dogs treated with tepoxalin). Three of the studies were controlled and followed a similar protocol and two of these studies used the final oral lyophilisate formulation. In two other studies, the final formulation was not used but micronized tepoxalin in gelatin capsules. Reference drugs were carprofen (2 studies) and meloxicam (1 study).

The studies followed a similar protocol. The inclusion criteria were broad, any dog showing pain and inflammation of musculoskeletal origin was eligible for inclusion. The major diagnoses in all studies were osteoarthritis, hip dysplasia, intervertebral disc syndrome and spondylosis. A wash out period of 5 days was considered sufficient for dogs treated earlier with anti-inflammatory drugs and 4 weeks for dogs treated with long-acting corticosteroid formulations. Pregnant females and dogs intended for breeding were not included. The dogs in the control groups were treated with carprofen or in another study with meloxicam. The first treatment was given at the veterinary clinic and was continued by the owner on the following 6 days. The dogs were examined on day 0 and day 6. The clinical examiner was blinded to treatment groups.

The clinical response was evaluated before and after 7 days of treatment, using a scoring system with a number of relevant parameters. The results showed significant improvement compared to pre-treatment scores for both test and reference drugs with no consistent differences between tepoxalin and reference drug. The combined overall improvement rate was 83 – 93%. Some dogs were excluded from the evaluation of efficacy due to over-dosage (>50%) or under-dosage (<75%) for a lack of availability of all strengths in the clinical studies. This may be considered as a deviation from full compliance with Good Clinical Practice.

Blood samples for haematological and clinical chemistry analyses were collected before and after treatment. No significant changes occurred in any of the parameters on a group basis, but a reduced haematocrit was observed in single dogs showing adverse reactions in the form of haemorrhagic diarrhoea.

The results were similar in all studies. After one week of tepoxalin treatment, all clinical parameters were significantly improved when compared to pretreatment. The improvements were statistically significant within each group ($p<0.001$). Only single parameters differed significantly between the groups. The combined ¨improved¨ and ¨vastly improved¨ overall evaluations represent 79% to 92% of the cases when scored by the owners and 83% to 93% when scored by the investigators.

It was concluded that the clinical efficacy of tepoxalin was equal to that of carprofen and meloxicam.

Three adverse reactions out of fifty-one dogs occurred during tepoxalin treatment. Hypersalivation for 24 hours was observed in one dog (out of fifty-one dogs), another dog showed increased appetite, and lethargy occurred in one dog (out of fifty-one) on days 2 and 3. Other side-effects were transient vomiting (4 dogs out of 74), mild gastrointestinal adverse reactions (6 dogs out of 87 dogs), diarrhoea/vomiting (10 dogs out of 100) and haemorrhagic diarrhoea (two dogs out of 100). Cessation

of therapy was not necessary. Other reactions were not considered to be treatment related. Appropriate information on these effects is given in the SPC and package insert.

Long term treatment

In April 2001, the Marketing Authorisation Holder submitted a type II variation for Zubrin to expand the approved 7 day indication to long-term use in the treatment of chronic osteoarthritis. The applicant in support of this variation submitted no new studies or other data, but provided an updated expert report that re-examines existing studies in support of the variation. The Committee concluded that the efficacy data from these studies were not sufficient to support the proposed indication of a life-long treatment of chronic musculoskeletal disorders. In order to support such claim, a new complete efficacy study would need to be provided. Therefore, the Committee agreed at that time that sufficient data were only provided to extend the maximum duration of treatment to 28 days. The following text was added to section 5.7 of the SPC: "The duration of treatment is dependent on clinical response. At weekly intervals, the patient should be reassessed to determine if further therapy is indicated. Duration of treatment should not exceed 4 consecutive weeks." Furthermore, an additional warning was added under section 5.3 (Undesirable effects) to discontinue treatment immediately, if side-effects occurr.

In 2006, the Marketing Authorisation Holder submitted a new European multicentre efficacy study over 56 days investigating the treatment of chronic musculoskeletal disorders. The efficacy of treatment with Zubrin was compared with that of meloxicam in dogs with clinical signs of pain and inflammation associated with chronic osteoarticular disease including e.g. hip dysplasia, osteoarthritis, intervertebral disc syndrome and spondylosis. The diagnoses were based on clinical signs, physical examination findings and diagnostic imaging evidence. Dogs with fractures and post-surgery cases were excluded. The first treatment was given at the clinic; further medication was administered by the animal owner. All dogs were examined clinically on treatment days 14, 28 and 56.

The improvement rates at days 14, 28 and 56 in the Zubrin group were 88%, 85% and 85%, respectively. There were no clinically significant changes of the haematology and biochemistry parameters during the treatment period. Overall, the improvement rates with Zubrin and the positive control were considered similar or slightly better for Zubrin. Apart from gastrointestinal reactions (mainly vomiting, diarrhoe), no treatment related side effects were observed in the treated animals.

Two deaths occurred in the Zubrin group on treatment days 56 and 29 caused by gastric dilatation-volvulus. None of these dogs had shown intolerance to treatment and these cases were not considered to be treatment-related, as pharmacovigilance data and earlier safety studies showed no relation between tepoxalin and this condition.

Based on the new data submitted (90-day target animal safety study and 56-day multicentre efficacy study), the Committee concluded to extend the indications to "Reduction of inflammation and relief of pain caused by acute and chronic musculoskeletal disorders".

The Committee also agreed to modify the warning in section 4.9 of the SPC (Amounts to be administered) recommending a re-examination of the dog after 7-10 days (rather than weekly) and deleting the previous restriction of a maximum treatment duration of 4 weeks.

V. RISK – BENEFIT ASSESSMENT AND CONCLUSIONS

Zubrin is an orally active non-steroidal anti-inflammatory drug with inhibitory effects on cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism. The posology is 10 mg/kg once daily for 7 days.

Tepoxalin is a white crystalline powder, which is practically insoluble in water. The synthesis of tepoxalin is a 3-stage process starting with a reaction between chloroacetophenone and succinic anhydride. The active substance was originally synthesised by Johnson & Johnson (J&J) and then the manufacturing process was transferred directly to Schering-Plough Avondale (SPA). The finished product is

manufactured at DDS Scherer, United Kingdom. The manufacturer in charge of batch release isSP Bray, Ireland. The process for the manufacturing of the finished product follows conventional pharmaceutical practices, which utilise a solution compounding step, filling into pre-formed blister pockets using a dosing system followed by freezing, lyophilisation and sealing. The Applicant proposed originally a shelf-life of 24 months, which was not accepted at the time of authorisation and a shelf-life of 18 months was considered acceptable. However, in a subsequent variation, the Applicant submitted further data allowing the extension of the shelf life to 2 years.

Although there are still some deficiencies in the documentation on pharmaceutical quality, the applicant has made several commitments to address outstanding quality issues after additional manufacturing experience has been gained.

Pharmacokinetic data showed strong inter-individual variations of tepoxalin and its metabolites in dogs, and inter-species differences in the elimination pathway. While tepoxalin in the dog is mainly (99%) excreted via faeces, in the mouse about 30% of the dose is eliminated via urine. It was therefore considered that dogs with mildly deficient renal function could be treated with tepoxalin. However, the Committee agreed to include a warning in the SPC and package insert regarding the use in dogs with markedly decreased renal function as the functioning of the kidney is very dependent on adequate cyclooxygenase activity.

Although the safe use of tepoxalin has been sufficiently demonstrated in older dogs, dogs below 6 months of age should not be treated since studies in dogs of this age group have not been provided. Adverse reactions seen in the clinical studies were mainly associated with known effects of NSAIDs on the gastrointestinal tract and have been included in the relevant sections of the SPC and product information. Reproductive toxicity was investigated in rats and rabbits but not in the target species. Hence, Zubrin oral lyophilisate is not recommended during pregnancy and lactation or in bitches intended for breeding.

In studies in humans, no serious toxicity would be expected following accidental ingestion of Zubrin oral lyophilisate in adults. Furthermore, the risk of accidental ingestion of several oral lyophilisates by a child (worst case scenario) has been sufficiently evaluated. An warning has been added to the SPC and package insert. Since the formulation might become very sticky upon wetting, an appropriate user warning is mentioned in the SPC and package insert.

The investigation of genotoxicity showed that tepoxalin was clastogenic in one *in vitro* test system but lacked effects in another *in vitro* test, thus not appearing to be a potent clastogen. This is further supported by the *in vivo* data. Requirement of further genotoxicity studies was considered not to add any information to the battery of tests already performed. It was concluded that no specific warning was warranted in the SPC.

The efficacy of Zubrin oral lyophilisate in dogs with inflammatory and painful musculo-skeletal disorders was investigated in four clinical studies of sufficient size performed in USA (3 studies) and Germany and Italy (1 study) comprising 639 dogs of which 357 dogs were treated with Zubrin. Reference drugs were carprofen (2 studies) and meloxicam (1 study). The results showed significant improvement compared to pre-treatment scores for both test and reference drugs with no consistent differences between tepoxalin and reference drug. Results were also provided from a blinded field study involving 86 dogs treated with doses of 5.0, 7.5 or 10 mg/kg of tepoxalin for 7 days. It showed superiority for the dose of 10 mg/kg, in which group 96% of the dogs improved significantly. The corresponding values for 5.0 mg/kg and 7.5 mg/kg were 71% and 76%, respectively.

Based on the original and complementary data presented the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC.

In 2006, the indication and duration of treatment with Zubrin were extended to allow the treament of chronic musculoskeletal disorders. This was based on the results of two new studies, a 90-day target animal safety study and 56-day multicentre efficacy study.

Following the withdrawal of the 30 mg presentation in 2005, the minimum weight of animals to be treated was increased from 3 kg to 5 kg bodyweight.