



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR) Bupivacaine (bovine species)

On 12 November 2020, the European Commission adopted a Regulation¹ establishing maximum residue limits (no MRL required classification) for bupivacaine in bovine species (calves up to 2 months of age only), 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.

Bupivacaine is intended for cutaneous and/or epilesional use in calves of less than 2 months of age for local anaesthesia after hot-iron disbudding at a maximum dose of 0.4 mg/kg bw bupivacaine hydrochloride.

Medical Ethics UK Ltd submitted to the European Medicines Agency an application for the establishment of maximum residue limits on 23 April 2019.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended, on 18 June 2020, the establishment of maximum residue limits for bupivacaine in bovine species.

Subsequently, the Commission recommended, on 29 September 2020, that maximum residue limits (no MRL required classification) in bovine species are established. This recommendation was confirmed, on 21 October 2020, by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 12 November 2020.

¹ Commission Implementing Regulation (EU) No 2020/1685, O.J. L 379, of 13 November 2020



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Bupivacaine
Therapeutic class:	NO ENTRY
Procedure number:	EMA/V/MRL/005009/FULL/0002
Applicant:	Medical Ethics UK Ltd
Target species:	Calves up to 2 months of age
Intended therapeutic indication:	Local anaesthesia
Route(s) of administration:	Cutaneous and/or epilesional

1. Introduction

Bupivacaine, also referred to as LAC-43 and Win 11,318, is an amino-amide local anaesthetic. It is a racemic mixture of its two enantiomers. In veterinary medicine, bupivacaine is authorised for use in companion animals and maximum residue limits in porcine species have been recommended by the CVMP (as shown in the table below). Bupivacaine is also authorised as a local anaesthetic for human use.

An application has been submitted by Medical Ethics UK Ltd. to the European Medicines Agency for the establishment of maximum residue limits for bupivacaine, to be used in a product in the form of bupivacaine hydrochloride monohydrate. Bupivacaine is intended to be used as a local anaesthetic for topical use after hot-iron disbudding of calves of less than 2 months of age, at a maximum dose of 0.4 mg/kg bw bupivacaine hydrochloride.

During the assessment of the present application for the establishment of MRLs for bovine, the CVMP finalised its evaluation of an application for the establishment of MRLs for bupivacaine in porcine species and recommended inclusion of the substance in table 1 of the Annex to Commission Regulation (EU) No 37/2010 as follows:

Pharmaco- logically active Substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Bupivacaine	NOT APPLICABLE	Porcine	No MRL required	NOT APPLICABLE	For use in piglets up to 7 days of age For cutaneous and epilesional use only	Local anaesthetic

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

Bupivacaine is an amide-type local anaesthetic consisting of an aromatic ring, an amide-linkage and a tertiary amine, which are the three common structural elements of local anaesthetics. In human medicine, bupivacaine is available as a solution for injection and can also be used topically. After injection, the target site is reached by passive diffusion. The lipophilic properties and the high protein binding capacity (95%) of bupivacaine result in a long-acting local analgesia in the target tissue, when compared to other local anaesthetics, e.g. lidocaine. When administered topically in the mucous membrane of the oral cavity of patients, analgesic effects begin after about 9 minutes and last for about 25–30 minutes. Bupivacaine, like other local anaesthetics, acts by reversibly binding to the voltage-dependent sodium and potassium channels. Its binding reduces the permeability for sodium and potassium ions in neural membranes, blocking the passing of action potentials, in nerve cell fibres and muscle cells and therefore blocking the local sensation of pain. It has been demonstrated that the blocking of cardiac sodium channels in isolated guinea pig ventricular myocytes was stereoselective, with the *R*-enantiomer being more potent than the *S*-enantiomer.

One study investigated the 'highest no effect dose' for local anaesthetic response to bupivacaine in horses. Reduction/abolition of horses' brisk pedal withdrawal reflex to a 90 °C skin stimulus was used as endpoint for pharmacological efficacy. Doses infiltrated around nerves of up to 0.25 mg/site of bupivacaine had no effect on withdrawal of the foot. Doses of 2 mg/site completely abolished the withdrawal reflex.

Pharmacokinetic properties (mainly in laboratory animals)

Absorption

There are no oral administration studies available on the pharmacokinetics of bupivacaine following internationally accepted guidelines in laboratory animals.

In rats, a single bupivacaine dose of 20 mg/kg bw was rapidly absorbed after intraduodenal administration, and a maximum plasma concentration of 7.9 µg/ml was reached within 15 minutes. In reproductive toxicity studies, satellite groups were included for toxicokinetic assessment. In rats, subcutaneous bupivacaine (racemic) doses of 10 mg/kg bw resulted in a plasma concentration of 0.5 µg/ml *S*-bupivacaine and 0.23 µg/ml *R*-bupivacaine in males and 0.2 and 0.17 in females, respectively. Subcutaneous levobupivacaine doses of 10 to 30 mg/kg bw, resulted in *S*-bupivacaine plasma concentrations of 0.24 to 1.02 µg/ml in females, and 0.54 to 1.47 µg/ml in males. After two weeks of treatment with subcutaneous levobupivacaine, toxicokinetic data were collected again. In females, *S*-bupivacaine plasma concentrations ranging from 0.48 to 1.35 µg/ml were detected, and 0.3 to 1.2 µg/ml in male animals. In rabbits, subcutaneous bupivacaine (racemic) doses of 5 mg/kg bw resulted in a plasma concentration of 0.142 µg/ml *S*-bupivacaine and 0.346 µg/ml *R*-bupivacaine. Levobupivacaine doses in rabbits of 5 to 20 mg/kg bw resulted in *S*-bupivacaine plasma concentrations of 0.35 to 1.12 µg/ml. In a study in humans, 10 healthy male individuals exposed orally to a single dose of bupivacaine lozenge (with doses ranging from 5–50 mg, corresponding to approx. 0.083 to

0.83 mg/kg bw) showed plasma levels of bupivacaine below 2 µg/ml, hence below the plasma level which corresponds to severe systemic effects on the central and the cardiac nervous system, such as seizures, convulsion, respiratory arrest and cardiac arrhythmias. In this study, a pharmacokinetic model was developed establishing maximum plasma levels ranging from 30–300 ng/ml for oral doses of 5 to 50 mg per person, which are reached approximately 1 hour after ingestion of a bupivacaine lozenge. The model was based on the analytical results from the test patients, however, the actual measured data (mean or median) were not provided in the publication.

From the studies provided, a quantitative estimation of systemic availability after oral exposure cannot be determined, and hence, for the purpose of this risk assessment, an absorption of 100% after oral administration is assumed.

Distribution

After subcutaneous administration of a single dose of 2 mg [³H]bupivacaine per kg/bw to rats or monkeys, radioactivity was detected mainly in the adrenal gland, brain, fat, heart, kidney, liver, lung, pancreas and spleen in rats, and in liver, kidney, lung and pancreas in monkeys. In piglets, following a topical administration into the incised scrotal sac, the highest accumulation, and slowest depletion of bupivacaine was from skin and fat tissues, which is to be expected from the lipophilicity of bupivacaine and low vascularity of fat tissue. The high lipophilicity of bupivacaine may indicate that the substance has a potential for bioaccumulation. In an intravenous study in pregnant rats, it was shown that maternal exposure to bupivacaine also leads to foetal exposure. Bupivacaine was detected in maternal as well as foetal plasma, brain, heart, liver and placenta immediately after infusion of 0.33 mg/kg bw per min over 15 min (corresponding to an overall dose of 4.95 mg/kg bw). Additional results from laboratory studies in rats and dogs, with intravenous administration of bupivacaine, indicate that plasma elimination is biphasic with a mean distribution half-life of 1.2 minutes and an elimination half-life of 37.7 minutes in rats, and a mean distribution half-life of 2.1 minutes and an elimination half-life of 39.1 minutes in dog.

Excretion

Biliary excretion appears to be more extensive in rats than in primates. In a study using radiolabelled [³H]bupivacaine administered subcutaneously, 78% of the dose (50% via urine and 28% via faeces) and 86% of the dose (79.9% urine and 6% via faeces) were excreted after 24 hours in rats and monkeys, respectively. In rats, about 80–97.8% of 3'-hydroxy- and 4'-hydroxybupivacaine (the two main metabolites detected) were excreted as glucuronide conjugates in the urine. In humans, urinary excretion of a racemic bupivacaine dose after epidural infusion amounts to 75%, with a range of 14.3–39.1% for (+)-(R)bupivacaine and 9.2–14.0% for (-)-(S)-bupivacaine. In humans, up to 6% of a dose applied intravenously is excreted unchanged via urine.

Metabolism

Metabolism of bupivacaine has been comprehensively assessed and reviewed. Bupivacaine is extensively metabolised in laboratory animals (rats, rabbits, monkeys) and in humans. Metabolism of bupivacaine occurs primarily in the liver. Oxidative dealkylation of the butyl side chain of the piperidine ring results in desbutyl bupivacaine, and *in vitro* results with human liver microsomes have shown that this process is mainly mediated by the cytochrome P450 3A4, and to a lesser extent by cytochromes P450 2D6 and 2C19.

In rat urine, 3'- and 4'-hydroxybupivacaine were the two major metabolites detected, with 3'-hydroxybupivacaine being detected in higher concentrations than 4'-hydroxybupivacaine after intraperitoneal injection. Desbutyl bupivacaine was also found, but in smaller quantities. In Wistar rats, 3'- and 4'-hydroxybupivacaine accounted for 73.6% of the administered dose. The occurrence of 6% pipercolic acid indicates that hydrolysis of the amide bond of desbutyl bupivacaine could also result in

formation of 2,6-xylidine. In another study in rats, further bupivacaine metabolites with hydroxylation of the piperidine ring and methylation, di- and trihydroxylation of bupivacaine were identified, but these were not further characterised. In another study, it was demonstrated that after an infusion of bupivacaine of 4.95 mg/kg bw in pregnant, near-term rats, the parent compound was metabolised within four hours. Concentrations of 3'-hydroxybupivacaine, 4'-hydroxybupivacaine and desbutyl bupivacaine were observed in maternal and foetal plasma, brain, heart, liver and placenta tissues.

The metabolites 3'- and 4'-hydroxybupivacaine, and desbutyl bupivacaine were also identified in human urine. In comparison with the fractions found in rats, in humans the fraction of 4'-hydroxybupivacaine is higher than that of 3'-hydroxybupivacaine. In this study, patients were dosed with epidural infusions of racemic bupivacaine and *R*- and *S*-enantiomers of desbutyl bupivacaine, 4'-hydroxybupivacaine, 3'-hydroxybupivacaine and bupivacaine were identified in urine. Since the total amount of *R*-enantiomers was somewhat higher than *S*-enantiomers, stereoselective clearance can be assumed. In the same study, individual differences in metabolic pathways were found: In three male patients *N*-dealkylation was the predominant route of metabolism, whereas in the other two patients it was hydroxylation.

In a study with pregnant women who received bupivacaine in combination with lidocaine, detection of 2,6-xylidine was not reported. Bupivacaine and the metabolite desbutyl bupivacaine were excreted into breast milk, and the milk/serum ratio at peak concentrations was 0.49 for bupivacaine and 1.47 for desbutyl bupivacaine. *N*-dealkylation and ring-hydroxylation reactions, however, do not necessarily result in splitting the amide bond or production of 2,6-xylidine. Hydrolysis of the amide bond and subsequent release of 2,6-xylidine is catalysed by human carboxylesterase HCES1a and is likely stereoselective.

In humans, 1.2% to 2.6% of a bupivacaine dose were metabolised to pipecolic acid. Though a minor pathway, it may indicate that at the same rate 2,6-xylidine might be formed. The author concluded that *N*-dealkylation of bupivacaine is necessary for amide hydrolysis and the extent of *N*-dealkylation and consequently amide hydrolysis of bupivacaine is low (1.6% in rats after intraperitoneal injection and 1.2 to 2.6% in humans after intramuscular injection). In humans, there is evidence of a first pass metabolism, indicated by a hepatic extraction ratio of 0.37 of bupivacaine.

Bupivacaine is also metabolised in livestock animals. In addition to the metabolites found in rats and in humans, 3'-hydroxydesbutyl bupivacaine and 4'-hydroxydesbutyl bupivacaine were found in the urine of sheep in one study, whereas in another study also in sheep, 99.99% of the bupivacaine dose was excreted unchanged. In cattle, the majority of a subcutaneous dose (98.6%) was excreted as desbutyl bupivacaine. No 2,6-xylidine was detected in cattle and sheep used in these studies. Metabolism of bupivacaine was also investigated in a horse. In this latter study, apart from 3'- and 4'-hydroxybupivacaine and *N*-desbutyl bupivacaine, oxidized bupivacaine with hydroxylation on the aliphatic side chain was detected. Formation of 2,6-xylidine was investigated but not detected. In piglets, a topical bupivacaine dose was metabolised to *N*-desbutyl bupivacaine, 3'-OH-bupivacaine and 2,6-xylidine.

2.1.2. Calculation of pharmacological ADI, if relevant

From animal data, no pharmacological point of departure could be derived which is relevant for oral exposure of consumers. Oral pharmacological data are available for humans and are discussed in section 2.1.1 'Overview of pharmacological properties' and 2.1.6 'Observations in humans'. Overall, the absence of cardiovascular effects after oral intake of bupivacaine by humans at 100 mg/person per day (1.67 mg/kg bw per day bupivacaine) can be used as point of departure (POD) for consumer risk assessment. The margin of exposure to pharmacologically active residues should be at least 10 (for intraspecies variability). Based on this, the pharmacological ADI is 0.167 mg/kg bw bupivacaine per day.

2.1.3. Overview of toxicology

Pharmacokinetic data in laboratory species and humans indicate the metabolism of bupivacaine may lead to the formation of the metabolite 2,6-xylylidine. This compound is a substance with the potential for genotoxicity *in vivo* and carcinogenic properties, as investigated in rats, as reported in the carcinogenicity section below.

Single dose toxicity

Published literature reports several adverse effects following a single administration of bupivacaine via several exposure routes and in various species. In laboratory animals, effects on the central nervous system include convulsions, and effects on the cardiac conduction system include increased cardiac output, increased heart rate and increased systemic arterial blood pressure, which can result in respiratory depression, cardiac arrest and death. The severity of acute effects increases with increasing plasma levels of bupivacaine, hence with increasing systemic availability and therefore is dependent on the route of administration. For instance, in mice, LD₅₀ values range from 7.8 mg/kg bw (intravenous administration) to 82 mg/kg (subcutaneous administration). The LD₅₀ values in rabbits and guinea pigs were in both species 50 mg/kg bw (from a subcutaneous administration with adrenalin in concentration of 1:200000, and intraperitoneal administration, respectively). Oral acute toxicity data is only available for rabbits (LD₅₀: 18 mg/kg bw), and this value is reported in the registry of toxic effects of chemical substances. However, the study protocol used or data on clinical observations is not available.

Repeated dose toxicity

There are no sub-acute, sub-chronic or chronic toxicity studies in experimental animals available using the oral route of exposure, hence a relevant NO(A)EL/LO(A)EL from oral exposure cannot be established. However, as the available data were generated using subcutaneous administration, systemic exposure can be assumed to exceed that which would occur following oral administration and consequently the effect and no effect levels reported below are accepted as conservative estimates of oral effects and no effect levels. Furthermore, chronic bupivacaine exposure of humans via the intrathecal route, resulting in far higher bupivacaine plasma levels than similar dose levels via the oral route, indicate no systemic drug-related long-term effects (see also section 2.1.7). Overall, omission of standard repeated dose studies in experimental animals was considered acceptable.

The available studies are of short duration and a low number of animals were used. Two available sub-acute (14 days) studies in rats and rabbits were carried out as dose range-finding studies for reproductive toxicity studies. In both studies, levobupivacaine was administered by subcutaneous injection to three animals per dose group. In rats, a NOAEL of 20 mg/kg bw per day could be derived based on convulsive episodes at the next higher dose level of 30 mg/kg bw per day. In rabbits, a LOAEL of 20 mg/kg bw per day based on slightly impaired mobility. Since these studies were dose range-finding studies, only clinical signs were observed, and haematology, clinical biochemistry or histopathological investigations were not performed.

New Zealand white rabbits (three per group) were treated twice a week for 4 weeks subcutaneously at 9–30 mg/kg bw with DepoFoam bupivacaine (which results in the continuous release of the active substance over time, preventing systemic peak exposures and extending analgesia), or with 9 mg bupivacaine hydrochloride/kg bw. One death was recorded in the high dose DepoFoam bupivacaine group, but the cause of death could not be determined. In the DepoFoam bupivacaine group, splenic, lymph node and thymic lymphoid depletion was observed, which was attributed to physiological stress. In addition, convulsions were observed occasionally after dosing at 9 (1/6) and 18 mg/kg bw (2/6). Convulsions were also observed in the bupivacaine hydrochloride group (5/6), from which animals

recovered within about 1 hour. No further effects on body weight, food consumption, haematology, coagulation, clinical chemistry, urinalysis, or organ weight endpoints were reported. A LOAEL of 9 mg/kg bw was set based on convulsions. In the same study, beagle dogs (three per group) were treated twice a week for 4 weeks subcutaneously either with 9–30 mg DepoFoam bupivacaine/kg bw or 9 mg bupivacaine hydrochloride/kg bw. No effects on clinical observations, body weight, food consumption, haematology, coagulation, clinical chemistry, urinalysis, or organ weight endpoints were observed in the treated animals. Electrocardiographic recordings showed no abnormalities due to treatment with bupivacaine DepoFoam or bupivacaine hydrochloride compared to control animals treated with a saline solution only. From the study in dogs, the authors derived a NOAEL of 30 mg/mg bw for DepoFoam bupivacaine. For bupivacaine hydrochloride, the NOEL was 9 mg/kg bw.

Reproductive toxicity, including developmental toxicity

No studies on fertility and reproductive performance ([multi-]generation study), and no developmental toxicity study with the oral route of exposure are available, hence a relevant NO(A)EL/LO(A)EL from oral exposure cannot be established. However, the available data, including subcutaneous studies of fertility and embryo-foetal development as well as pre- and postnatal development in rats (including mating of the F1 generation to give F2) and rabbits, provide sufficiently detailed information to conclude on reproductive, including developmental toxicity of bupivacaine and consequently omission of standard oral reproductive and developmental toxicity studies as referred to in VICH GL 22 and VICH GL 32 is considered acceptable.

Fertility and embryo-foetal development were investigated in a subcutaneous study with 10, 20, 30 mg/kg bw per day levobupivacaine and 10 mg/kg bw per day bupivacaine. 24 male animals per group were treated 4 weeks before pairing, throughout pairing until the day of necropsy in week 12. Twenty-four female animals per group were treated 2 weeks prior to pairing, throughout pairing and until gestation day 17. Females were terminated on gestation day 20. Deaths of males occurred in the high (2/24), and low (1/24) dose levobupivacaine groups, and in the bupivacaine (1/24) group. Deaths of females occurred in the high (3/24), and low (1/24) dosing group without clear causes of deaths. In the high dose group of levobupivacaine, reduced numbers of *corpora lutea* (18%), reduced number of implantations (19%) and reduced total number of foetuses (19.5%) were the only observed effects. In males, no differences in sperm motility, count, proportion of morphological abnormalities or concentration occurred compared to controls. In the bupivacaine group, a reduced mean sperm concentration was observed. For effects on fertility, a NOEL of 20 mg/kg bw levobupivacaine and a LOEL of 10 mg/kg bw for bupivacaine can be derived. The observed foetal effects occurred in the high dose levobupivacaine group and comprised a statistically significant increase in the percentage of foetuses with external and visceral variations (18.9 vs. 13.4% in control), low incidence of dilatation of olfactory ventricles (1 vs. 0% in control [not statistically significant]), increase in numbers of incompletely ossified nasals (3.9 vs. 1% in control) and misshapen *sternebrae* (2.6 vs. 0% in control) and slight increase in number of foetuses with malformations (1.6 vs. 0.25% in control [not statistically significant]). With bupivacaine, there was an increase in the percentage of foetuses with external and visceral variations (20.7 vs. 13.4% in control) and low incidence of dilatation of olfactory ventricles (0.6 vs. 0% in control [not statistically significant]). The authors concluded that the latter effect might be considered treatment-related, as it had not been experienced before in the test facility. For levobupivacaine and bupivacaine, a maternal NOAEL of 30 mg/kg bw per day and a LOEL of 10 mg/kg bw per day can be derived, respectively. For foetal effects, a NOAEL of 20 mg/kg bw and a LOAEL of 10 mg/kg bw per day was derived from the study.

In a study in Sprague-Dawley rats (no standard guideline provided), no effects on litter size, offspring loss or weight of the offspring at birth and on days 7 and 21 after parturition were reported after subcutaneous injection of up to 18 mg bupivacaine/kg bw per day from gestation day 15 to day 15 after

parturition (main study). In a preliminary pilot study with a subcutaneous injection treatment of up to 24 mg/kg bw per day from gestation day 15 to day 3 after parturition, postnatal loss occurred at doses where also maternal toxicity was observed, i.e. 50% postnatal death was observed at the highest dose of 24 mg/kg, but no detailed data for the other dose groups are available. The authors of the study attributed foetal deaths to decreased maternal care, as corresponding dams showed severe clinical effects and poor care of the newborn. It is noted that litter size, pup and litter weight and pup loss at birth were the only investigated foetal endpoints. Maternal endpoints included: clinical signs, mortality, body weights and food consumption, duration of gestation, labour and delivery. Based on the provided information, a maternal NOAEL of 5.5 mg/kg bw and a foetal NOAEL of 18 mg/kg bw per day can be derived from the main study. From the pilot study, a maternal LOEL of 14 mg/kg bw can be established, which corresponds to the lowest concentration tested but for which no foetal toxicological reference value was available.

A study in CrI:CDBR rats investigated pre- and postnatal development after subcutaneous administration of 10–30 mg levobupivacaine/kg bw per day and 10 mg bupivacaine/kg bw per day. A dose of 10 mg/kg bw per day (levobupivacaine) and 30 mg/kg bw per day (levobupivacaine) caused maternal deaths (1/24 and 3/24, respectively). Gestation, post-implantation survival, live birth and viability indices and physical and functional development of the offspring to weaning were similar to the control group for both substances. F1 males had reduced bodyweights in all treatment groups compared to controls, being statistically significant for 10 mg/kg bw bupivacaine and 10 mg/kg bw levobupivacaine. Subsequent growth of the high dose offspring (levobupivacaine) until day 21 postpartum was slightly less than that of controls, the difference in female and combined weights being statistically significant at weaning. For foetal toxicity/teratogenicity, the NOEL was reported as 20 mg/kg bw per day for levobupivacaine based on reduced body weights and slightly reduced growth and a LOEL of 10 mg/kg bw per day for bupivacaine was reported for F1 females during gestation and F1 males.

A study in rabbits investigated embryo-foetal development after subcutaneous administration of 0, 5, 10 and 20 mg levobupivacaine/kg bw or 5 mg racemic bupivacaine/kg bw from gestation day 7 to gestation day 19. Deaths occurred in the high dose (3/24) and low dose (1/24) groups with levobupivacaine, and in the bupivacaine group (1/24) due to convulsions, an abscess or as a result of abortion. The highest dose of levobupivacaine caused convulsive episodes in 8/24 dams, a bodyweight loss of 1.6%, which was reversible after the end of treatment, and lower food consumption in dams. For maternal toxicity, a NOEL of 10 mg/kg bw per day was established for levobupivacaine and 5 mg/kg bw per day for bupivacaine. A foetal NOAEL of 10 mg/kg bw per day for levobupivacaine was reported based on a higher incidence of pre-implantation loss and a higher proportion of male fetuses compared to control; a foetal LOEL of 5 mg/kg bw per day for bupivacaine, based on the only tested concentration and a higher proportion of male fetuses compared to control, was also established.

According to the US FDA product label for a bupivacaine hydrochloride injection product, an increase in embryo-foetal deaths was observed in rabbits at the high dose (22.2 mg/kg bw) in the absence of maternal toxicity. A NOAEL of 5.8 mg/kg bw was established in the product literature based on this effect.

In a behavioural study in rhesus monkeys of the perinatal effects of bupivacaine, cognitive development, fine motor maturation and spontaneous behaviour of 11 infants (9 males and 2 females) were investigated for a year after a maternal intravenous dose of 1.2 mg bupivacaine hydrochloride/kg bw. Treatment of dams with bupivacaine resulted in impaired cognitive development, delayed behaviour maturation patterns, prolonged increase in motor disturbance behaviours, and a very low level of vigilance in one infant, compared to the control group. Only one dose was tested, and the results have to be considered an effect level.

Genotoxicity

In bacterial gene mutation tests with bupivacaine, one performed according to OECD TG 471 under GLP-compliant conditions, clear negative results were obtained with and without two metabolic activation systems (induced rat and hamster liver) up to a dose of 10 mg/plate. A further bacterial gene mutation assay resulted in *'no substantial increases in a revertant colony in the presence or absence of S9 mix'*. This information is only available as a summary (no independent assessment possible) and no further details on the test method or detailed results were given. In an *in vitro* mouse lymphoma assay (MLA), levobupivacaine gave clearly negative results with and without metabolic activation. In the MLA with bupivacaine, while overall negative results were obtained, there was a marginal (2-fold) increase in mutation frequency, and this was seen without S9 in one experiment at the highest concentration, which was also cytotoxic. A chromosomal aberration test with bupivacaine hydrochloride in human peripheral blood lymphocytes, carried out according to OECD TG 473 under GLP-compliant conditions, was clearly negative with and without metabolic activation. In a further chromosomal aberration test in cultured human peripheral blood lymphocytes using levobupivacaine, it was concluded that the test was negative for structural chromosome aberrations, whereas the numerical aberrations were statistically significantly different from controls. The report concludes that *'the biological importance of this observation is not clear'*. No details on the method or results were given (no independent assessment possible).

An oral (gavage) *in vivo* micronucleus assay in the bone marrow of mice with bupivacaine hydrochloride following a US National Toxicological Program protocol concluded that bupivacaine does not cause chromosomal or numerical aberrations *in vivo* up to a dose of 100 mg/kg bw. This is supported by two further *in vivo* bone marrow micronucleus assays in mice (no details on the guideline provided) with bupivacaine and levobupivacaine, which were also reported to be negative. A *Drosophila* wing spot test, for which no validated test method/guideline is currently available, gave inconclusive results for bupivacaine and levobupivacaine.

Overall, the negative results indicate that bupivacaine is not genotoxic.

The genotoxicity of the bupivacaine metabolite 2,6-xylydine has been investigated separately. The metabolite 2,6-xylydine showed weakly positive responses in bacterial mutagenicity tests using *Salmonella typhimurium* strain TA100 (and once in TA1535) in the presence of S9 mix in some tests, while, in other tests, the results were negative. 2,6-xylydine induced chromosomal aberrations in hamster ovary and lung cells *in vitro* at high concentrations or under cytotoxic conditions, and induced gene mutations in eukaryotic test systems *in vitro*. *In vivo*, 2,6-xylydine was considered non-clastogenic in micronucleus assays, in the peripheral blood and bone marrow of mice after oral doses up to 375 mg/kg bw. However, no plasma level was measured in these studies. An oral *in vivo* unscheduled DNA synthesis assay performed in rats with 2,6-xylydine was clearly negative in liver cells up to a dose of 850 mg/kg bw. However, exposure of liver was not monitored.

A transgenic gene mutation assay was positive in nasal tissue of mice after doses of 100 mg of 2,6-xylydine/kg bw, administered once weekly by oral gavage for four weeks. DNA extraction was carried out from nasal tissue, bone marrow and liver. A two-fold increase in mutation frequency of lacZ and cII genes in nasal tissue was reported; transitions of the base pairs AT to GC and transversions of the base pairs GC to AT were observed, but the authors question their biological relevance because the statistical significance was not reported and no historical control mutant frequency data were presented. Two *in vivo* comet assays with 2,6-xylydine were reported to be positive as well. In one comet assay study, 2,6-xylydine was orally administered at 200 mg/kg bw four times at weekly intervals, and positive results were obtained in the lung, kidney, and liver at 3 hours after the last dosing but not 24 hours after the last dosing. In the second comet assay in mice, a single dose of 350 mg/kg was administered orally. Statistically significant increases in comet tail length were seen in the stomach and bladder 8 hours after dosing only and were not significant at 3 and 24 hours after dosing. Significant increases in comet tail

length were also seen at 3 and 8 hours (but not 24 hours) in brain, and, at 8 and 24 hours, in lung tissue.

In vitro studies with the 2,6-xylylidine metabolite *N*-(2,6-dimethylphenyl)hydroxylamine showed that genotoxic effects are present in prokaryotic and eukaryotic test systems using synthesized *N*-(2,6-dimethylphenyl)hydroxylamine. A comet assay was positive for *N*-(2,6-dimethylphenyl)hydroxylamine. The authors attributed DNA damage to redox cycling of intracellularly bound aminophenol/quinonimine structures generating reactive oxygen species, which may point to 2,6-xylylidine exhibiting genotoxic (clastogenic) effects only under certain activation conditions and beyond certain levels of exposure. This was not further verified.

Carcinogenicity

No carcinogenicity studies using bupivacaine are available. Findings in *in vivo* and *in vitro* genotoxicity assays were overall negative and there is evidence of anti-proliferative properties of bupivacaine. Although, repeated dose toxicity studies with sub-chronic or chronic exposure are not available, no concern for carcinogenicity is inferred and no carcinogenicity study is considered necessary.

2,6-xylylidine, a metabolite of bupivacaine in piglets, has been associated with carcinogenicity in rats. In a carcinogenicity test, rats received 2,6-xylylidine in the feed at nominal doses of 0, 300, 1000 and 3000 ppm (corresponding to an average dose of 0, 14.7, 49.3 and 151.2 or 0, 19.9, 69.7 or 219.4 mg/kg bw per day for males or females, respectively) for two years. A statistically significant decrease in mean bodyweight gains was seen at 3000 mg/kg in both sexes and at 1000 mg/kg in females. Increase in adenomas and carcinomas of the nasal cavities were found in rats of both sexes of the 1000 and 3000 mg/kg groups. Unusual rhabdomyosarcomas and malignant mixed tumours of the nasal cavity were observed in both sexes of the high dose group. No relevant statistically significant subcutaneous fibromas and fibrosarcomas were noted at any dose, however, subcutaneous fibrosarcomas were observed in three high dose females, one high dose male, one mid dose female, one low dose male and one control female. Also, an increased incidence of neoplastic nodules in the liver of female rats (but not in males) was observed. Hepatocellular carcinomas were observed in one control, one mid dose and one high dose female rat. In males, a high dose rat had a neoplastic nodule, and a control and a mid-dose male had a hepatocellular carcinoma. No NOEL was established in the study. Based on the tumour incidence of 81.8% in male animals (adenomas, adenocarcinomas, carcinomas combined), a T25 (the dose that caused tumours in 25% of animals) of 46.2 mg/kg bw per day was derived. It was noted that actual exposure levels of the rats are likely to be below the nominal doses in feed. It was evident in a stability test that open storage of the feed at room temperature over 7 days resulted in losses of 9% (day 1) to 43.9% (day 7) of the test substance. The nominal doses and the resulting T25 need to be corrected for the possible losses of 2,6-xylylidine in feed over the full study duration. As it is not known which is the most relevant value, correction for the range of losses would result in a T25_{corrected} range of 25.9 to 42.0 mg/kg bw per day as points of departure for risk assessment (the lower value being the most conservative).

Studies of other effects including immunotoxicity and neurotoxicity

No studies have been carried out with bupivacaine to show potential immunotoxicity. However, in the case of this compound, these studies are not considered necessary because there is no signal that would indicate concern of immune system damage from literature.

2.1.4. Calculation of the toxicological ADI or alternative limit

A low observed effect level of 5 mg/kg bw per day based on a higher incidence of pre-implantation loss and a higher proportion of male fetuses compared to control was observed in a developmental toxicity study in rabbits. This is currently the lowest toxicological point of departure to be used for quantitative risk assessment for exposure to bupivacaine residues. The margin of exposure between the point of departure for developmental effects in rabbits and exposure to bupivacaine residues should be at least 200 (2 for extrapolation from LOEL to NOEL, 10 for intra- and 10 for interspecies variability). A toxicological ADI for bupivacaine based on this point of departure is therefore established as 0.025 mg/kg bw per day or 1.5 mg/person per day. This toxicological ADI does not consider the carcinogenic effects of the metabolite 2,6-xylylidine; this is considered separately below.

In calves, 2,6-xylylidine, currently considered to have a potential for genotoxicity *in vivo* and carcinogenic properties in rats, was detected as a metabolite of bupivacaine after application of a dose of 0.4 mg bupivacaine hydrochloride/kg bw for a 50 kg calf. Therefore, bupivacaine treatment may lead to exposure to 2,6-xylylidine via food derived from treated cattle and might also be formed through metabolism in humans, although to a low extent.

Given the occurrence of the carcinogenic metabolite 2,6-xylylidine, a toxicological reference value for exposures to this metabolite is required. Based on the T25_{corrected} range of 25.9 to 42.0 mg/kg bw per day (see above) and using a factor of 250,000 to linearly extrapolate to a negligible risk of 1 in 10⁶ (non-threshold mechanism of action), the resulting substance-specific acceptable intake is in the range of 6.2 to 10.1 µg/person per day for consumers.

2.1.5. Overview of microbiological properties of residues

Bupivacaine was shown to have antimicrobial activity to bacterial strains which may colonize the human intestine. Mean MIC concentrations ranged from 0.25 to 2 mg/ml in a study where antimicrobial activity of bupivacaine was tested, mainly in oral bacterial strains and a few intestinal bacterial strains. Racemic bupivacaine appears to have somewhat higher potency in antimicrobial activity than levobupivacaine. No studies on disruption of the colonisation barrier and the increase of the population of resistant bacteria were provided. The observed MIC values for bupivacaine are in a high concentration range (mg/ml) and clearly outside the range of MICs that would normally be considered relevant for the calculation of microbiological ADI.

2.1.6. Calculation of microbiological ADI

MIC values of bupivacaine are in a range (mg/ml) not considered relevant for consumer risk assessment. Derivation of a microbiological ADI is not required.

2.1.7. Observations in humans

In humans, a single intravenous dose of bupivacaine (corresponding to 2.1 µg/ml plasma) did not cause adverse effects such as convulsions nor depressive effects on central circulation or respiration. However, higher plasma levels (2.62 µg/ml levobupivacaine and 2.25 µg/ml racemic bupivacaine) were reported to cause an increase in blood pressure and affected the electrical conduction system of the heart (increase in PR and QT intervals), with levobupivacaine having a less pronounced effect when compared to that of racemic bupivacaine. The effect of a bupivacaine lozenge as topical pharyngeal anaesthetic was investigated in 51 patients undergoing non-sedated upper gastrointestinal endoscopy. With a single oral dose of 25 mg, patients experienced significantly less discomfort compared to the control group receiving

the standard therapy, a pharyngeal lidocaine spray at a single dose of 30 mg. None of the patients reported side effects in the study. In a study from 2017, 25 head and neck cancers patients received lozenges with 25 mg for the treatment of oral mucositis. The median daily intake was 100 mg per person. The reported adverse effects can be attributed to the local pharmacological effect of bupivacaine, i.e. dysphagia, odynophagia, hyperalgesia and hypersalivation. No further effects were noted. Another study investigated the effects of a 25 mg bupivacaine lozenge treatment on oral mucositis in 10 patients with head and neck cancer. Maximum pain reduction was observed after a mean time of 44 or 77 minutes in the oral cavity or the pharynx, respectively, with a significant or slight pain reduction that lasted up to 180 minutes in the oral cavity. No further side effects were reported.

In a randomized, double-blinded, crossover trial, the effects of bupivacaine on symptoms of oral pain, xerostomia, and taste alterations were investigated in 18 patients with burning mouth syndrome. Patients were advised to take 3 lozenges daily for 2 weeks, containing either 5 mg bupivacaine (corresponding to 15 mg/day) or placebo. Slight, but statistically significant decrease in oral burning pain was seen in patients treated with bupivacaine lozenges. Additional side effects were tolerable burning or stinging sensation, swallowing discomfort and ceased or altered taste sensation. After oral exposure of up to 25 mg bupivacaine for up to 14 days, there appear to be no effects on the central nervous system or cardiovascular system, as seen in studies following other exposure routes, particularly intravenous exposures at lower doses. These results are in line with the findings of a study from 2017, which indicated that a plasma level below 2 µg/ml does not result in effects on the central nervous system and in the cardiovascular system. In cancer patients, no long-term neurotoxicity effects were reported from prolonged intrathecal bupivacaine exposure (approximately 3 months, and in single cases for over 1 year). This includes post-mortem neurohistopathologic results. No clear analgesic effect was found in patients who underwent tonsillectomy, with swabs or gauzes soaked with solutions of 0.5% bupivacaine (corresponding to 25 or 50 mg bupivacaine HCl) applied topically to the surgical site. In the studies with bupivacaine lozenges, an oral dose of 5 mg per person was locally analgesic and is therefore considered as local pharmacological LOAEL. Systemically, absence of cardiovascular or central nervous system side effects in humans up to an oral dose of 100 mg per person for up to 7 days was used for establishing a pharmacological NOEL.

Additional information is available from studies with parenteral administration routes, mainly intrathecal administration with bolus doses or slow intrathecal infusion, where protective effects with regard to thromboembolism after surgery were observed. In patients receiving epidural anaesthesia with bupivacaine, a reduction of platelet aggregation could be observed at therapeutic dose levels up to 3 hours after surgery. No further effects on coagulation or fibrinolysis were observed. Withdrawal of the local anaesthetic results again in an increase in post-surgery thromboembolism. *In vitro*, bupivacaine, in clinically relevant concentrations of 1–10 µmol/l (0.3–3 µg/ml), increased the activated clotting time and inhibited TXA2 signalling, pointing to an active role in antithrombotic action of bupivacaine. Considering that intrathecal exposure to bupivacaine results in far higher bupivacaine plasma levels than similar dose levels via the oral route, these effects may not be relevant after oral intake of residues from food. This is supported by the absence of effects on haematology and coagulation in rabbits and beagle dogs on the last day of subcutaneous dosing of DepoFoam bupivacaine or bupivacaine up to 30 mg/kg bw.

Information on human sub-chronic and chronic exposures to bupivacaine is also available from studies with repeat dose bolus injection and/or continuous prolonged perineural, epidural or intrathecal infusion, with exposures to bupivacaine doses of 10 to 300 mg/kg bw per day and in combination with opioids. The most sensitive adverse effects relate to unwanted motor paralysis or autonomic effects due to direct local anaesthetic action on intraspinal or sympathetic nerve tissue. In a double-blinded, randomized, crossover, multicentre study performed to assess safety and efficacy of intrathecal bupivacaine in 24 patients, 0, 4, 6 or 8 mg bupivacaine/person per day were administered together with a constant opioid dose for four consecutive months. Only one patient reported mild numbness in his lower extremities,

without weakness at a dose of 8 mg/day. In a retrospective analysis of 51 patients with cancer pain, side effects were absent (including sensory deficits, motor complaints, and signs of autonomic dysfunction or neurotoxicity) below a daily dosage of 30 mg bupivacaine by continuous infusion. A mean bupivacaine dose of 46 mg (range 20–75 mg)/person per day (median dose of 54 mg/day) over a mean duration of 57 days (range 13 to 87 days) administered via a catheter in the cervical or upper thoracic spine, resulted in neurological effects such as leg weakness and arm weakness/numbness in 4/6 patients. Further reported adverse effects were not attributed to bupivacaine but to the co-treatment with diamorphine, clonidine and/or baclofen. Overall, it may be concluded that chronic human dosage rates by the intrathecal route of up to approx. 30 mg/day (0.5 mg/kg/day) are not associated with systemic drug-related adverse events. However, derivation of a NO(A)EL from the sub-(chronic) studies in humans is not possible due to co-administration of at least one further active substance.

2.1.8. Findings of EU or international scientific bodies

No relevant evaluations by EU or international scientific bodies were identified.

2.1.9. Overall conclusions on the ADI and alternative toxicological reference value

Bupivacaine

Based on the absence of cardiovascular effects after oral intake by humans at 100 mg/person per day (1.67 mg/kg bw per day) and an uncertainty factor of 10 (for intraspecies variability), the pharmacological ADI was calculated as 0.167 mg/kg bw per day. Almost all available studies in experimental animals as well as humans were carried out using non-representative routes of exposure (i.e. epidural, intrathecal, subcutaneous), resulting in higher systemic exposures compared to the oral route. It can be concluded that an oral repeat dose study in experimental animals is therefore unlikely to lower the pharmacological or toxicological ADI.

MIC values of bupivacaine are in a range (mg/ml) not considered relevant for consumer risk assessment. Derivation of a microbiological ADI is not required.

A low observed effect level of 5 mg/kg bw per day based on a higher incidence of pre-implantation loss and a higher proportion of male fetuses compared to control was observed in a developmental toxicity study in rabbits. This is the lowest toxicological point of departure available for use in the quantitative risk assessment for exposure to bupivacaine residues. With an uncertainty factor of 200 (2 for extrapolation from LOEL to NOEL, 10 for intra- and 10 for interspecies variability), the toxicological ADI for bupivacaine is 0.025 mg/kg bw per day or 1.5 mg/person per day. The study the toxicological ADI is based on was carried out using the subcutaneous application route and the ADI is therefore considered a conservative (worst case) estimate.

As the lowest ADI for bupivacaine is the toxicological ADI, this is used for quantitative risk assessment.

2,6-xylidine

Given the occurrence of the carcinogenic metabolite 2,6-xylidine as residue in edible tissues as well as from human metabolism of bupivacaine, a toxicological reference value for exposures to this metabolite is required. Based on the T25_{corrected} range of 25.9 to 42.0 mg/kg bw per day and using a factor of 250,000 to linearly extrapolate to a negligible risk of 1 in 10⁶, the resulting substance-specific acceptable intake for 2,6-xylidine is in the range of 6.2 to 10.1 µg/person per day for consumers.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

In a non-GLP-compliant pilot study, three male cattle were treated subcutaneously with a bupivacaine solution at a dose of 5 mg/kg bw and samples of blood, urine and tissues were taken. Two steers were slaughtered at 72 hours and one at 7 days after treatment. Samples were analysed using a LC-MS/MS method. No information on the validation of the method was provided, LODs as well as LOQs were not reported and it is not mentioned which metabolites of bupivacaine were included in the analyses. *N*-desbutyl bupivacaine was the major metabolite of bupivacaine in cattle plasma, urine and tissues. Bupivacaine was still detectable in muscle, fat, liver and kidney at 72 hours and 7 days post administration.

This study used a combination product containing bupivacaine, lidocaine, cetrimide and adrenaline. The dose of bupivacaine applied was approximately ten times the dose intended to be applied in the field. As the study had several shortcomings in terms of missing GLP-compliance, low number of animals and missing validation data, it was not further taken into account.

One combined metabolism and residue depletion study in the target animals (i.e. calves) at the intended dose was provided by the applicant. The GLP-compliant study was conducted in 48 calves with horn buds (even mix of males and females, Holstein-Frisian/beef cross, 28.4 to 46.2 kg bw, 4 to 6 weeks old). Animals were allocated to 12 treatment groups. Groups 1 to 9 were disbudded on day 0 with a hot iron and treated with a combination product containing lidocaine, bupivacaine, cetrimide and adrenaline (gel formulation) within one minute of disbudding. Groups 10 to 12 were disbudded on day 8 with a hot iron and treated with a product containing bupivacaine hydrochloride monohydrate 5.28 mg/ml (equivalent to bupivacaine base 4.43 mg/ml, aqueous solution) within one minute of disbudding. Animals in group 9 were used as spare animals only.

Disbudding was performed using a hot iron (diameter approximately 2 cm) which was applied directly to the horn bud for approximately 3 seconds. The iron was then rotated and removed either taking the underlying skin and tissues with it or leaving residual circular tissue which was readily removed manually. The resultant wound consisted of circular ring of 'burnt' tissue and a central 'denuded' wound. The formulations were applied directly to the fresh wound as well as to the surrounding burnt tissue, each 50% of the dose per site (i.e. 2 ml per horn bud).

Substantial run-off from the treatment site was found in all treatment groups. The mean percentage run-off from the animals treated with the combination product was 23.8% and for those treated with bupivacaine was 42.1%.

Several samples from blood, urine and faeces were collected from animals in groups 1 to 8 in a time interval from 15 minutes up to 4 days post treatment and in groups 10 to 12 in a time interval from 15 minutes up to 24 hours post-treatment. Tissue samples (liver, kidney, loin muscle, perirenal fat, heart muscle), plasma, urine and faeces were collected post-slaughter at 5 minutes, 4 hours, 12 hours, 24 hours, 2 days, 4 days, 7 days and 28 days after treatment in groups 1 to 8. Tissue samples (liver, kidney, loin muscle, perirenal fat, heart muscle) were collected post-slaughter at 5 minutes, 12 and 24 hours after treatment in groups 10 to 12.

In groups 1 to 8 (treated with the combination product), bupivacaine peak levels in plasma were measured at six hours. For urine and faecal samples bupivacaine residues peaked at four hours. In groups 10 to 12, bupivacaine residues peaked at four hours in plasma, 12 hours in urine and 24 hours in faecal samples.

In urine, at 4 hours, the major compound found was lidocaine, with appreciable amounts of monoethylglycinexylidide, 3-OH-lidocaine, glycinexylidide, lidocaine *N*-oxide, 2,6-xylidine, bupivacaine, 3-OH-bupivacaine and *N*-desbutyl bupivacaine. The majority of these substances was found in the 4-hour to 4-day urine.

The major compound found in faeces at 4 hours was lidocaine. Bupivacaine and its metabolites were present at low concentrations, as were the metabolites of lidocaine, including 2,6-xylidine. In tissue samples of groups 1 to 8, bupivacaine residues peaked at four hours.

In groups treated with the single substance product (groups 10–12), unmetabolized bupivacaine was the predominant residue in plasma with a peak concentration of 14 µg/l at 4 hours. *N*-desbutyl bupivacaine was a minor metabolite and there were only trace levels of 3-OH-bupivacaine and 2,6-xylidine (< 0.5 µg/l). In urine, 3-hydroxybupivacaine was the major metabolite, with C_{max} being 140 µg/l at 12 hours. Concentrations of bupivacaine and 2,6-xylidine were very low, i.e. < 5 µg/l and < 1.2 µg/l, respectively.

In groups 10 to 12, bupivacaine levels peaked at 12 hours in tissue samples except in kidney samples, which peaked at 24 hours. In hepatic tissue, *N*-desbutyl bupivacaine was the major metabolite, 3-hydroxybupivacaine was a minor metabolite and 2,6-xylidine was a trace, or very minor metabolite, below limits of detection or quantification.

N-desbutyl bupivacaine was also the predominant residue in kidney tissues. However, unmetabolized bupivacaine was the major residue present in fat. 2,6-xylidine was only present at levels below limits of detection or quantification at all time points in all tissues other than a single recording of 0.24 µg/kg in fat at 12 hours.

The study provided was in line with VICH GL 46 concerning the administration of the drug via the intended route of administration, i.e. application to the wound site after hot iron disbudding. As only selected metabolites were measured, the study was not a full residue study according to this GL.

Bodyweights of animals reported were implausibly low for calves at an age of 4 to 6 weeks. Certain amounts of the applied doses run-off from the treatment site and there was high inter-individual variability in the remaining proportion of the applied products, with higher mean run-off values for the bupivacaine hydrochloride mono-product. In almost all animals, the remaining dose was much lower than the intended one, most likely leading to lower residue concentrations than those that would have occurred if the intended dose had remained in place.

Results for three different metabolites (*N*-desbutyl bupivacaine, 3-OH-bupivacaine, 2,6-xylidine) and for the parent substance in plasma, urine, faeces as well as in the relevant target tissues were provided. Results indicate that bupivacaine is subject to absorption after application to the wound site.

Further results of the study are reported in section 2.2.2 below.

2.2.2. Residue depletion studies

No radiometric residue depletion studies were conducted.

Two studies on residue depletion of bupivacaine in calves were provided by the applicant. The first study was a combined metabolism and residue depletion study described in detail in section 2.2.1 above. Only results relevant for the assessment of residue depletion are mentioned in this section.

Residues of bupivacaine and metabolites (*N*-desbutyl bupivacaine, 3-OH-bupivacaine, 2,6-xylidine) were found in all edible tissues in animals treated with the combination product (groups 1 to 8).

The applicant calculated estimates for consumer exposure based on data from groups 1 to 8 using the

sum of bupivacaine residues in edible tissues plus concentrations of 3-OH-bupivacaine and *N*-desbutyl bupivacaine per standard food basket. The resulting estimates were 2.46, 1.87, 2.78, 0.53 and 0.48 µg/person/day at 4, 12, 24, 48 and 96 hours, respectively.

Data from groups treated with bupivacaine only (groups 10–12) demonstrate that residue concentrations of bupivacaine, 3-OH-bupivacaine and *N*-desbutyl bupivacaine were very low at time points up to and including 24 hours. Residue concentrations of 2,6-xylidine were either below the LOD or between the LOQ (0.2 µg/kg) and the LOD (0.07 µg/kg), except for one value, i.e. 0.24 µg/kg in fat at 12 hours.

Also, for these groups, the intake of residues from calf tissues at 12 and 24 hours for bupivacaine plus 3-OH-bupivacaine and *N*-desbutyl bupivacaine as well as for 2,6-xylidine was calculated. Consumer exposure to bupivacaine plus metabolites was 0.044 µg/person/day at 12 hours and 0.031 µg/person/day at 24 hours after treatment. Consumer exposure to 2,6-xylidine was estimated to be 1.54 µg/person/day at 12 hours and 1.12 µg/person/day at 24 hours.

However, based on the high and variable amounts of the products which were subject to run-off and differing remaining doses per animal, the storage instability in bupivacaine, 3-hydroxybupivacaine, *N*-desbutyl bupivacaine and 2,6-xylidine and the implausibly low animal weights (28.4 to 46.2 kg) for calves of 4 to 6 weeks of age, results from this study were not further taken into account for consumer exposure assessment.

In another GLP-compliant residue depletion study, twenty-four calves (dairy breed or cross thereof, 32.2–65.0 kg bw, 2–7 weeks of age) were randomly allocated to six treatment groups.

Animals were disbudded immediately (~30 seconds) prior to treatment. Disbudding was performed using the hot iron method: With the calf adequately restrained, the heated hot iron was applied over the horn bud and rolled several times such that a ring of tissue around the bud was cauterised through the full thickness of the skin. Cauterised tissue was removed, providing maximum absorptive bed of wounded tissue.

Groups 1 to 3 were treated with a combination product (gel formulation) containing lidocaine, bupivacaine, cetrimide and adrenaline at a dose of 4 ml per animal, i.e. 20 mg bupivacaine hydrochloride per animal or 0.4 mg/kg bw for a calf of 50 kg bw. In groups 4 to 6, a gel formulation containing bupivacaine, adrenalin and cetrimide was used. The formulation, in combination with care in application, prevented run-off and the maximum intended label dose of the product remained in contact with the fresh surface of the disbudding wound and immediately adjacent skin.

Animals were slaughtered at 6 hours (groups 1 and 4), 24 hours (groups 2 and 5) and 48 hours (groups 3 and 6) after treatment and samples from skeletal muscle (loin), kidney, liver, perirenal and subcutaneous fat were collected from each animal.

In all groups and in all tissues, concentrations of bupivacaine consistently decreased from 6 hours after treatment to 48 hours after treatment. Although doses were identical, concentrations at 6 hours after treatment were lower in groups 1 to 3 compared to groups 4 to 6.

Highest concentrations of the metabolites 3-OH-bupivacaine and *N*-desbutyl bupivacaine were found in liver and kidney tissues in all treatment groups. Almost all measured concentrations of 3-OH-desbutylbupivacaine (except liver and kidney) and 4-OH-desbutylbupivacaine were below the respective LOQs.

In the groups treated with the product containing lidocaine and bupivacaine (groups 1 to 3), concentrations of 2,6-xylidine were even higher than concentrations of bupivacaine. Whereas in the groups treated with the product containing bupivacaine only (groups 4 to 6), almost all concentrations of 2,6-xylidine in all tissues were below the respective LOQs.

To allow for comparison with relevant reference values, the amount of bupivacaine plus metabolites as well as the amount of 2,6-xylidine per food basket for the concentrations measured at 6, 24 and 48 hours in groups 4 to 6 were calculated.

At the earliest time point measured (6 hours post treatment), the total intake of residues (bupivacaine plus metabolites including 2,6-xylidine²) would be 6.68 µg/person/day or 0.11 µg/kg bw/day, respectively.

The concentrations of 2,6-xylidine (0.05 µg/person/day) plus the additional proportion of bupivacaine (plus other metabolites) that could give rise to 2,6-xylidine via metabolism in man (0.08 µg/person/day), leading to a total of 0.13 µg/person per day at 6 hours after treatment, is considerably below the substance-specific reference value (lower limit of the acceptable reference range 6.2 µg/person/day) at all times after treatment.

Selection of marker residue and ratio of marker to total residues

As a 'No MRL required' status is recommended, identification of a marker residue and establishment of a ratio of marker to total residues is not required.

2.2.3. Monitoring or exposure data

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

2.2.4. Analytical method(s) used in residue studies

Two analytical LC-MS/MS methods have been developed and validated for the determination of bupivacaine in tissues, fluids and faeces. The methods are presented in an internationally recognized format and have been validated in line with the current requirements of Commission Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009.

Bupivacaine and its metabolites are quantified using ultra high performance liquid chromatography (UHPLC) coupled with a tandem mass spectrometer. The test method for determination of residues of bupivacaine and its metabolites in bovine tissues, fluids and faeces was validated according to Annex 3 'Protocol for residue method validation' of VICH GL 49. Validation data provided are compliant with the requirements according to VICH GL 49.

Some results from storage stability studies were not in accordance with the stipulations of VICH GL 49. A remarkable degradation of 2,6-xylidine and 3-OH-bupivacaine was observed in liver, kidney, muscle and fat after 3–4 months even when stored at -18 °C. Similarly, in the short-term stability studies, a remarkable degradation of the metabolites 2,6-xylidine and 3-OH-bupivacaine, 3-OH-desbutylbupivacaine and 4-OH-desbutylbupivacaine in certain tissues was observed even when stored at -18 °C. This is likely to affect the results of the residue depletion studies in tissues. However, the results of the short term stability studies (2 and 4 weeks) show that, despite the observed instability of the analytes, there is a significant amount of the analyte left after two and four weeks (in the residue depletion study the maximum storage interval at approximately -18 °C from sampling to extraction was 14 days). Overall, with regard to the portion of the utilized ADI and substance-specific reference values (see section 2.2.2), the impact of the instabilities on the residue depletion studies is not considered to

² Strictly speaking, to calculate total residues in the food basket, a correction factor should be introduced to correct differences in molecular weights between bupivacaine metabolites and the parent substance. However, as molecular weights of the metabolites are similar to that of bupivacaine and residue concentrations were low, the resulting difference to the calculation is small and would not have an impact on the assessment.

influence the consumer safety assessment to a significant extent.

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

The substance is not intended for use in dairy animals and therefore potential effects in dairy products were not investigated. In addition, microbiological effects were only observed at very high concentrations which are highly unlikely to occur.

2.2.6. Findings of EU or international scientific bodies

No relevant evaluations by EU or international scientific bodies were identified.

3. Risk management recommendations

3.1. Availability of alternative medicines and other legitimate factors

Availability of alternative medicines

There are limited treatments available which can be used for anaesthesia/treatment of pain associated with the disbudding of calves.

Technological aspects of food and feed production (potential effects on the microorganisms used for industrial food processing)

Calf meat is not further processed to food or feed products using microorganisms.

Conditions of use

Use of bupivacaine needs to be restricted to calves less than two months of age and to cutaneous and epilesional application routes as these were the conditions used in the residue studies provided.

Other factors that should, if applicable, be taken into consideration in support of the MRL recommendation:

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

3.2. Elaboration of MRLs

The toxicological ADI for bupivacaine is considered the overall ADI to be used for the risk assessment and is 0.025 mg/kg bw per day or 1.5 mg/person per day. It is considered appropriate to reserve 20% of the ADI in case future uses of the substance would result in residues in other food commodities, particularly milk. Consequently, the amount of the ADI that can be considered in relation to residues in tissues is 1200 µg/person/day. In addition, the acceptable intake for the carcinogenic metabolite 2,6 xylidine that is present in edible bovine tissue is calculated to be 6.2 µg/person per day.

Residue depletion data demonstrate that, at the earliest time point measured (6 hours post treatment), the total intake of residues (bupivacaine plus metabolites including 2,6-xylidine) would be 6.68 µg/person/day or 0.11 µg/kg bw/day, based on the standard food basket. This corresponds to a margin of approximately 180 between the maximum potential intake of total residues at 6 hours the portion of the ADI that can be considered in relation to residues in tissues (1200 µg/person/day). The margin increases to 263 at 24 hours after treatment and to 863 at 48 hours after treatment. These

margins are considered sufficient to account for any potentially unmeasured additional metabolites as well as for shortcomings in the validation of the analytical method. It is accepted that consumer exposure will remain below the ADI at all times after treatment.

Concerning the risk assessment for the carcinogenic metabolite 2,6-xylylidine, direct exposure to this substance plus exposure to the additional portion of bupivacaine (and metabolites) that could give rise to 2,6-xylylidine via metabolism in man, corresponding to an overall total of 0.13 µg/person per day at 6 hours after treatment, is considerably lower than the substance-specific reference value of 6.2 µg/person/day at all times after treatment.

In view of the fact that exposure to residues of bupivacaine can be expected to be substantially below the ADI at 6 hours after dosing and that, at this timepoint, exposure to the genotoxic metabolite 2,6-xylylidine will also be below the relevant reference value, a 'No MRL required' classification is considered appropriate for bupivacaine. This conclusion can only be applied to the conditions used in the residue studies provided and consequently use of bupivacaine in cattle should be restricted to cutaneous and epilesional use in calves up to 2 months of age.

4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits established for bupivacaine on the basis of residue data in calves, to other food producing species. Bupivacaine may be metabolised to a greater extent in older calves and consequently a recommendation cannot be made to establish an MRL entry applicable to all cattle. Extrapolating the available data to species other than bovine would be associated with an even greater degree of uncertainty and consequently, based on the available data, the Committee considers that extrapolation of MRLs to other food producing species cannot be recommended.

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

Having considered that:

- the toxicological ADI of 1.5 mg/person per day was established as the overall ADI and used for quantitative risk assessment of consumer exposure to bupivacaine residues,
- an acceptable, virtually safe, exposure level of 2,6-xylylidine of 6.2 µg/person per day based on substance-specific data was derived,
- the quantitative risk assessment based on bupivacaine residues in calves' tissues resulted in a consumer exposure estimate well below the ADI at all timepoints investigated (i.e. from 6 hours after dosing),
- the quantitative risk assessment based on levels of 2,6-xylylidine in calves' tissues plus levels of 2,6-xylylidine produced by metabolism of bupivacaine in humans resulted in a consumer exposure estimate below the acceptable exposure level at all timepoints investigated (i.e. from 6 hours after dosing),
- metabolism of bupivacaine can only be assessed in calves up to 2 months of age based on the study data provided and a corresponding restriction is therefore considered appropriate,
- the available data only allowed assessment of residues resulting from epilesional and cutaneous application and a restriction to these routes of administration is therefore considered necessary,

the Committee concludes that the establishment of maximum residue limits for bupivacaine in bovine is not necessary for the protection of human (consumer) health and therefore recommends the inclusion of bupivacaine in table 1 of the Annex to Commission Regulation (EU) No 37/2010 as follows:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Bupivacaine	NOT APPLICABLE	Bovine	No MRL required	NOT APPLICABLE	For use in calves up to 2 months of age only For cutaneous and epilesional use only	Local anaesthetic

The theoretical maximum daily intake of residues from bovine tissues represents approximately 0.5% of the ADI. There is a margin of approximately 48 between the potential total consumer exposure to 2,6-xylidine and the reference value of 6.2 µg/person per day.

6. Background information on the procedure

Submission of the dossier 23 April 2019

Steps taken for assessment of the substance

Application validated:	15 May 2019
Clock started:	16 May 2019
List of questions adopted:	12 September 2019
Consolidated response to list of questions submitted:	20 March 2020
Clock re-started:	23 March 2020
CVMP opinion adopted:	18 June 2020