10 December 2015
EMA/CVMP/262442/2015
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Sisapronil (bovine and caprine species)

On 17 November 2015 the European Commission adopted a Regulation[1] establishing maximum residue limits for sisapronil in bovine and caprine 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.

Sisapronil is intended for use as a long-acting injectable ectoparasiticide for control of cattle ticks.

Zoetis Belgium SA submitted to the European Medicines Agency an application for the establishment of maximum residue limits, on 27 November 2013.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 15 January 2015 the establishment of maximum residue limits for sisapronil in bovine species and the extrapolation of these maximum residue limits to caprine species.

On 27 January 2015, Zoetis notified the European Medicines Agency of its intention to request a re-examination of the CVMP opinion and the grounds for the re-examination were submitted on 9 March 2015.

Following consideration of the grounds for the re-examination the Committee for Medicinal Products for Veterinary Use adopted a final opinion on 7 May 2015.

Subsequently the Commission recommended on 23 September 2015 that maximum residue limits in bovine and caprine species are established. This recommendation was confirmed on 14 October 2015 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 17 November 2015.

[1] Commission Implementing Regulation (EU) No 2015/2062, O.J. L 301, of 18 November 2015

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Summary of the scientific discussion for the establishment of MRLs

Substance name:	Sisapronil
Therapeutic class:	Antiparasitic agents / Agents (acting) against ectoparasites
Procedure number:	EMEA/V/MRL/003915/FULL/0001
Applicant:	Zoetis Belgium SA
Target species:	Bovine
Intended therapeutic indication:	Control of cattle ticks
Route(s) of administration:	Subcutaneous

1. Introduction

Sisapronil is a new synthetic pharmaceutical molecule designed for the control of ectoparasites on cattle. The molecule is a member of the phenylpyrazole class of compounds. The chemistry of sisapronil differs from the older classes of antiparasitics, particularly the macrocylic lactones and synthetic pyrethroids.

Sisapronil is intended for use as a long-acting injectable ectoparasiticide for control of cattle ticks. It also aids in the control of bot fly larvae, hornfly and screwworm. Sisaponil is to be administered by a single subcutaneous injection at a dose of 1 ml/50 kg bw (2 mg/kg bw).

Upon injection, sisapronil is stored in the body fat of animals and is slowly released into the animal's circulatory system and skin. Sisapronil binds tightly to ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes in insects and acari. This mechanism of action results in lethal uncontrolled activity of the central nervous system of insects and acari.

Sisapronil is not used in human medicine.

The evaluation performed for the re-examination of the initial CVMP opinion is described in section 4 of this document with the overall recommendation provided in section 5. During the re-examination a small number of corrections were made to the original EPMAR.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

The claimed mechanism of action (GABA inhibition) was not supported by any documentation. It is however recognised that in a single oral dose neurofunctional study in the rat at doses of 100 to 1000 mg/kg bw neurotoxicity (reflected by alterations in both a functional observational battery and motor activity parameters) was observed at doses of 500 and 1000 mg/kg bw. The observed NOEL was 100 mg/kg bw, which is well above the anticipated human exposure, and well above the proposed toxicological NOAEL. Based on this it can be concluded that the toxicological ADI will adequately cover

consumer safety concerns resulting from the pharmacological mechanism of action (GABA inhibition) of sisapronil.

Pharmacokinetic properties (mainly in laboratory animals)

The oral pharmacokinetics of sisapronil were evaluated as part of the toxicology studies in rats (dose range finding study, single dose neurofunctional evaluation, 28-day oral toxicity study, 13-week oral toxicity study and 1-year oral toxicity study) and dogs (28-day and 90-day oral toxicity studies). Plasma concentrations of sisapronil in cynomolgus monkeys were measured after single intravenous and oral administration.

In the rat and dog, following oral administration at doses up to 10 and 25 mg/kg bw/day, respectively, plasma concentration of sisapronil increased with increasing dose but in a less than dose proportional manner. There was substantial accumulation in plasma concentration following repeated administration, due to the long half-life of sisapronil in plasma (e.g., 14 days in the rat). Oral bioavailability in cynomolgus monkeys was 6.5% and the observed elimination half-life in plasma was 12.4 days.

A mass balance study in rats showed that a residue with the same retention time as the bovine liver residue M3 (the only residue other than parent sisapronil detected in bovine tissues, see Section 2.2.1) was present also in the liver of rats following a single oral dose of 50 mg [^{14}C]sisapronil/kg bw, where it represented 10 to 18% of the total residues. This indicates that rats in the toxicological studies were exposed to the bovine liver residue M3 to which humans can be exposed via edible tissues from cattle treated with sisapronil. MS/MS analysis indicated that metabolite M3 is structurally similar to parent sisapronil but attempts to identify the metabolite further were unsuccessful. A residue with the same retention time as the two co-eluting bovine urine residues M1 and M2 (see Section 2.2.1) was shown to represent an important part of the total residues also in urine of orally treated rats. The metabolites M1 and M2 were characterised as having undergone oxidation and conjugation with glucoronic acid but they were not identified further. The mean recovery of the radioactive dose in the liver 5 days following administration was 3.3 to 3.4% whereas 0.9 to 2.3% and 41.2 to 46.5% of the radioactive dose was excreted in urine and faeces, respectively, during the 6-day collection period. Thus, 95 to 98% of the total excreted residues were recovered in faeces.

2.1.2. Calculation of pharmacological ADI, if relevant

Based on the available data, pharmacological effects are not expected at doses in the same range as toxicological effects. Consequently, in accordance with the Guideline on the approach to establish a pharmacological ADI (EMA/CVMP/SWP/355689/2006), there is no need for establishing a pharmacological ADI.

2.1.3. Overview of toxicology

Single-dose toxicity

Single dose toxicity of sisapronil was investigated in a series of non-GLP studies in the mouse and rat, as well as in one GLP study in the rat.

Sisapronil was of low acute toxicity following oral, intravenous and subcutaneous administration. In mice, single intravenous doses up to 3 mg/kg bw and single oral doses up to 30 mg/kg bw produced no adverse effects. In rats, single oral doses up to 10 mg/kg bw produced no adverse effects. Single oral doses of up to 200 mg/kg bw in rats were generally well tolerated with a temporary decrease in

food consumption at doses below 200 mg/kg bw in one study and clinical signs of hunched posture and piloerection for up to 2 days at a dose of 175 mg/kg bw in another study. The oral LD_{50} in the rat was determined to be 551.5 mg/kg bw.

Repeated dose toxicity

Short-term studies

In a 28-day repeated dose oral toxicity study in the dog with doses of 0, 1, 5 or 25 mg/kg bw/day effects on body weight and food consumption were observed. Target organs were the liver, thyroid and thymus. Increased liver weights were correlated with increased glycogen in hepatocytes. Vacuolar degeneration of individual hepatocytes was also observed. Decreased thymus weights in females correlated with an increased incidence of minimal to mild decreased lymphoid cellularity. Minimal thyroid follicular cell hypertrophy was also observed, however, these changes were not associated with definitive effects on thyroid hormone levels. Based upon these findings the NOEL was 1.0 mg/kg bw/day.

In a 28-day repeat dose oral toxicity study in the rat with doses of 0.1, 1 or 10 mg/kg bw/day, the target organs for toxicity were the liver and thyroid. Increased liver weights and hepatocellular hypertrophy were consistent with increased levels of metabolising enzymes. Increased thyroid weights and hypertrophy/hyperplasia of thyroid follicular epithelium were accompanied by increased thyroid stimulating hormone and decreased thyroxine levels. The NOEL was 0.1 mg/kg bw/day. Considering the results in the 90-day and 1-year rat studies the findings in the 10 mg/kg bw/day groups are considered adverse. Therefore the NOAEL in this study was 1.0 mg/kg bw/day.

A 2-month oral dose-range finding study in rats with doses up to 100 mg/kg bw/day was performed in order to define the maximum tolerated dose in a potential reproduction toxicology study. Central nervous system toxicity (tremor, hyperreactivity and tense posture) was observed following doses of 20 mg/kg bw/day and above.

Long-term studies

A rat 90-day repeated dose study at oral doses of 0, 0.1, 0.3, 1.0 or 10 mg/kg bw/day showed effects on liver and thyroid weights accompanied by histopathological findings and effects on hormone levels. These observations are consistent with the shorter repeated dose studies in the rat. The NOEL is 0.3 mg/kg bw/day, based on very slightly increased absolute and relative liver weights were observed in the 1.0 mg/kg bw/day females.

Consistent with the shorter repeated dose study in dogs the target organs for toxicity were the liver and the thyroid in a repeated dose study where dogs received doses of 0.3, 1.0 or 10 mg/kg bw/day for 90 days. Increased liver weights were accompanied by increased glycogen in hepatocytes. Vacuolar degeneration of individual hepatocytes was also observed. Minimal thyroid follicular cell hypertrophy was observed, however without increased thyroid weight or changes in thyroid hormones. It is nevertheless reasonable to assume that the mechanism of thyroid follicular cell hypertrophy in the dog is similar to the rat. There was no progression over time from 28 days to 90 days and the effect is considered as an adaptive response. and the effects observed at 0.3 mg/kg bw/day were considered not adverse, therefore a NOAEL of 1.0 mg/kg bw/day was derived from this study.

Consistent with all other repeated dose toxicity studies in rats and dogs, the target organs for toxicity were the liver (hepatocellular hypertrophy) and the thyroid in a 1-year oral toxicity study in the rat. Thyroid follicle cell adenomas were observed in 1, 2, 1, 3, and 8 males, and in 0, 2, 1, 3 and 2 females for treatment groups 0, 0.1, 0.3, 1.0 and 10 mg/kg bw/day, respectively. However, effects on the thyroid were considered rat specific and not relevant for the human risk assessment and so were

disregarded (see comments in relation to the mode of action study, below). Overall, it was considered that a clear NOAEL cannot be established from this study. Based on liver effects (increased absolute liver weights in females and increased liver cell hypertrophy in males at 0.3 mg/kg bw) 0.1 mg/kg bw/day is established as the NOEL and is considered to be the only limit dose that can be established from this study.

An assessment of the mode of action for sisapronil-induced thyroid tumour formation suggests that sisapronil induces UDP-glucuronosyltransferase (UDPGTs). The first step leading to tumor susceptibility is a decrease in circulating T4 (thyroxine) concentrations. Serum levels are decreased because sisapronil induces hepatic UDPGT activity toward T4 and T3 (triiodothyronine), increasing the conjugation of thyroid hormones in liver. In response to the hypothyroid state, thyroid stimulating hormone (TSH) synthesis and release is stimulated, and this is the key event that leads to thyroid follicular cell growth and hyperplasia. In response to the hypothyroid state and increased TSH (thyroid stimulating hormone) levels, the thyroid gland is stimulated to produce more T4, and over time, there is compensatory thyroid gland enlargement. The thyroid gland remains enlarged throughout up to 1 year of treatment, suggesting a chronic stimulation of function.

The available data provide evidence that increased hepatic clearance of T3 and T4 causes chronic stimulation of the thyroid–pituitary axis, seen acutely as an increase in TSH secretion, with long-term stimulation of the thyroid gland as a chronic consequence leading to tumour development. There is evidence for sisapronil that increased clearance of hormones in the liver leads to the long-term stimulation of the thyroid gland and increases the risk of thyroid tumour formation in rats. A 30-day mode of action study was performed in rats using oral administration. Sisapronil was administered at dose levels of 0, 1, 7.5, 10 or 15 mg/kg/bw day for 7, 14 or 30 days and fipronil was used as positive control. Observed effects on liver and thyroid weights, histological changes in liver and thyroid, lowering of thyroid hormone levels, increases in thyroid stimulating hormone and increased levels of metabolizing enzymes is indicative of the anticipated mode of action for sisapronil and supports the theory that thyroid tumour formation involves the disruption of homeostasis of the thyroid-pituitary axis by this mechanism. The rat is reported to be very susceptible to thyroid neoplasia secondary to hypothyroidism and modest changes in thyroid hormone homeostasis will promote tumour formation. Data in humans suggest that prolonged TSH stimulation of the thyroid is unlikely to induce malignant changes. This conclusion is consistent with the decision tree for the classification of substances causing thyroid tumours in rodents established by the European Chemicals Bureau. It is agreed that the rat is a sensitive species for tumour formation in the thyroid by this mechanism and therefore the findings in the thyroid are not appropriate endpoints upon which to base the ADI.

Reproductive toxicity, including developmental toxicity

The potential adverse effects of sisapronil on reproduction were assessed in a 2-generation rat study following oral (gavage) doses of 0.3, 2.0 and for F0 only, 15 mg/kg bw/day. Consistent with repeat dose toxicity findings in the other rat studies the liver and thyroid were the target organs in the F0 and F1 adult animals with a NOAEL at 0.3 mg/kg bw/day. There were findings of follicular cell hypertrophy and hyperplasia at 0.3 mg/kg bw/day with a dose-dependent increase in severity with nodular hyperplasia and follicular cell adenoma observed in the 15 mg/kg bw/day group males. No effects were observed on F0 reproductive parameters. In the F1 animals lower fertility and ovarian follicle counts were observed with a NOAEL at 0.3 mg/kg bw/day. Impaired follicular development could be attributable to hypothyroidism and the lower ovarian follicle count was likely due to the liver and thyroid effects noted. Male and female F1 pup birth weights and body weight gains were lower in the 15 mg/kg bw/day group compared to the control group. Postnatal survival was also severely affected in the high dose F1 pups with a NOAEL of 2.0 mg/kg bw/day.

Developmental toxicity studies were conducted in the rat and the rabbit following oral (gavage) doses of 0.3, 2 and 20 mg/kg bw/day on gestation days 6 to 20 and 0.3, 2 and 20 mg/kg bw/day on gestation days 7 to 28, respectively. Maternal toxicity in both the rat and the rabbit was observed as reduced food consumption and decreased body weight gain or mean body weight loss in the high dose animals. As a consequence lower mean foetal weights were observed in the rat, and in addition abortion and premature delivery was observed in the rabbit. There was no evidence of teratogenicity or other developmental toxicity. The NOAELs for maternal toxicity and foetal toxicity were considered to be 2.0 mg/kg bw/day in both rats and rabbits.

Genotoxicity

Sisapronil was tested for genotoxicity in the standard battery of tests including an *in vitro* bacterial reverse mutagenicity assay, an *in vitro* test for chromosomal effects in mammalian cells and an *in vivo* bone marrow micronucleus assay in rats. Sisapronil did not induce gene mutations with or without metabolic acvtivation, in the bacterial mutation assay. Sisapronil did not demonstrate clastogenic activity, with or without metabolic activation in human lymphocytes and was negative in the *in vivo* rat micronucleus assay. Based on the negative results observed in these assays it can be concluded that sisapronil is not genotoxic.

Carcinogenicity

No data from carcinogenicity studies were provided. The lack of carcinogenicity testing is accepted as sisapronil is not genotoxic, there is a plausible mode of action for the observed thyroid follicular hypertrophy/hyperplasia, the adenomas observed in the rat are not considered relevant for the human risk assessment, and none of the lesions associated with relevant structural alerts of sisapronil (identified by DEREK modeling) were observed in the 1-year rat study.

Studies of other effects including neurotoxicity and immunotoxicity

The neurotoxicity of sisapronil was evaluated in an oral single dose study in the rat at doses of 100 to 1000 mg/kg bw. Neurotoxicity (reflected by alterations in both functional observational battery and motor activity parameters) was observed from 500 mg/kg bw. The observed NOEL was 100 mg/kg bw. Based on the results of the acute and subchronic toxicology studies in rats and dogs, and target safety studies in cattle, it was concluded that sisapronil does not exert neurological effects at dose levels lower than those that elicit other toxicological effects.

Sisapronil was evaluated for skin and ocular irritation in the albino rabbit. Sisapronil was not irritating or corrosive to skin or eyes.

The potential of sisapronil to induce skin sensitization was assessed in the mouse local lymph node assay. Sisapronil was found not to be a skin sensitizer.

2.1.4. Calculation of the toxicological ADI or alternative limit

Summary of NOAELs/NOELs and all relevant studies

Species	Study type and duration	NOAEL (mg/kg/day)	NOEL (mg/kg/day)	Comments
Rat	28-day repeat-dose	1.0	0.1	-
Rat	90-day repeat-dose	1.0	0.3 (F), 1.0 (M)	Very slightly increased absolute and relative liver weights

Species	Study type and duration	NOAEL (mg/kg/day)	NOEL (mg/kg/day)	Comments
				observed in the 1.0 mg/kg/day females.
Rat	1-year repeat-dose	not determined	NOEL for liver effects 0.1	
Dog	28-day repeat-dose	5 (M)/ 1.0 (F)	1.0	-
Dog	90-day repeat-dose	1.0	0.3	-
Rat	2-generation reproductive toxicity study	0.3	Not determined.	LOEL for systemic toxicity = 0.3 mg/kg due to thyroid follicular cell hypertrophy and hyperplasia at 0.3 mg/kg. Not relevant for human risk assessment.

The NOEL of 0.1 mg/kg bw/day, based on liver effects seen in the one year repeated dose study in rats, is considered to be the appropriate starting point from which to derive the toxicological ADI. Using the standard uncertainty factor of 100 results in a toxicological ADI of 0.001 mg/kg bw and day (0.06 mg/person and day).

2.1.5. Overview of microbiological properties of residues

Sisapronil is not classified as an antimicrobial agent and it is not structurally related to antimicrobial agents used in human or animal medicine. Therefore, data on microbiological properties are not considered necessary.

2.1.6. Calculation of microbiological ADI

As sisapronil is not expected to possess antimicrobial activity the establishment of a microbiological ADI is not considered relevant.

2.1.7. Observations in humans

No data on the effects of sisapronil in humans were available.

2.1.8. Findings of EU or international scientific bodies

Sisapronil has not been evaluated by any other EU or international scientific bodies.

2.1.9. Overall conclusions on the ADI

The establishment of a pharmacological ADI and a microbiological ADI are not considered necessary for this substance. The toxicological ADI of 0.001 mg/kg bw (0.06 mg/person) is considered the relevant ADI for use in the consumer safety evaluation.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

The absorption, distribution, metabolism and excretion of [^{14}C]sisapronil were investigated in a pivotal total radioactive residues study in which cattle were given a single subcutaneous injection of 2 mg [^{14}C]sisapronil/kg bw. Blood samples for analysis of total radioactive residues were taken at 1 to 90 days after administration. Excreta were collected on days 10 to 12, 30 to 32 and 60 to 62 and tissues were collected at 10 to 90 days after administration for analysis of total radioactive residues and residue profiling. Total radioactive residues were analysed by liquid scintillation counting and residue profiling was performed by liquid and solid phase extraction followed by HPLC fractionation and liquid scintillation counting.

Plasma kinetics of sisapronil estimated from data obtained in two studies in which cattle were given single subcutaneous or intravenous injections of 2 mg sisapronil/kg bw were reported (non-GLP). Blood samples were taken at 1 to 84 or 140 days following administration. Plasma was analysed for sisapronil by LCMS/MS and pharmacokinetic parameters were estimated using non-compartmental methods.

Overall, the pharmacokinetic data in cattle demonstrate that residues of sisapronil are retained in tissues, with the highest levels present in fat, up to at least 90 days following a subcutaneously injected dose of 2 mg [^{14}C]sisapronil/kg bw and that unchanged sisapronil constitutes the major part of the total radioactive residues in all tissues except the liver. Distribution and excretion of total radioactive residues were shown to be similar in cattle given a subcutaneous injection of sisapronil and rats given an oral dose of sisapronil. Furthermore, a residue with the same retention time as the bovine liver residue M3 was present also in the liver of orally treated rats (see section 2.1.1).

Absorption

In the total radioactive residues study, the plasma concentrations of total radioactive residues showed that [^{14}C]sisapronil was released and absorbed from the injection site with a peak at 5 days after administration and that radioactive residues were present in plasma up to and including the last sampling point, 90 days after administration.

The studies of unlabelled compound showed that absorption of sisapronil was prolonged with a mean time to maximum plasma concentration of 12 to 16 days (and a mean maximum plasma concentration of 72 to 75 ng/ml) following subcutaneous injection and that sisapronil was present in plasma up to and including the last sampling point 140 days after administration. Subcutaneous bioavailability of sisapronil was estimated to be close to 100%.

Distribution

Total radioactive residues were detected in all edible tissues during the 90-day collection period. Combined renal and omental fat contained the highest levels of total radioactive residues at all time points. Mean concentrations decreased from 10195 µg equivalents/kg at 10 days after administration to 891 µg equivalents/kg at 90 days after administration. The decrease was consistent except for a temporary increase at 60 days after administration. A similar pattern of total radioactive residues depletion was generally observed also for the other edible tissues, with total radioactive residue concentrations being greater in the liver than in the kidney, and greater in the kidney than in the loin muscle.

The total radioactive residue concentrations in the injection site fluctuated over the sampling period with a peak mean level of 1061 µg equivalents/kg at 20 days after administration and a mean level of 436 µg equivalents/kg at the last sampling time 90 days after administration. The injection site surround tissue contained a mean total radioactive residue level of 418 µg equivalents/kg at 10 days after administration which had decreased to 40 µg equivalents/kg 90 days after administration.

The total radioactive residue concentrations in the injection site from day 30 to day 60 after administration (e.g., 169.4 to 187.8 µg equivalents/kg with a mean of 175.5 µg equivalents/kg on day 30) were markedly lower than the marker residue concentrations in the injection site from day 30 to day 60 after administration of sisapronil in the marker residue depletion study (e.g. 11800 to 73900 µg/kg with a mean of 29600 µg/kg on day 30) whereas the results for the other tissues were within the same range. Based on the results of the residue profiling in the total radioactive residue study, intact [^{14}C]sisapronil was stated to represent the major part of the total radioactive residues in all tissues except the liver. Mean injection site residue levels were comparable by day 90 and hence their use in determining the marker to total residues ratios is considered appropriate.

The highest mean recovery was observed in the liver, which contained 1.6% of the administered dose 10 days after administration.

The studies of unlabelled compound showed that the mean apparent volume of distribution following intravenous injection was high (24 l/kg/day).

Elimination

The studies of unlabelled compound showed that mean apparent plasma clearance following intravenous injection was low (0.87 l/kg/day) with a mean terminal half-life of 19 days. Mean terminal half-life and mean residence time following subcutaneous injection were estimated as 19 to 23 days and 32 to 48 days, respectively.

Metabolism

Based on the residue profiling, bovine liver was the only tissue in which residues other than the parent compound could be detected at amounts of at least 10% of total radioactivity. The bovine liver residue M3 represented 19 to 45% of total residues. A residue with the same retention time as the bovine liver residue M3 was present also in the liver of orally treated rats, where it represented 10 to 18% of the total residues (see Section 2.1.1). Several attempts to isolate and identify the bovine liver residue M3 from liver collected from the total radioactive residues study as well as from two non-radiolabeled residue depletion studies were undertaken but without success. As the results of the residue profiling analyses indicate that rats in the laboratory safety tests were exposed to the bovine liver residue M3, identification of the M3 residue is not considered critical.

The results of the residue profiling showed that unchanged sisapronil constitutes the major part of the total radioactive residues in all tissues except the liver. In fat, kidney and loin muscle, the percentage of sisapronil made up 97 to 100%, 96 to 100% and 90 to 100% of total radioactive residues, respectively. In the liver, sisapronil made up 51 to 74% of total radioactive residues.

The co-eluting residues M1 and M2 generally represented a major part of the total urinary residues. A residue with the same retention time was also seen in urine of orally treated rats (see section 2.1.1).

Excretion

The results from the total radioactive residues study in cattle showed that 0.3% and 16% of the total subcutaneously dosed radioactivity was excreted in urine and faeces, respectively, over the 9-day

collection period. Thus, 98% of the total excreted dose was excreted in faeces, which implies a relatively low importance of urinary excretion for the elimination of sisapronil.

2.2.2. Residue depletion studies

In a residue depletion study in cattle, levels of sisapronil were investigated in edible tissues at 30-day intervals up to 240 days following a subcutaneous injection of 2 mg sisapronil/kg bw.

Sisapronil was shown to be present in all edible tissues up to and including the last sampling point at day 240. A general pattern with a peak mean concentration at 30 days after administration followed by a slow decline was observed for all tissues except injection site muscle, for which the depletion of sisapronil was faster than for other tissues from 30 to 120 days following administration. The core injection site muscle contained the highest levels of sisapronil on day 30 and day 60 with a mean level of 29600 µg/kg on day 30 declining to 76.5 µg/kg on day 240. Otherwise the highest mean levels were seen in fat (7520 µg/kg on day 30 and 564 µg/kg on day 240) followed by, in decreasing order, liver (759 µg/kg on day 30 and 60.3 µg/kg on day 240), kidney (465 µg/kg on day 30 and 43.2 µg/kg on day 240), small intestine (232 µg/kg on day 30 and 45.5 µg/kg on day 240) and hindquarter muscle (172 µg/kg on day 30 and 32.4 µg/kg on day 240).

Selection of marker residue and ratio of marker to total residues

The results of the pivotal total radioactive residues depletion study in cattle showed that the parent compound sisapronil represented the major part of the total residues in all edible tissues and was quantifiable in all edible tissues up to the last collection at 90 days after administration. Sisapronil was therefore selected as the marker residue.

Based on the results of the residue profiling (see Section 2.2.1) a marker to total residues ratio of 1.0 was considered appropriate for fat and kidney. For muscle and liver, the conservative marker to total ratios of 0.90 and 0.50, respectively, are considered appropriate. The figures for the ratios of marker to total radioactive residues are based on selected tissue samples and not from all animals and on residues representing 10% or more of total radioactivity, which supports a more conservative approach.

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

An analytical method for the determination of residues of sisapronil in tissues of cattle based on liquid chromatography separation with MS/MS detection was available. Sisapronil and internal standard (sisapronil-[$^{13}C_2$, ^{15}N]) were isolated from tissues using a two step liquid extraction and the supernatant was injected onto a column for separation and the substance was detected by tandem mass spectrometry.

The calibration range was 5 to 1000 µg/kg. The lower limit of quantification was 5.00 µg/kg and the upper limit of quantification (ULOQ) was 1000 µg/kg for all tissues. It should be noted that concentrations in fat may exceed the ULOQ of 1000 µg/kg. Based on the presented validation data for high concentration fortified samples it has been demonstrated that the dilution process works.

The inter-run (between day) precision, expressed as inter-run coefficient of variation (% CV), were calculated for all obtained values and the values were considered as individual values and not as the number of replicates in each run multiplied by the number of runs. Since the measured values of individual coefficient of variation samples fulfilled the acceptance criteria, the statistical results are however not expected to influence the validity of the validation.

The relevant EU Reference Laboratory has reviewed the analytical method and is in agreement with the above review.

2.2.5. Findings of EU or international scientific bodies

Sisapronil has not been evaluated by any other EU or international scientific bodies.

3. Risk management considerations

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

As the substance is not expected to possess antimicrobial activity no effects on microorganisms used for industrial food processing are expected.

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

Sisapronil is a lipophilic compound that can easily pass membranes, as shown by the results of the residue studies. While studies in milk producing or egg laying animals have not been performed, it can be expected that residues would be present in milk or eggs for a long time in view of the very long half-life of the substance. This may imply the need for prolonged withdrawal periods for those food commodities. Due to its lipophilic nature, treatment of honey bees with sisapronil would be expected to lead to high levels of residues in wax, which argues against its use in this species.

In the absence of a maximum residue limit for milk, sisapronil should not be used in animals intended to produce milk for human consumption.

No additional relevant factors were identified for consideration of the risk management recommendations.

3.3. Elaboration of MRLs

The following maximum residue limits in bovine tissues are recommended based on the depletion profile seen in cattle administered sisapronil at the recommended dose:

Muscle: 45 µg/kg
Fat: 600 µg/kg
Liver: 60 µg/kg
Kidney: 45 µg/kg

Detailed calculation of theoretical daily intake of residues

Edible tissue or products	Daily consumption (kg)	MRL proposal µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product (µg)
Muscle	0.30	45	0.90	15
Fat	0.05	600	1.0	30
Liver	0.10	60	0.5	12
Kidney	0.05	45	1.0	2.25
			Total	59.25
			%ADI	99%

Based on these MRLs the total maximum theoretical daily intake from cattle tissues equates to 59.25 µg, which represents 99% of the ADI.

It is recognised that the proposed MRLs will prevent any further extension of the MRLs to other food commodities such as milk, eggs and honey. This is considered justified for this substance as its highly lipophilic nature means that its development for use in treating animals producing milk, eggs or honey for human consumption is unlikely.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for sisapronil in bovine species to other food producing species and commodities. Taking into account the current scientific knowledge the recommendations on extrapolation is justified as follows:

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
Sheep	No	Existing data indicate that the pattern of metabolites seen in rats, dogs and cattle is similar with the predominant residue being the parent compound. Based on this existing inter-species metabolism data, and that sheep and cattle are related species (ruminants), the assumption could be made that the parent compound would be the predominant residue in sheep as well. 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. As sheep meat is consumed on a regular basis and in large quantities, species specific data are considered necessary in order to quantify the possible impact on consumer safety of exposure to residues. No analytical method for monitoring of residues in sheep tissues was available for evaluation.
Goats	Yes	Existing data indicate that the pattern of metabolites seen in rats, dogs and cattle is similar with the predominant residue

		being the parent compound. Based on this existing inter-species metabolism data, and that goats and cattle are related species (ruminants), the assumption can reasonably be made that the parent compound will be the predominant residue in goat tissues and consequently can be accepted as the marker residue. 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 lack of species specific data are compensated for by the fact that consumption of goat meat, and consequently exposure to residues in goat meat, is limited. Therefore the same MRL values as established in cattle can be recommended in goats without compromising the safety of the consumer. No data are available to demonstrate that the analytical method proposed for monitoring of residues in cattle tissues is applicable for monitoring of residues in goat tissues but there is no reason for believing that it would not be.
Horses and rabbits	No	Existing data indicate that the pattern of metabolites seen in rats, dogs and cattle is similar with the predominant residue being the parent compound. Based on this existing inter-species metabolism data, the assumption could be made that the parent compound will be the predominant residue in horse and rabbit tissues. However, horses and rabbits (non-ruminants) are not considered to be closely related to cattle (ruminants), and due to the highly lipophilic nature of sisapronil, assumptions should not be made about the likely similarity of the residue profiles in these unrelated species. No specific pharmacokinetic or residue data were available for horses or rabbits 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. No data are available to demonstrate that the analytical method proposed for monitoring of residues in cattle tissues is applicable for monitoring of residues in horse or rabbit tissues.
Pigs	No	Existing data indicate that the pattern of metabolites seen in rats, dogs and cattle is similar with the predominant residue being the parent compound. Based on this existing inter-species metabolism data, the assumption could be made that the parent compound would be the predominant residue in pigs as well. However, pigs (non-ruminants) are not considered to be closely related to cattle (ruminants), and due to the highly lipophilic nature of sisapronil assumptions should not be made about the likely similarity of the residue profiles in these unrelated species. No specific pharmacokinetic or residue data were available for pigs 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. As pig meat is consumed on a regular basis and in large quantities, species specific data are considered necessary in order to quantify the possible impact on consumer safety of exposure to residues. No analytical method for monitoring of residues in pig tissues was available for evaluation.
Poultry (including eggs)	No	Metabolism can be significantly different in poultry compared to cattle. 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. In addition, as the recommended tissue MRLs use up 99% of the ADI, extrapolation to eggs is not possible. No analytical method for monitoring of residues in poultry tissues or eggs 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. In addition, it is expected that the use in lactating animals is not practical in view of the very long half-life of sispronil. Furthermore, as the recommended tissue MRLs use up 99% of the ADI, extrapolation to milk is not possible. No analytical method for monitoring of residues in milk was available for evaluation.
Salmonidae and other fin fish	No	Metabolism is generally less complicated in fish than in cattle. As the marker residue in cattle is the parent compound, and based on existing inter-species metabolism data, the assumption could be made that the parent compound would be the predominant residue in Salmonidae and other fin fish. However, fin fish are not considered to be closely related to cattle (ruminants), and due to the highly lipophilic nature of sisapronil assumptions should not be made about the likely similarity of the residue profiles in these unrelated species. No specific pharmacokinetic or residue data were available for these species 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. No analytical method for monitoring of residues in fin fish

		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. In addition, the very lipophilic nature of sisapronil can lead to accumulation in the wax, and therefore it is less suitable for use in beehives. No data are available to demonstrate that the analytical method proposed for monitoring of residues in cattle tissues is applicable for monitoring of residues in honey.

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

Having considered that:

- the toxicological ADI of 0.06 µg/person was established as the overall ADI for sisapronil,
- the parent compound sisapronil was retained as the marker residue,
- the ratio of marker to total residues calculated at 90 days after administration was 1.0 in fat and kidney, 0.90 in muscle and 0.50 in liver,
- extrapolation of the maximum residue limits recommended for cattle tissues to caprine tissues is considered appropriate,
- a validated analytical method for the monitoring of residues of sisapronil in edible bovine tissues is available,
- although it was not specifically demonstrated, the analytical method for monitoring of residues in bovine tissues is expected to be basically applicable for monitoring of residues in caprine tissues;

the Committee recommends the establishment of maximum residue limits for sisapronil in bovine tissues. Furthermore, and with reference to article 5 of Regulation (EC) No 470/2009, the Committee agreed to extrapolate the conclusions to caprine tissues, in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Sisapronil	Sisapronil	Bovine, caprine	45 µg/kg 600 µg/kg 60 µg/kg 45 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption.	Antiparasitic agent/Agents against ectoparasites

Based on these MRLs, the total theoretical maximum daily intake (TMDI) from bovine or caprine tissues is 59.25 µg/person, which equates to 99% of the ADI.

4. Re-examination of the CVMP opinion of sisapronil

Following receipt of the CVMP opinion for sisapronil, the applicant submitted detailed grounds for the re-examination of the opinion on 09 March 2015. These focused on the selection of the NO(A)EL in the 1-year oral toxicity study in rats, which is key for the derivation of the overall ADI, which in turn impacts on the MRLs.

4.1 Detailed grounds for re-examination submitted by the applicant

Further statistical analyses of the critical effects observed in the 1-year oral toxicity study in rats were presented in writing and at an oral explanation.

In relation to the increased absolute liver weights observed at 0.3 mg/kg bw/day in females, it was clearly shown that this effect was correlated to body weight and was not substance related, by using an analysis of covariance. The same analysis showed that the effects on liver weight were significant at 1.0 and 10 mg/kg bw/day.

Regarding liver cell hypertrophy, the data relating to this finding had not been subjected to statistical analysis when presented with the original application. With the grounds for re-examination, results of statistical analyses using a number of different nonparametric tests were provided. All tests clearly indicated that the results of the 0.3 mg/kg bw/day group were not statistically significantly different from the controls. Liver cell hypertrophy attained statistical significance at 10 mg/kg bw/day.

Based on the new analyses presented, it was argued that the NOEL from this study is 0.3 mg/kg bw/day, and that the ADI can be based on this value.

It was further argued that the applicant's originally proposed MRLs (200, 100, 100, and 2000 µg/kg for the marker residue sisapronil in bovine liver, kidney, muscle, and fat, respectively) can be established in view of the higher ADI.

4.2 Overall conclusions on grounds for re-examination

The CVMP assessed the detailed grounds for re-examination.

From the various repeat dose toxicity studies presented, it is clear that effects on the liver (characterised by increases in liver weight, hepatocellular hypertrophy and hepatocellular vacuolation) are treatment-related and that incidence increases with increasing dose. From a review of the raw data, it appears that the first signal for liver effects (increases in liver weight, hepatocellular hypertrophy) appears at 0.3 mg/kg bw/day in the 1-year oral toxicity study. However, the results of the new statistical analyses indicate that the findings in the 0.3 mg/kg bw/day group were not statistically significantly different from the controls.

Having considered:

- the absence of a statistically significant effect on liver weight at 0.3 mg/kg/day;
- the absence of a statistically significant effect on hepatocellular hypertrophy at 0.3 mg/kg bw/day;
- the absence of hepatocellular hyperplasia at any test dose (that is, changes in liver not progressing to proliferative change);

The CVMP can accept 0.3 mg/kg bw/day as the NOEL for the 1-year oral toxicity study and as the overall NOEL.

The NOEL of 0.3 mg/kg bw/day is considered to be the appropriate starting point from which to derive the toxicological ADI. Applying the standard uncertainty factor of 100 to this NOEL results in a toxicological ADI of 0.003 mg/kg bw (equivalent to 180 µg for a 60 kg person).

In light of this revised ADI, the following MRLs for the marker residue sisapronil are recommended:

Muscle: 100 µg/kg
Fat: 2000 µg/kg
Liver: 200 µg/kg
Kidney: 100 µg/kg

These MRLs reflect the distribution in the edible tissues and take into account the ratios of marker to total residues.

Detailed calculation of theoretical daily intake of residues

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product (µg)
Muscle	0.30	100	0.90	33
Fat	0.05	2000	1.0	100
Liver	0.10	200	0.5	40
Kidney	0.05	100	1.0	5
			Total	178.3
			%ADI	99%

Using the standard food basket, the MRLs add up to a maximum consumer intake of 99% of the ADI. This leaves no scope for extension of the MRLs to other food commodities, but considering the very long half-life of sisapronil, it's use in animals producing milk or eggs for human consumption would not be practical as it would require that large amounts of milk and eggs be discarded.

On this point, it should be noted that, in the absence of a milk MRL and noting that the substance has a long half-life (slow depletion from the body), there may be a need to restrict use of the substance in dairy animals for a substantial period of time prior to calving and the production of milk for human consumption. Likewise, even if, in future, MRLs could be extended to chicken tissues, the use in laying hens is expected to be largely restricted, if possible at all.

In view of the fact that the substance is not considered viable for use in milk producing and egg producing animals and considering the very long half-life of the substance, there is no clear benefit in restricting the portion of the ADI available for tissues, particularly as this would serve to reduce the viability of the substance for use in meat producing animals.

5. Recommendations following re-examination

Having considered that:

- the toxicological ADI of 180 µg/person was established as the overall ADI for sisapronil,
- the parent compound sisapronil was retained as the marker residue,
- the ratio of marker to total residues calculated at 90 days after administration was 1.0 in fat and kidney, 0.90 in muscle and 0.50 in liver,
- extrapolation of the maximum residue limits recommended for cattle tissues to caprine tissues is considered appropriate,
- a validated analytical method for the monitoring of residues of sisapronil in edible bovine tissues is available,
- although it was not specifically demonstrated, the analytical method for monitoring of residues in bovine tissues is expected to be basically applicable for monitoring of residues in caprine tissues,

the Committee recommends the establishment of maximum residue limits for sisapronil in bovine tissues. Furthermore, and with reference to article 5 of Regulation (EC) No 470/2009, the Committee agreed to extrapolate the conclusions to caprine tissues, in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Sisapronil	Sisapronil	Bovine, caprine	100 µg/kg 2000 µg/kg 200 µg/kg 100 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption.	Antiparasitic agents / Agents against ectoparasites

Based on these MRLs, the total theoretical maximum daily intake (TMDI) from bovine or caprine tissues is 178.3 µg/person, which equates to 99% of the ADI.

6. Background information on the procedure

Submission of the dossier	27 November 2013
Steps taken for assessment of the substance	
Application validated:	11 December 2013
Clock started:	12 December 2013
List of questions adopted:	10 April 2014
Consolidated response to list of questions submitted:	7 August 2014
Clock re-started:	11 August 2014
List of outstanding issues adopted:	9 October 2014
Clock re-started:	17 December 2014
CVMP opinion adopted:	15 January 2015

Submission of grounds for re-examination:	9 March 2015
Oral explanation:	9 April 2015
CVMP opinion adopted:	7 May 2015