10 April 2015
EMA/CVMP/118717/2015
Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report regarding the request for an opinion under Article 30(3) of Regulation (EC) No 726/2004

In relation to the potential risk for the consumer resulting from the use of lidocaine in food producing species

Procedure no: EMEA/V/A/092

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1. Background information on the procedure

1.1. Request for CVMP opinion

On 21 December 2012, The Netherlands presented to the European Medicines Agency a request for an opinion in accordance with Article 30(3) of Regulation (EC) No 726/2004 from the Committee for Medicinal Products for Veterinary Use (CVMP) on the risk for the consumer resulting from the use of lidocaine in food producing species.

1.2. Steps taken during the procedure

- During the January 2013 CVMP meeting, the following was agreed:
  - Dr Bruno Urbain was appointed rapporteur.
  - Dr Cristina Muñoz Madero was appointed co-rapporteur.
  - The procedure started at the January 2013 CVMP meeting and a timetable was adopted.
  - For the assessment of the key questions on genotoxicity/carcinogenicity it was agreed to involve an Ad Hoc Expert Group (AHEG).
- An AHEG was established during the March 2013 CVMP meeting.
- The rapporteur's assessment report was circulated to all CVMP members on 15 April 2013.
- The co-rapporteur's critique to the rapporteur's assessment report was circulated to all CVMP members on 29 April 2013.
- During the 14-16 May 2013 CVMP meeting the Committee discussed the rapporteur's assessment report including the co-rapporteur's critique and adopted a list of questions to the AHEG.
- An AHEG meeting was held on 10 June 2013.
- On 11 June 2013 the overall conclusions from the AHEG meeting and the final AHEG report were presented to the CVMP. The clock was stopped to await the results from a new residues study being conducted by The Netherlands.
- The results from the residues study were submitted on 17 March 2014 and the clock re-started.
- On 16 April 2014 the updated rapporteur's assessment report was circulated to all CVMP members.
- The co-rapporteur's critique to the updated rapporteur's assessment report was circulated to all CVMP members on 22 April 2014.
- During the 6-8 May 2014 CVMP meeting the updated rapporteur's assessment report including the co-rapporteur's critique was discussed.
- The procedure was also discussed during the 3-5 June 2014, 9-11 September 2014, 9-11 December 2014, 13-15 January 2015, 10-12 February 2015 and 10-12 March 2015 CVMP meetings.
- On 12 September 2014 the Committee sent a request for advice on risk management considerations to the European Commission.
- On 14 November 2014 the European Commission provided their advice to the CVMP.

- On 20 February 2015 the revised rapporteur's assessment was circulated to all CVMP members.
- On 10 April 2015 the CVMP adopted an opinion in accordance with Article 30(3) of Regulation (EC) No 726/2004.

2. Scientific discussion

2.1. Introduction

Lidocaine was assessed by the CVMP for the purpose of establishing maximum residue limits (MRLs) in 1999 (see published summary report EMEA/MRL/584/99-FINAL). The evaluation concluded that numerical MRL values were not required for the protection of the consumer and therefore the substance is included in Table 1 of the Annex of Commission Regulation (EU) No 37/2010 with a "no MRL required" classification but with the use restricted to *equidae* and for local/regional anaesthesia only.

Lidocaine can therefore only be used in *equidae* or exceptionally in other species in line with the provisions of Article 11 of Directive 2001/82/EC (under the so-called "cascade"). The "cascade" is a legal provision that, in general terms, allows veterinarians under specified conditions to use products that are authorised for another species if no medicinal product has been authorised for the treatment of a specific condition in the concerned animal species. It is reported that products containing lidocaine are in fact widely used in major food producing species such as cattle and pigs under the cascade due to the lack of authorised anaesthetics in those animal species.

The CVMP previously concluded that MRLs could not be established in food producing species other than horses because the metabolism in these species was unknown. A particular concern was a metabolite of lidocaine, 2,6-xylidine, but data on the occurrence of this metabolite was not available for most food producing species. 2,6-Xylidine was shown to have genotoxic characteristics *in vivo* (see CVMP MRL Summary Report).

Recent research indicates that 2,6-xylidine is the main metabolite produced by primary hepatocytes and liver microsomes from pigs and cattle when exposed to lidocaine *in vitro* (Thuesen and Friis, 2012). The presence of 2,6-xylidine in urine of pigs and cattle was confirmed after intravenous administration of lidocaine (personal communication C. Friis, 2012).

The Dutch authorities considered that the data available indicate a reason for concern with regard to consumer safety and that in order to decide if any actions are necessary at EU level, e.g., related to the marketing authorisations and/or the MRL status of lidocaine, a common understanding of the scientific conclusions on the hazards and consumer risks resulting from the use of lidocaine in food producing species was required.

Therefore, the CVMP was requested to give an opinion on the risk for the consumer resulting from the use of lidocaine in food producing species.

The questions raised by The Netherlands to the CVMP were as follows:

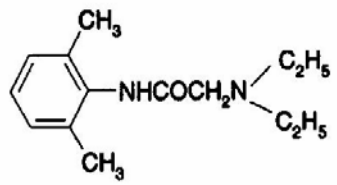
1. Can the CVMP confirm that 2,6-xylidine is a genotoxic carcinogen? From the information available, it appears that 2,6-xylidine is carcinogenic (carcinoma's at multiple sites), however, genotoxicity studies suggest that not 2,6-xylidine but actually a further metabolite is responsible for the genotoxic action, as the results of *in vitro* studies were positive with metabolic activation only.

2. It appears that humans can metabolise lidocaine into 2,6-xylidine. Therefore, even if 2,6-xylidine is not formed in the food producing species (as in horses), the exposure to the parent substance lidocaine via food of animal origin may eventually cause consumer exposure to 2,6-xylidine. Does the CVMP consider that there is a consumer risk for genotoxic and/or carcinogenic effects following exposure to the parent substance lidocaine?
3. What is the consumer risk of exposure to lidocaine-related residues in food resulting from the use of lidocaine in horses, pigs and cattle? Please note that withdrawal periods in horses would be 0 days and the use in other species 28 days for slaughter and 7 days for milk (according to the current rules of the cascade).
4. Does the CVMP consider it necessary to take risk management measures? If yes, what risk management measures and communication does the CVMP consider appropriate?
5. As 2,6-xylidine can also be formed from xylazine, does the CVMP see any consumer safety concern after human exposure to xylazine and/or its metabolites?

2.2. CVMP assessment of the risk for the consumer resulting from the use of lidocaine in food producing species

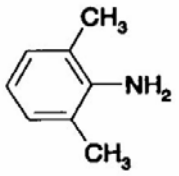
2.2.1. Discussion of Question 1

Lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide] (see Fig.1) is a water-soluble local-regional anaesthetic agent. In humans and animals lidocaine is metabolised to various metabolites, one being 2,6-xylidine (2,6-dimethylaniline or DMA, see Fig. 2), which has been reported to be genotoxic.



Lidocaine

Figure 1. Molecular structure of lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide]



2,6-Xylidine

Figure 2. Molecular structure of 2,6-xylidine (2,6-dimethylaniline or DMA)

In 1993 the International Agency for Research on Cancer (IARC) classified 2,6-xylidine as a group 2B substance ("possibly carcinogenic to humans"). Based on animal carcinogenicity data and other relevant data (induction of haemoglobin adducts, covalent binding to DNA, genotoxicity), IARC concluded that there is inadequate evidence in humans for the carcinogenicity of 2,6-xylidine and that there is sufficient evidence in animals for the carcinogenicity of 2,6-xylidine.

The CVMP Summary report on lidocaine notes that 2,6-xylidine is a mutagenic agent *in vitro* and has genotoxic characteristics *in vivo*.

Biotransformation of lidocaine

The literature on lidocaine metabolism was extensively reviewed by the US National Toxicology Program (NTP) in 2000.

Lidocaine is almost completely metabolised in all species studied, with the liver and intestine (after oral administration) as the primary sites of metabolism. Three main types of metabolic reactions have been identified: aromatic hydroxylation, N-dealkylation and amide hydrolysis, followed by conjugation (Figure 3). Lidocaine undergoes N-dealkylation to form monoethylglycinexylidide (MEGX), which is further dealkylated to glycinexylidide (in humans this dealkylation is primarily mediated by Cytochrome P 450 3A4 [CYP 3A4]) or hydrolysed to 2,6-xylidine. Glycinexylidide is further hydrolysed to form 2,6-xylidine.

In human liver slices, MEGX and 2,6-xylidine were identified as the major metabolites after 4 hours of incubation with lidocaine. The metabolism in different species is qualitatively, but not quantitatively, similar.

In cows 2,6-xylidine is an important metabolite following treatment with lidocaine. 2,6-xylidine was clearly detected in plasma and urine (Hoogenboom *et al.*, 2014).

The metabolism of lidocaine in pig, cattle and rat was investigated *in vitro* using preparations of liver microsomes and primary hepatocytes (Thuesen and Friis, 2012). In both pig and cattle MEGX and 2,6-xylidine were the major metabolites formed in microsomes as well as hepatocytes. In rat MEGX was the major metabolite formed in both microsomes and hepatocytes. In pig and cattle minor metabolites include 3-hydroxy-lidocaine, 4-hydroxy-2,6-xylidine and glycinexylidide. Minor metabolites formed by rat microsomes and hepatocytes were 3-hydroxy-lidocaine, 2,6-xylidine and glycinexylidide. The metabolite 4-hydroxy-2,6-xylidine was produced by rat microsomes but not hepatocytes.

In horse MEGX was the major metabolite formed in microsomes, while 2,6-xylidine was not formed (Friis, personal communication).

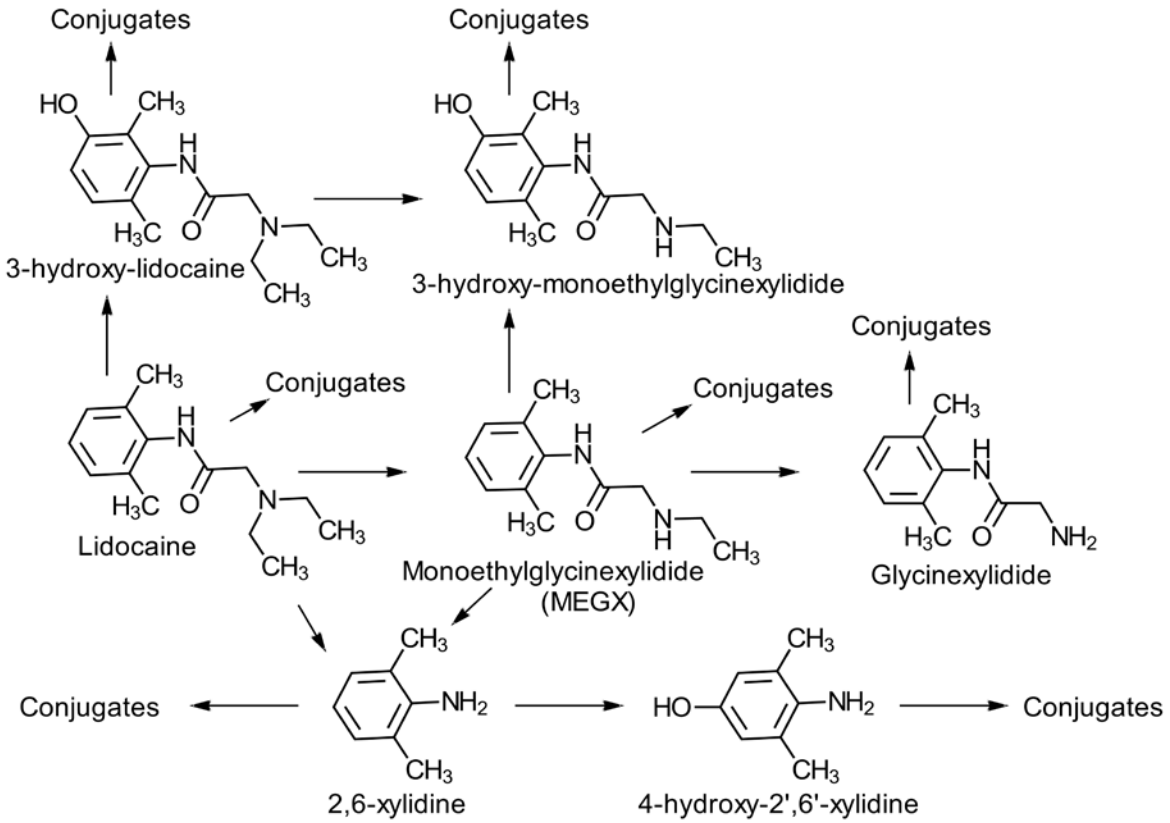


Figure 3: Potential biotransformation routes for lidocaine (according to Friis, 2015 *in press*).

Bioactivation of 2,6-xylidine

CYP-mediated hydroxylation of 2,6-xylidine results in 4-amino-3,5-dimethylphenol (DMAP) (also known as 4-hydroxy-2',6'-xylidine and 4-hydroxy-2,6-dimethylamine) which, as a conjugate in urine, represents about 80% of the lidocaine dose in dogs and rats (Short, Joseph and Hardy, 1989). CYP-mediated monooxygenation of 2,6-xylidine may also lead to the formation of N-(2,6-dimethylphenyl)hydroxylamine (DMHA). DMHA can react with DNA directly or via esterification (Gan, Skipper and Tannenbaum, 2001; Marques *et al.*, 2002) and DMAP is potentially genotoxic following nonenzymatic oxidation to an iminoquinone (Gan, Skipper and Tannenbaum, 2001).

In DNA reacted with N-acetoxy-2,6-dimethylaniline, a reactive acetate ester of DMHA, four adducts were detected (dG-C8-2,6-dimethylaniline, dG-N(2)-2,6-dimethylaniline, dG-O(6)-2,6-dimethylaniline and dA-N(6)-2,6-dimethylaniline) (Goncalves *et al.*, 2001).

CYP 2A6 and CYP 2E1 are known CYPs responsible for the formation of reactive electrophilic intermediates from 2,6-xylidine.

In humans, CYP 2E1 and 2A6 are expressed in the liver and represent approximately 6 and 7% of the total CYP-content. The extrahepatic location of these isoforms in humans has also been investigated. The expression of CYP 2A6 was reported at very low levels in the nasal mucosa (Koskela *et al.*, 1999). In a review by Ding and Kaminsky (2003) CYP 2A6 was reported in the nasal mucosa, lung and trachea and CYP 2E1 was reported in the lungs and oesophagus.

The possible activation pathways for 2,6-xylidine are shown in Figure 4 below.

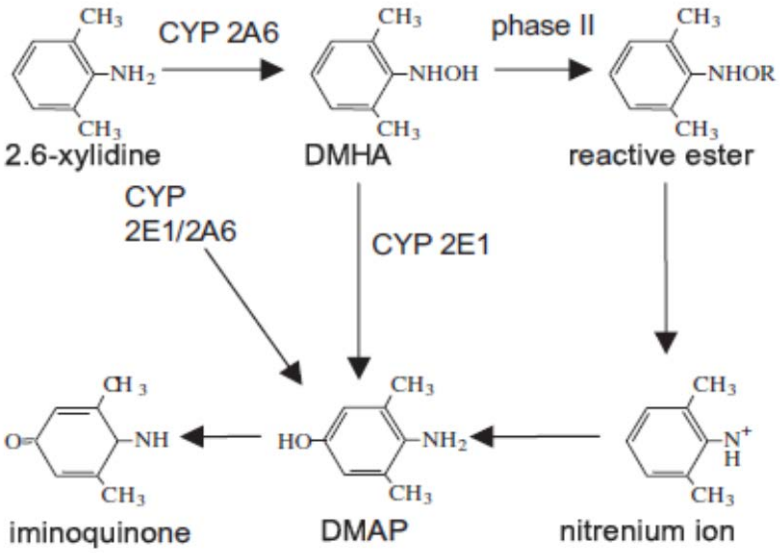


FIG. 1. Proposed activation pathways of 2,6-xylidine. Oxidation of 2,6-xylidine by CYP2A6 or CYP2E1 or by CYP2A6 forms DMAP and DMHA, respectively. DMHA has the potential to react with DNA or hemoglobin. It may also undergo phase II conjugation to reactive esters that can decompose spontaneously to a reactive nitrenium ion, which can react with DNA and other macromolecules. The nitrenium ion can also react with H_2O to form DMAP. The latter can undergo a non-enzymatic oxidation to an iminoquinone, which is a strong electrophile. DMHA can also be rearranged to DMAP by a CYP2E1-catalyzed reactions (modified after Gan *et al*., 2001).

Figure 4. Possible activation pathways for 2,6-xylidine (from Tydén, Tjälve and Larsson, 2004).

In conclusion, the key metabolites formed from 2,6-xylidine by phase 1 metabolism, DMHA and DMAP, may lead to the formation of reactive intermediates like a nitrenium ion by phase 2 (mainly acetylation) metabolism or an iminoquinone. These reactive intermediates have the potential to covalently bind to DNA.

Genotoxic potential

Reports of a large number of tests on the genotoxicity of lidocaine and 2,6-xylidine are available.

Studies in bacterial systems have shown inconsistent results. Negative results have been reported with and without metabolic activation while positive results have been reported in studies in presence of metabolic activation. Kirkland *et al.* (2012) concluded that 2,6-xylidine was not mutagenic in the Ames tests with numerous variations of metabolic conditions.

In vitro tests for gene mutation and chromosomal effects in mammalian cells were positive.

Several studies performed in the rat demonstrate that after oral and intravenous administration of 2,6-xylidine, DNA adducts were formed in the nasal mucosa, the mucosa of the upper alimentary and respiratory tract, liver and other tissues.

Although induction of DNA adducts has previously been considered as the mechanism of genotoxic/carcinogenic action, recent studies indicate that reactive oxygen species (ROSs) can play a role in the genotoxicity of 2,6-xylidine.

2,6-xylidine can be further metabolised to DMHA and DMAP. As detailed above these metabolites lead to the formation of reactive intermediates like a nitrenium ion or an iminoquinone, which have the potential to covalently bind to DNA.

Overall, the data appear to indicate that 2,6-xylidine has a potential to induce genotoxicity *in vivo*. It is not known whether a threshold exists for genotoxicity.

A non-exhaustive list of genotoxicity studies performed with lidocaine and its metabolites is provided in Table 1.

Table 1. Tests for genotoxicity of lidocaine, 2,6-xylidine and other metabolites.

Test	Test system	Results	Reference	Comments
Bacterial test	Ames test/ *Salmonella typhimurium,* strain TA100 N-hydroxylated metabolite of 2,6-xylidine (DMHA)	positive	Beland *et al.,* 1997	
	Ames test/ *Salmonella typhimurium,* strains TA100 and TA98 +/- S9 mix lidocaine	negative	Waskell, 1978	
	Ames test/ *Salmonella typhimurium,* strain TA1538 +/- S9 mix lidocaine/ monoethylglycinexylidine/ N-hydroxylidocaine/ N-hydroxy-monoethylglycinexylidine/ 2,6-xylidine/ 2,6-dimethylphenylhydroxylamine	negative	Nelson, Nelson and Trager, 1978	
	Ames test/ *Salmonella typhimurium,* strain TA1538 + S9 mix 2,6-xylidine	positive	From unpublished report submitted as part of original MRL application	
	Ames test/ *Salmonella typhimurium,* strain TA100 + S9 mix 2,6-xylidine	negative	Zimmer *et al.*, 1980	

	Ames test/ *Salmonella typhimurium,* strain TA100 + S9 mix N-hydroxylated metabolite of 2,6-xylidine (DMHA)	positive	Nohmi *et al.,* 1983	yield of the mutagenic metabolite increased with increasing S9 content
	Ames test/ *Salmonella typhimurium,* + S9 mix 2,6-xylidine	equivocal	Zeiger *et al.,* 1988	weak or questionable mutagenicity of 2,6-xylidine in the presence of 5-10% of Aroclor induced hamster liver S9, but positive mutagenicity when the S9 concentration was increased to 30%.
	Ames test/ *Salmonella typhimurium,* strain TA100 + S9 mix 2,6-xylidine	positive	Kugler-Steigmeier, 1989	positive when S9 concentration was raised from 10 to 20%
	Ames test Conventional strains and strains overexpressing acetyltransferase rat S9, human S9 2,6-xylidine/DMHA/metabolites	negative	Kirkland, 2012	2,6-xylidine not mutagenic even with metabolic activation. Formation of DMHA by induced rat liver S9 and human S9 was shown to occur, and to be concentration and time-dependent.
In vitro test for gene mutation in mammalian cells	Mouse lymphoma tk gene mutation assay 2,6-xylidine	positive	From unpublished report submitted as part of original MRL application	
	Glutamic-pyruvate transaminase (gpt) test in Chinese hamster ovary cells. 2,6-xylidine	positive	Chao *et al., 2012*	The authors found that the main mechanism of mutagenic action was by means of reactive oxygen species (ROS) generation instead of covalent DNA adducts.
In vitro test for chromosomal effects in mammalian cells	Sister chromatid exchange in Chinese hamster ovary cells 2,6-xylidine	positive	Galloway *et al.,* 1987	In presence of precipitated compound

	Chromosomal aberrations test in Chinese hamster ovary cells 2,6-xylidine	positive	Galloway *et al.,* 1987	In presence of precipitated compound
	Chromosomal aberrations test in Syrian hamster embryo cells. lidocaine	negative	Hagiwara *et al.,* 2006	
	DNA adducts salmon testes DNA 2,6-xylidine	positive	Goncalves *et al.*, 2001	4 different adducts identified
In vitro/in vivo rat liver unscheduled DNA synthesis (UDS)	Unscheduled DNA synthesis in rat hepatocytes 2,6-xylidine	negative	Mirsalis *et al.*, 1989	
In vivo test for gene mutation in insects	Wing somatic mutation and recombination test in Drosophila melanogaster larvae lidocaine	negative	Schneider *et al., 2009*	
In vivo test for chromosomal effect	DNA adducts in rats / nasal tissues 2,6-xylidine	equivocal	Short, Joseph and Hardy, 1989	negative only after treatment with a single dose of ^{14}C-2,6-xylidine, while after a 9-day pre-treatment with non-labelled compound the test was positive
	DNA adducts in rats / liver, nasal mucosa, urinary bladder Lidocaine, 2,6-xylidine	positive	Duan, 2008	
	DNA adduct in rats/ nasal tissues, upper alimentary and respiratory tract 2,6-xylidine	positive	Tydén, Tjälve and Larsson, 2004	
	DNA adducts in rats / nasal tissues 2,6-xylidine	positive	Jeffrey, Iatropoulos and Williams, 2006	

	DNA adduct in mice / urinary bladder, liver, colon, kidney, lung, pancreas 2,6-xylidine	positive	Skipper *et al.,* 2006.	Adducts detected mainly in urinary bladder and liver, less frequently in other tissues
	Comet assay in mice 2,6-xylidine	positive	Sasaki *et al*., 1999	DNA damages in skin, urinary bladder, lung and brain
	Micronuclei in mouse polychromatic erythrocytes 2,6-xylidine	negative	Parton, 1988, 1990	
	Preferential killing of DNA repair deficient *Escherichia coli* bacteria *in vivo* using a host-mediated assay in the mouse 2,6-xylidine	negative	From unpublished report submitted as part of original MRL application	

Carcinogenic potential

The major mechanism of carcinogenicity of aromatic amines such as 2,6-xylidine is via metabolism by cytochromes P450 to the N-hydroxyl derivatives that may be further metabolized by conjugation to yield reactive metabolites. DNA binding has been reported for 2,6-xylidine and its metabolites. Alteration of DNA is recognised as a possible mechanism by which compounds such as these exert some of their carcinogenicity.

Carcinogenicity studies

2,6-xylidine has been tested for its carcinogenic potential in a rat study (NTP, 1990), where it was administered in the diet prior to mating, during pregnancy, lactation and weaning of the offspring and then for 2 years in the diet of the offspring. The dose levels were 0, 300, 1000 and 3000 ppm.

In this study, the epithelium of the nasal cavity was the primary site of both neoplastic and non-neoplastic (acute inflammation, epithelial hyperplasia, and squamous metaplasia) lesions. Carcinomas or adenocarcinomas occurred in high dose males and high and mid dose females. Papillary adenomas occurred in high and mid dose males and high dose females. The carcinomas were highly invasive and metastases to the brain were observed in several rats in the high dose groups. Furthermore, rhabdomyosarcoma, a rare tumour in the nasal cavity, was observed in the high dose group.

In addition, increased incidences of subcutaneous fibroma and fibrosarcoma in both sexes and an increased incidence of neoplastic nodules in the liver (neoplastic nodules were seen in 0, 2, 4 and 7% of controls, low, mid and high dose animals, respectively) in females were observed in the study.

Although findings of DNA adducts do not represent a definite indication of carcinogenicity, there was a good correlation between the localisation of DNA adducts in the rat genotoxicity studies and the tumour sites in the carcinogenicity study.

The results of the carcinogenicity study have been questioned for several reasons, mainly due to the non-standard design (pre- and postnatal exposure) and possible evaporation of the drug from the feed mixture causing direct exposure of the nasal cavity by inhalation.

The animals were exposed *in utero*, via maternal milk and via the feed from 3 weeks of age and throughout their lives. It is not known to what extent 2,6-xylidine crosses the placenta or is excreted into milk and how much of the amount excreted into milk is deactivated via chemical reaction in the milk (2,6-xylidine is highly reactive with lactose and glucose). Thus, both the actual exposure and the influence of the non-standard design are unknown.

The degree of evaporation from the diet and possible exposure by inhalation is unknown. Stability data indicate that 3000 ppm of 2,6-xylidine in the feed was quite stable for 28 days in an open container, whereas 40% of the 2,6-xylidine in the 1000 ppm diet was lost within one week. The stability of the low dose of 300 ppm of 2,6-xylidine in the feed is not known. The results from the stability study of the 1000 ppm diet indicate that the mid-dose animals received a substantially lower oral dose than that intended and possibly also inhaled some of the compound. If there is an inverse concentration dependent stability problem, the low dose will have been much lower than intended.

It has therefore been proposed that the effects on the nasal cavity may be related to a local response to inhaled 2,6-xylidine. However, the formation of DNA adducts after both oral and intravenous administration of 2,6-xylidine has been demonstrated in the rat (Tydén, Tjälve and Larsson, 2004; Jeffrey, Iatropoulos and Williams, 2006). These results show that the nasal olfactory and respiratory mucosa and the mucosa of several other tissues have the capacity to bioactivate 2,6-xylidine after

both intravenous and oral administration. Furthermore, it should be noted that subcutaneous fibromas/fibrosarcomas are typical of monocyclic arylamines (Weisburger *et al.*, 1978).

From this study it seems that 2,6-xylidine is carcinogenic in the rat, inducing highly malignant tumours at the site where the highest levels of DNA adducts were seen in genotoxicity studies, as well as tumours typical of monocyclic arylamines. Thus, the tumour formation in the nasal tissue is likely to be due to a genotoxic mechanism. However, the study does not allow the determination of a no observed effect level (NOEL) for carcinogenicity.

Overall, the genotoxicity studies show the presence of DNA adducts at the site of tumour induction, which implies a link between exposure to 2,6-xylidine and cancer incidence, mediated by covalent binding to DNA in the target cells.

Expert consultation on the genotoxicity and carcinogenicity of 2,6-xylidine

In addition to the evaluation described above, the CVMP convened an ad hoc expert group specifically to consider the genotoxicity and carcinogenicity of 2,6-xylidine. The group was asked to respond to four specific questions. These are presented below along with the responses:

1. Could the AHEG confirm that 2,6-xylidine is genotoxic?

Response from the AHEG:

Studies in bacterial systems show inconsistent results. Chromosomal aberrations, DNA strand breaks and gene mutation *in vitro* studies in mammalian cells were positive. Although no induction of micronuclei was observed, several *in vivo* studies measuring DNA adducts were positive both in rats and mice in both target and non-target tissues for cancers (examples include olfactory mucosa, mucosa of the upper alimentary tract and the respiratory tract as well as in the liver and bladder). *In vivo* induction of DNA breaks as measured by Comet assay was shown in stomach, urinary bladder, lung and brain.

Therefore 2,6-xylidine has a potential of genotoxicity *in vivo*.

2. If yes, does a threshold exist for genotoxicity?

Response from the AHEG:

Based on the conclusions on genotoxicity, risk assessment should follow a non-threshold approach awaiting further research in this field.

3. Could the AHEG confirm that 2,6-xylidine is carcinogenic?

Response from the AHEG:

The *in vivo* NTP bioassay clearly demonstrates that 2,6-xylidine administered in the diet in rats is a carcinogen. The main tumours observed were carcinoma of the nasal cavity as well as subcutaneous fibroma and fibrosarcoma. It is noticed that the DNA adducts were observed in the same target tissues where tumours were observed and hence 2,6-xylidine can be considered a genotoxic carcinogen.

4. If yes, is it possible to derive a NOEL for carcinogenicity?

Response from the AHEG:

No, tumours were observed at the lowest tested dose level of 300 ppm (LOEL). Even with this LOEL no proper risk assessment can be performed because of non-controlled exposure of the rats due to contradictory findings on stability of the chemical in feed.

CVMP response to question 1 from The Netherlands:

Question:

Can the CVMP confirm that 2,6-xylidine is a genotoxic carcinogen? From the information available, it appears that 2,6-xylidine is carcinogenic (carcinomas at multiple sites), however, genotoxicity studies suggest that not 2,6-xylidine but actually a further metabolite is responsible for the genotoxic action, as the results of *in vitro* studies were positive with metabolic activation only.

CVMP response:

It is confirmed that 2,6-xylidine is a genotoxic carcinogen in rats and it is assumed that no threshold exists for genotoxicity. No NOEL has been established for carcinogenicity.

2,6-Xylidine can be further metabolised to DMHA and DMAP. These metabolites lead to the formation of reactive intermediates like a nitrenium ion or iminoquinone. These reactive intermediates have the potential to covalently bind to DNA.

2.2.2. Discussion of Question 2

Preliminary to the answer, it should be noted that the published CVMP Summary report for lidocaine indicates that 2,6-xylidine is formed in horses since unaltered 2,6-xylidine accounted for 2.5% radioactivity in urine from treated horses. The answer to this question should be read together with the information provided in response to question 1.

The oral bioavailability of lidocaine in human is 30-35% (de Boer *et al*., 1979).

Lidocaine is almost completely metabolised in all species studied, with the liver and intestine (after oral administration) as the primary sites of metabolism. In human liver slices, MEGX and 2,6-xylidine were identified as the major metabolites after 4 hours of incubation with lidocaine. Thus, the possibility that 2,6-xylidine would be bioactivated in the human liver as well as in extrahepatic tissues cannot be excluded.

Given that 2,6-xylidine is considered to be a genotoxic carcinogen, there is a potential consumer risk for genotoxic and/or carcinogenic effects following exposure of humans to the parent substance lidocaine.

CVMP response to question 2 from The Netherlands:

Question:

It appears that humans can metabolise lidocaine into 2,6-xylidine. Therefore, even if 2,6-xylidine is not formed in the food producing species (as in horses), the exposure to the parent substance lidocaine via food of animal origin may eventually cause consumer exposure to 2,6-xylidine. Does the CVMP consider that there is a consumer risk for genotoxic and/or carcinogenic effects following exposure to the parent substance lidocaine?

CVMP response:

Lidocaine may undergo metabolism to 2,6-xylidine in humans in intestines and liver. By consequence, even if 2,6-xylidine would not be formed in the food producing species, the exposure to the parent substance lidocaine via food of animal origin may eventually cause consumer exposure to 2,6-xylidine. Therefore there is a potential risk for genotoxic and carcinogenic effects to the consumer following the exposure to lidocaine.

2.2.3. Discussion of Question 3

To answer the question there is a need (1) to estimate consumer exposure to lidocaine and its metabolites and (2) to compare the estimated exposure to a safe level. In the case of lidocaine, estimating both exposure and a safe level are problematic. Indeed there are few pharmacokinetic and residue data to assist in estimating consumer exposure. Moreover a safe level cannot be determined in the standard way as the available data do not allow an acceptable daily intake (ADI) to be established.

A. Estimating consumer exposure

Absorption, distribution, metabolism, and excretion (ADME) data

Information is available in man, rats, guinea pigs, dogs, horses, pigs and cattle.

Lidocaine is reported to be rapidly absorbed following administration by the major routes. Oral bioavailability is low (approximately 30–35% in humans) because of extensive first-pass hepatic metabolism, with the parent substance being rapidly eliminated via hepatic oxidative pathways. Lidocaine and its metabolites are rapidly excreted in the animal species studied, mainly via the renal route. The amounts of 2,6-xylidine excreted in urine after administration of lidocaine are low (except in guinea pigs). 2,6-xylidine has been shown to be excreted in bovine milk (Opinion of the Norwegian Scientific Committee for Food Safety, 2005).

In a study by Puente and Josephy (2001), seven Holstein cows were injected with lidocaine before surgery at doses of 2.9–3.9 mg/kg. Milk samples were obtained prior to surgery and at a single time point 2.5–6 hours after lidocaine injection. The metabolite 2,6-xylidine was detected in milk at levels ranging from 14.5–66 µg/kg.

Residue depletion study in cattle

A residue study has been undertaken in The Netherlands (Hoogenboom *et al.*, 2014) to investigate the residues of lidocaine and in particular the presence 2,6-xylidine in tissues and milk of cattle following parenteral administration representing the field use.

The study included eight high-yielding multiparous dairy cows (Holstein Friesian) that were treated with lidocaine by injection in the abdominal muscles in the same manner as for a caesarean section. The cows had not been treated with any anaesthetics for 4 weeks before the experiment.

Treatment with lidocaine

Infiltration anaesthesia was given over 5 minutes by injecting a total of 150 ml of a solution of lidocaine hydrochloride (20 mg/ml) and adrenaline (10 µg/ml). The anaesthetic was divided into five 30 ml injections. Each injection was given subcutaneously and intramuscularly at five different sites over an imaginary 20 cm incision line, and covered an area of 4 x 20 cm^2 in the left paralumbar fossa.

Measurements and sampling

For four animals (animals 1 to 4), blood samples were taken from the jugular vein immediately before the time of injection, and 30, 60, 120 and 180 minutes after injection. Cows were milked immediately before the time of injection and 30, 60, 120 and 180 minutes after injection. The animals were slaughtered 3.5 hours after lidocaine administration. The injection sites, meat, liver and kidney were collected.

For the remaining four animals (animals 5 to 8) blood samples were taken from the jugular vein immediately before the time of injection, and at 10, 20, 30, 60, 90, 120, 180, 240 and 360 minutes and 9, 12, 24, 36 and 48 hours after the injection. Cows were milked immediately before the time of

injection and 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 hours after injection. The animals were slaughtered 48.5 hours after lidocaine administration. The injection sites, meat, liver and kidney were collected.

Faeces and urine were collected separately and sampled immediately before the time of injection. Following injection, samples were collected and weighed according to the following intervals: 0–3, 3–6, 6–9, 9–12, 12–24, 24–36 and 36–48 hours.

An LC-MS/MS based analytical method was used to determine the levels of lidocaine, 2,6-xylidine, MEGX, 3-hydroxy-lidocaine, 4-hydroxy-lidocaine, DMAP and lidocaine-N-oxide. The availability of deuterated standards allowed the accurate quantification of the compounds.

Results

Plasma

Lidocaine was the most abundant compound present followed by 2,6-xylidine. Other metabolites including MEGX, the N-oxide, and DMAP were observed in some samples but at much lower concentrations.

Milk

Lidocaine reached highest levels around 1–2 hours after administration with some variation between cows. The highest observed level was around 0.6 mg/kg. Levels were still high after 3 hours but decreased to non-detectable levels at 48 hours after treatment.

2,6-Xylidine was also detected at considerable levels in milk from some cows, in some cases at levels similar to those of lidocaine. 2,6-Xylidine was still detectable in milk at 48 hours but at levels below 1 µg/kg. In addition to these two compounds MEGX, the N-oxide and 3-hydroxy-lidocaine could be detected although at much lower levels. DMAP was detected within the first hours but reached non-detectable levels within 24 hours.

Urine

2,6-Xylidine was by far the most abundant metabolite. Also the 3- and 4-hydroxy-metabolites of lidocaine were detected, in most cases at higher levels than lidocaine. MEGX was detected at relatively low levels.

Renal excretion of parent lidocaine was concluded to be negligible, accounting for about 0.01 to 0.08% of the lidocaine dose. Renal excretion of primary metabolites was two orders of magnitude greater, accounting for about 4 to 9% of the lidocaine dose. As lidocaine and its metabolites were not expected to be excreted in the faeces to any significant extent (see below) this left a large part of the lidocaine dose unaccounted for.

However, scrutiny of chromatograms revealed two further metabolites. One, present at low levels, was identified as the N-oxide of lidocaine.The second was identified as DMAP. In the absence of a deuterated standard the levels of this substance could not be accurately calculated. Studies with spiked urine samples indicated that levels of this metabolite were underestimated by a factor 10–40, due to very poor recovery. DMAP was therefore by far the most important lidocaine metabolite identified in this study, in terms of relative contribution to the excretion in urine. Further studies would be needed to accurately quantify this metabolite.

Faeces

No results were available. The report notes that lidocaine residues are not expected to be found in faeces, as Keenaghan and Boyes (1972) showed negligible faecal excretion of lidocaine in rats. It was assumed that the same holds for dairy cows.

Injection site

Very high levels of lidocaine (around 300 mg/kg) were found at the injection site in animals slaughtered 3.5 hours after injection. By 48.5 hours levels had decreased by more than 1000-fold, to 56–390 µg/kg.

2,6-Xylidine was also detected at 3.5 hours, but at 1000-fold lower levels than lidocaine itself. At 48.5 hours levels of 2,6-xylidine were around 1 µg/kg to non-detectable.

MEGX, the 3- and 4-hydroxy-metabolites of lidocaine and the N-oxide were also detected after 3.5 hours. MEGX and the N-oxide were also detectable after 48.5 hours.

Muscle meat

Lidocaine levels at 3.5 hours were around 68–190 µg/kg. At 48.5 hours lidocaine was still detectable but at levels of around 1–2 µg/kg in 3 animals and around 10 µg/kg in one animal.

2,6-Xylidine was present at levels similar to lidocaine at 3.5 hours but below the limit of quantification (LOQ) after 48.5 hours. Other metabolites were present at much lower levels at 3.5 hours and around or below LOQs at 48.5 hours.

Liver

Lidocaine was present at low levels in liver, whereas 2,6-xylidine was by far the most abundant metabolite at 3.5 hours and was still present at levels around 10 µg/kg in 3 out of 4 animals at 48.5 hours. MEGX was detected in all 4 animals at 3.5 hours and the 3-hydroxy metabolite in 3 out of 4 animals, but levels of these metabolites were below 1 µg/kg. It was suggested that post-mortem degradation of lidocaine might partly explain the rather low levels of lidocaine compared to 2,6-xylidine in liver.

Kidneys

In animals slaughtered after 3.5 hours, lidocaine and 2,6-xylidine were present at similar levels of around 300–500 µg/kg. All other metabolites were detected but at much lower levels. Lidocaine was still detected at 48.5 hours in two animals, in which low levels of 2,6-xylidine were also present. MEGX was detected in one animal. Other metabolites were not detectable.

Table 2. Mean concentrations* of lidocaine and its metabolites in various tissues (µg/kg).

Time (h)	Tissue	Lidocaine	2,6-xylidine	3-hydroxy-lidocaine	4-hydroxy-lidocaine	MEGX	N-oxide	DMAP
3.5	Injection site	305733.4	325.2	23.0	5.9	232.8	296.3	nd
	Muscle	119.4	49.6	0.3	nd	2.0	1.2	5.2
	Liver	0.8	180.2	0.4	nd	0.2	nd	nd
	Kidney	325.5	382.3	5.8	0.6	3.5	0.2	10.3
48.5	Injection site	228.6	0.7	nd	nd	8.5	0.9	nd
	Muscle	3.6	nd	nd	nd	0.4	0.0	nd
	Liver	nd	10.5	nd	nd	nd	nd	nd
	Kidney	5.3	0.5	nd	nd	0.4	nd	nd

* The means are only based on the detected levels.
From Hoogenboom *et al.* (2014)

Discussion of the results of the residues study

The authors conclude that the study confirms the results of the *in vitro* study by Thuesen and Friis (2012), indicating that 2,6-xylidine is an important metabolite in cows treated with lidocaine. 2,6-xylidine was clearly detected in plasma, being present at levels often similar to those of lidocaine during the 24 hours following injection. 2,6-xylidine was initially the most abundant metabolite in urine.

The overall recovery of residues in this study was low, at 6 to 9%. This was considered to be largely because levels of DMAP in urine were markedly underestimated. The authors also note that, in some animals, part of the lidocaine dose may have been injected into the rumen, which may have resulted in slightly reduced levels of lidocaine at the injection site. Finally, given the rapid metabolism of lidocaine, the possibility that the principle biotransformation products were further metabolised to compounds not detected with the analytical method cannot be ruled out.

In non-injection site muscle and in kidney, 2,6-xylidine was present at similar levels to lidocaine. It was also present at the injection site but at clearly lower levels than the parent compound. In liver 2,6-xylidine was the most abundant residue, with only low levels of lidocaine present. The authors noted that this might have been due to post-mortem degradation, as samples were not snap-frozen. In milk, 2,6-xylidine was also detected, often at levels approaching those of lidocaine itself. A further metabolite present in considerable/non-negligible concentrations in several tissues was identified as lidocaine-N-oxide.

The study authors conclude that cattle milk and meat contain high levels of 2,6-xylidine shortly after administration of lidocaine. Concentrations of lidocaine peaked at 20–30 minutes in plasma, at 60–120 minutes in milk and at 180 minutes in urine. Concentrations of metabolites peaked at similar time

points or 30–60 minutes later. In non-injection site muscle, kidney and milk levels of 2,6-xylidine had decreased to non-detectable levels or levels close to the detection limit of 0.4 µg/kg. In liver, levels of 2,6-xylidine at 48.5 hours were still around 10 µg/kg (approximately 17-fold lower than in liver samples collected at 3 hours). In injection site samples, 2,6-xylidine was detected at 48.5 hours but at levels around the LOQ (and 450-fold lower than at 3.5 hours). MEGX and lidocaine-N-oxide were also detected in injection site samples at 48.5 hours, but at far lower levels than at 3.5 hours. Lidocaine itself was still present at the injection site at 48.5 hours at an average level of 229 µg/kg (more than 1000-fold lower than at 3.5 hours).

CVMP comment

Data from this new residue depletion study in cattle indicate that lidocaine and related residues are present in edible tissues and in milk at early time-points after treatment.

The concentration of lidocaine at the injection site was considerably higher (305 mg/kg at 3.5 hours and 229 µg/kg at 48.5 hours) than in other tissues.

In milk, lidocaine levels were still high after 3 hours but decreased to non-detectable levels (LOQ was 0.18 µg/kg, LOD not reported) 48 hours after treatment. 2,6-xylidine was also detected at quite similar levels to lidocaine and was still detectable at 48 hours but at levels below 1 µg/kg (the mean for 3 cows being approximately 0.5 µg/kg).

Pharmacokinetic modelling

In order to better understand the pharmacokinetics of lidocaine and (the sum of) its metabolites (2,6-xylidine, MEGX and 3-OH-lidocaine; 4-OH-lidocaine was not detected in milk and meat) the study authors developed a physiologically based pharmacokinetic (PBPK) model using the above experimental data as well as published data (Sellers *et al.*, 2009) for model verification purposes.

The model was developed from earlier work by Pilari and Huisinga (2010) and consisted of a 2-compartment model with a peripheral compartment (representing adipose tissue, muscle, bone and skin) and a central compartment (representing blood and all remaining tissues).

A bi-exponential absorption model was considered to best reflect the available data for milk and plasma and was used to derive the half-lives shown in the table below.

Table 3. Absorption half-lives (h) for lidocaine ($t_{1/2,1}$ and $t_{1/2,2}$) and initial and terminal half-lives (h) for lidocaine ($t_{1/2,init}$ and $t_{1/2,term}$) and the lidocaine gram-equivalent sum of metabolites.

Animal number	Lidocaine				Metabolites	
	$t_{1/2,1}$	$t_{1/2,2}$	$t_{1/2,init}$	$t_{1/2,term}$	$t_{1/2,init}$	$t_{1/2,term}$
5	0.39	2.1	0.33	2.2	0.14	3.0
6	0.22	3.0	0.40	3.1	0.08	0.7
7	0.26	3.2	0.28	2.6	nd[a]	nd
8	0.27	2.6	0.32	2.1	0.03	1.1

a: not determined because of absurd small parameter value for $P_{M,p}$.
From Hoogenboom *et al.* (2014)

The data in plasma and milk for animals 1, 2, 3 and 4, that were sacrificed after 3.5 hours, were compared to the concentration-time curves for cows 5, 6, 7 and 8. It was concluded that lidocaine concentrations in plasma were slightly underestimated by the model, as were the metabolite concentrations in milk after 3.5 hours.

The concentration values in injection site, kidney, liver and muscle after 3.5 hours were used to develop a bi-phasic absorption model.

At the injection site the measured concentrations after 48.5 hours (in cows 5, 6, 7 and 8) ranged from 56 to 390 µg lidocaine/kg, i.e. amounts corresponding to 0.004 to 0.014% of the injected lidocaine dose. While these values indicate almost complete absorption from the injection site, the model calculated fractions of the lidocaine dose remaining at the injection site 48.5 hours after administration to be even smaller, in the range of 3.1×10^{-6} to 1.4×10^{-3}% of the lidocaine dose.

The table below compares predicted and observed residue levels for kidney and liver (central compartment tissues) and muscle (a peripheral compartment tissue) and shows that lidocaine concentrations in both compartments and the sum of the metabolite concentrations in the peripheral compartment were overestimated by the computer model. The sum of the metabolite concentrations in the central compartment is comparable to the experimental data.

Table 4. Comparison of model and experimental data in kidney, liver and muscle at time 3.5 h after injection. Metabolites are expressed as lidocaine equivalents.

	Central (µg/kg)			**Peripheral (µg/kg)**	
	Model	**Kidney**	**Liver**	**Model**	**Muscle**
Lidocaine	1200–1600	310–330	nq	480–750	68–180
Metabolites	240–850	520–980	170–710	170–1000	38–230

nq: not quantifiable.
From Hoogenboom *et al.* (2014)

The model was used to calculate, for each cow, the time period required to allow depletion of lidocaine and its metabolites to below the limits of quantification. The results are shown in the table below.

The model calculations indicate that lidocaine and lidocaine metabolites could still be detected in milk 48 hours after administration, but by 60 hours no lidocaine or metabolites are expected to be detectable in milk anymore.

Detectable levels of lidocaine and lidocaine metabolites are expected to be present in meat (peripheral compartment) and organs (central compartment) after 24 hours but not after 48 hours.

Table 5. Model calculation of the concentration (µg/kg) of lidocaine and lidocaine metabolites in plasma, milk and the central and the peripheral compartments of cows administered a single s.c./i.m. dose of 3 g of lidocaine, for time points 3.5, 8, 24 h or 2, 3, 7 and 28 days.

Time	Compartment	Lidocaine				Metabolites			
		Cow 5	Cow 6	Cow 7	Cow 8	Cow 5	Cow 6	Cow 7	Cow 8
3.5 h	plasma	110	58	83	79	180	140	200	190
	milk	390	200	210	190	180	190	350	190
	Central	1900	1400	1400	1800	3000	3400	3400	4500
	peripheral	810	810	610	500	880	1400	1100	1200
8 h	plasma	25	18	33	24	63	31	70	64
	milk	110	64	110	98	100	76	170	130
	Central	430	420	560	560	1100	740	1300	1500
	peripheral	240	320	320	220	470	240	440	410
24 h	plasma	0.24	0.64	1.3	0.44	2.2	1.1	3.0	1.2
	milk	20	12	30	19	24	10	43	26
	Central	4.1	15	22	10	37	27	51	28
	peripheral	2.6	13	16	5.2	22	8.7	17	8.2
2 d	plasma	<	<	<	<	<	<	<	<
	milk	<	0.23	0.62	<	0.42	<	0.88	<
	Central	<	<	<	<	<	<	<	<
	peripheral	<	<	<	<	<	<	<	<
3 d	plasma	<	<	<	<	<	<	<	<
	milk	<	<	<	<	<	<	<	<
	Central	<	<	<	<	<	<	<	<
	peripheral	<	<	<	<	<	<	<	<
7 d	plasma	<	<	<	<	<	<	<	<
	milk	<	<	<	<	<	<	<	<
	Central	<	<	<	<	<	<	<	<
	peripheral	<	<	<	<	<	<	<	<
28 d	plasma	<	<	<	<	<	<	<	<
	milk	<	<	<	<	<	<	<	<
	Central	<	<	<	<	<	<	<	<
	peripheral	<	<	<	<	<	<	<	<

< : below LOQ (0.18 µg/kg for lidocaine, 0.37 µg/kg for 2,6-xylidine).
From Hoogenboom *et al.* (2014)

CVMP comment

The pharmacokinetic model enables a prediction of the necessary timeframe to reduce the concentration of lidocaine and its residues to a non-detectable level in milk (60 hours) and tissues (48 hours). At 48 hours only 0.015 % of the injected amount was measured at the injection site. However it is not possible to predict at what time the residues would be non-detectable at the injection site.

Time required for total / partial elimination of a drug

The time needed to eliminate a drug from an animal's body can be predicted on the basis of:

- the elimination rate during the terminal phase of the elimination, as represented by the terminal elimination half-life, which is equal for all tissues and body fluids;
- the total number of molecules administered, which can be calculated from:
  - the total treatment dose given to the animal
  - the bodyweight of the animal
  - the molar weight of the drug substance
  - Avogadro's number

By applying the terminal elimination half-life to the total number of molecules administered, the time needed to eliminate a defined number of molecules can be estimated. The defined number of molecules to be eliminated can be set as all molecules (total elimination) or as some other amount, such as an amount that would leave residues equivalent to the threshold of toxicological concern (TTC) (see below) in the animal's body.

The following equation describes the elimination process in the terminal phase:

$$D(t) = D(0) \times e^{-t/t_{el}} \qquad \text{equation 1}$$

In which:

D = number of molecules
t_{el} = terminal elimination time

The equation can be converted to find the time needed to ensure a given total residue burden in the body:

$$T = \frac{\ln\left(\left(\frac{dose \times duration}{1000}\right) \times bw \times \frac{A}{M}\right) - \ln(B) \times 1.44 \times t_{1/2}}{24} \qquad \text{equation 2}$$

In which:

T = time (days)
dose = total dose (g/kg bw/day)
duration = treatment duration (days)
bw = bodyweight (kg)
A = Avogadro's number = 6.0×10^{23}
M = Molar mass (g/mol)
$t_{1/2}$ = terminal elimination half-life (h)

And in which $B = \left(\frac{safe\ amount\ in\ \mu g}{M}\right) \times A \times 10^{-6}$ equation 3

In which:

M = Molar mass (g/mol)
A = Avogadro's number = 6.0×10^{23}

The output of equation 2 is always rounded to a whole number of days.

The terminal half-lives for lidocaine, 2,6-xylidine and MEGX were calculated based upon urinary/milk excretion data generated in the Dutch residues study (Hoogenboom *et al.*, 2014, see table 6). These data reveal terminal half-lives that are greater than those calculated on the basis of plasma data.

Table 6. Terminal half-lives calculated after fitting the data to the 2-compartment model developed as part of the Dutch study (Hoogenboom *et al.*, 2014).

Compound	Tissue	Half-life (h)	Remark
Lidocaine	Plasma	3,2	Only detected during the first 24 hours
Lidocaine	Urine	3,5	A slower phase was detected, but could not be fitted
Lidocaine	Milk	18,3	
2,6-xylidine	Plasma	3,3	Only detected during the first 24 hours
2,6-xylidine	Urine	11,0	
2,6-xylidine	Milk	23,9	
MEGX	Plasma	1,6	Only detected during the first 5 hours
MEGX	Urine	nd	No linear relationship between excretion rate and plasma concentration
MEGX	Milk	2,9	Only detected during the first 10 hours

Bearing in mind that eventually all terminal phases (reflecting the absorption rate from the site of injection) theoretically should run parallel, and that the calculated half-lives are only rough estimates (due to the fact that only 1–2 data points are available), the mean half-life of the slow elimination phase detected from milk and urine data was calculated and is considered to be 17.7 hours. One of the restrictions of using this approach is that there should be a linear relation between plasma concentration and urinary excretion rate, which was established to be the case after further analysis of the data.

Since most of the dose of lidocaine would be eliminated via the urine as 2,6-xylidine or DMAP, as a worst case, 3000 mg of 2,6-xylidine (based on the fact that the dose administered to cattle was 3000 mg lidocaine) would be the starting dose from which to calculate the elimination time.

Based on the data from the residues study and the subsequent analysis, using only conventional pharmacokinetic models/principles, the following total elimination time for lidocaine and 2,6-xylidine could be calculated for a dose of 3000 mg (approximately 4 mg/kg), given via multiple intramuscular injections to cows.

For lidocaine and 2,6-xylidine, the total elimination time (i.e. the time taken for the last molecule to leave the cow) would be 52 days. This time is based on a worst case scenario in which the cow receives the maximum dose of 4 mg/kg and elimination proceeds at a uniform (slow) elimination rate characterised by a half-life of 17.7 hours.

The calculation can be refined to take into account the first rapid absorption phase, as indicated in all plasma curves, and the slow terminal phase (with $t_{1/2}$ of 17.7 hours) that would become apparent after 20–24 hours (see for example the excretion of 2,6-xylidine in urine in Figure 5 below). Most of the dose of lidocaine would have been eliminated during the first phase, i.e. on the first day after dosing, leaving approximately 0.1% of the 3000 mg dose entering the slow phase of absorption. The total elimination time for the 3 mg entering the slow phase is 44 days, leading to a total elimination time of 45 days.

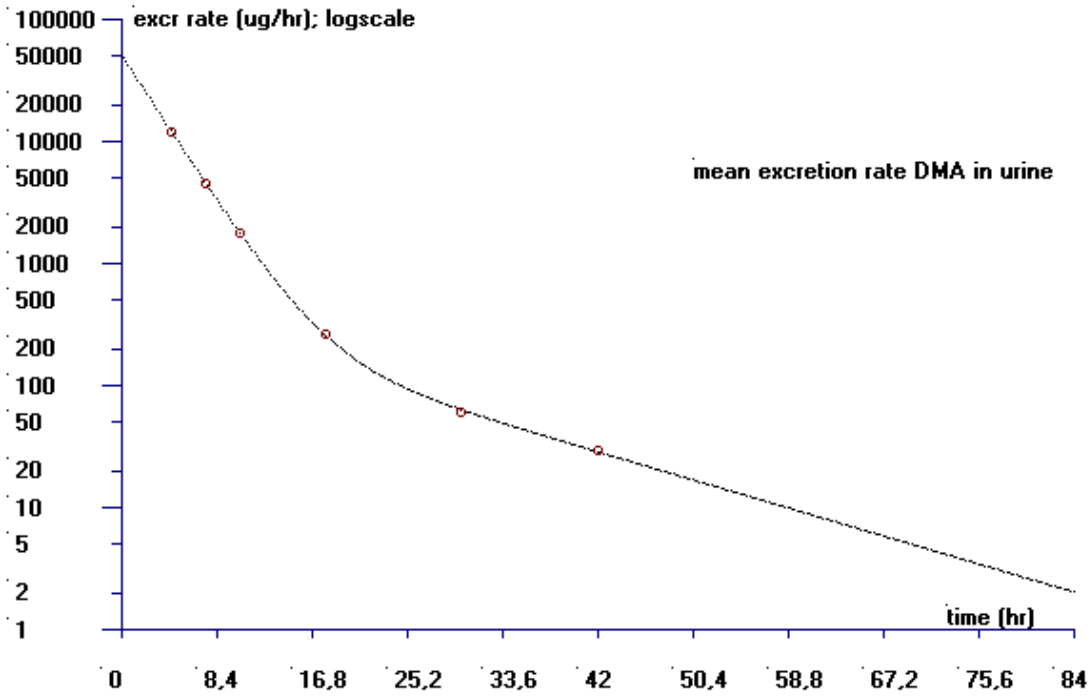


Figure 5. Mean (n=4) time dependent course of the renal excretion rates of 2,6-xylidine (DMA) (µg/hour), fitted according to a conventional 2-compartment model

B. Determining a safe level for lidocaine and its metabolites

As stated in the CVMP summary report for lidocaine, from the available data neither a pharmacological nor a toxicological NOEL could be identified, and thus an ADI cannot be calculated.

In the absence of an ADI, the CVMP explored a number of alternative approaches for determining a safe exposure level for the consumer.

Norwegian Food Safety Authority approach

In 2005 the Norwegian Scientific Committee for Food Safety published an assessment performed to determine whether the cascade withdrawal periods for lidocaine containing products used in cattle, swine, sheep and goat could be shortened. The assessment focused on the potential consumer exposure to 2,6-xylidine and used the margin of exposure (MOE) approach to characterise the risk of exposure to this metabolite. This is the approach recommended by the European Food Safety Authority (EFSA, 2005) for risk assessment of substances with genotoxic and carcinogenic properties and to which consumers may be exposed via food. EFSA considers that, in general, for genotoxic and carcinogenic substances a MOE of 10,000 or above would be of low concern from a public health point of view and might be considered as a low priority for risk management actions.

In its derivation of a MOE the Norwegian Scientific Committee for Food Safety used the T_{25}, representing the dose corresponding to a 25% incidence of tumours, as the reference point against which to compare potential consumer exposure. The T_{25} was calculated for different nasal cavity tumours in rats fed with 150 mg 2,6-xylidine/kg bw for 102 weeks in the NTP study discussed earlier in this report (NTP, 1990). The lowest T_{25} was calculated as 63.5 mg/kg bw.

The Norwegian Committee estimated consumer exposure to 2,6-xylidine based on numbers of relevant disease cases in food animals in 2004 for which lidocaine could have been used in Norway, and

considered, as a worst case scenario, that all lidocaine calculated to have been administered in the target animal species would have been metabolised to 2,6-xylidine. It was also assumed that exposed meat was “diluted” in the total amount of meat of the relevant species and that exposed milk was diluted in the total volume of milk produced. In this way an average daily intake of 2,6-xylidine was calculated.

Worst-case MOEs were calculated as 992,000 for milk, 148,000 for meat and 794,000 for meat and milk. These values are all considerably greater than 10,000, suggesting that exposure to 2,6-xylidine from lidocaine used in food producing animals would be of low concern from a public health point of view. The Norwegian Scientific Committee for Food Safety concluded that the cascade withdrawal periods for milk (7 days) and meat (28 days) could be shortened.

CVMP comment

The CVMP considers that it is not appropriate to set MRLs for substances that are both genotoxic and carcinogenic. This is consistent with the EFSA position, which is that substances that are genotoxic and carcinogenic should not be approved for deliberate addition to foods or for use earlier in the food chain, if they leave genotoxic and carcinogenic residues in food. However, the MOE is an appropriate tool for use in characterising the risk associated with unintended consumer exposure to such substances and so could be useful in relation to the risk assessment of lidocaine-derived genotoxic residues.

However, the CVMP considers that it is not possible to derive a MOE for 2,6-xylidine as a reference point against which to compare potential consumer exposure (the T_{25}) cannot be adequately derived from the NTP carcinogenicity study. This is because of uncertainties associated with the actual doses received by the animals in that study, which appear to have been lower than intended. Consequently, the 150 mg/kg bw exposure cannot be used to calculate the T_{25}.

TTC approach

The Threshold of Toxicological Concern (TTC) approach is a risk assessment tool establishing human exposure threshold values for chemicals below which there is a very low probability of adverse effects to human health (European Union, 2012 SCCS/SCHER/SCENIHR). EFSA (2012) considers that the approach is applicable to substances for which the chemical structure is known but for which there are few or no relevant toxicity data. It is considered to be a useful tool for priority setting and for deciding whether exposure is so low that the probability of adverse health effects does not warrant the generation of further data. For substances with a structural alert for genotoxicity EFSA recommends a TTC of 0.15 µg/person/day as the threshold below which human exposure should remain.

The TTC approach has not previously been applied in relation to residues of veterinary medicinal products in food. It is used to set limits for genotoxic impurities in medicinal products for human use (CHMP/ICH guideline M7), where a TTC value of 1.5 µg/person/day is considered as a threshold below which patient exposure must remain. The TTC value used by the EMA in relation to human patient exposure to genotoxic impurities is 10 fold higher than the value used by EFSA because it is considered that the human patient will receive an overall health benefit from taking the medicine, and so can accept the increased risk (the consumer of food containing genotoxic residues will receive no such health benefit).

The CVMP considers that, while the TTC approach does not provide a surrogate MRL, it is appropriate for use as a tool to estimate the acceptability of the risk experienced by consumers exposed to residues of lidocaine. The appropriate TTC value to apply in this context is considered to be the value applied by EFSA, i.e. 0.15 µg/person/day.

The residues study by Hoogenboom *et al.* (2014) in cattle provides data that can be used to estimate potential consumer exposure, for comparison with the TTC value. These data indicate that lidocaine and related residues are present in edible tissues and in milk at early time-points after treatment. Pharmacokinetic modelling enables the prediction of the necessary timeframe to reduce the concentration of lidocaine and its residues to non-detectable levels in milk and tissues (60 and 48 hours, respectively). However, the sensitivity of the detection method is not sufficient to allow estimation of the timeframe required in order for residue intake to fall below the TTC. When residues are at the LOQs of analytical method (lidocaine: 0.18 µg/kg for tissues and milk, 2,6-xylidine: 0.37 µg/kg for tissues and milk), the total residue intake (lidocaine plus 2,6-xylidine) would be 1 µg/person. This represents approximately 6 times the TTC value of 0.15 µg/person.

Overall approach taken to address question 3

The CVMP considered that conservative estimates of time taken to eliminate residues from the body could be achieved using the total/partial elimination approach, calculated using the terminal half-lives derived from the residues study by Hoogenboom *et al.* (2014). Furthermore, the CVMP considered that the TTC of 0.15 µg/person represents a level below which exposure should remain. The Committee used the total/partial elimination approach to determine the time required for residues in the animal's body to deplete to 0.15 µg. If the total level of residues in the animal's body is 0.15 µg, then it follows that it would be impossible for a consumer to be exposed to this quantity of residues by ingesting a standard foodbasket containing 500 g of tissues and 1.5 litres of milk (the consumer would only be exposed to this level of residues if he/she ingested the entire animal). The CVMP also calculated the time required for total elimination of all residues from the animal's body.

Using the terminal half-life (17.7 days) to calculate the time required for elimination from the dose administered (3000 mg) down to the TTC (0.15 µg) yields an elimination time of 17 days (this is a worst case calculation in which it is assumed that the cow receives the maximum dose of 4 mg/kg, that the entire dose is converted to 2,6-xylidine, and that elimination proceeds at a uniform (slow) elimination rate characterised by a half-life of 17.7 hours). If the initial rapid elimination phase is also considered, then an elimination time of 15 days is calculated.

Separate elimination times were calculated for milk, using the longest estimated terminal half-life for milk of 24 hours (the longest half-life was used for milk while an average half-life was used for tissues as specific values were available for milk but not for tissues, and these specific values are more conservative than the average value). The results are presented in the table below, along with the carcass elimination times (calculated using the mean terminal half-life of 17.7 hours). For both milk and tissues two estimates are provided: one in which it is assumed that elimination proceeds at a uniform rate characterised by the terminal half-life, and a refined estimate which recognises that approximately 99.9% of the dose is eliminated during the first rapid elimination phase, with only the remaining 0.1% being eliminated at the slower rate.

The CVMP considers that it is more appropriate to derive elimination times recognising that 99.9% of the dose will be eliminated within the first day after dosing.

Table 7. Time to total elimination and time to elimination down to the TTC (days). Carcass elimination times were derived using the mean calculated terminal half-life (17.7 hours) while milk elimination times were calculated using the longest terminal half-life calculated (24 hours).

		Time to elimination (days) based on application of terminal half-life to the total dose	**Refined time to elimination (days), based on application of the terminal half-life to the portion of the dose not eliminated during the rapid elimination phase**
Total elimination	Carcass	52	45
	milk	70	61
Elimination down to the TTC (0.15 µg)	Carcass	17	11
	milk	24	15

Using the same principles, the level of residues remaining in the carcass and milk may also be calculated at any given time after administration (see table 8 below).

Table 8. Calculated residue levels remaining in the carcass and in milk following application of minimum cascade withdrawal periods, based on application of the terminal half-life to the portion of the dose not eliminated during the rapid elimination phase

	Residues remaining after 7 days (µg)	**Residues remaining after 28 days (µg)**
Carcass	4.2	0.00000001
milk	23	0.00001

From the above table it can be concluded that the minimum withdrawal period for use of a veterinary medicinal product under the cascade for meat of 28 days:

- does not result in the total elimination of residues from the body; however the remaining residues (i.e. in the entire body of the cow) make up only 10 pg, which can be considered negligible;
- results in elimination of residues down to a level below the TTC of 0.15 µg.

Regarding milk, the minimum withdrawal period for use under the cascade of 7 days:

- does not result in the total elimination of residues from the body; remaining residues in the body may make up 23 µg;
- does not result in elimination of residues in the body down to a level below the TTC of 0.15 µg; as indicated in the previous table, elimination of total body residues down to the TTC is calculated to require 15 days.

In relation to residues in porcine meat, no residue data are available and it is therefore not possible to calculate the level of residues that will remain in the animal's body at the minimum cascade withdrawal period of 28 days. However, *in vitro* studies indicate that the metabolism is comparable to that in cattle and it is expected that the minimum cascade withdrawal period of 28 days for meat is sufficient to ensure that residues deplete to negligible levels. Furthermore, considering that lidocaine will be used

for castration within the first weeks of life, therefore at a time far from slaughter (5–6 months), the risk to the consumer is considered negligible.

In relation to horses, no residue data are available. However, as indicated in the published CVMP summary report, metabolism is extensive and rapid. Furthermore, new *in vitro* data further demonstrate that the production of 2,6-xylidine in horses is less significant than in cattle.

Lidocaine will be used for nerve and joint anaesthesia on the lower leg, and in the scrotum prior to castration of stallions, and for occasional wound suturing.

In the absence of residue data in horses it is not possible to conclude with 100% certainty whether a withdrawal period of 0 days is safe. However in the absence of any new residue data, considering very limited use currently authorised for lidocaine and the extensive metabolism, and the fact that new *in vitro* data have further demonstrated that2,6-xylidine formation in horses is less significant than in cattle, the risk to the consumer can be considered negligible.

CVMP response to question 3 from the Netherlands:

Question:

What is the consumer risk of exposure to lidocaine-related residues in food resulting from the use of lidocaine in horses, pigs and cattle? Please note that withdrawal periods in horses would be 0 days and the use in other species 28 days for slaughter and 7 days for milk (according to the current rules of the cascade).

CVMP response:

Data from a new residue depletion study in cattle indicate that lidocaine and related residues are present in edible tissues and in milk at early time-points after treatment. However, modelling data indicate that by the minimum cascade withdrawal period of 28 days the total amount of residues remaining in the animal's body will be in the picogram range; even if an entire carcass could be ingested by a single consumer, exposure to residues would remain below the TTC of 0.15 µg.

Regarding milk, the minimum cascade withdrawal period of 7 days does not result in the total elimination of residues or in the elimination of total body residues down to the TTC. To ensure that total residues in the cow's body are below this level requires an interval of 15 days between use of lidocaine and the taking of milk for human consumption. At this time point there is no risk to the consumer.

For pigs no residue data are available and it is therefore not possible to calculate residue levels that will remain following the cascade withdrawal period. However, since metabolism is comparable to that in cattle, it is expected that the minimum cascade withdrawal period of 28 days for meat is sufficient to ensure that residues deplete to negligible levels. Furthermore, considering that lidocaine is used for castration within the first weeks of life, therefore far from slaughter, the risk to the consumer is considered negligible.

For horses, in the absence of residue data it is not possible to conclude with 100% certainty whether the withdrawal period of 0 days is safe. However in the absence of any new residue data, considering the very limited use currently authorised for lidocaine and the extensive metabolism, and considering that new *in vitro* data have further demonstrated that 2,6-xylidine formation in horses is less significant than in cattle, the risk to the consumer can be considered negligible.

2.2.4. Discussion of Question 4

Risk management considerations

Volume 8 of the Rules Governing Medicinal Products in the European Union states that:

"Substances or metabolites that cause genotoxicity in vivo are not permitted for use in veterinary medicines intended for food-producing animals due to the uncertainty in establishing a threshold for this effect."

This principle was established many years ago, well before implementation of the current MRL regulation (Regulation (EC) No 470/2009). While the CVMP and the European Commission remain of the view that use of genotoxic carcinogens in veterinary medicinal products for administration to food producing species should be avoided, Regulation (EC) No 470/2009 requires consideration of a number of relevant provisions. The regulation specifies that the CVMP opinion shall consist of a scientific risk assessment and risk management considerations when establishing MRLs, with information on issues to consider provided in Article 4(2) of Regulation (EC) No 470/2009:

"The scientific risk assessment and the risk management recommendations shall aim to ensure a high level of human health protection, whilst also ensuring that human health, animal health and animal welfare are not negatively affected by the lack of availability of appropriate veterinary medicinal products."

In addition Article 7 of Regulation 470/2009 states that:

"The risk management recommendations shall be based on the scientific risk assessment performed in accordance with Article 6 and shall consist of an assessment of the following:

(a) the availability of alternative substances for the treatment of the relevant species or the necessity of the substance evaluated in order to avoid unnecessary suffering for animals or to ensure the safety of those treating them;

(…)"

The CVMP considers that, in this case, the availability of alternatives is a critical issue as lidocaine is a substance for which no suitable alternatives are known to exist.

In terms of efficiency (potency, duration and onset of action) and recognised side effects, there are no known alternatives that can be considered adequate for the replacement of lidocaine in veterinary medicine. Lidocaine currently fulfils a need within large animal practice for surgery and diagnostic procedures, a need that cannot be adequately fulfilled by other local anaesthetics such as procaine, which has different physicochemical properties, a different toxicity profile in large animals and for which there is a lack of scientific studies assessing clinical efficacy. Indeed, the use of lidocaine in large animals has allowed the possibility for 'standing' conscious surgeries in large animals, which is a necessity for large animal practices, and one that has greatly improved animal welfare and safety for procedures such as caesareans and castrations. Lidocaine, with its relatively strong potency and long duration of action (e.g., compared to procaine), also makes a key contribution to the safety of the veterinarian, who may be placed at an unacceptable risk of serious physical injury if undertaking some large animal procedures using alternative anaesthetics.

Thus, if lidocaine was not available for use in food producing animals, some surgical procedures would become difficult or impossible to perform. General anaesthesia may be viewed as a potential alternative, yet with a number of associated inherent risks and complications.

The lack of suitable alternatives could have critical consequences for the treatment of a large number of food-producing animals with an inevitable detrimental impact in farming and animal husbandry.

It is also noteworthy that lidocaine is authorised for use in human medicine and some lidocaine products have over-the-counter status for both cutaneous and oral treatments of very mild diseases such as sore throat. In the evaluation of human medicines the risk of adverse reactions is considered against the benefit of the treatment for the patient and consequently the level of risk that can be accepted is greater than that which can be accepted for consumers of food derived from animals treated with veterinary medicinal products (where consumers receive no benefit from the presence of residues). The CHMP has concluded that the use of lidocaine is acceptable for short-term and local treatments. The fact that the benefit-risk of lidocaine in human medicines is considered positive for oral and dermal treatments and the wide availability of lidocaine products should be borne in mind when reflecting on the risk associated with residues derived from foodstuffs from animals treated with lidocaine for regional/local anaesthesia.

The published MRL Summary report already includes:

- that 2,6-xylidine is a mutagenic agent *in vitro* and has genotoxic characteristics *in vivo*;
- the results of the carcinogenicity study carried out with 2,6-xylidine (even if no conclusion on carcinogenicity is given);
- that neither a pharmacological nor a toxicological NOEL could be identified, and thus an ADI cannot be calculated;
- the available pharmacokinetic data in horses show a rapid metabolism and extensive excretion.

The re-assessment of the genotoxicity and carcinogenicity data does not change the original conclusions. Moreover new *in vitro* data have revealed a different metabolic pathway in horses suggesting a less significant production of 2,6-xylidine in this species.

Considering the very limited use currently authorised (for local/regional anaesthesia only), the extensive metabolism and the fact that new *in vitro* data suggests less significant production of 2,6-xylidine in horses than in other species, the risk of consumer exposure to residues of lidocaine in horse meat is considered very low. In view of this, the current MRL classification ('No MRL required' for horses and for local/regional anaesthesia only) for lidocaine remains appropriate and no risk mitigation measures are considered necessary. In addition it is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure.

The use of authorised veterinary medicinal products containing lidocaine in food producing species other than horses is generally allowed according to the rules of the cascade. Article 11(2) of Directive 2001/82/EC specifies that:

"…Unless the medicinal product used indicates a withdrawal period for the species concerned, the specified withdrawal period shall be not less than…:

- 7 days for milk,

- 28 days for meat from poultry and mammals including fat and offal…"

In some Member States the use is conditional upon special license. The Norwegian Food safety Authority has made an exemption from cascade withdrawal periods and allows a zero day withdrawal period for milk and a 24 hour withdrawal period for slaughter.

In the USA, lidocaine is widely used in cattle with no withdrawal period. Based on the response to question 3 (see section 2.2.3), for cattle, considering that the estimated amount of lidocaine residues in the cow's body is negligible (about 10 pg) it can be considered that the minimum cascade withdrawal period of 28 days for meat is appropriate. Therefore, no new risk management measures are needed.

Regarding milk, the minimum cascade withdrawal period of 7 days is not sufficient to ensure that total residues remaining in the cow's body will be below the TTC of 0.15 µg. To ensure that total residues in the cow's body are below this level requires an interval of 15 days between use of lidocaine and the taking of milk for human consumption. At this timepoint there is no risk to the consumer.

For pigs no residue data are available. However since metabolism is comparable to cattle, it is expected that the minimum cascade withdrawal period of 28 days is sufficient to allow depletion of residues to a safe level. Considering that lidocaine will be used for castration at a time far from slaughter, the risk to the consumer is considered negligible.

Communication

There are authorised lidocaine-containing products for horses, dogs and cats. Warnings could be implemented in the product information of these products. However there is no legal basis to make recommendations on off-label use. It therefore seems more appropriate to inform users via specialised literature for veterinarians or via dedicated websites.

CVMP response to question 4 from The Netherlands:

Question:

Does the CVMP consider it necessary to take risk management measures? If yes, what risk management measures and communication does the CVMP consider appropriate?

CVMP Response:

For horses, considering the very limited use currently authorised (for local/regional anaesthesia only), the extensive metabolism and the fact that new *in vitro* data suggests less significant production of 2,6-xylidine in horses than in other species, the risk of consumer exposure to residues of lidocaine in horse meat is considered very low. In view of this, the current MRL classification ('No MRL required' for horses and for local/regional anaesthesia only) for lidocaine remains appropriate and no risk mitigation measures are considered necessary. In addition it is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

For cattle, considering that the estimated amount of lidocaine residues in the cow's body is negligible (about 10 pg) it can be considered that the minimum cascade withdrawal period of 28 days is appropriate. Therefore, no new risk mitigation measures are needed.

Regarding milk, the safety associated with the minimum cascade withdrawal period of 7 days is uncertain as this time period is not sufficient to ensure that total residues remaining in the cow's body will be below the TTC of 0.15 µg. To ensure that total residues in the cow's body are below this level requires an interval of 15 days between use of lidocaine and the taking of milk for human consumption. At this timepoint there is no risk to the consumer.

For pigs no residue data are available. However, considering that metabolism in pigs is comparable to that in cattle, it is expected that the minimum cascade withdrawal period of 28 days is sufficient to ensure elimination of residues to a safe level. Moreover considering that the use for castration takes place at a time far from slaughter, the risk to the consumer is considered negligible.

It would seem appropriate to inform lidocaine users via specialised literature for veterinarians or via dedicated websites that an interval of 15 days between use of lidocaine and the taking of milk for human consumption is recommended.

2.2.5. Discussion of Question 5

Xylazine was evaluated by the CVMP in 1999, resulting in a "No MRL required" status for bovine and *equidae* tissues only – the MRL entry specified that the substance was not for use in animals producing milk for human consumption (see published summary report EMEA/MRL/611/99-FINAL-corrigendum). A further evaluation was undertaken in 2002, resulting in the removal of the restriction relating to use of the substance in animals producing milk for human consumption (see published summary report EMEA/MRL/836/02-FINAL-corrigendum). Consequently, xylazine currently has a "No MRL required" status in bovine and *equidae* tissues and milk. No ADI has been established for xylazine.

In its 2002 summary report the CVMP concluded that:

- xylazine is used in a small number of individual animals for non-regular treatments,
- the treated animals are unlikely to be sent for slaughter during or immediately after treatment,
- xylazine is very rapidly and extensively metabolised in cattle tissues and milk and is very rapidly excreted,
- depletion of xylazine in cattle tissues and milk was very rapid and residues in cattle derived food were well below doses of possible consumer concern at the first day post dosing already,
- 2,6-xylidine is not found in cattle urine, tissues and milk and no metabolites derived from cleavage of the thiazine and the phenyl ring or decomposition of the thiazine ring are present in cattle tissues and milk.

For cattle, the existing MRL assessment addresses the risk of consumer exposure to residues of 2,6-xylidine and concludes that there is no consumer safety concern as the metabolite was not detected.

Regarding possible consumer exposure to the parent xylazine, no information is available on the oral bioavailability in humans. Very little information is available on biotransformation of xylazine in humans. Metabolites were identified in urine. Xylazine was N-dealkylated and S-dealkylated, oxidized, and/or hydroxylated to 12 phase I metabolites. The phenolic metabolites were partly excreted as glucuronides or sulfates. All phase I and phase II metabolites identified in rat urine were also detected in human urine. In rat urine after a low dose as well as in human urine after an overdose, mainly the hydroxy metabolites were detected (Meyer and Maurer, 2013).

While bioavailability and biotransformation of xylazine following oral ingestion by humans has not been characterised, in rats oral absorption of xylazine is close to 100%. Consequently, significant absorption may also be expected in humans. Moreover, 2,6-xylidine has been identified in urine following parenteral administration of xylazine to humans.

According to the 2002 summary report, the combined potential consumer intake of xylazine from cattle tissues and milk was 25 µg (0.4 µg/kg) at 12 hours post dose and 8 µg (0.13 µg/kg) at 24 hours post dose. The withdrawal period for xylazine-containing products is 1 day for meat and zero days for milk.

While the risk for genotoxic effects following ingestion of xylazine cannot be completely ruled out, considering the low exposure level expected one day after treatment of cattle and considering the extensive metabolism, the risk is considered negligible. It is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

The metabolic pathway in horses is qualitatively similar to that observed in rats, namely hydroxylation of the phenyl ring, conjugation with glucuronic acid, oxidation/opening of the thiazine ring. Since decomposition of the phenyl ring is a prerequisite for the formation of 2,6-xylidine, the presence of this metabolite in horse would be possible.

While there are no data indicating the presence of this metabolite in edible tissues following use of xylazine in horses, it has been shown that 2,6-xylidine is formed *in vivo* in horses. Spyridaki *et al.* (2004) detected this metabolite in the urine of horses using a validated GC-MS method; the substance was detected from 1 hour up to approximately 13 hours after intravenous injection of 160–210 mg. However, in light of the extensive metabolism in horses the risk is considered negligible. It is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

CVMP response to question 5 from The Netherlands:

Question:

As 2,6-xylidine can also be formed from xylazine, does the CVMP see any consumer safety concern after human exposure to xylazine and/or its metabolites?

CVMP Response:

Since 2,6-xylidine has not been detected in cattle milk and tissues, there is no consumer safety concern for this metabolite.

The bioavailability and biotransformation of xylazine in humans following oral administration has not been characterised. However, as oral absorption in rats is nearly 100%, absorption by the oral route may be expected in humans. In addition 2,6-xylidine has been detected in urine following parenteral administration of xylazine in humans.

Therefore there is a risk for genotoxic effects to the consumer following the exposure to xylazine. However considering the low exposure level (8 to 25 µg) one day after treatment of cattle and the extensive metabolism in cattle and horses, the risk is considered negligible. It is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

3. Overall conclusions

The CVMP considered the procedure under Article 30(3) of Regulation (EC) No 726/2004 in relation to the potential risk for the consumer resulting from the use of lidocaine in food producing species. The Committee was asked five specific questions, which are presented below along with the responses. In order to reach these responses the Committee:

- reviewed the available data relating to the metabolism of lidocaine and the generation of potentially genotoxic metabolites in animals and humans
- reviewed the existing data on genotoxicity and carcinogenicity and convened an ad hoc expert group specifically to advise on this matter
- reviewed new residues data generated in cattle along with residue predictions based on a physiologically based pharmacokinetic model
- considered different approaches for evaluating the risk associated with exposure to lidocaine residues in the absence of standard health based reference values like an ADI
- considered the availability of alternatives to lidocaine for use in large animals and the need for risk mitigation measures in order to minimise potential consumer exposure to lidocaine residues
- considered possible exposure of consumers to 2,6-xylidine as a result of the use of xylazine.

Questions posed by The Netherlands and CVMP responses:

Question 1:

Can the CVMP confirm that 2,6-xylidine is a genotoxic carcinogen? From the information available, it appears that 2,6-xylidine is carcinogenic (carcinomas at multiple sites), however, genotoxicity studies suggest that not 2,6-xylidine but actually a further metabolite is responsible for the genotoxic action, as the results of in vitro studies were positive with metabolic activation only.

CVMP response:

It is confirmed that 2,6-xylidine is a genotoxic carcinogen in rats and it is assumed that no threshold exists for genotoxicity. No NOEL has been established for carcinogenicity.

2,6-xylidine can be further metabolised to DMHA and DMAP. These metabolites lead to the formation of reactive intermediates like a nitrenium ion or iminoquinone. These reactive intermediates have the potential to covalently bind to DNA.

Question 2:

It appears that humans can metabolise lidocaine into 2,6-xylidine. Therefore, even if 2,6-xylidine is not formed in the food producing species (as in horses), the exposure to the parent substance lidocaine via food of animal origin may eventually cause consumer exposure to 2,6-xylidine. Does the CVMP consider that there is a consumer risk for genotoxic and/or carcinogenic effects following exposure to the parent substance lidocaine?

CVMP response:

Lidocaine may undergo metabolism to 2,6-xylidine in humans in intestines and liver. By consequence, even if 2,6-xylidine would not be formed in the food producing species, the exposure to the parent substance lidocaine via food of animal origin may eventually cause consumer exposure to 2,6-xylidine. Therefore there is a potential risk for genotoxic and carcinogenic effects to the consumer following the exposure to lidocaine.

Question 3

What is the consumer risk of exposure to lidocaine-related residues in food resulting from the use of lidocaine in horses, pigs and cattle? Please note that withdrawal periods in horses would be 0 days and

the use in other species 28 days for slaughter and 7 days for milk (according to the current rules of the cascade).

CVMP response:

Data from a new residue depletion study in cattle indicate that lidocaine and related residues are present in edible tissues and in milk at early time-points after treatment. However, modelling data indicate that by the minimum cascade withdrawal period of 28 days the total amount of residues remaining in the animal's body will be in the picogram range; even if an entire carcass could be ingested by a single consumer, exposure to residues would remain below the TTC of 0.15 µg.

Regarding milk, the minimum cascade withdrawal period of 7 days does not result in the total elimination of residues or in the elimination of total body residues down to the TTC. To ensure that total residues in the cow's body are below this level requires an interval of 15 days between use of lidocaine and the taking of milk for human consumption. At this time point there is no risk to the consumer.

For pigs no residue data are available and it is therefore not possible to calculate residue levels that will remain following the cascade withdrawal period. However, since metabolism is comparable to that in cattle, it is expected that the minimum cascade withdrawal period of 28 days for meat is sufficient to ensure that residues deplete to negligible levels. Furthermore, considering that lidocaine is used for castration within the first weeks of life, therefore far from slaughter, the risk to the consumer is considered negligible.

For horses, in the absence of residue data it is not possible to conclude with 100% certainty whether a withdrawal period of 0 days is safe. However in the absence of any new residue data, considering the very limited use currently authorised for lidocaine and the extensive metabolism, and considering that new *in vitro* data have further demonstrated that 2,6-xylidine formation in horses is less significant than in cattle, the risk to the consumer can be considered negligible.

Question 4:

Does the CVMP consider it necessary to take risk management measures? If yes, what risk management measures and communication does the CVMP consider appropriate?

CVMP response:

For horses, considering the very limited use currently authorised (for local/regional anaesthesia only), the extensive metabolism and the fact that new in vitro data suggests less significant production of 2,6-xylidine in horses than in other species, the risk of consumer exposure to residues of lidocaine in horse meat is considered very low. In view of this, the current MRL classification ('No MRL required' for horses and for local/regional anaesthesia only) for lidocaine remains appropriate and no risk mitigation measures are considered necessary. In addition it is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

For cattle, considering that the estimated amount of lidocaine residues in the cow's body is negligible (about 10 pg) it can be considered that the minimum cascade withdrawal period of 28 days is appropriate. Therefore, no new risk mitigation measures are needed.

Regarding milk, the safety associated with the minimum cascade withdrawal period of 7 days is uncertain as this time period is not sufficient to ensure that total residues remaining in the cow's body will be below the TTC of 0.15 µg. To ensure that total residues in the cow's body are below this level

requires an interval of 15 days between use of lidocaine and the taking of milk for human consumption. At this timepoint there is no risk to the consumer.

For pigs no residue data are available. However, considering that metabolism in pigs is comparable to that in cattle, it is expected that the minimum cascade withdrawal period of 28 days is sufficient to ensure elimination of residues to a safe level. Moreover considering that the use for castration takes place at a time far from slaughter, the risk to the consumer is considered negligible.

It would seem appropriate to inform lidocaine users via specialised literature for veterinarians or via dedicated websites that an interval of 15 days between use of lidocaine and the taking of milk for human consumption is recommended.

Question 5:

As 2,6-xylidine can also be formed from xylazine, does the CVMP see any consumer safety concern after human exposure to xylazine and/or its metabolites?

CVMP response:

Since 2,6-xylidine has not been detected in cattle milk and tissues, there is no consumer safety concern for this metabolite.

The bioavailability and biotransformation of xylazine in humans following oral administration has not been characterised. However, as oral absorption in rats is nearly 100%, absorption by the oral route may be expected in humans. In addition 2,6-xylidine has been detected in urine following parenteral administration of xylazine in humans.

Therefore there is a risk for genotoxic effects to the consumer following the exposure to xylazine. However considering the low exposure level (8 to 25 µg) one day after treatment of cattle and the extensive metabolism in cattle and horses, the risk is considered negligible. It is also noted that the likelihood of animals being sent for slaughter immediately after treatment is very low, which will further reduce the risk of consumer exposure to residues.

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