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SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Tildipirosin (bovine, caprine and porcine species)

On 3 March 2014 the European Commission adopted a Regulation[1] establishing maximum residue limits for tildipirosin in bovine, caprine and porcine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Tildipirosin is used for the prevention and treatment of bacterial respiratory disease and is administered by subcutaneous or intramuscular injection.

Provisional maximum residue limits had previously been established[2] for tildipirosin in bovine, caprine and porcine species with an expiry date of 1 January 2012.

Intervet International BV submitted its responses to the list of questions further to the establishment of provisional MRLs to the European Medicines Agency on 15 June 2011.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended, on 15 September 2011, the establishment of maximum residue limits for tildipirosin in bovine, caprine and porcine species.

On 25 October 2011 the European Commission requested the Committee to reconsider its opinion of 15 September 2011 and in particular to reconsider the text relating to the injection site, recommended for inclusion in the “Other provisions” of Table I of the Annex to Commission Regulation (EU) 37/2010.

On 8 December 2011 the Committee adopted a revised opinion recommending the establishment of maximum residue limits for tildipirosin in bovine, caprine and porcine species, maintaining the previous recommendation.

On 5 October 2012 the European Commission requested the Committee to reconsider its opinion of 8 December 2011, in particular focusing on the provisions relating to the injection site and the feasibility of residue controls.

On 18 July 2013 the Committee for Medicinal Products for Veterinary Use adopted a revised opinion recommending the establishment of maximum residue limits for tildipirosin in bovine, caprine and porcine species.

[1] Commission Implementing Regulation (EU) No 201, O.J. L62, of 04.03.2014

[2] Commission Regulation (EU) No 759, O.J. L233, of 25.08.2010

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Subsequently the Commission recommended on 14 January 2014 that maximum residue limits in bovine, caprine and porcine species are established. This recommendation was confirmed on 4 February 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 3 March 2014.

Summary of the scientific discussion for the establishment of MRLs

Substance name:	Tildipirosin
Therapeutic class:	Anti-infectious agents/Antibiotics/Macrolides
Procedure number:	EU/09/166/INT
Applicant:	Intervet International BV
Target species:	Bovine and porcine species
Intended therapeutic indication:	Prevention and treatment of bacterial respiratory disease in cattle and pigs
Route (s) of administration:	Bovine: subcutaneous Pigs: intramuscular

1. Introduction

Tildipirosin (20,23-di-piperidinyl-mycaminosyl-tylonolide, tildipirosin, Cas No 328898-40-4) is a semisynthetic derivative of the naturally occurring 16-membered macrolide tylosin. Tildipirosin is intended for parenteral treatment and prevention of respiratory disease in cattle and pigs. Tildipirosin is administered as a single-dose injection: subcutaneously in cattle and intramuscularly in pigs. The anticipated optimal clinical dose is 4 mg/kg bw.

Tildipirosin is not used in human medicine.

Tildipirosin was included in Commission Regulation (EU) No 37/2010[3] as indicated in the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Tildipirosin	Tildipirosin	Bovine Caprine	400 µg/kg 200 µg/kg 2000 µg/kg 3000 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption. The MRL for muscle does not apply to the injection site, where residue levels should not exceed 11500 µg/kg. Provisional MRLs expire on 1 January 2012.	Macrolide
		Porcine	1200 µg/kg 800 µg/kg 5000 µg/kg 10000 µg/kg	Muscle Skin+fat Liver Kidney	The MRL for muscle does not apply to the injection site, where residue levels should not exceed 7500 µg/kg. Provisional MRLs expire on 1 January 2012.	

Further to the establishment of provisional MRLs in August 2010, the applicant submitted on 15 June 2011 additional data on the validation of the analytical method proposed for the monitoring of residues in cattle and pig tissues and to demonstrate that the analytical method is applicable to goat tissues, in order to allow the establishment of final maximum residue limits.

Based on its evaluation of the data submitted by the company the Committee adopted an opinion on 15 September 2011, recommending the establishment of final maximum residue limits for tildipirosin in bovine, caprine and porcine species, at the same level and with the same other provisions as established following its opinion recommending provisional MRLs.

On 25 October 2011 the European Commission requested the Committee to reconsider its opinion of 15 September 2011 and to amend the part of the opinion that includes a residue limit for the injection site in the “other provisions” of Table I of the Annex to Commission Regulation (EU) 37/2010.

On 8 December 2011, having further reflected on the issues, the Committee concluded that the request to reconsider its opinion did not provide sufficient scientific or safety grounds to justify a change to its previous recommendation, and so adopted an opinion reconfirming its recommendation of 15 September 2011.

[3] OJ No L 15 of 20.01.2010

On 5 October 2012 the European Commission requested the Committee to reconsider its opinion of 8 December 2011, in particular focusing on the provisions relating to the injection site and the feasibility of residue controls.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

As for other macrolides, the antimicrobial activity of tildipirosin is due to its binding to the ribosomal 50S subunit of bacterial cells thereby inhibiting bacterial protein synthesis. The *in vitro* antimicrobial activity against Gram-negative and Gram-positive pathogens indicates that tildipirosin is effective against a range of bacterial pathogens frequently associated with bovine and swine respiratory disease. Comparison of minimum inhibitory versus bactericidal concentrations shows that generally the antimicrobial action of tildipirosin is bacteriostatic.

The binding of tildipirosin to proteins is limited with about 17% for mouse plasma and 30% for pigs and cattle plasma, and calf bronchial fluid. Similar to other macrolides, tildipirosin exhibits highest *in vitro* activity at basic pH, whereas acidic pH results in a marked attenuation of activity.

Considering the structural similarities between tildipirosin and tilmicosin (both are 16-membered macrolides) cardiotoxic potential was investigated. Studies in dogs were performed to evaluate possible effects of tildipirosin on blood pressure, heart rate, and electrocardiogram (ECG) lead II. Tilmicosin was used as the comparator compound. The lowest effective dose of tildipirosin was 20 mg/kg bw administered once by intramuscular injection, which induced a slight but significant decrease in pulse pressure. Tilmicosin was shown to induce a similar but more pronounced pulse pressure response as well as increases in heart rate and diastolic blood pressure and shortening of ECG lead II PR and QT intervals. In this study comprising four dogs only, the NOEL for cardiovascular effects of tildipirosin after single intramuscular administration was 10 mg/kg bw. If it is assumed that tildipirosin and tilmicosin share the same secondary pharmacological and hence electrophysiological properties, it can be postulated that tildipirosin would exert cardiovascular effects at higher doses which were not investigated in this study.

In a maximum tolerated oral dose study in dogs, no changes in the ECG were noted following single (300 mg/kg bw) or repeated (150 or 200 mg/kg bw daily for two days and 200 mg/kg bw daily for seven days) administration of tildipirosin. Electrocardiograms were recorded before and 30 minutes after treatment. However, significant changes in ECG were observed in a 4-week oral toxicity study at the highest dose of 180 mg/kg bw/day after one and four weeks (changes included QT prolongation, shortening of PQ interval, and increases in heart rate). As blood pressure was not measured no conclusions could be drawn on pulse pressure in this high dose range. Increases in heart rate and ECG changes (QT prolongation, shortening of PQ interval) were also observed in dogs receiving 60 mg/kg bw/day after nine and thirteen weeks in a 13-week oral toxicity study. No cardiovascular effects were observed following repeated doses of 6 and 20 mg/kg bw.

In addition, tildipirosin at doses of 10 and 20 mg/kg bw was shown to induce local swelling comparable to tilmicosin (this was not considered relevant for the determination of an ADI).

In the intramuscular studies the NOEL for cardiovascular effects was 10 mg/kg bw.

Pharmacokinetic properties (mainly in laboratory animals)

Absorption of tildipirosin was rapid after oral administration to Wistar rats and Beagle dogs. Blood plasma concentrations of tildipirosin declined with elimination half-lives of 6.6 to 17.3 hours. Tildipirosin plasma concentrations did not differ significantly between genders. The oral bioavailability of tildipirosin was substantially higher in dogs than in rats. No potential for accumulation of tildipirosin was evident by comparison of single and repeated once daily administration.

Tissue distribution, metabolism and elimination were studied in rats and dogs. Rats were orally treated for seven consecutive days at two doses (25 µg/kg bw/day and 25 mg/kg bw/day). The rats were slaughtered at 6 hours after last administration and samples of liver, kidney, muscle and fat from the high dose group and the colon from each animal were taken. Urine and faeces were collected daily, separately for each animal. In addition, rats were orally treated at a single dose of 400 mg ^{14}C-tildipriosin/kg bw and slaughtered 3 hours after administration. Beagle dogs were orally treated for seven consecutive days with 25 mg ^{14}C-tildipirosin/kg bw/day and slaughtered 6 hours after the last administration. Tissue samples (liver, kidney, muscle and fat) were collected. Urine and faeces from dogs were collected daily.

Tissue and excreta samples (urine, faeces) from all studies were analysed for total radioactivity. No information was available on biliary excretion. Selected tissue and excreta samples were extracted and the extracts analysed via radio-HPLC to determine the metabolite profiles in each species. The metabolites were identified by HPLC-MS/MS with concurrent radio-detection. Tildipirosin was determined in all samples by co-chromatography.

Rats: The major route of excretion in rats was via faeces. Approximately 92% of the administered dose for male and female rats, for the low dose group and 85% and 83% of the administered dose for male and female rats, respectively, for the high dose group were recovered in faeces. A component that co-chromatographed with tildipirosin was detected in faeces in both the low and high dose group accounted for maximum to approximately 3% of the administered dose. The predominant metabolite in rat excreta was the sulphate conjugate of reduced tildipirosin (M7). M7 accounted, in rat faeces, for 61% and 35% of the administered dose in males and females, respectively, in the low dose group and 42% and 44% in males and females, respectively, in the high dose group. In urine and colon contents, the sulphate conjugate of reduced and hydrated (or ring open) tildipirosin (M4) was identified. In colon contents M7 was detected as the major metabolite and accounted for a maximum, in the high dose group, of about 47% total radioactive residues (TRR). In rat tissue samples (liver and kidney) unchanged tildipirosin was detected and M7 was determined in both tissues as the major metabolite. Two further minor metabolites (about 10% TRR) were detected and identified in kidney and liver tissue.

In the additional rat study at 400 mg ^{14}C-tildipirosin/kg bw the animals were sacrificed 3 hours after administration and liver and kidneys were removed for extraction and chromatographic analysis. Using mass spectrometry the metabolites desmethyl-tildipirosin, S-cysteine conjugate of reduced tildipirosin and dihydroxy-tildipirosin were detected in rat.

Dogs: The major route of excretion in dogs was via faeces. About 65% and 62% of the administered dose in male and female dogs, respectively, were recovered in faeces. Urinary excretion was relatively minor (7% and 11% of the administered dose in males and females, respectively). The major metabolite in excreta samples was identified as M7 and accounted, in faeces, for 27% and 34% of the administered dose in male and female dogs, respectively. Unchanged tildipirosin was also detected in both urine and faeces (6% in urine and 16% of the administered dose in faeces). Unchanged tildipirosin was the major component found in dog liver samples accounting for 50% and 57% TRR in males and females, respectively. Unchanged tildipirosin was also the major component found in dog kidney samples, accounting for 53% and 55% TRR in males and females, respectively. A further

metabolite assigned as desmethyl-tildipirosin was detected in significant concentrations in liver and kidney (about 10% TRR and 10% TRR, respectively).

In summary, after repeated oral administration of ^{14}C-tildipirosin to rats and dogs the highest radioactivity was detected in colon, followed by liver, kidney, brown fat and muscle. Excretion of radioactivity was mainly through faeces (up to 92% in rats and 65% in dogs). The excretion of tildipirosin in urine was very low. Data on biliary excretion were not available and the physiological origin of the major metabolites observed in faeces was therefore unclear.

It is postulated that in rats and dogs the metabolism of tildipirosin proceeds by cleavage of the mycaminose sugar moiety, by reduction and sulphate conjugation with subsequent hydration (or ring opening), by demethylation, by mono- or dihydroxylation (in rats with subsequent sulphate conjugation of the reduced and dihydroxylated metabolite, or in rats and dogs with subsequent dehydration) and by S-cysteine conjugation (in dogs also S-glutathione conjugation). In rats and dogs, unchanged tildipirosin was found in liver, kidney, faeces, and urine as a minor constituent only, whereas metabolites predominated.

Metabolites present in liver, kidney and urine of cattle and pigs were also detectable in rats and dogs. Therefore, although the fraction of various metabolites varied among species, the metabolite profiles of target and laboratory animals are qualitatively similar (see also Section 3.1. below).

2.1.2. Calculation of pharmacological ADI, if relevant

At dose levels lower than those required to elicit toxicity, there was no evidence from findings in oral repeated dose toxicity studies in rats and dogs that tildipirosin exerts secondary pharmacodynamic activity on organs of concern (renal, gastrointestinal, neurological, cardiovascular, or respiratory system). However, based on the structural similarities between tildipirosin and tilmicosin the cardiotoxic potential of tildipirosin was investigated in single dose studies in dogs using intramuscular administration. The NOEL for cardiovascular effects following intramuscular administration was seen to be 10 mg/kg bw. Based on these findings it can be concluded that tildipirosin does not exert secondary pharmacodynamic effects at doses that would require the establishment of a pharmacological ADI.

2.1.3. Overview of toxicology

Acute toxicity

The potential acute systemic effects of tildipirosin have been investigated in three single oral dose toxicity studies and one single intravenous dose toxicity study in rodents. In mice, no adverse effects were noted after oral administration of 1700 mg/kg bw. In rats, slightly to moderately ruffled fur, slight to moderate sedation, hunched posture, slightly poor coordination, eye-lids half-closed or completely closed and serious rhinorrhea were seen (2000 mg/kg bw orally). Adverse findings in rats were reversible. No macroscopic findings were detected at necropsy. After intravenous application in mice (6.25, 12.5, 25 and 50 mg/kg bw), deaths occurred at 12.5 mg/kg bw and higher immediately after treatment. These animals showed a severe loss of coordination, slight to severe convulsions, deep respiration, ventral recumbency, gasping and writhing. At necropsy, no macroscopic findings were noted. In conclusion, the results of the studies showed that tildipirosin can be considered to be associated with low levels of acute toxicity at oral doses of up to 2000 mg/kg bw, and to be associated with an intermediate level of acute toxicity in mice following intravenous administration ($6.25 < LD_{50} < 12.5$ mg/kg bw).

Repeated dose toxicity in rats

Oral gavage repeated dose toxicity studies were carried out in rats. In the 4 week rat study groups of 5 male and 5 female Spraque Dawley rats received tildipirosin (as 1.6-tartrate) by oral gavage at doses of 0, 40, 160 or 640 mg/kg bw per day. These doses correspond to 25, 100 and 400 mg/kg bw active substance (free base). At the highest dose calcium concentrations in females were reduced. At 100 and 400 mg/kg bw/day, the following effects were observed dose dependently: slight signs like stilted gait and/or squatting posture in females; slightly decreased total protein and globulin concentrations predominantly in females; increased absolute testes weight but no histopathological correlates. Neurotoxicological parameters, i.e. open field observations and assessment of sensory function, as well as forelimb and hindlimb grip strength, remained unaffected by the administration of the test item in all dose groups. Motor activity was increased in treated female rats at all dose groups compared to study control group. However, this finding is an incidental observation without biological relevance since no clear dose-dependency could be observed and dose group values differ only marginally from normal historical range. A NOEL of 25 mg/kg bw/day was established.

In the 3-month rat study (20, 60 or 400 mg/kg bw/day, 10 animals per sex and dose level) changes were noted at the two higher dose levels. At 400 mg/kg bw/day food consumption and body weight gain were markedly depressed, white blood cell population was slightly to markedly increased as well as liver enzyme concentrations and urine volume. Organ weights for heart, liver, kidney, spleen, thyroid and adrenal gland were increased, while organ weight for thymus was decreased. In 7 of 10 animals reddish/dark red foci in fundus or forestomach were observed. Histopathology revealed mainly vascular and epithelial vacuolation and hyperplasia in a large number of organs and tissues as well as depletion/atrophy of lymphoid system organs (spleen and thymus). At the mid dose of 60 mg/kg bw/day exophthalmus and increased salivation was observed. Additionally, in one male and one female, vacuolation and hyperplasia in the pharyngeal epithelium was noted. At 20 mg/kg bw/day no toxic signs were observed and this dose was defined as the NOEL.

Repeated dose toxicity in dogs

In a maximum tolerated dose study, tildipirosin was administered by gelatin capsules to dogs. In phase 1 (1 male and 1 female), descending doses of 300, 200 and 150 mg/kg bw/day for up to 2 days per dose resulted in slightly reduced food consumption and body weight in the female. The single dose of 300 mg/kg bw/day caused marked clinical signs in the female, mainly indicative of an effect on the central nervous system (CNS). Doses of 200 mg/kg bw/day resulted in similar, but less severe signs in the male and female on the second day of treatment. At 150 mg/kg bw/day, salivation and vomiting were noted in the female on the second day of treatment. Most signs generally appeared about 1-2 hours after treatment and lasted for up to 4 hours. In phase 2 (2 males and 2 females), treatment at 200 mg/kg bw/day for 7 consecutive days resulted in similar CNS effects throughout the treatment period, as well as in reduced food consumption and body weight in both sexes. There were no changes recorded in the ECG or at necropsy. It is concluded that the maximum tolerated dose in dogs was 200 mg/kg bw/day.

In a 4-week toxicity study, tildipirosin was administered to dogs at doses of 0, 20, 60 or 180 mg/kg bw/day (4 dogs per sex and dose) for 28 days. At 20 mg/kg bw/day tremor and arteritis/periarteritis in the heart were observed, each in one animal. At 60 mg/kg bw/day, the tildipirosin-related signs were limited to increased GLDH (glutamate dehydrogenase concentration) and minimal to slight degenerative changes of various organs. At 180 mg/kg bw/day, the following signs were observed: clinical signs indicative of an effect on the CNS and gastrointestinal tract; moderately reduced food consumption with body weight loss; non-pigmented tapetum lucidum in all dogs; in ECG markedly increased heart rate, increased QT interval, moderately decreased PQ interval and slightly decreased P-wave duration; increased red blood cell count, haemoglobin concentration and haematocrit; slightly

decreased platelet count; markedly increased ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) concentrations; increased GLDH, protein, albumin and calcium concentrations; reddish discoloured mesenteric lymph nodes; increased weight of kidney, liver, gall bladder, pituitary, thyroid and adrenal gland; in histopathology minimal to slight degenerative changes of various organs. A NOEL could not be established as effects were seen at all levels. The lowest dose of 20 mg/kg bw/day was considered to be a LOEL.

In a 13-week toxicity study, tildipirosin was administered by capsule to dogs at doses of 0, 6, 20 or 60 mg/kg bw/day (4 dogs per sex and dose) for 90 days. Treatment related effects observed at 20 mg/kg bw/day were increased GLDH and protein concentrations as well as arterial vacuolation in the tongue and smooth muscle vacuolation in the oesophagus. At 60 mg/kg bw/day the following were observed: occasionally decreased food consumption with intermittently reduced body weight gain in females from week 5 onwards; non-pigmented tapetum lucidum; in ECG increased heart rate and QT interval, slightly decreased PQ interval; slightly increased ALAT and GLDH concentrations; increased protein and albumin concentrations; increased weight of liver, gall bladder, pituitary and thyroid gland; in histopathology, vacuolation of tunica media in larger arteries, of smooth muscle fibres as well as of various other cells in several organs and tissues, hyaline droplets in renal tubular epithelia, increased haemopoietic activity in spleen, and colloid content in thyroid gland. Altered behaviour (restlessness) was noted at all doses with no clear dose dependency. At the low dose of 6 mg/kg bw/day altered behaviour was seen in all female dogs and in one male in the second half of the treatment period. Sporadic signs of recumbency, tremor, whimpering and panting were also noted. These effects were judged as not being treatment related because these behavioural findings were not found to be reproducible in long-term oral dog study. A NOEL of 6 mg/kg bw/day was established.

In a 55-week repeat-dose toxicity study in dogs, tildipirosin was administered by capsule to dogs at doses of 0, 4, 10 or 50 mg/kg bw/day (4 dogs per sex and dose) for 55 weeks. At 4 and 10 mg/kg bw/day, no tildipirosin-related signs were observed in either sex. At 50 mg/kg/day treatment resulted in degenerative changes in the smooth muscle fibres in many organs and tissues. The severity of smooth muscle vacuolation was comparable in all organs, including the nervous tissue. The changes were associated with increased liver enzyme activity (ALAT, GLDH), plasma protein concentrations, and weights of some organs (pituitary gland, thyroid gland, gallbladder, liver and spleen) as well as decreased food consumption and body weight gains. Morphologic signs of neurotoxicity such as single swollen neurons, swollen axonal cones, single acute neuronal deaths (shrunken neurons) as well as single fibre degeneration of the sciatic nerve in several males without any glial reaction were observed. Similar lesions were seen in the retina. Furthermore, there was vacuolation of epithelial cells in many organs, myocardial vacuolation as well as vacuolated cells in various other tissues and organs. Other findings consisted of thyroid gland with follicular cell hypertrophy and colloid mineralization, spleen with lymphoid atrophy and thymus with increased severity of atrophy, exocrine pancreas with minimal to moderate fatty change, kidney with increased incidence and severity of tubular basophilia as well as minimal increased incidence of pyelitis, and liver with minimal phagocytosis of eosinophilic material in Kupffer cells in all animals. A NOEL of 10 mg/kg bw/day was established.

Tolerance in target species

A single parenteral administration of tildipirosin solution for injection in the target animal species at 10 times (cattle, subcutaneously) and 5 times (pigs, intramuscularly) the maximum recommended clinical dose was tolerated without clear toxicological endpoints. Effects such as discomfort during and after injection, swellings at the injection site and increased creatine phosphokinase, white blood cell count, neutrophils and monocytes were attributed to the reactions at the site of administration.

Effects on reproduction including developmental effects

The potential toxic effects of tildipirosin on reproduction have been investigated in rats in one dose-range finding (0, 20, 60 and 400 mg/kg bw/day) and one pivotal two-generation study (0, 20, 80 and 320 mg/kg bw/day). The NOEL for parental toxicity was 20 mg/kg bw/day based on organ effects in the P and F1 generations at 80 mg/kg bw/day. Absolute weight and organ to body weight ratio of the left thyroid gland in males of P-generation was increased. Histopathology data showed vacuolation in the epididymis in the P and F1 generation males, in the thyroid gland of the P generation males and in the oviducts in both generations. The NOEL for reproductive effects was considered to be 80 mg/kg bw/day based on a decreased number of implantations in both generations associated with fewer live pups at 320 mg/kg bw/day. The NOEL for developmental toxicity was 80 mg/kg bw/day based on a slight delay in incisor eruption and changes in organ weights in F1/F2 pups at a dose of 320 mg/kg bw/day. No teratogenic potential was observed at any dose level.

The potential toxic effects of tildipirosin in pregnant females and on embryo-foetal development were investigated in a dose-range finding study in rabbits (0, 15, 30, 75 mg/kg bw/day) and a pivotal study in rabbits (0, 10, 30, 90 mg/kg bw/day) as well as in dose-range finding and pivotal studies in rats (0, 20, 60, 400 mg/kg bw/day and 0, 30, 120, 480 mg/kg bw/day, respectively). For rabbits, a further, non-GLP dose tolerability study in non-pregnant females (50, 100 mg/kg bw/day) was performed prior to the prenatal development toxicity studies. In both species, no effects on embryo-foetal development were observed below maternal toxic dose levels (reduced food consumption and body weight gain). Effects were mainly consistent between both species, but an increase of post-implantation loss was only noted in rats at 120 and 480 mg/kg bw/day. Rabbits showed a lower NOEL for foetal body weight as a result of reduced maternal food consumption and body weight gain compared with rats.

In conclusion, 30 mg/kg bw/day was established as a NOEL for maternal toxicity and for foetal toxicity. No teratogenic potential of tildipirosin was observed in any study at any dose level.

Mutagenicity

Tildipirosin was tested in a comprehensive series of mutagenicity test systems: a bacterial reverse mutation assay, an *in vitro* mammalian cell mutation assay (mouse lymphoma), an *in vitro* chromosome aberration assay in human lymphocytes and an *in vivo* mouse micronucleus test. Tildipirosin did not induce gene mutations with or without metabolic activation, in either the bacterial or the mammalian cell mutation assays. Tildipirosin did not demonstrate clastogenic activity, with or without metabolic activation in human lymphocytes and was negative in the *in vivo* mouse micronucleus assay. The results indicate that tildipirosin is not genotoxic.

Carcinogenicity

No data from carcinogenicity studies were provided. Due to the absence of a chemical relationship to known carcinogens, the negative results of genotoxicity assays and the lack of carcinogenic potential of other macrolide antibiotics it is assumed that tildipirosin is devoid of a carcinogenic risk.

Neurotoxicity

No specific studies on neurotoxicity were provided. Based on the results of the acute and subchronic toxicology studies in rats and dogs, and target species safety studies in cattle and pigs, it was concluded that tildipirosin does not exert neurological effects at dose levels lower that those that elicit other toxicological effects.

Immunotoxicity

Tildipirosin was evaluated for skin and ocular irritation in the albino rabbit. Tildipirosin was not irritating or corrosive to skin and eyes. The potential of tildipirosin to produce sensitization following

topical exposure was evaluated in guinea pigs given a combination of intradermal injections and topical applications (maximisation study design). A positive reaction was produced in nearly all test animals (10 of 10 and 8 of 10) at the 24 and 48-hour scoring intervals respectively, indicating that tildipirosin can be considered to be a contact sensitizer in guinea pigs.

2.1.4. Calculation of the toxicological ADI or alternative limit

Tildipirosin is devoid of mutagenic potential and the absence of structural alerts or pre-neoplastic lesions in subchronic and chronic toxicity studies suggested that the substance does not possess carcinogenic potential. No effects on embryo-foetal development were observed below maternally toxic doses and there was no evidence of any teratogenic potential of tildipirosin. Effects were mainly consistent across the two species tested (rabbits and rats). From the prenatal development studies the lowest NOEL of tildipirosin in pregnant rats and rabbits was 30 mg/kg bw/day. In repeated dose toxicity studies, dogs were shown to be more sensitive to the administration of tildipirosin than rats.

A NOEL of 6 mg/kg bw/day was established in the 13-week-toxicity study in dogs and a NOEL of 10 mg/kg bw/day in the 55-week-study in dogs, the most appropriate species. Considering all the available data from repeat dose toxicity studies in dogs, it can be concluded that the LOEL was 20 mg/kg bw/day, and that consequently the NOEL was 10 mg/kg bw/day. The toxicological ADI is therefore proposed to be calculated from the NOEL of 10 mg/kg bw/day established from the chronic dog toxicity study. Taking into account an uncertainty factor of 100 the toxicological ADI was established as 100 µg/kg/day i.e. 6000 µg/person/day.

2.1.5. Overview of microbiological properties of residues

Disruption of the colonisation barrier

The information provided to address the question of microbiological effects of tildipirosin included *in vitro* testing of bacteria representing the human gut flora, testing in an *in vivo* rat model to provide excretion and metabolism data for orally administered tildipirosin, a microbiological assay to investigate microbiological activity in colon contents and a literature review on the impact of pH on the microbiological activity of tildipirosin.

MICs of tildipirosin were determined according to CLSI standards and VICH GL 36 against 10 isolates from 10 bacterial groups sourced from the faecal microbiota of healthy unmedicated human volunteers. The MICs ranged from 0.5 to greater than 128 µg/ml. The MIC_{50} values were 2 µg/ml for *Clostridium* and *Fusobacterium*, 4 µg/ml for *E. coli*, 8 µg/ml for *Enterococcus*, 16 µg/ml for *Bifidobacterium*, 32 µg/ml for *Eubacterium*, *Bacteroides fragilis*, and other *Bacteroides* species, and >128 µg/ml for Gram-positive anaerobic cocci ("*Peptostreptococcus*") and *Lactobacillus*. All tested genera apart from the intrinsically resistant *Lactobacillus* spp. and *Peptostreptococcus* spp. (MIC_{50}>128 µg/ml) were considered as appropriate input data for the calculation of MIC_{calc} according to CVMP/VICH/467/03-FINAL-corr. The resulting MIC_{calc} was 5.2 µg/ml.

The fraction of the dose to which gut bacteria would be exposed was investigated in an *in vivo* study in rats treated orally with [^{14}C]-tildipirosin at 2 different doses over 7 days (25 µg/kg bw/day and 25 mg/kg bw/day). The animals were slaughtered at 150 hours after first administration (plus 6 hours after last administration) and excreta as well as colon contents were analyzed for total radioactive residues (TRR) and metabolite profiles. The data showed that tildipirosin is predominately excreted in faeces with this route of elimination corresponding to about 83% to 92% of the total dose. Colon contents contained an amount of radioactive residues approximately equivalent to 43% of the last administration 6 hours after the low oral dose of 25 µg/kg bw/day, and 41% of the last dose 6 hours after the high oral dose of 25 mg/kg bw/day. Urinary excretion corresponded to only about 1% of the

dose. The major portion of the dose recovered in colon contents was present in metabolised form. Unchanged tildipirosin was a minor component and accounted for between 5% and 8% of the total residues. Sulphate conjugates of reduced tildipirosin represented most of the tildipirosin derived radioactivity, with the major metabolite (M7) comprising between 32% and 52% of the residues.

There was no information on the microbiological potential of metabolites and their physiological origin was not further clarified (host origin and excreted via bile or produced by gut flora). In the absence of these data, it is conservatively assumed that all metabolites have the same microbiologically activity as parent compound and are of gastrointestinal origin.

In order to obtain an estimate of the extractable portion of tildipirosin derived substances, faecal samples obtained from the *in vivo* ^{14}C-tildipirosin studies in rats were repeatedly extracted with polar organic solvents (methanol). The results indicated that only a maximum of 65 to 71% of drug related residues would be extractable and potentially present as free active substance. Though the nature of the non-extractable fraction was not specifically investigated, the results obtained by the extraction method indirectly suggest that a considerable portion of the ^{14}C-tildipirosin related residues were, if not covalently bound, then at least strongly associated with faecal matter.

The samples of rat ^{14}C-tildipirosin labelled colon contents were also tested for microbiological activity against *Micrococcus luteus* ATCC 9341 following extraction in phosphate buffer (pH 7.3). The limit of detection (LOD) for the assay was determined as 1.315 mg/kg. None of the colon content samples showed antimicrobial effects despite the presence of tildipirosin and tildipirosin-related residues in the ppm range, as determined in the radiotracer assay (tildipirosin circa 65 mg/kg and TRR circa 1000 mg/kg). This result might be interpreted as suggesting that tildipirosin and its residues would not be microbiologically active in the presence of faecal matter. However, critical validation parameters such as extraction efficiencies for the microbiological assay, were not satisfactorily examined and, thus, results were difficult to interpret in quantitative terms. Results for some spiked faecal slurry supernatants (at 3000 µg/l) indicated, however, that tildipirosin is at least partially inactivated in the presence of soluble colon contents with an average decrease of about 16 to 18% of activity.

As with other macrolides, the activity of tildipirosin is pH sensitive and the basic amine groups are protonated under acidic conditions resulting in a potential loss of antimicrobial potency under acidic conditions. A number of studies in humans have shown that whilst pH in the human colon varies over right, mid, and left colon, it remains acidic for the majority of the colonic transit with an average pH around 6.5 - 6.7. Supporting quantitative data on pH dependency and *in vitro* microbiological activity of tildipirosin against various food-borne pathogens and commensal organisms of cattle and pig origin showed an increase of MICs by two log2 dilution steps in the majority of species when the pH was lowered from 7.3 to 7.0, and by at least three log2 dilution steps when the pH was lowered further to 6.7. These findings were further supported by a supplementary pH study testing 31 bacterial strains, including *Escherichia coli*, *Enterococcus* spp., *Clostridium* spp., and *Fusobacterium* spp., isolated from humans faecal microbiota. The results demonstrated that a reduction in pH from 7.3 to 6.7 produced a considerable reduction in the antibacterial activity of tildipirosin against aerobic bacteria (MIC increase of 8 to 16 fold) and a moderate reduction for tested anaerobic bacteria when the pH was slightly lowered from 7.0 to 6.7 (minimum MIC increase of 2 fold).

Increase of the population of resistant bacteria

Two studies were submitted addressing the issue of resistance development of tildipirosin. Results from one study did not show any resistance to tildipirosin in strains of *Camphylobacter* isolated from faeces of healthy cattle and pig. In a more complex study the frequency of horizontal conjugative resistance transfer at sub-inhibitory concentrations of tildipirosin was measured relative to the comparator macrolides erythromycin, azithromycin and tulathromycin. Tildipirosin did not increase the frequency of horizontal conjugative resistance transfer compared with the reference macrolides. These

results lead to the assumption that tildipirosin has the same mode of action and the same potential of resistance development as other 16-membered macrolides. No microbiological ADI with respect to resistance development was calculated.

Overall, these studies suggest that resistance to tildipirosin is not more likely to develop than to other macrolides. It is accepted that calculation of a microbiological ADI with respect to resistance development is not necessary.

Potential effects on microorganisms used in industrial processing of foodstuffs

The substance is not intended for dairy cattle and therefore potential effects in dairy products were not investigated.

2.1.6. Calculation of microbiological ADI

A value for the microbiological ADI was based on MIC determinations for relevant genera of human gut flora, as described in CVMP/VICH/467/03-FINAL-corr, and calculated as follows:

$$ADI_{micro} = \frac{5.2\ \mu g/ml \times 220\ g/day}{[0.43 \times 0.71 \times 0.5] \times 60\ kg\ person} = 124.91\ \mu g/kg\ bw/day$$

Where:

- MIC_{calc} derived from the lower 90% confidence limit for the mean MIC_{50} of the most relevant genera was 5.2 µg/ml;
- The fraction of the oral dose which is available for colonic microorganisms was conservatively estimated based on the following factors:
  - a factor of 0.43 to take account of the % of oral dose recovered from the colon, based on *in vivo* results,
  - a factor of 0.71 to take account of the free available fraction of the colonic residues, based on extensive extraction with organic solvents,
  - a factor of 0.50 to take account of the impact of acidic colonic pH on tildipirosin activity (at least 50% reduction of microbiological activity under pH conditions in the colon);
- The weight of a person is 60 kg;
- The mass of colon content is 220 g per day.

The approach is considered suitably conservative, particularly in light of the cautious assumption that all metabolites are equally active as the parent compound and exclusively produced via direct exposure of gut flora.

The estimated microbiological ADI is 7.5 mg/person (7495 µg/person).

2.1.7. Observations in humans

No data are available on the effects of tildipirosin in humans.

2.1.8. Findings of EU or international scientific bodies

No data on tildipirosin are available from EU or international scientific bodies.

2.1.9. Overall conclusions on the ADI

Secondary pharmacodynamic effects of tildipirosin are not considered to be such that a pharmacological ADI would need to be derived. The toxicological ADI of 6 mg/person (6000 µg/person) was based on the NOEL of 10 mg/kg bw/day from a 55-weeks toxicity study in dogs, the most appropriate species, applying an uncertainty factor of 100. The microbiological ADI was calculated to be 7.5 mg/person (7495 µg/person) based on the approach detailed in CVMP/VICH/467/03-FINAL-corr and is higher than the toxicological ADI.

The toxicological ADI of 6 mg/person (6000 µg/person) is therefore established as the overall ADI.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

Studies in both target species were performed to investigate the pharmacokinetic profile of tildipirosin in blood plasma, bronchial fluid and lung tissue. Plasma data in cattle after single subcutaneous injections of 2, 4 or 6 mg tildipirosin/kg bw demonstrated rapid absorption from the site of injection (mean time of maximum concentration (T_{max}) <1 hour) and a relatively long persistence of tildipirosin in the animals as shown by plasma concentrations above the lower limit of quantification (LOQ) of 10 ng/ml up to 21 days after treatment. The pharmacokinetic profile was also characterized by a long terminal plasma half life ($T_{1/2}$ > 6.5 days) and a long mean residence time ($MRT_{(0\text{-}LOQ)}$ > 5.5 days). The mean area under the concentration versus time curve from time zero to the last sampling time associated with a quantifiable concentration ($AUC_{(0\text{-}LOQ)}$) increased dose dependently (14683, 21017, and 30350 h*ng/ml). The plasma pharmacokinetic profile in pre-ruminant calves (5-8 weeks of age) and ruminant cattle was found to be similar. In pigs, after single intramuscular injection of 2, 4 or 6 mg tildipirosin/kg bw plasma data demonstrated rapid absorption from the site of injection (mean T_{max} < 0.5 hours) and plasma concentrations above the limit of qualification (LOQ) of 10 ng/ml up to 14 days after treatment in most animals and at all doses. Furthermore, the pharmacokinetic profile was characterized by a long $T_{1/2}$ (> 4 days) and a long $MRT_{(0\text{-}LOQ)}$ (> 3.5 days). The mean $AUC_{(0\text{-}LOQ)}$ increased dose dependently (5773, 11012, and 13373 h*ng/ml).

Using an equilibrium dialysis method, the *in vitro* protein binding of tildipirosin in bovine and pig blood plasma and in bovine bronchial fluid was determined as approximately 30%. Protein binding was not tildipirosin concentration dependent in the range tested, which was 0.1 to 50 µg tildipirosin/ml plasma and 1 to 10 µg tildipirosin/ml bronchial fluid.

Metabolism and comparative metabolism

Several GLP radio-labelled studies were conducted in target animals and in laboratory animals. In these metabolism studies ^{14}C-tildipirosin was subcutaneously and intramuscularly administered to cattle and pig and orally to Han Wistar rats and Beagle dogs. The target animal species cattle and pig were administered projected therapeutic doses of 4 mg/kg bw and 5 mg/kg bw, respectively[4].

Pigs: The highest mean percentage of administered dose recovered was 62% in faeces and 17% in urine of pig at 14 days after administration. In contrast to the findings in laboratory animals unchanged tildipirosin was determined as the major component in excreta of pig as well as in all tissue

[4] For details of the study parameters carried out in cattle and pigs see at 2.2.2 Residue depletion studies.

samples. Other tissue metabolites were desmethyl tildipirosin and S-cysteine conjugate of reduced tildipirosin. The metabolite profiles obtained from pooled male and female tissue and excreta samples showed no differences.

Cattle: The highest mean percentage of administered dose recovered was 40% in faeces and 24% in urine of cattle at 14 days after administration. Consistent with the metabolic pattern in pigs the main component found in urine and faeces of cattle was unchanged tildipirosin. In urine M7 and the sulphate conjugates of reduced and hydrated (or ring open) tildipirosin (M4) were also detected. In all tissue samples unchanged tildipirosin was detected as the major component. In liver and injection site desmethyl-tilidipirosin and S-cysteine conjugate of reduced tildipirosin were also found. No differences between the metabolite profiles were obtained from male and female tissue and excreta samples.

In pigs and cattle the metabolism of tildipirosin proceeds by loss of the mycaminose sugar moiety, by reduction and sulphate conjugation and subsequent hydration or ring opening, demethylation, mono- or dihydroxylation, dehydration and by S-cysteine and S-glutathione conjugation.

The main route of excretion in all species is via faeces and urinary excretion is relatively minor (independent of whether the compound is administered by the parenteral or oral route). All metabolites found in target animals have also been observed in laboratory animals. Whereas in laboratory animals the main metabolite detected in excreta and tissues was M7, in target animals the major component of radioactivity was unchanged tildipirosin. It was not clear whether this is mainly attributable to a route of administration or species effect. Some minor metabolites (content <10% TRR) in samples of all animals were not identified. Overall, the results of the metabolite profiling show qualitative interspecies consistency in metabolic pathways in target and laboratory animal species. Therefore, it was concluded that the laboratory species (rat and dog) used in the toxicity studies were auto-exposed to the same range of compounds as humans consuming edible tissues of treated cattle and pigs would be exposed to.

2.2.2. Residue depletion studies

Two radiometric residue depletion studies were conducted in cattle.

In a pilot study 8 cattle (87.5 to 112.5 kg bw) were treated with a single subcutaneous injection at a target dose of 4 mg ^{14}C-tildipirosin/kg bw formulated as 18% w/v aqueous solution of pH 7.5. Concentrations of total radioactive residues expressed as tildipirosin equivalents/kg and of parent compound (tildipirosin) were determined in edible tissues and injection site at 7, 28, 14 and 35 days after administration. Highest total residues were detected in liver (16577 to 6708 µg equivalents/kg), kidney (13992 to 4238 µg equivalents/kg) and injection site (19119 to 3879 µg equivalents/kg) followed by fat (2637 to 719 µg equivalents/kg) and muscle (701 to 181 µg equivalents/kg). Muscle and fat always contained the lowest concentration of tildipirosin equivalents and were not considered to be target tissues. Using a validated online SPE-HPLC-MS/MS method (LOQ: 50 µg/kg) parent compound (tildipirosin) was detectable in all tissues and at all sacrifice days, in high concentrations in kidney, liver and injection site, where concentrations of tildipirosin depleted from 12430 to 2546 µg/kg in kidney, from 9841 to 2368 µg/kg in liver and from 9429 to 3369 µg/kg at the injection site. In fat and muscle tildipirosin concentration depleted from 3476 to 548 µg/kg and 417 to 107 µg/kg, respectively.

In a pivotal study 28 cattle (141.5 to 227 kg bw) received a single subcutaneous injection at a target dose of 4 mg ^{14}C-tildipirosin/kg bw formulated as 18% w/v aqueous solution of pH 5.5. Concentrations of total radioactive residues expressed as tildipirosin equivalents per kg and of parent compound (tildipirosin) were determined in edible tissues and injection site at 3, 7, 21, 35, 49 and 63 days after administration. Highest total residues were detected in liver (28852 to 4552 µg equivalents/kg), kidney

(21613 to 1443 µg equivalents/kg) and injection site (42030 to 2388 µg equivalents/kg) followed by fat (789 to 124 µg equivalents/kg) and muscle (1176 to 76 µg equivalents/kg). Muscle and fat always contained the lowest concentration of total residues and were not considered to be target tissues. Using a validated online SPE-HPLC-MS/MS method (LOQ: 50 µg/kg) parent compound (tildipirosin) was detectable in all tissues and at all sacrifice days, in high concentrations in kidney, liver and injection site, where concentrations of tildipirosin depleted from 12323 to 971 µg/kg in liver, from 16193 to 586 µg/kg in kidney and from 30780 to 1614 µg/kg at the injection site. In fat and muscle concentration of parent tildipirosin depleted from 815 to 99 µg/kg and from 737 to less than 50 µg/kg, respectively.

In a non-radiometric residue depletion study, 28 cattle (298 to 348 kg bw) received a single subcutaneous injection in the neck at a target dose of 4 mg tildipirosin/kg bw (intended formulation for marketing). At 9, 18, 27, 36, 45, 54 and 63 days after administration tissue samples were analysed for parent compound (tildipirosin), the proposed marker residue, using an online SEP-HPLC-MS/MS method (LOQ: 50 µg/kg). At the initial sampling time, highest mean concentrations of tildipirosin were present at the injection site (core tissue: 36693 µg/kg) followed by kidney (8600 µg/kg) and liver (5524 µg/kg) and lowest mean concentrations were present in fat (460 µg/kg) and muscle (324 µg/kg). At day 63 after administration, mean concentration had declined to 1333/2124 µg/kg at the injection site (core/surrounding tissue), around 600 µg/kg in kidney and liver and below the limit of quantification in fat and muscle.

Two radiometric residue depletion studies were conducted in pigs.

In a pilot study 8 pigs (14 to 20 kg bw) were treated with a single intramuscular injection in the neck area at a target dose of 5 mg ^{14}C-tildipirosin/kg bw formulated as 4% w/v aqueous solution of pH 7.5. Concentrations of total radioactive residues expressed as tildipirosin equivalents/kg and of parent compound (tildipirosin) were determined in edible tissues and injection site at 7, 14, 21 and 28 days after administration. Highest total residues were detected in kidney (12922 to 1796 µg equivalents/kg), liver (7430 to 1734 µg equivalents/kg) and injection site (6436 to 796 µg equivalents/kg) followed by skin+fat (331 to 75 µg equivalents/kg) and muscle (454 to 62 µg equivalents/kg). Muscle and skin+fat always contained the lowest concentration and were not considered to be target tissues. Using a validated online SPE-HPLC-MS/MS method (LOQ: 50 µg/kg) parent compound (tildipirosin) was detectable in all tissues and at all sacrifice days, in high concentrations in kidney, liver and injection site, where concentrations depleted from 9992 to 1072 µg/kg in kidney, from 4846 to 845 µg/kg in liver and from 4086 to 575 µg/kg at the injection site. In skin+fat and muscle, concentrations of tildipirosin depleted from 389 to less than 65 µg/kg and 285 µg/kg to below the LOQ, respectively.

In a pivotal study 20 pigs (14 to 21.5 kg bw) received a single intramuscular injection in the neck area at a target dose of 5 mg ^{14}C-tildipirosin/kg bw formulated as 4% w/v aqueous solution of pH 5.5. Concentrations of total radioactive residues expressed as tildipirosin equivalents/kg and of parent compound (tildipirosin) were determined in edible tissues and injection site at 3, 7, 14, 21 and 28 days after administration. Highest total residues were detected in kidney (20225 to 2026 µg equivalents/kg) and liver (9310 to 2794 µg equivalents/kg) and injection site (8464 to 730 µg equivalents/kg). Skin+fat (741 to 69 µg equivalents/kg) and muscle (946 to 57 µg equivalents/kg) always contained the lowest concentrations and were not considered to be target tissues. Using a validated online SPE-HPLC-MS/MS method (LOQ: 50 µg/kg) parent compound (tildipirosin) was detectable in all tissues and at all sacrifice days, in high concentrations in kidney, liver and injection site, where concentrations depleted from 12425 to 883 µg/kg in kidney, from 3849 to 577 µg/kg in liver and from 5395 to 246 µg/kg at the injection site. In skin+fat and muscle concentration of tildipirosin depleted from 559 µg/kg to below the limit of quantification and 537 µg/kg to below the limit of quantification, respectively.

In a non-radiometric residue depletion study, 24 pigs (30.1 to 38.4 kg bw) received a single intramuscular injection in the neck area at a target dose of 4 mg tildipirosin/kg bw (intended formulation for marketing). At 4, 8, 12 and 16 days after administration tissue samples were analysed for parent compound (tildipirosin), the proposed marker residue, using an online SPE-HPLC-MS/MS method (LOQ: 50 µg/kg). At the initial sampling time, highest mean concentrations of tildipirosin were present in the kidney (11320 µg/kg) followed by the injection site (core tissue: 8649 µg/kg), liver (4145 µg/kg), skin+fat (721 µg/kg) and were lowest in muscle (328 µg/kg). At day 16 after administration, mean concentrations had declined to 2390 µg/kg in kidney, 1761 µg/kg at the injection site (core tissue), 1928 µg/kg in liver, 184 µg/kg in skin+fat and 68 µg/kg in muscle.

Selection of marker residue and target tissues

From the results of the radiolabelled studies it was concluded that the parent compound was a major component in all tissues. Parent compound represented, over a wide time period, around 30% to 90% of total residues in tissues of cattle (from day 3 to 63) and 20% to 80% in pig (from day 3 to 28 days). Therefore, parent compound (tildipirosin) was proposed as the appropriate marker residue for both species, cattle and pig.

The ratios of marker to total residues have been determined in cattle for each tissue type over a time period of 3 to 63 days. Ratios were in the range of 0.520 to 0.158 (mean 0.281) in liver, 0.749 to 0.406 (mean 0.592) in kidney, 1.03 to 0.74 (mean 0.892) in fat, 0.852 to 0.593 (mean 0.699) in injection site muscle and 0.627 to 0.473 (mean 0.532) in normal muscle (3 to 21 days).

The ratios of marker to total residues have been determined in pigs for each tissue type over a time period of 3 to 28 days. Ratios were in the range of 0.489 to 0.207 (mean 0.350) in liver, 0.614 to 0.435 (mean 0.537) in kidney, 0.804 to 0.678 (mean 0.746) in skin+fat (3 to 21 days), 0.691 to 0.337 (mean 0.508) in injection site muscle and 0.570 to 0.512 (mean 0.544) in normal muscle (3 to 21 days). No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.4. Analytical method for monitoring of residues

A validated off-line SPE-HPLC/MS/MS method for determination of the marker residue, tildipirosin, in bovine tissues (muscle, fat, liver, kidney), and in porcine tissues (muscle, skin+fat, liver, kidney) is available according to the current requirements of Volume 8 of The Rules Governing Medicinal Products in the European Union. For caprine tissues, an analytical method, in line with the requirements for minor species as detailed in Volume 8, is available.

The limits of detection in bovine muscle, liver, kidney and fat were 7 µg/kg, 3 µg/kg, 44 µg/kg and 27 µg/kg, respectively, and the limits of detection in porcine muscle, kidney and skin+fat were 51 µg/kg, 37 µg/kg and 53 µ/kg, respectively. The limits of quantification were 200 µg/kg and 600 µg/kg in muscle, 150 µg/kg in fat and fat+skin and 250 µg/kg in liver and kidney of bovine and porcine tissues, respectively.

In caprine tissues the limits of quantification were 200 µg/kg in muscle, 100 µg/kg in fat, 1000 µg/kg in liver and 1500 µg/kg in kidney. The method is considered validated for monitoring residues in muscle, fat, liver and kidney according to current requirements.

2.2.5. Findings of EU or international scientific bodies

No data on tildipirosin are available from EU or international scientific bodies

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

The substance is not intended for use in dairy cattle and therefore potential effects in dairy products were not investigated.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

In its opinion of 15 September 2011 the Committee considered that:

Residue depletion data demonstrate that tildipirosin levels in carcass tissues (i.e. fat, skin+fat and muscle) other than injection site muscle were low compared to levels in liver and kidney, in both cattle and pig. If a muscle MRL were set using the conventional approach (i.e. based on residue levels seen in non-injection site muscle), the value would be such that the withdrawal period for any injectable product containing tildipirosin would not be practicable. On a number of previous occasions the Committee has elected not to recommend an MRL for muscle for injectable substances that deplete very slowly from the injection site. For products containing active substances for which no muscle MRL has been established, withdrawal periods are calculated using the "ADI approach", which ensures that at the selected withdrawal period, residues in a food basket including 300 g of injection site muscle are below the ADI, so ensuring consumer safety. However, the absence of an MRL for muscle represents a serious problem for residue control laboratories as muscle is often the tissue selected for residue monitoring. Furthermore, meat imported into the EU often takes the form of lean cuts of muscle. The absence of an MRL for muscle means that it is not possible to control residues levels in imported meat of this type.

Given the points raised in the above paragraph, the CVMP chose to recommend a muscle MRL derived in the conventional way but highlighted that this value was not suitable for monitoring residues at the injection site. An alternative value, that corresponds to the maximum level of residues that would be expected in the injection site at the anticipated withdrawal period (hereafter referred to as the Injection Site Residue Reference Value – ISRRV) was also calculated, as follows: the theoretical maximum daily exposure was calculated on the basis of recommended MRLs for liver, kidney and fat (skin+fat in the case of pig) and the resulting value was compared to the ADI. The ISRRV was then derived in a manner that would allow for residues in 300g of muscle to correspond to the remaining portion of the ADI. The withdrawal period should be derived in a manner that ensures that residues at the injection site will be below this value (11500 µg/kg for cattle (and goats) and 7500 µg/kg for pigs) and that residues in non-injection site muscle, liver, kidney and fat will be below the MRLs for these tissues. In this way, the withdrawal period would not be longer than is necessary in order to ensure consumer safety.

On 25 October 2011 the European Commission, having considered the CVMP opinion of 15 September 2011, requested the Committee to reconsider its recommendation and to amend the part of the opinion that includes a residue limit for the injection site in the "Other provisions" of Table I of the Annex to Commission Regulation (EU) 37/2010.

The Committee, having reflected on the issues, considered that:

- even for injectable substances that deplete slowly from the injection site, such as tildipirosin, MRLs should in principle be established for muscle;
- the existing system of residue surveillance would be facilitated if a single residue limit in muscle were established;
- setting a single low MRL value for muscle would result in a considerably lengthened withdrawal period where this is not justified on the basis of consumer safety. In practice this approach would unnecessarily reduce the availability of veterinary medicinal products;
- for substances that deplete slowly from the injection site, establishing a single muscle residue limit based on residue levels that can be expected in injection site muscle would result in an MRL that is of limited value for residue control as the vast majority of muscle samples will not be injection site muscle;
- establishing no MRL for muscle would leave residue monitoring authorities with no reference value against which to compare residues in muscle and could result in punitive treatment of meat producers if the absence of an MRL were interpreted as an indication that residues at any level were non compliant;
- establishment of two residue limits for muscle presents difficulties for implementation of residue control measures according to current monitoring practices; these difficulties may be overcome, for example, by residue monitoring in a non-muscle tissue, by residue monitoring in a muscle into which injections are not given, or by taking two separate muscle samples for analysis.

In its deliberations the Committee reiterated the view that its previous recommendation fully ensured consumer safety. The Committee considered that the request to reconsider its opinion did not provide sufficient scientific or safety grounds to justify a change to its previous recommendation and, in its opinion of 8 December 2011, confirmed it previous recommendation.

The CVMP opinion was discussed at a meeting of the Standing Committee on Veterinary Medicinal Products, held on 27 September 2012. Following the discussions at that meeting the Commission, on 5 October 2012, again requested the Committee to reconsider its recommendation and to give further consideration to alternative approaches for addressing injection site residues and to the feasibility of residue controls.

Having considered the issue at length the Committee notes that:

- it would possible to calculate a muscle MRL based on either (i) residues expected in injection site muscle or (ii) residues expected in non-injection site muscle;
- a muscle MRL based on approach (i) (residue levels expected in injection site muscle) would be of little relevance for residue control authorities. This is because injection sites are scarce while non-injection site muscle is abundant and consequently, except on rare, chance occasions, non-injection site muscle will be sampled by residue control authorities. So in the vast majority of samples residues would be far below the injection site levels used to derive the MRL, even if the withdrawal period were not respected. So compliance with the muscle MRL would provide no information on whether the withdrawal period had been respected or on whether residue levels in other tissues comply with their respective MRLs;
- from a residue control point of view, it would make far more sense to base the muscle MRL on approach (ii) (residue levels that can be expected in non-injection site muscle) as this will be representative of muscle sampled on all but very rare, chance occasions. From a consumer safety perspective this is also the preferred option, as non-injection site muscle is the muscle regularly consumed;

- Annex I of Regulation (EC) No 854/2004, in Section II, Chapter V, indicates that "meat is to be declared unfit for human consumption if it: …(i) contains residues or contaminants in excess of the levels laid down in community legislation" (ie above the MRL);
- in practice, as injection sites will not always be easily identifiable, it cannot be assumed that they will always be removed from the food chain.

In light of the above the CVMP concludes that the muscle MRL should be set in such a way as to maximise both its relevance for residue control purposes and its ability to protect consumer health – i.e. it should be derived based on residue levels that can be expected in non-injection site muscle.

However, because it cannot be assumed that injection sites will always be removed from the food chain there is also a need to ensure that residues at the injection site are present at levels that do not represent a risk to the consumer. An Injection Site Residue Reference Value (ISRRV) that specifies the level of residues at the injection site that can be considered safe should therefore be derived. However, this value is no longer proposed for inclusion in Regulation (EU) No 37/2010 and is not intended for use in routine residue surveillance. Rather it provides a value to be used by competent authorities when setting withdrawal periods for tildipirosin containing products. The withdrawal period must ensure that residues in non-injection site muscle, as well as in liver, kidney and fat, are below the MRLs and that residues at the injection site are below the ISRRV. In this way the withdrawal period will ensure that, even if a consumer were to ingest an injection site, consumer exposure to residues would not represent a health risk.

The CVMP notes that Article 1 of Directive 2001/82/EC defines the withdrawal period as the period necessary to ensure that foodstuffs do not contain residues in excess of the MRLs. While efforts may be made to remove injection sites, in those instances where they enter the food chain undetected, residues may be present at levels that exceed the muscle MRL. While these residues will not represent a consumer safety concern, the chance sampling of an injection site by a residue control authority would lead to a non-compliant residue finding and possible punitive action against the farmer. Such action would be unfair given that the non-compliant finding would not represent non-compliance with the withdrawal period.

In relation to residue monitoring, in some cases, residue control authorities will have access only to muscle tissue, in which case only muscle tissue will be available for residue monitoring (this is particularly the case for meat imported into Europe). However, where the entire carcass is available, the CVMP recommends that for the purpose of monitoring residues of tildipirosin, fat (skin+fat for pigs), liver or kidney should be sampled in preference to muscle. This is because residues in these tissues deplete more slowly than residues in muscle and so will provide a better basis for verifying compliance with the withdrawal period.

3.3. Elaboration of MRLs

MRL values for cattle and pig liver, kidney and fat (cattle) and skin+fat (pig) are proposed based on the residue depletion data, distribution of residues between target tissues and taking into account the toxicological ADI of 6000 µg/person.

Ratios of marker to total residues were determined on the basis of the residue data around the time point when the amount of total residues in the edible portion (0.1 g liver, 0.05 g kidney, 0.05 g fat, including 0.3 g injection site muscle instead of normal muscle) drops below the ADI of 6000 µg (day 21 in cattle and day 7 in pig).

For cattle, using average ratios of marker to total residues of 0.28 in liver, 0.59 in kidney, 0.91 in fat and 0.53 in normal muscle, MRLs of 2000 µg/kg, 3000 µg/kg and 200 µg/kg and 400 µg/kg for liver, kidney, fat and muscle, respectively, were derived.

As described in the preceding section, an Injection Site Residue Reference Value (ISRRV) was then derived in a manner that would allow for residues in 300 g of muscle to correspond to the unused portion of the ADI, based on the fact that the theoretical maximum daily exposure calculated on the basis of the MRLs for liver, kidney and fat correspond to approximately 16% of the ADI. The ISRRV calculated in this way is 11500 µg/kg. Withdrawal periods for injectable tildipirosin products should ensure that residue levels present in non-injection site muscle, liver, kidney and fat do not exceed the MRLs for muscle, liver, kidney and fat, respectively and that residue levels present in injection site muscle do not exceed the ISRRV.

For pigs, using average ratios of marker to total residues of 0.42 in liver, 0.59 in kidney, 0.77 in skin+fat and 0.54 in normal muscle, MRLs of 5000 µg/kg, 10000 µg/kg and 800 µg/kg and 1200 µg/kg for liver, kidney, skin+fat and muscle, respectively, were derived.

As for cattle, an ISRRV was then derived in a manner that would allow for residues in 300 g of muscle to correspond to the unused portion of the ADI, based on the fact that the theoretical maximum daily exposure calculated on the basis of the MRLs for liver, kidney and fat correspond to approximately 35% of the ADI. The ISRRV calculated in this was 7500 µg/kg. Withdrawal periods for injectable tildipirosin products should ensure that residue levels present in non-injection site muscle, liver, kidney and fat do not exceed the MRLs for muscle, liver, kidney and fat, respectively and that residue levels present in injection site muscle do not exceed the ISRRV.

Tildipirosin is not intended for use in dairy cattle and therefore residue depletion data in milk were not provided. As a consequence no MRL is recommended for milk and the use of the substance shall be restricted to animals that do not produce milk for human consumption.

Calculation of theoretical daily intake of residues

Cattle

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	400	0.53	226.4
Fat	0.05	200	0.91	10.99
Liver	0.10	2000	0.28	714.29
Kidney	0.05	3000	0.59	254.24
Estimated total daily intake (µg/person)				1205.92
ADI (µg/person)				6000 20.09 % of ADI

Pig

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	1200	0.54	666.67
Fat	0.05	800	0.77	51.95
Liver	0.10	5000	0.42	1190.48
Kidney	0.05	10000	0.59	847.46
Estimated total daily intake (µg/person)				2756.56
ADI (µg/person)				6000 45.9 % of ADI

Based on the recommended MRLs, the theoretical maximum daily intake from tissue of cattle (and goat) and pig, calculated using the recommended maximum residue limits, represents 20% and 46% of the ADI. However, when the calculation is performed using the ISRRVs of 11500 µg/kg for cattle (and goats) and 7500 µg/kg for pigs, consumer intake represents approximately 98.5% of the ADI in both cases.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/2009 the CVMP considered the possibility of extrapolating the maximum residue limits established for tildipirosin on the basis of residue data in cattle and pigs to other food producing species and food commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
Goats	Yes	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, the assumption was made that the parent compound will be the predominant residue in the goat and consequently it was accepted as the marker residue for goats as well as for cattle and pigs. Although no specific pharmacokinetic or residue data were available for goats and therefore the ratio of marker to total residues could not be derived, the limitations resulting from the lack of species specific data are compensated for by the fact that consumption of goat's meat, and consequently consumer exposure to residues in goat's meat is limited. Therefore the same MRL values as established in cattle can be recommended in goats without compromising the safety of the consumer. Additionally, an analytical method suitable for the monitoring of tildipirosin residues in goat tissues is available.

Sheep	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar. Based on this existing inter-species metabolism data, and that cattle and sheep are related species (ruminants) the assumption could be made that the parent compound would be the predominant residue in sheep and so would be a suitable marker residue. However, no specific pharmacokinetic or residue data were available for sheep and therefore the assumption related to the marker residue could not be confirmed and the ratio of marker to total residues could not be derived. Sheep meat is consumed on a regular basis and in large quantities. Species specific data are therefore considered necessary to allow adequate evaluation of the risk to consumer safety posed by residues in sheep tissues. No analytical method for monitoring of residues in sheep tissues was available for evaluation.
Poultry (including eggs)	No	Metabolism can be significantly different in poultry compared to cattle and pigs. Consequently species specific metabolism and residue data are considered necessary to allow adequate evaluation of the risk to consumer safety posed by residues in poultry-derived food commodities. No analytical method for monitoring of residues in poultry tissues (or eggs) was available for evaluation.
Horses	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar and it can be expected that the parent compound would be a suitable marker residue in horses. However, no data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pigs is applicable for monitoring of residues in horse tissues.
Rabbits	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and pigs is similar and it can be expected that the parent compound would be a suitable marker residue in rabbits. However, no data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pigs is applicable for monitoring of residues in rabbit tissues.
Fin fish	No	Metabolism is generally less complicated in fish than in cattle and pigs. Consequently, if the marker residue is the parent compound in cattle and pigs it can be assumed that the parent compound would also be a suitable marker residue in fish meat. However, no analytical method for monitoring of residues in fish meat was available for evaluation.
Milk	No	No data are available that would allow conclusions to be drawn on the appropriate marker residue or marker to total residues ratio to use in milk. Milk is consumed on a regular basis and in large

		quantities and consequently data on residues in this commodity are considered necessary in order to allow adequate evaluation of the risk to consumer safety posed by residues in milk. No analytical method for monitoring of residues in milk was available for evaluation.
Honey	No	Residue depletion in honey does not occur through metabolism and consequently conclusions drawn from data in other food products cannot be extrapolated to honey. Honey specific data are required in order to allow adequate evaluation of the risk to consumer safety posed by residues in honey. No data are available to demonstrate that the analytical method used for monitoring of residues in cattle and pig tissues is applicable for monitoring of residues in honey.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- an ADI of 100 µg/kg bw/day or 6000 µg/person/day has been established;
- liver, kidney and the injection site muscle were the main target tissues for tildipirosin derived residues in cattle and pigs; residue concentrations were always low in fat and non-injection site muscle of both species;
- the parent compound was identified as the marker residue in cattle and pigs and is also considered to be the appropriate marker residue in goats;
- total residue in standard edible tissues of cattle and pigs (including injection site residues) were below the ADI at day 21 and day 7 after administration for cattle and pigs, respectively; marker to total residue ratios were established at these time points.
- for cattle average ratios of marker to total residues were 0.28 in liver, 0.59 in kidney, 0.91 in fat, 0.53 in normal muscle and 0.70 in injection site muscle. For pig average ratios of marker to total residues were 0.42 in liver, 0.59 in kidney, 0.77 in skin+fat, 0.54 in normal muscle and 0.59 in injection site muscle;
- the Commission and residue control authorities consider that, in order to ensure the feasibility of residue controls, a single official residue limit for muscle must be published in Regulation (EU) No. 37/2010;
- Injection Site Residue Reference Values (ISRRVs) of 11500 µg/kg 7500 µg/kg are established for cattle and pigs, respectively, and the value for cattle is applicable also to goats – these values should be taken into account when deriving withdrawal periods;
- for the purpose monitoring of residues of tildipirosin it is recommended that, where the entire carcass is available, fat (skin+fat for pigs), liver or kidney should be sampled in preference to muscle as residues in these tissues deplete more slowly than residues in muscle and so will provide a better basis for verifying compliance with the withdrawal period;

- an analytical method for monitoring of residues in cattle, pigs and goat tissues validated according to the current requirements is available;

the Committee recommends the establishment of maximum residue limits for tildipirosin in accordance with the following table:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Tildipirosin	Tildipirosin	Bovine, caprine	400 µg/kg 200 µg/kg 2000 µg/kg 3000 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Anti-infectious agents/ Antibiotics
		Porcine	1200 µg/kg 800 µg/kg 5000 µg/kg 10000 µg/kg	Muscle Skin and fat in natural proportions Liver Kidney	NO ENTRY	

4. Background information on the procedure

Submission of the dossier	5 March 2009
Steps taken for assessment of the substance	
Application validated:	19 March 2009
Clock started:	20 March 2009
List of questions adopted:	17 June 2009
Consolidated response to list of questions submitted:	10 November 2009
Clock re-started:	11 November 2009
CVMP opinion on provisional MRLs adopted:	10 December 2009
Submission of responses to the List of questions:	15 June 2011
CVMP opinion adopted:	15 September 2011
Commission request for reconsideration of opinion	25 October 2011
Revised CVMP opinion adopted:	8 December 2011
Commission request for reconsideration of opinion:	5 October 2012
Revised CVMP opinion adopted:	18 July 2013