22 August 2014
EMA/CVMP/656490/2013
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Cabergoline (bovine)

On 19 June 2014 the European Commission adopted a Regulation[1] establishing maximum residue limits for cabergoline in bovine, 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 (CVMP).

Cabergoline is intended for use in dairy cows for the reduction of udder involution duration during the drying-off period in the dairy cow and is administered as a single intramuscular injection.

CEVA Santé Animale submitted an application for the establishment of maximum residue limits to the European Medicines Agency on 21 September 2012.

Based on the data in the dossier, the CVMP recommended on 12 December 2013 the establishment of maximum residue limits for cabergoline in bovine species.

Subsequently the Commission recommended on 27 March 2014 that maximum residue limits in bovine species are established. This recommendation was confirmed on 17 April 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 19 June 2014.

[1] Commission Implementing Regulation (EU) No 677/2014, O.J. L 180, of 20.06.2014

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Cabergoline
Therapeutic class:	Agents acting on the reproductive system
Procedure number:	EU/12/202/CEV
Applicant:	Ceva Santé Animale
Target species:	Bovine
Intended therapeutic indication:	Reduction of udder involution duration during drying-off period in the dairy cow
Route(s) of administration:	Intramuscular

1. Introduction

Cabergoline is a D2 dopamine receptor agonist intended for use in dairy cows for reduction of udder involution duration during the drying-off period. The product is to be administered as a single intramuscular dose of 10 µg/kg bw into the neck, 60 days prior to calving.

Cabergoline is used in human medicine in the treatment of hyperprolactinaemic conditions (such as inhibition of puerperal lactation, prolactinomas) and as a second-line drug for the treatment of Parkinson's disease. In companion animals it is reported to be used for the treatment of false pregnancy in dogs and for suppression of lactation in bitches and queens.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties

Data from several experiments in the 1980s have shown that cabergoline inhibits prolactin secretion and consequently lactation due to its selective action on D2 receptors in the pituitary gland, which has clearly been demonstrated in rats, dogs and rabbits.

Inhibitory effects of cabergoline on prolactin secretion affected basal secretion as well as conditions of hypersecretion of prolactin. In lactating rats, a decreased serum prolactin level was seen at 12.5 µg cabergoline /kg bw and inhibition of lactation occurrred after the second administration of daily doses of 25 µg/kg bw of cabergoline, in a study in which cabergoline was administered for 4 days. In male rats, basal prolactin secretion was inhibited at 10 µg/kg bw and higher. At 3 µg/kg bw, no effects on prolactin secretion were observed in the male rat.

The lactating bitch has been shown to be more sensitive than the rabbit and rat: the average daily weight gains of puppies declined with increasing levels of cabergoline, causing complete suppression of lactation at 2.5 µg/kg bw. The NOEL for effects on lactation has been identified as 1 µg/kg bw. No impact on sexual hormones was identified in immature rats of either sex: luteinising hormone

secretion is not influenced. No androgenic, anti-androgenic, oestrogenic or anti-oestrogenic activities were seen.

No antithyroid effects were detected in rats. Cabergoline showed only weak affinity with noradrenergic α1 and α2 receptors, but did not influence norepinephrine metabolism. Cabergoline did not influence serotonin metabolism in the rat.

Many studies were performed to investigate the activity of cabergoline on the central nervous system in rats. The central dopamine system was slightly affected in rats at oral doses higher than those proven effective in inhibition of prolactin secretion.

An oral study was performed to investigate the emetic action of carbergoline in dogs. Emesis was noted after the lowest dose used (5 µg/kg bw) and the effect was more frequent at doses of 10 or 20 µg/kg bw. No NOEL could be drawn from this study since 5 µg/kg bw (the lowest dose used) caused emesis in 1 of 6 dogs.

Further investigations showed that other secondary pharmacodynamic effects of cabergoline occur at doses somewhat higher than those inhibiting prolactin secretion: a NOEL of 8 µg/kg bw was established for gastric emptying in rats. In dogs, no emesis or cardiovascular effects were seen at doses up to 2.5 µg/kg bw/day, which were proven to be effective at inhibiting prolactin secretion. The dog was the most sensitive species in respect to cardiovascular effects of cabergoline. No effect on diuresis was observed at 20 µg/kg bw in rats.

In humans, the lowest effective single dose is 0.2 mg/person, corresponding to about 3 µg/kg bw. When given twice weekly, a dose of 0.125 mg/person cabergoline has been shown to be effective in inhibition of prolactin secretion in some patients with hyperprolactinaemia. This dose corresponds to 2 µg/kg bw.

A study in male healthy subjects showed a dose-related decrease of serum prolactin at single oral doses of 0.2 mg/person and higher with inhibition of spontaneous circadian rhythm. The effect was of rapid onset and of long duration. No effects on growth hormone, thyroid stimulating hormone, luteinising hormone, cortisol or vital signs, ECG and laboratory profile were observed. Nasal stuffiness and headache occurred at 0.4 and 0.6 mg/ person. No effects were seen at 0.1 mg/person (n=3), which was considered a NOEL, equivalent to 1.7 µg/kg bw.

Pharmacokinetic properties (mainly in laboratory animals)

Cabergoline was rapidly absorbed after oral administration in rats and dogs, mainly via the ileum and jejunum. Peak plasma concentrations of total radioactivity of cabergoline were observed within 0.25 to 1 hour after single oral administration. Oral bioavailability was at least 50%. Area under the curve (AUC) and C_{max} increased approximately in proportion to the dose.

Blood plasma concentrations of total radioactivity declined with elimination half-lives of 17 to 40 hours, depending on the study and possibly the method of radioactive labelling of cabergoline. Unchanged drug plasma levels decreased with a plasma half-life of approximately 10 hours.

More than 60% of administered cabergoline was bound to plasma proteins *in vivo*.

Radiolabelled cabergoline was rapidly and extensively distributed in tissues and organs. After a single oral administration, peak levels of radioactivity were attained at 8 hours in most tissues and declined to about 15 to 20 % of peak values at 72 hours. The pituitary showed a somewhat slower decrease of radioactivity. Highest concentrations of cabergoline were found in the liver, followed by the pituitary gland, adrenals, spleen, kidneys and lungs (based on the AUC (0 to 72 hours) tissue to plasma ratio). At 168 hours, residues were still detected in most of the tissues.

The concentrations in the brain were generally low. Placental transfer of ^{14}C-cabergoline was found to be very low, whereas milk showed relevant concentrations.

Fifty to ninety percent of total radioactivity was eliminated in urine and faeces during the first 24 hours and was nearly complete at 96 hours after oral administration in rats. The main route of elimination of cabergoline was faecal (total excretion greater than 80%), largely as a result of extensive biliary excretion. A relevant amount of cabergoline underwent enterohepatic circulation.

Residues in urine accounted for 4 to 15% of the administered dose (oral or intravenous) in rats and 13 and 22% of the dose in monkey and man, respectively. Urinary excretion in rat and monkey was practically complete within 48 to 72 hours. In humans, elimination was slower, and there was a larger fraction excreted in urine and a correspondingly smaller fraction in faeces.

Cabergoline was rapidly metabolised. In plasma, the metabolites reached their peak levels within 1 to 2 hours after administration, whereas unchanged drug reached peak plasma levels at 8 hours.

In urine of humans, rats and monkeys, the carboxylic acid derivative 6-demethyl-6-allyldihydrolysergic acid arising from cleavage of the amide adjacent to the ergoline nucleus was the main metabolite. The relative concentration of 6-demethyl-6- allyldihydrolysergic acid decreased in the 24 to 72-hour urine, whereas the concentration of unchanged cabergoline increased. Other metabolic pathways of cabergoline resulted in the metabolites des-ethylcarbamoyl cabergoline and des-dimethylaminopropyl cabergoline. These metabolites were also detected in ADME and residue depletion studies in urine of cattle. Furthermore, an unidentified metabolite referred to as M2 was found in relevant amounts in liver and kidneys in cattle. M2 was also seen to be produced by rat liver microsomes and so is considered toxicologically covered in safety studies in rats. Exhalation was not a relevant excretion route for cabergoline.

During repeated (oral) treatment for 21 days, steady state plasma radioactivity concentration was found to be reached after 14 daily administrations. The mean concentrations of radioactivity were 1.8 times higher compared with those seen after single administrations. In the tissues, steady state cabergoline concentrations were reached between 14 and 21 daily administrations. The highest concentration was found in the liver 1 hour after the 21st administration, the highest relative increase (compared to single dose administration) was observed in the pituitary. The ratio of radioactivity concentrations between tissue and plasma increased with dosing time. Eight weeks after the last administration, the radioactivity decreased below the detection limit in about 1/3 of the tissues, and remained more than 10% of maximum level in several tissues. The total amount and ratio of excretion between urine and faeces was approximately the same as after single oral treatment. Steady state combined urinary and faecal excretion of ^{14}C-cabergoline was reached by the 7th administration.

2.1.2. Calculation of pharmacological ADI

In laboratory animals, the dog was the most sensitive species in respect to primary pharmacodynamic effects of cabergoline. A pharmacological NOEL of 1 µg/kg bw was identified based on inhibited prolactin secretion (suppressed lactation) in lactating bitches.

Since human data are considered most relevant for human risk assessment, it was agreed to use the pharmacological NOEL of 1.7 µg/kg bw derived from a study on healthy male subjects and based on decreased serum prolactin and inhibition of spontaneous circadian rhythm. Nevertheless, the use of an uncertainty factor of 10 is considered not sufficient, as there are some deficiencies regarding the design of the study: only male patients were used and there is high interindividual variability for normal prolactin secretion, which may not be sufficiently covered by a low number of test persons, especially at 50 and 100 µg/person (n=3). Therefore, an additional uncertainty factor of 5 is used.

A pharmacological ADI of 0.034 µg/kg bw i.e. 2 µg/person/day is established based on the NOEL of 1.7 µg/kg bw and applying an uncertainity factor of 50.

2.1.3. Overview of toxicology

Single-dose toxicity

Single oral dose toxicity studies have been performed in mice showing LD_{50} values of 331 mg/kg for female and 557 mg/kg for male mice. In rats, the LD_{50} was 588 mg/kg for females and 644 mg/kg for males. The main signs of toxicity were sialorrhea and prostration in rats and hyperactivity and convulsions in mice. Oral doses of 10 mg/kg produced no signs of toxicity in rabbits (two animals) and monkeys (one animal). After intravenous administration, LD_{50} values were reported as 38 mg/kg in female mice, 33 mg/kg in male mice, and 34 mg/kg in rats.

Repeated dose toxicity

Five oral repeated dose toxicity studies were provided in the dossier: two 90-day studies in rats, subchronic and chronic studies in monkeys and a 52-week study in rats.

In a study from 1983, cabergoline was administered to rats daily for 90 days by oral gavage at 3 dose levels (50, 500 and 5000 µg/kg/day). Each dose group consisted of 15 male and 15 female rats. There were no compound related mortalities. In females at the highest dose, a decreased body weight gain despite an increased food intake was observed. Skin lesions were seen, mostly in the high dose groups, and were more pronounced in rats with signs of neurological excitation. Some minor effects were seen in haematology and on urinalysis. The main effects seen in clinical chemistry are considered to be a dose-dependent reduction of cholesterol concentrations in females and an increase of urea in all dose groups in males and in the high dose females. The main target organ was the ovary. The adrenals and kidneys were also affected. An increase in absolute and relative ovarian weights at the highest dose was observed. Pituitary weights were decreased in all female dose groups in males of the mid and high dose groups. An increase in number of corpora lutea was observed in all dose groups. Zona fasciculate and reticularis of the adrenal cortex were hypertrophic and hyperplastic in rats of the mid and high dose groups. Incidence and severity of nephrocalcinosis was increased in the females of the high dose group. Increased ovarian weights, decreased pituitary weights and reduced cholesterol concentrations in females and decreased urea in males were observed at 50 µg/kg/day. As effects were observed at all doses tested no NOEL could be determined in this study.

In a further study (2011) in rats, cabergoline (solution in 1% ethanol in water) was administered daily for 90 days by oral gavage at 4 dose levels (0.05, 0.5, 5 and 50 µg/kg/day). Each dose group consisted of 10 male and 10 female rats. There were no compound related mortalities and no effects on food consumption or body weights. There were no effects on haematology, on urinalysis or on the oestrous cycle in female rats. There was a slight but significant reduction of cholesterol concentrations in females at the highest dose. The main target organ was the ovary. An increase in absolute and relative ovarian weights at the highest dose was observed. There were no other gross or microscopic changes related to compound intake, and no effects on ophthalmoscopy or neurological aspects of animal behaviour. The NOEL in this study was 5 µg/kg bw/day.

Additionally, cabergoline was daily administered to Sprague-Dawley rats for 12 months by oral gavage at 3 dose levels (50, 400, 3200 µg cabergoline as base/kg bw/day). Each dose group consisted of 35 male and 35 female animals. No dose-related deaths were noted (highest mortality in the mid dose groups: 2 males/7 females), but the mortality from the tenth month onward was clearly higher in females than in males. Dose-dependent slight decreases in body weights were seen in females at the high and mid doses. Slightly higher food intake in high dose females was observed. Dose-related dirty,

sometimes ruffled fur was noted intermittently in the two higher dose groups. Some minor effects were seen in haematology. Even at the lowest dose the following effects on clinical chemistry were seen in females: a dose-dependent reduction of cholesterol concentrations, calcium and albumin. The main target organ was the ovary. Dose-related increases were seen in ovary and adrenal weights, whereas the pituitary weights were dose-dependently decreased. An increase in number of corpora lutea was observed in all dose groups. Zona fasciculate and reticularis of the adrenal cortex were hypertrophic and hyperplastic in rats of the mid and high dose groups. Incidence and severity of nephrocalcinosis was increased in the females of the high dose group. The LOEL was 50 µg/kg bw/day, based on increased ovarian weights, decreased pituitary weights and reduced cholesterol concentrations in females and decreased urea in males. As effects were observed at all doses tested no NOEL could be determined in this study.

In Cynomolgus monkeys, cabergoline was administered at doses of 32, 320, and 3200 µg/kg bw by gavage to three males and three females per group for 13 weeks. At the highest dose (3200 µg/kg cabergoline per kg bw), high substance related mortality was observed (4 out of 6 animals died). The surviving animals showed anorexia and hyperkinesias, changes in haematology and chemistry, increase in the adrenals weights and at histopathology examination, hyperplasia of the zona fasciculate cells and reduced cytoplasmic vacuolization of the adrenals glands. At the mid dose (320 µg/kg cabergoline per kg bw) and at the end of the treatment period (week 13), an increased percentage of granulocytic neutrophils and decreased percentage of lymphocytes and an increase of beta-globulin fraction were observed. A NOEL of 32 µg/kg bw was established.

Cabergoline was administered daily by oral gavage in a 0.8% hydroxypropylmethylcellulose gel to monkeys for 52 weeks at 3 dose levels (50, 250, 1250 µg cabergoline as base/kg bw per day). Each dose group consisted of 8 male and 8 female animals. No treatment related effect was noted at the doses of 50 and 250 µg/kg bw in both sexes, whereas one male monkey was sacrificed due to its moribund condition at 1250 µg/kg bw. In the high dose male group, findings included repeated vomiting, slight increase of reticulocytes and increased values for sodium and potassium. A NOEL of 250 µg/kg bw was calculated.

Reproductive toxicity, including developmental toxicity

Two oral reproductive toxicity studies were performed in the rat.

In a preliminary one generation study cabergoline was administered daily for 2 weeks before mating, during mating, gestation, and lactation until day 20 post partum at dose levels of 0, 1.5, 3, 6, and 12 µg/kg bw. At 12 µg/kg bw/day, there was a reduction of the fertility index, an increase of the post-implantation loss and a reduction in mean pup body weight. At 6 µg/kg bw/day, an increase in mean post-implantation loss and a tendency toward a lower mean pup body weight at the end of lactation was observed. Consequently, the NOEL for fertility and maternal toxicity was 6 µg/kg bw/day and the NOEL for pup development is considered to be 3 µg/kg bw/day.

In the main two generation reproduction toxicity study cabergoline was administered orally by gavage to 50 animals per group (25 males and 25 females) at a dose of 0, 3, 6 or 12 µg/kg bw/day, 10 weeks prior to pairing, during pairing, through gestation and lactation until weaning of the pups (day 21 post partum). Selected pups from the F1 generation were selected and treated with cabergoline once daily by oral gavage for 10 weeks, from day 22 post partum until the start of the pairing period, during pairing, gestation and lactation until weaning of the pups (F2). F2 pups were sacrificed on day 4 post partum or at weaning. Cabergoline administration caused effects on body weight/ body weight gain: in F0 males, a tendency to lower body weight gains until week 20 was observed for the highest dose group. Females in the highest dose group showed a statistically significant reduced body weight gain measured at weeks 6 to 7 during the premating phase (n=25). Mean food consumption was lower than

control in the high dose group during the lactation period. However during gestation and lactation, the number of pregnant females in that group was considered not to be sufficient to allow a reliable conclusion on effects of cabergoline. No conclusion could be drawn on food consumption in the high dose group. In F1 pups in the highest dose group, litter mean body weight was reduced during lactation. Furthermore, mean food consumption was lower from day 7 post partum at 12 µg/kg bw/day and during the last week of the lactation period at 6 µg/kg bw/day. In female F2 pups, a lower mean body weight gain was seen from day 7 post partum, leading to a lower mean body weight at the end of the lactation period at 6 µg/kg bw/day and higher. The fertility index was clearly reduced in the highest dose group, with the effect being more pronounced in the F0 generation (fertility index of 12%) than in the F1 generation (54%). The high number of non pregnant animals was attributed to the treatment with cabergoline. Due to the low number of pregnant F0 females achieved in the highest dose group it was not possible to draw any definitive conclusions on the effects seen during the gestation and lactation periods, at pathological examination, or on the F1 generation. However, an effect of the test item during the lactation period on food consumption, parental body weight gain and on pup body weight gain was strongly suspected. Under the experimental conditions of this study, the dose level of 6 µg/kg bw/day was considered to be the NOEL for fertility in F0 and F1 generations. For maternal toxicity and developmental toxicity a NOEL of 3 µg/kg/day is established based on reduced food consumption of F1 dams during lactation and reduced bodyweight at the end of lactation in female F2 pups.

Developmental studies were performed in two species: rats and rabbits.

In the rat in a preliminary study, cabergoline was administered daily to pregnant rats at doses of 0, 6.25, 12.5, 25 and 50 µg/kg bw from gestation day 5 to gestation day 19. A complete inhibition of implantation at 50 µg/kg bw/day and a marked increase in the pre-implantation loss at 25 µg/kg bw/day were observed. No adverse effects considered related to test item administration were noted at the external examination of the foetuses or placenta. Therefore the dose levels selected for the main developmental toxicity study in the rat were set at 10, 20 and 40 µg/kg bw per day.

An oral gavage developmental toxicity study was performed in rats: 28 mated females per group received 10, 20 or 40 µg/kg bw cabergoline per day from day 6 to 19 of gestation. No mortalities occurred. There were no effects on food consumption in maternal animals, but effects on body weights between gestation day 6 and 9 and between gestation day 18 and 20 were observed at 20 µg/kg bw/day (and higher). The mid dose produced a slight increase in the numbers of females with complete pre-implantation losses. At the highest dose, 24/28 dams showed complete intrauterine mortality caused by pre-implantation and/or early embryonic losses. There were only 2 viable litters for evaluation from high dose dams. There was no increased incidence of visceral or skeletal abnormalities, although the numbers for evaluation were low at the high dose. The NOELs in this study were 10 µg/kg bw/day for maternal toxicity and 20 µg/kg bw/day for developmental effects. There was no evidence for a teratogenic effect of cabergoline in the rat.

In a preliminary dose range study cabergoline was administered daily to pregnant rabbits at dose levels of 0, 0.5, 1, 5, and 10 mg/kg bw from gestation day 6 to 27. Although body weight gains for the whole period in test groups were statistically not significantly different from those of the control group, a dose dependent reduction of mean body weight gain between gestation day 6 and 9 was observed in all treated groups. This was associated with a decreased mean food consumption in all treated groups. The increase of early embryonic loss at 10 mg/kg bw/day and post implantation loss at both highest dose groups was not seen in the main study and can therefore be considered not to be treatment related. No further substance related effects were seen.

In the pivotal study, groups of pregnant rabbits were given oral doses of 0, 0.5, 2 or 10 mg/kg bw/day from days 6 to 27 of gestation. Effects on body weight gain were observed immediately after the onset

of treatment between gestation day 6 and 9: a body weight loss was seen in does in the high dose and mid dose groups. Already at 0.5 mg/kg bw, a decreased mean body weight gain was seen. These effects were associated with a dose-dependent decrease in mean food consumption. No further substance related effects were observed. Based on the effects on body weight gain after onset of the cabergoline administration, no NOEL for maternal toxicity could be established. A NOEL of 10 mg/kg bw/d was determined for developmental toxicity based on the absence of relevant findings at all doses. No teratogenic effect was observed.

Genotoxicity

Cabergoline was tested in standard test battery for its genotoxic potential.

In a bacterial reverse mutation assay (Ames test) four strains of *Salmonella typhimurium,* TA98, TA100, TA1535, TA1537 and one strain of *Escherichia coli,* WP2 *uvrA,* were used in three independent experiments using the plate incorporation method or the pre-incubation method. In all tests, none of the observed reversion colony numbers were above the respective biological threshold value. Carbergoline is considered non mutagenic in the bacterial reverse mutation assay.

In a mouse lymphoma assay, L5178Y TK+/- cells were exposed to different concentrations of cabergoline, with or without metabolic activation (S9). In the 3 hour incubation test cells were exposed to the following cabergoline concentrations: 200; 100; 50; 40; 30; 20; 15; 10; 5; 2.5; 1.25 and 0.625 µg/ml. In the 24 hour incubation test the following concentrations were used: 100; 50; 40; 30; 20; 15; 10; 5; 2.5; 1.25 and 0.625 µg/ml. Treatment with the test item did not result in a statistically or biologically significant dose dependent increase in mutation frequency either in the presence or absence of S9.

In an *in vivo* micronucleus test, male mice were administered oral doses of cabergoline at 400, 500 or 600 mg/kg bw 24 hours before sacrifice and 600 mg/kg bw 48 hours before sacrifice. Bone marrow was sampled from femurs and prepared for microscopic analysis. The frequencies of micronucleated polychromatic erythrocytes in animals in the test groups were comparable to the values found in the corresponding negative control group.

Based on these data it can be concluded that cabergoline is devoid of any genotoxic potential.

Carcinogenicity

No data from carcinogenicity studies were provided. This is considered acceptable since cabergoline has no structural similarity with known carcinogens, no pre-neoplastic lesions have been identified in toxicity studies and no mutagenic potential was detected in the standard battery of tests.

Studies of other effects including immunotoxicity and neurotoxicity

No signs of immunotoxicity and neurotoxicity were observed in repeat dose toxicity studies. Therefore, no specific studies were performed.

2.1.4. Calculation of the toxicological ADI or alternative limit

The lowest NOEL of 3 µg/kg bw has been identified in a 2 generation reproduction study in rats.

Applying an uncertainty factor of 100, a toxicological ADI of 0.03 µg/kg bw i.e 1.8 µg/person has been established.

2.1.5. Overview of microbiological properties of residues

The microbiological properties of cabergoline have not been specifically investigated. This is acceptable as cabergoline has been used in human medicine for almost 20 years and there is no indication that it possesses antimicrobial activity.

2.1.6. Calculation of microbiological ADI

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

2.1.7. Observations in humans

Cabergoline is used therapeutically for its inhibitory effect on prolactin secretion for the treatment of prolactinomas, for the inhibition of puerpal lactation and for the treatment of parkinsonism. Its safety profile in humans is well known. The most common undesirable effects are asthenia, fatigue, dizziness /vertigo, headache, depression, sleep disturbance, somnolence, abdominal pain, dyspepsia, gastritis, nausea, constipation, vomiting, hot flushes and breast pain. The only major adverse effect that has occurred in patients given cabergoline and other dopamine agonists is valvular heart disease and cardiac valve regurgitation, when given at daily doses greater than 3 mg and for periods of at least 6 months.

There were no effects of cabergoline in 226 pregnant women exposed to 0.25 and 2 mg for periods of 3 weeks or longer. Neither foetal exposure to cabergoline through early pregnancy, nor doses of around 1 mg from near to conception to the end of gestation showed effects on the outcome of pregnancy.

The lowest effective single dose is 0.2 mg/person, corresponding to about 3 µg/kg bw. When given twice weekly, a dose of 0.125 mg/person has been shown to be effective in inhibition of prolactin secretion in some patients suffering of hyperprolactinaemia. This dose is equivalent to 2 µg/kg bw.

2.1.8. Findings of EU or international scientific bodies

Evaluations from EU or other international bodies were not available.

2.1.9. Overall conclusions on the ADI

A pharmacological ADI of 0.034 µg/kg bw i.e. 2 µg/person/day and a toxicological ADI of 0.03 µg/kg bw i.e. 1.8 µg/person/day were established. The lower of these two ADIs, i.e. 1.8 µg/person/day, was established as the overall ADI. It is noted that the value of the pharmacological ADI is very close to that of the toxicological ADI and provides support for the position that the toxicological ADI is a suitable reference value for use in the consumer safety assessment.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

The pharmacokinetics of cabergoline were studied in two absorption, distribution, metabolism and elimination (ADME) studies following a single intramuscular injection of 10 µg ^{14}C-cabergoline in

lactating cattle. The animals were slaughtered at 12, 72 and 120 hours and at 10 and 20 days after treatment, respectively. Urine, faeces, plasma milk and tissues were sampled and analysed with the following methods: liquid scintillation counting or combustion/LSC for determination of total radioactivity (TRR), thin-layer chromatography (TLC) for co-chromatogaphy of urine with cabergoline and reference substances and LC-MS/MS for qualitative and quantitative analysis of ^{14}C-cabergoline and metabolite ^{14}C- des-ethylcarbamoyl cabergoline in tissues.

Cabergoline was shown to be rapidly absorbed, distributed and excreted. The maximum concentration of total residues in plasma was reached between 0.5 and 6 hours after administration. Parent cabergoline in plasma peaked between 4 and 8 hours after administration and declined to below detectable concentrations at 120 hours after administration.

The highest concentrations of ^{14}C-total residues were measured in injection site, lungs, kidney, bile and liver at 12 hours after administration. ^{14}C-residues declined quickly, with the highest elimination rates in the first 3 days. ^{14}C-total residue concentrations in fat, skeletal muscle and milk were very low. Overall recovery of ^{14}C-cabergoline accounted for 86.15% of the dose with 14.10% and 56.46% of the dose excreted in the urine and faeces, respectively. The percentage of the dose recovered in selected tissues accounted for 15.59% of the dose.

The metabolism of cabergoline in cattle was investigated in plasma, urine and tissues (liver, kidney) . Plasma and milk were analysed for parent cabergoline and the metabolite des-ethylcarbamoyl cabergoline. No investigations in faeces were provided. Results indicated that cabergoline is rapidly and extensively metabolised.

In pooled urine (0 to 120 hours) parent compound was not detected but several minor metabolites were found where each component represented less than 10% of the dose. Identified urinary metabolites were des-ethylcarbamoyl cabergoline, des-dimethylaminopropyl cabergoline, and a carboxylic acid derivative of cabergoline which accounted for up to 1.5%, 0.5% and 5.5% of the dose, respectively. These metabolites were also found in the urine of rats.

In plasma and milk parent cabergoline was detected in very low quantities. The metabolite des-ethylcarbamoyl cabergoline could not be detected in plasma (less than the limit of quantification of 0.00234 µg/l) or milk (less than the LOQ of 0.025 µg/l).

Three tissue metabolites M1, M2 and M3 were found. The major metabolite M2, which could not be chemically identified, represented 22 – 27% of total extractable metabolites in liver and 10% of total extractable metabolites in kidney up to 5 days after administration. The other metabolites, the metabolite M1 (also not identified) and M3 (corresponding to a carboxylic acid derivative of cabergoline and also observed in urine of rats) were minor components in liver and kidney and represented less than 10% of total extractable metabolites.

Several studies were performed with the aim of identifying the M2 metabolite present in liver and kidney of cows. The following methods were used: LC-MS/MS analysis of four different analytical reference compounds, attempted chemical synthesis of M2, production of M2 metabolite by biomimetism, metabolism study in microsomes from rat and cow (*in-vitro*) and production of M2 metabolite following intramuscular administration to the cow.

All attempts to synthesise M2 failed and structural identification was therefore not possible. Production of pure M2 *in vitro* and *in vivo* was limited to small amounts of the metabolite.

However, it was possible to show, by HPLC MRM and MS/MSMS analysis, that the metabolites produced by rat and cow microsomes were identical to M2 metabolite from cow liver and urine samples. The peak observed at the relative retention time in rat and bovine microsomes samples was identical to that of M2 naturally observed in cow liver and urine.

The metabolite fraction from microsome supernatants from the rat were additionally used to test for pharmacological activity on D2 receptors in comparison to cabergoline. It was demonstrated that the M2 metabolite did not add significantly to cabergoline activity on D2 receptors (ie it had negligible activity).

In the above mentioned ADME studies a high and persistent concentration of radioactivity was observed in liver and kidney. The extraction recovery of total radioactive residues in liver decreased with increasing sample time and declined from 69.9 % at 0.5 hours after administration to 42% at 3 days, 33% at 5 days, 23% at 10 days to 15% at 20 days after treatment. Results indicated a relatively high portion of non-extractable residue, already at early time points after administration of ^{14}C-cabergoline (58% at day 3 after administration).

2.2.2. Residue depletion studies

In radiometric absorption, distribution, metabolism and elimination (ADME) studies in dairy cows (n=6) tissues and milk were investigated following a single intramuscular injection of approximately 10 µg ^{14}C-cabergoline per kg bw. The animals were slaughtered at 12, 72 and 120 hours and in a second study at 10 and 20 days after treatment. Liver, kidney, muscle, fat and injection site (core and surround) were collected. Milk was sampled once from each animal at sacrifice. ^{14}C-total radioactivity was determined using liquid scintillation counting. Parent ^{14}C-cabergoline was quantified using a validated LC-MS/MS method. The limit of quantification was 0.125 µg/kg in liver, 0.250 µg/kg in kidney, 0.075 µg/kg in muscle, 0.050 µg/kg in fat and 0.00625 µg/l in milk.

Mean concentrations of total radioactivity were highest in kidney (140 µg equivalents/kg), liver (87.5 µg equivalents/kg) and at the injection site (189 to 217 µg equivalents/kg) at 12 hours after administration. Only low concentrations were detected in fat (1.7 µg equivalents/kg) and muscle (4.56 µg equivalents/kg) at this early time point after administration. Radioactivity continuously depleted over the study period. Depletion was rapid in kidney (37.4, 22.6, 11.2 and 4.12 µg equivalents/kg) and injection site core (19.5, 18.9, 37.1 and 6.25 µg equivalents/kg) but less in liver (80.8, 76.3, 44.6 and 23.7 µg equivalents/kg) at 3, 5, 10 and 20 days after administration respectively.

Parent ^{14}C-cabergoline was detected in relevant concentrations in all edible tissues. Highest mean concentrations of parent drug were detected 12 hours after administration in kidney (92.6 µg/kg), liver (31.4 µg/kg) and injection site core (180.3 µg/kg), and declined rapidly to concentrations of 16.6 µg/kg, 5.6 µg/kg and 7.8 µg/kg at 72 hours after administration, respectively and further to values below the limit of quantification of the analytical method at day 20 after administration.

Mean total ^{14}C-radioactivity concentration in milk samples were low, at 0.678 µg equivalents/kg at 12 hours, 0.579 µg equivalents/kg at 72 hours and 0.387 µg equivalents/kg at 120 hours. Mean concentrations of ^{14}C-cabergoline in milk accounted for 0.340 µg equivalents/kg at 12 hours and declined to 0.127 and 0.0777 µg equivalents/kg at 72 and 120 hours, respectively.

Two radiometric residue studies were conducted in pregnant dairy cows (each n=4). Milk was investigated following a single intramuscular injection of approximately 9 to 11 µg ^{14}C-cabergoline per kg bw at drying off, on average forty to sixty days before calving (range: 32 to 56 days). Milk was collected as colostrum and at the first milk release for the first 10 days following calving. All cows were milked twice a day. Concentrations of total ^{14}C-radioactivity expressed as µg cabergoline equivalents/kg were very low after calving and in the range of 0.025 to 0.050 µg equivalents/kg at day 1 and declined to less than 0.017 to 0.021 µg equivalents/kg at day 2. After this time (up to day 10 after calving) concentrations of radioactivity in milk were below the limit of detection (i.e. less than

0.017 µg equivalents/kg). Using LC-MS/MS analysis the concentration of parent ^{14}C-cabergoline in milk was below the limit of quantification (0.00625 µg/kg) at all times from the first milking onwards. The major urinary metabolite des-ethylcarbamoyl cabergoline could not be detected in milk (limit of quantification: 0.025 µg/kg).

In a non-radiolabelled residue depletion study in pregnant dairy cows (n=10) milk was investigated following a single intramuscular injection of approximately 10 µg cabergoline per kg bw at the beginning of the drying off period, on average 63 days before calving (range: 46 to 91 days). Milk was collected as colostrum and at the first milk release for the first 14 days following calving. All cows were milked twice a day. The concentration of cabergoline and its metabolite des-ethylcarbamoyl cabergoline in milk were determined by a validated LC-MS/MS method. The limit of quantification (LOQ) was 0.050 µg/kg for cabergoline and 0.200 µg/kg for des-ethylcarbamoyl cabergoline. Neither the parent drug, cabergoline, nor its metabolite des-ethylcarbamoyl cabergoline were detectable from the first milking to the 28th milking after calving.

In a non-radiolabelled residue depletion study in dairy cows (n=20) liver, kidney muscle, fat and injection site (core and surround) were investigated following a single intramuscular injection of average 8.6 µg cabergoline per kg bw (maximum injection volume of 5 ml per site). The animals were slaughtered at 3, 6, 11, 16 and 21 days after treatment. Cabergoline was quantified using a validated LC-MS/MS method. The limit of quantification was 0.125 µg/kg in liver, 0.250 µg/kg in kidney, 0.075 µg/kg in muscle and 0.050 µg/kg in fat. Cabergoline concentrations were highest in kidney and liver at all slaughter days and declined from maximum 29.91 µg/kg and 9.04 µg/kg at day 3, respectively to below the limit of quantification at day 21 in all samples. Residue concentrations in fat and muscle were significantly lower and depleted from maximum concentrations of 1.09 µg/kg and 1.37 µg/kg at day 3, respectively to below the limit of quantification at day 11 post application. Residue concentrations at the injection site (core and surround) were below the limit of quantification in all samples at day 16 for the first time.

Investigation of bioavailability of bound residues

A study was conducted to determine the bioavailability of non-extractable (bound) residues in liver using the method of Gallo-Torres (1972). However, the sensitivity of the test system to detect orally ingested ^{14}C-cabergoline derived 'bound' residues at 10 days after administration to rats was considered to be insufficient. No quantitative conclusions on the amount of bioavailable/non-bioavailable residues from the bound residues could be drawn as the total dose of approximately 10 µg ^{14}C-cabergoline/kg administered per rat over three consecutive days was only one order of magnitude higher than the limit of quantification of the combustion-LSC used. Even if a significant proportion of 'bound' residues was bioavailable from liver tissue and 'accumulated' in rat liver, it could not be regarded as assured that concentrations above limit of quantification would have been present in rat liver at 10 days after treatment (when samples of liver were taken).

The available data also do not allow the quantification of bioavailable ^{14}C-cabergoline, excreted in faeces after biliary secretion. As no measurements in bile were conducted, no conclusion is possible on whether ^{14}C-cabergoline recovered in faeces stems from unabsorbed non-bioavailable substance in the gastrointestinal tract or has been absorbed and biliary excreted.

Although study results do not rule out the possibility that a fraction of the orally administered ^{14}C-cabergoline dose might become bioavailable in rats, they do at least indicate that the total amount of ^{14}C-cabergoline would not become bioavailable. Given these reservations, the studies can be accepted as an indication of possible incomplete bioavailability of residues from liver tissues, but they do not allow quantification of the amount of "bound" residues.

Selection of marker residue and ratio of marker to total residues

The parent compound cabergoline can be accepted as an appropriate marker residue as it comprises the majority of the tissue residues and is detected over a sufficiently long period after administration (up to ten days). Residues were greatest in liver and kidney.

For tissues the marker to total ratios at 0.5, 3, 5 and 10 days were calculated as follows: 0.36, 0.07, 0.04, 0.005 for liver, 0.66, 0.44, 0.28, 0.105 for kidney, 0,88, 0.73, 0.473, 0.058 for muscle, 0.54, 0.39, 0.111 and "not determined" for fat, and 0.95, 0.40, 0.303, 0.014 for injection site (core). For milk the marker to total ratios at 0.5, 3 and 5 days have been calculated with 0.50, 0.22 and 0.20, respectively. The ratios of marker to total residues declined with time and ratios at day 5 represented the latest study day with the marker residue cabergoline measured in all target tissues. Ratios at day 5 are considered suitable to be used to calculate the amount of total residues.

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 cabergoline in tissues and milk of cattle based on HPLC with MS/MS detection was available. Cabergoline was isolated from tissues and milk using a liquid/liquid extraction and purified by a chromatographic separation. The quantification was performed by tandem mass spectrometric detection.

The method was clearly described and was validated according to Volume 8 of The rules governing medicinal products in the European Union. The limit of quantification for cabergoline in tissues was 0.075 µg/kg for muscle, 0.125 µg/kg for liver, 0.250 for kidney, 0.050 for fat and 0.050 µg/kg for milk representing the lowest concentration levels which were determined in the recovery experiments with sufficient accuracy and precision.

The relevant European reference laboratory was consulted on the analytical method and agreed with the conclusions above.

2.2.5. Findings of EU or international scientific bodies

None available.

3. Risk management considerations

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

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

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

No such considerations were identified.

3.3. Elaboration of MRLs

MRL values for cattle liver, kidney, muscle and fat are recommended based on the residue depletion data and ratios of marker to total residues at day 5 as follows:

Tissue	MRL
Muscle	0.15 µg/kg
Fat	0.10 µg/kg
Liver	0.25 µg/kg
Kidney	0.50 µg/kg
Milk	0.10 µg/kg

Calculation of theoretical daily intake of residues

Study results indicate that the amount of bioavailable residues in liver tissues is lower than 100% but do not allow deriving quantitative values. Therefore, the amount of total residues for liver tissue was calculated based on the uncorrected ratio of marker to total residues.

Tissues	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio Marker/Total residue*	Amount total residues (µg)
Muscle	0.30	0.15	0.471	0.095
Fat	0.05	0.10	0.111	0.045
Kidney	0.05	0.50	0.280	0.089
Liver	0.10	0.25	0.044**	0.575
Milk	1.5	0.10	0.202	0.742
			Total	1.546
			% ADI	85.8

* ratio calculated at day 5 (study ST-ADME/646.0/1049)
** not corrected for extractable residues

Proposed MRLs do not reflect the tissue distribution as further lowering of MRLs for fat and muscle is not possible based on the limits of quantification set.

Ratios of marker to total residues at day 5 were used to calculate the amount of total residues, as this is the last day with measurable concentrations of marker residue. As ratios decline with time, ratios would be even lower at the time point at which the theoretical maximum daily intake (TMDI) falls below the ADI and therefore the amount per edible tissue would be somewhat higher. But this will be compensated for by the fact that only 86% of the ADI is utilized with the MRLs proposed and the amount of bioavailable residues from liver tissues is likely to be lower than 100%.

Residue depletion data demonstrate that residues in kidney can be expected to deplete to the relevant MRL more slowly than residues in other tissues including the injection site. Consequently, the CVMP recommends that, where the entire carcass is available, monitoring of residues of cabergoline should focus on kidney in preference to other tissues, as compliance with the kidney MRL can be expected to indicate that residues in other tissues will also be compliant with their respective MRLs.

3.4. Considerations on possible extrapolation of MRLs, if applicable

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 cabergoline in bovine to other food

producing species and commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as described below.

Cabergoline inhibits prolactin secretion and lactation. As this effect is considered only to be relevant to mammals, extrapolation to non-mammalian species is not considered.

Animal species/ food commodities	Extrapolation possible (Yes/No)	Justification
Sheep Goat Horses Pigs Rabbits Milk from species other than cattle	No	Existing data indicate that the metabolic pathway seen in rats, monkeys, cattle and humans is qualitatively similar. However, as evident from cattle data, the marker residue is present at very low concentrations in tissues and milk and MRLs were therefore based on 2x LOQ of the analytical method. Also the ratios of marker to total residues are generally very low and quickly decrease over time. This high pharmacokinetic/metabolic variability would introduce unacceptable uncertainty in an extrapolation of the MRLs to other species and consequently would not allow consumer safety to be ensured. In addition, no specific pharmacokinetic or residue data were available to confirm the marker residues in food producing species other than cattle. No analytical method for monitoring of residues in other food producing species tissues was available for evaluation.

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

Having considered that:

- the toxicological ADI of 1.8 µg/person per day is considered the overall ADI for cabergoline;
- the parent drug cabergoline was identified as the marker residue;
- the ratios of marker to total residues were calculated at 5 days as 0.471 for muscle, 0.111 for fat, 0.044 for liver, 0.280 for kidney and 0.202 for milk respectively;
- liver and kidney were the tissues with highest cabergoline residues, while residue concentrations in muscle, fat and in milk were very low;
- there were non extractable (bound) residues in liver, however the fraction of bioavailable “bound” residues after oral ingestion could not be precisely determined based on available data;
- an analytical method based on LC/MS/MS for the determination of cabergoline in edible tissues and milk of cattle is available which is validated in accordance with the requirements of Volume 8 taking into account the MRL values proposed;
- for the purposes of monitoring of residues of cabergoline it is recommended that, where the entire carcass is available, kidney should be sampled in preference to other tissues as residues in kidney

are expected to deplete to the MRL more slowly than residues in other tissues and so will provide a better basis for verifying compliance with the withdrawal period;

the CVMP recommends the establishment of maximum residue limits for cabergoline in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Cabergoline	Cabergoline	Bovine	0.10 µg/kg 0.25 µg/kg 0.50 µg/kg 0.15 µg/kg 0.10 µg/kg	Fat Liver Kidney Muscle Milk	NO ENTRY	Prolactin inhibitor

Based on these MRLs, the total theoretical maximum daily intake (TMDI) from tissue of cattle was 1.546 µg/kg which accounts for 86 % of the overall ADI.

4. Background information on the procedure

Submission of the dossier	21 September 2012
Steps taken for assessment of the substance	
Application validated:	10 October 2012
Clock started:	11 October 2012
List of questions adopted:	7 February 2013
Consolidated response to list of questions submitted:	13 September 2013
Clock restarted:	14 September 2013
CVMP opinion adopted:	12 December 2013