



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

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

On 12 November 2020, the European Commission adopted a Regulation¹ establishing maximum residue limits (no MRL required classification) for bupivacaine in porcine species (piglets up to 7 days 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 piglets up to 7 days of age for local anaesthesia during and after castration at a maximum dose of 8.4 mg/animal.

Medical Ethics UK Ltd submitted to the European Medicines Agency an application for the establishment of maximum residue limits on 22 June 2018.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended, on 20 February 2020, the establishment of maximum residue limits for bupivacaine in porcine species.

Subsequently the Commission recommended, on 29 September 2020, that maximum residue limits (no MRL required classification) in porcine 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/0001
Applicant:	Medical Ethics UK Ltd
Target species:	Piglets up to 7 days 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. In veterinary medicine, bupivacaine is authorised for use in companion animals, usually administered by injection. 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 in the amount of 5 g/l (i.e. 4.2 g/l bupivacaine), as racemic mixture (50:50) of its two enantiomers R(+) bupivacaine and S(-) bupivacaine (levobupivacaine).

Bupivacaine is intended to be used as a local anaesthetic for cutaneous and epilesional² use during and after castration of piglets under 7 days of age, at a maximum dose of 2 ml of the product (8.4 mg bupivacaine).

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

² i.e. administration onto a lesion

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 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, and 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, and 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 pipercolic 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. For information on metabolism in piglets see section 2.2.

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 2.1.7. '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 xylidine. 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 performed 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 effect and no effect levels. Furthermore, chronic bupivacaine exposure of humans via 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 bupivacaine was administered by subcutaneous injection to three animals per dose group. In rats a NOAEL 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 with 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, hematology, coagulation, clinical chemistries, 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 chemistries, 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 percentage of foetuses with external and visceral variations (18.9% vs. 13.4. in control), low incidence of dilatation of olfactory ventricles (1% (not statistical significant) vs. 0 in control), 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% (not statistically significant) vs. 0 in control). 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 LOEL 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 was 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 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 LOEL of 10 mg/kg bw per day for bupivacaine 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 body weight 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 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 S-9 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, was clearly negative with and without metabolic action. 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 are 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.

Carcinogenicity

No carcinogenicity studies using bupivacaine are available. Findings in *in vivo* and *in vitro* genotoxicity assays were overall negative and there is some 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 xylidine, a metabolite of bupivacaine in piglets, has been associated with carcinogenicity in rats. In a carcinogenicity test, rats received 2,6 xylidine 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 body weight gains were 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 rat (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 TD25 (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% (day1) to 43.9% (day 7) of the test substance. The nominal doses and the resulting TD25 need to be corrected for the possible losses of 2,6 xylidine 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 TD25_{corr} 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 xylidine; this is considered separately, below.

In piglets, 2,6 xylidine, 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 4 mg/kg bw (see section 2.2.1 below). Therefore, bupivacaine treatment may lead to exposure to 2,6 xylidine via food derived from treated pigs and is also formed through metabolism in humans, although to a lesser extent.

Given the occurrence of the carcinogenic metabolite 2,6 xylidine, a toxicological reference value for exposures to this metabolite is required. Based on the TD_{25corrected} 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 concentration 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 that of 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 unsedated 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, 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 was 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, (0.3-3µg/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 hematology 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 subchronic and chronic exposures to bupivacaine is also available from studies with repeat dose bolus injection and/or continuous prolonged peri-neural, epidural or intra-theal 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 blind, randomized, crossover, multicenter 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, 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 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 amount of 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 TD_{25corrected} 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

The applicant provided two metabolism studies in the target animals (piglets).

A GLP-compliant study was conducted in 12 entire male piglets (commercial hybrid, 4 to 6 weeks old, 6-10.5 kg bw). Animals were treated using 0.5% solution bupivacaine (as bupivacaine hydrochloride, 5.28 mg/ml equivalent to 5 mg/ml anhydrous bupivacaine hydrochloride). Piglets up to 4 kg bw were treated at a dosage of 0.8 ml/kg bw (4.2 mg bupivacaine hydrochloride per kg bw), and piglets of 4 kg bw and above were treated at a fixed volume of 3.4 ml. The dosage used is well in the range of the intended dose of bupivacaine but below the maximum dose of 5.0 mg hydrochloride /kg bw.

The calculated dose of bupivacaine was applied into the incised scrotum with the dosage divided equally per side of the castration. After 20 seconds, the testes were removed via normal procedure. Samples of blood, urine, faeces and edible tissues were collected and analysed for residues of bupivacaine, 2,6 xylidine, N-desbutyl bupivacaine and 3-OH bupivacaine at 15, 45, 90 minutes and then at several time points between 4 to 96 hours after treatment.

The major compound found in plasma was bupivacaine, which was also found in urine. However, 3-OH bupivacaine was confirmed to be the main metabolite of bupivacaine in piglets, being present in high concentrations in both urine and faeces. In addition, significant concentrations of N-desbutyl bupivacaine and unmetabolised bupivacaine were also excreted in the urine.

2,6 xylidine was also present at quantifiable levels in liver, muscle, skin and faeces (all concentrations were below 1 µg/kg bw), with slightly higher levels in kidney (1.41 µg/kg at 10 hours post treatment, 1,21 µg/kg bw at day 1 post treatment), and urine (3.32 µg/l at 10 hours, post treatment). Levels in muscle were below the LOQ (0.2 µg/kg) at 10 and 24 hours after treatment, and below the limit of detection (0.07 µg/kg) at day 4.

The amount of 2,6 xylidine calculated per standard food basket indicates that consumer exposure levels are below the acceptable exposure level (lower limit of the acceptable reference range 6.2 µg/person/day) at 10 hours, day 1 and day 4 after treatment. However, according to literature data a small amount of additional 2,6 xylidine resulting from the metabolism in humans of ingested piglet meat containing bupivacaine residues needs to be added (1.2% of bupivacaine and further metabolites).

A second metabolism study was conducted with a combination product containing bupivacaine plus lidocaine, adrenaline and cetrimide and applied to 9 study groups, and with bupivacaine (as hydrochloride) 0.5% solution applied to 3 study groups. Lidocaine and metabolites as well as cetrimide residues were found in samples from bupivacaine-only treated animals, indicating cross-contamination. Results from this study were considered as unreliable and not taken further into account.

2.2.2. Residue depletion studies

No radiometric residue depletion studies were conducted. The applicant provided results from two non-radiolabelled residue depletion studies. One was conducted with a combination product containing bupivacaine plus lidocaine, cetrimide and adrenaline and the second study was conducted using a combination of bupivacaine, cetrimide and adrenaline only.

In the first study, thirty entire male piglets (1.35-2.91 kg bw, commercial hybrid) were randomly allocated to six groups of four animals each (group 1-6), and one group of six animals (group 7, treated spare animals). Piglets were treated with the product (lidocaine 50.5 g/l; bupivacaine 5.02 g/l; cetrimide

5.06 g/l, adrenaline 0.0495 g/l) topically for either tail docking, or castration.

For castration (groups 1-5), piglets up to 4 kg bw were treated at a dose of 0.6 ml/kg bw (3 mg bupivacaine hydrochloride per kg bw) divided equally per side of the castration. Piglets of 4 kg bw and above were treated at a fixed volume of 2.4 ml divided equally per side of the castration. The product was applied into the incised scrotum and piglets were castrated 20 seconds later. The dosage used is within the intended dose range but considerably lower than the highest intended dose of 5.0 mg bupivacaine hydrochloride per kg bw. The requirement according to VICH GL 46, that the dose administered to the study animals should be the highest intended dose, was not met.

In piglets in group 6, the product was applied to the wound within one minute after tail docking at a dosage of 0.3 ml (1.5 mg bupivacaine hydrochloride per kg bw).

Several samples from blood, urine and faeces were collected from animals in Groups 5 and 7 in a time interval from 5 minutes up to 4 days post-treatment. Additionally, blood, urine and faecal samples were collected from all animals at sacrifice.

Triplicate samples of skeletal muscle (loin), liver, kidney and skin with subcutaneous fat in natural proportions from over the shoulder were collected from each animal at 5 minutes (group 1), 4 hours (group 2), 12 hours (group 3), 24 hours (groups 4 + 6) and 7 days (group 5) after treatment. Additional sampling from treated spare animals (group 7) at 42 days post-treatment was conducted due to the persistence of most metabolites beyond the 7 days post-treatment sampling time point and the desire to trace metabolite levels to below limit of quantification for withdrawal time calculations.

Samples were analysed for parent compounds and the metabolites 2,6 xylidine, 3-OH bupivacaine and N-desbutyl bupivacaine (measurements for residues of lidocaine/metabolites, cetrimide are not reported here).

Residues of bupivacaine were found in all edible tissues with highest concentrations at 12 hours after treatment. Concentrations in skin and fat were higher compared to other edible tissues. Concentrations of 2,6 xylidine were in the same order of magnitude as the bupivacaine concentrations, while concentrations of 3-OH bupivacaine and N-desbutyl bupivacaine were considerably lower in all tissues and at all time points.

The amount of total residues as well as the presence, number and nature of further metabolites are unknown. No adjustment was made for the shortcomings in the analytical method and the dosage being lower than the maximum intended dose.

The available data indicate that the sum of bupivacaine, 3-hydroxy bupivacaine and N-desbutyl bupivacaine present in the standard food basket reaches a highest value of 249 µg at 12 hours after treatment.

A combination product was used in the residue depletion study described above, and both lidocaine as well as bupivacaine may release 2,6 xylidine. Since it cannot be discriminated which proportion of 2,6 xylidine concentrations originates from which parent substance, the study cannot be used to estimate consumer exposure to 2,6 xylidine originating from bupivacaine alone.

The second residue depletion study was conducted in three groups of four piglets each (1.86-2.77 kg bw, commercial hybrid). For castration, animals were treated with a combination product containing 5.0 mg/ml bupivacaine hydrochloride, 5.0 mg/ml cetrimide, and 0.451 mg/ml adrenaline bitartrate at a dose of 0.8 ml/kg bw. Piglets were sacrificed at 6 hours, 24 hours and 48 hours after treatment and samples of skeletal muscle (loin), liver, kidney and skin with subcutaneous fat in natural proportions from over the shoulder were collected.

Samples were analysed for bupivacaine, N-desbutyl bupivacaine, 3-OH bupivacaine, 2,6 xylidine, 3-OH-

desbutyl bupivacaine and 4-OH-desbutylbupivacaine in the matrices concerned using a LC-MS/MS analytical method.

Highest residues concentrations were measured for bupivacaine and 3-OH bupivacaine. In all tissues and for all metabolites, residue concentrations decreased from 6 hours after treatment to 48 hours after treatment. Literature data showed that no further metabolic pathways are relevant in the target species, indicating that the toxicological relevance of the metabolites is comparable to those seen in laboratory animals.

The calculated total intake of residues (bupivacaine plus metabolites including 2,6 xylidine) at 6 hours post treatment was 161.94 µg/person/day or 2.69 µg/kg bw/day based on the standard food basket.

Selection of marker residue and the ratio of marker to total residues

Selection of a marker residue and of a ratio of marker to total residues are not needed, as the utilized portion of the ADI is sufficiently low to allow for a "No MRL required" status – see section 3.2 below.

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) for monitoring of residues

The applicant developed a residue analytical method allowing for the determination of bupivacaine and its metabolites, and provided a validation report.

Bupivacaine and its metabolites were quantified using Ultra-High Performance Liquid Chromatography (UHPLC) with a tandem mass spectrometer. For bupivacaine and N-desbutyl bupivacaine other chromatography conditions were used compared to those for 2,6 xylidine and 3-OH bupivacaine. The limit of detection for bupivacaine and its metabolites was 0.07 µg/kg (intended and estimated LOD). The limit of quantification for bupivacaine and its metabolites in tissues was 0,2 µg/kg (intended and estimated LOQ). The statistically determined limits of detection and quantification differed from the estimated and intended values.

Validation data for the test method for the determination of residues of bupivacaine and its metabolites in porcine tissues, fluids and faeces was generated according to Annex 3 "Protocol for Residue Method Validation" of VICH guideline 49 but are not fully compliant with the requirements of the guideline. High dispersion of measurement values occurred in particular at low concentrations, resulting in higher measurement uncertainties. Some non-compliances also occurred at higher concentrations. The applicant performed a new partial (not complete) validation study. The newly acquired validation data were merged with a part of the original validation data and the overall results were better in accordance with stipulation of VICH guideline 49. In addition, the estimated limits of detection and limits of quantification differ from the statistically determined ones, but both estimated and statistically determined values are in the same concentration range and the influence on the overall results is unlikely to be significant. In summary, a full new validation of the method with all validation parameters in line with the requirements of VICH guideline 49 was not available. However, keeping in mind the considerations and conclusions in sections 3.2 'Elaboration of MRLs' and 5. 'Conclusions and recommendation for the establishment of maximum residue limits' of this document, the shortcomings with regard to the validation of the analytical test method, are considered to have only a minor influence on the reported results of the residues studies and are likely to be compensated for by the overall margins of exposure obtained (see section 3.2 below).

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 castration of piglets.

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

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

Conditions of use

Use of bupivacaine needs to be restricted to piglets up to 7 days of age. Available data indicate that pigs at higher age more extensively metabolise the substance, potentially resulting in higher concentrations of 2,6 xylidine. Furthermore, use needs to be restricted to the cutaneous and epilesional routes as this is the scenario used in the available studies and assessed by the CVMP. Use of bupivacaine via injection may require higher doses or may lead to a different residue depletion pattern and may, therefore, require additional risk management measures in order to ensure consumer safety.

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 porcine tissue is calculated to be 6.2 µg/person per day (worst case scenario).

Residue depletion data demonstrate that a total intake of residues, including bupivacaine plus its metabolites at 6 hours post treatment would be 161.94 µg/person per day, or 2.69 µg/kg bw per day based on the standard food basket. The margin between the amount of measured residues per food

basket and the proportion of the ADI that can be considered in relation to residues in tissues (1200 µg/person/day) is approximately 7.4 at 6 hours after treatment and approximately 17.5 at 24 hours after treatment. Concerning risk assessment for the carcinogenic metabolite 2,6 xylidine, the exposure of 2,6 xylidine at 6 hours after treatment is compared to the substance specific reference value of 6.2 µg/person per day. A figure of 1.2% was used for the proportion of bupivacaine (and metabolites) that could give rise to 2,6 xylidine via metabolism in man (1.940 µg/person per day) and was, therefore, added to the concentrations of 2,6 xylidine measured in the relevant tissues (0.25 µg/person per day). The resulting total potential human exposure (2.185 µg/person per day) is below the reference values for 2,6 xylidine of 6.2 µg/person per day at all examined timepoints, i.e. from 6 hours onwards.

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 (margin of 7.4) and that, at this timepoint, exposure to the genotoxic metabolite 2,6 xylidine 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 should be restricted to cutaneous and epilesional use in piglets up to 7 days 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 piglets, to other food producing species. As discussed in section 3.1 bupivacaine may be metabolised to a greater extent in older pigs and consequently a recommendation cannot be made to establish an MRL entry applicable to all pigs. Extrapolating the available data to species other than pigs 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 xylidine of 6.2 µg/person per day based on substance specific data was derived,
- the quantitative risk assessment based on bupivacaine residues in piglet 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 xylidine in piglet tissues plus levels of 2,6 xylidine 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 in neonatal piglets is considerably lower than that in pigs at higher age and a corresponding restriction to use in neonatal animals is therefore considered appropriate,
- the available data only allowed assessment of residues resulting from cutaneous and epilesional application and a restriction to this route of administration is therefore considered necessary,

the Committee concludes that the establishment of maximum residue limits for bupivacaine in porcine 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:

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

The theoretical maximum daily intake of residues from porcine tissues represents approximately 11% of the ADI. Assuming slaughter of piglets just 6 hours after dosing there is a margin of approximately 3 between the potential total consumer exposure to 2,6 xylydine and the reference value of 6.2 µg/person per day.

1. Background information on the procedure

Submission of the dossier:	22 June 2018
Steps taken for assessment of the substance	
Application validated:	11 July 2018
Clock started:	12 July 2018
List of questions adopted:	8 November 2018
Consolidated response to list of questions submitted:	17 April 2019
Clock re-started:	22 April 2019
List of outstanding issues adopted:	20 June 2019
Consolidated response to outstanding issues submitted:	20 January 2020
Clock re-started:	21 January 2020
CVMP Opinion adopted:	20 February 2020