The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

XYLAZINE HYDROCHLORIDE
(Extension to dairy cows)

SUMMARY REPORT (2)

1. Xylazine [2-(2,6-dimethylphenylamino)-5,6-dihydro-4*H*-1,3-thiazine, CAS No 7361-61-7] is a thiazine derivative used in veterinary medicine as the hydrochloride for sedation, analgesia, muscle relaxation and for anaesthetic premedication. It is available as 2% injectable solution and as a dry substance with solvent for intravenous or intramuscular injection. Depending on the route and indication, recommended dose levels are in the range of 0.016 to 0.3 mg/kg bw in cattle and 0.6 to 1 mg/kg bw in horses.

   The available pharmacological and toxicological studies were too limited to establish a pharmacological or toxicological ADI. Pharmacological effects were reported in the most sensitive species (cattle) at parenteral doses of 16 µg/kg bw. In humans, the first pharmacological effects were produced at oral doses of 170 µg/kg bw and the first acute toxic effects were produced at oral doses of 700 µg/kg bw.

   Currently, xylazine is included in Annex II of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Animal species	Other provisions
Xylazine hydrochloride	Bovine, *Equidae*	Not for use in animals from which milk is produced for human consumption

   An application has now been submitted for the extension of MRLs for xylazine hydrochloride to dairy cows. The substance would be administered as a single intramuscular injection of up to 0.3 mg/kg bw as xylazine base.

2. Non-GLP pharmacokinetic studies were performed in rats, dogs, cattle, horses and sheep. In rats, the oral absorption was found to be nearly 100%. Following intravenous or oral administration of radiolabelled xylazine, the compound was rapidly distributed in various tissues. About 70% of the total radioactivity was eliminated via kidney and 30% via faeces with a biological half-life of 2 to 3 hours. After intravenous or intramuscular administration to sheep, horses, cattle and dogs at recommended dose levels, xylazine was rapidly and extensively distributed with a distribution half-life of 1 to 6 minutes and an apparent volume of distribution of 1.9 to 2.7 l/kg bw. The compound was rapidly eliminated with an elimination half-life of 22 to 58 minutes and this was probably related to an intensive metabolism rather than to a rapid renal excretion. After intramuscular injection of ^{14}C-labelled xylazine at a dose of 0.33 mg/kg bw to cattle, peak radioactivity concentrations were found in plasma during the first 1.5 hours (peak 0.46 mg/l) and declined within 10 hours to approximately 0.05 mg/l.

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3. In rats, the compound was rapidly metabolised to approximately 20 metabolites with only 8% of the administered radioactivity being eliminated in the urine in unchanged form. In a GLP-compliant study 8 rats were treated orally with ^{14}C-xylazine hydrochloride (labelled in the aniline moiety) at a dose of 5 mg/kg (free base). Radioactivity was predominantly excreted in urine (68.3% to 78.4% within 24 hours). Urinary metabolites were separated by thin layer chromatography. The urinary radioactivity was mainly associated with polar conjugates, which were enzymatically deconjugated to 5 major metabolites. These included products of hydroxylation of the phenyl ring and subsequent conjugation with glucuronic acid and products derived by oxidation and opening of the thiazine ring.

   In horses, xylazine was transformed into a number of polar and non-polar products. The metabolic pathway was qualitatively similar to that observed in rats, namely hydroxylation of the phenyl ring, conjugation with glucuronic acid, oxidation/opening of the thiazine ring.: The major metabolites were present as glucuronide conjugates in extracted urine.

   In GLP-compliant studies, the qualitative and quantitative metabolism of xylazine was investigated in cattle urine and tissues and in cattle milk using a single intramuscular dose of ^{14}C-xylazine at 0.3 mg/kg bw (^{14}C-phenyl ring label). Metabolite profiles in ß-glucuronidase treated and untreated urine (urinary excretion was 85% of the dose within 24 hours post dose) was investigated by thin layer chromatography. A total of 10 metabolites constituting more than 90% of urinary radioactivity were detected. The 5 major components, which comprised about 80% of the urinary radioactivity were isolated and structurally identified by HPLC/MS as glucuronide conjugates of phenyl ring hydoxylated xylazine and conjugated and/or unconjugated derivatives involving oxidation and opening of the thiazine ring. The most abundant compound was the conjugated oxidation product 2-(3- and/or 4-hydroxy-2,6-dimethylanilino)-4-oxo-5,6-dihydro-1,3-thiazine. A combination of thin layer chromatography and HPLC analysis indicated the absence of parent xylazine and 2,6-xylidine in the urine.

4. Metabolic profiles were investigated by HPLC and thin layer chromatography (TLC) in cattle tissues at 4 hours (all edible tissues) and 24 hours post dose (liver only, radioactivity was too low for analysis in the remaining tissues) and in cattle milk from the first milking (7 hours) post dose. Metabolites were identified by co-chromatography with ß-glucuronidase treated urine samples, and authentic standards of parent xylazine and 2,6-xylidine (only in urine). The pattern of metabolites in tissues and milk appeared to be comparable to that in the urine. Unchanged xylazine was a significant component at 4 hours post dose but disappeared rapidly to account for only 4% of the liver radioactivity after 24 hours. In milk parent xylazine was a major component and represented approximately 25 % of the total radioactive residues in the first milking (7 hours) after dosing. The critical substance 2,6-xylidine was not detected in tissues and milk neither qualitatively in thin layer chromatography systems nor by quantitative LC combined with MS (limits of detection: 5 µg/kg and 1 µg/l, respectively). These data for milk, tissues and urine indicated that biotransformation of xylazine in cattle does not involve cleavage at the amine bridge between the thiazine and phenyl ring or complete decomposition of the thiazine ring, i.e. metabolic steps which are prerequisites for the formation of 2,6-xylidine, a substance with genotoxic and carcinogenic potential. In a pre-GLP metabolism study using thin layer chromatography and colourimetric detection (2 to 4 hours post dosing) 2,6-xylidine was reported to occur as metabolite in cattle urine.

5. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has considered xylazine at the 47th meeting. At that time, based on the unresolved issue of whether the genotoxic and carcinogenic compound 2,6-xylidine is formed as metabolite in cattle tissues, JECFA was unable to conclude on the assessment of xylazine. The new metabolism data described here, indicating that 2,6-xylidine is not formed in cattle tissues and milk, were not available to JECFA for their evaluation.

6. In a GLP-compliant residue study 3 calves and 1 cow were each injected with ^{14}C-labelled xylazine (labelled in the thiazine moiety) at the highest recommended dose rate of 0.33 mg/kg bw and were slaughtered 10, 24, 48 and 74 hours later. At 10 hours total residue concentrations were 410 µg equivalents/kg in kidney, 240 µg equivalents/kg in liver and 190 µg equivalents/kg in the injection site. At 24 hours the total residue concentrations were 110 µg equivalents/kg in kidney, 120 µg equivalents/kg in liver and 140 µg equivalents/kg in the injection site, at 48 hours 120 µg equivalents/kg in liver, while in all other tissues and at 74 hours residue concentrations were below the limit of quantification of 100 µg equivalents/kg. In milk, total residue concentrations were 60 µg/l at 12 hours, 50 µg/l at 24 hours, 30 µg/l at 36 hours, 20 µg/l at 48 hours, 10 µg/l at 60 hours and below the limit of quantification of 10 µg/l at 72 hours. The concentration of xylazine in milk was not determined in this study.

7. In a GLP-compliant total residue study using an adequate number of 16 cattle were given an intramuscular dose of ^{14}C-labelled xylazine hydrochloride (labelled in the phenyl moiety) at 0.3 mg/kg bw. Groups of 4 animals were slaughtered at 4 hours and 1, 2 and 6 days post dose. Highest total residue concentrations were measured in liver, kidney and injection site at 4 hours post dose. Residues depleted rapidly in liver from 500 µg equivalents/kg (4 hours) to 93 µg equivalents/kg (1 day), 61 µg equivalents/kg (2 days) and 29 µg equivalents/kg (6 days) and in kidney from 872 µg equivalents/kg to 41 µg equivalents/kg, 20 µg equivalents/kg and 10 µg equivalents/kg. Injection site residues declined from 8322 µg equivalents/kg to 15 µg equivalents/kg within the first day and were below the limit of detection 6 days post dose. In muscle and fat total residues were below the limit of detection (8 µg equivalents/kg) 1 day post dose (these results were comparable with those obtained in the previous study where the thiazine ring was labelled).

8. In a GLP-compliant total residue study in milk 8 dairy cows were treated with an intramuscular dose of ^{14}C-labelled xylazine hydrochloride (labelled in the phenyl moiety) at 0.3 mg/kg bw. Milk was collected twice daily for a period of 7 days. Mean total residue concentrations in high and low milk yield cows respectively were 46.2 and 63.4 µg /l in the 1st milking, 11.7 and 18.7 µg /l in the 2nd milking, 4.7 and 8.1 µg /l in the 3rd milking, 0.8 and 2.1 µg /l in the 4th milking, less than 0.5 µg/l and 1.1 µg / l in the 5th milking. From the 6th milking on total residue concentrations in all samples were at or below the limit of detection (0.5 µg/l). Parent xylazine was determined by TLC and concentrations were low in the range of 2 - 21 µg/l at the 1st milking post dose already.

9. There was one older GLP-compliant residue study in milk with 6 dairy cows receiving a single intramuscular dose of (unlabelled) xylazine of 0.3 mg/kg bw. Milk was collected twice daily up to 11 days and analysed at different time intervals. Xylazine was only found in the 1st milking in 3 out of 6 samples at concentrations of 19, 16 and 12 µg/l. In all other samples concentrations were below the limit of quantification (10 µg/l).

10. Potential consumer intake of xylazine derived residues in the 500 g standard food package from cattle tissues at 24 hours post dose was as low as 14.1 µg total residues and 0.6 µg parent compound (ratio parent compound to total residues of approximately 4% at 24 hours post dose). Consumer intake of xylazine residues from the 1.5 litre standard portion of cattle milk of the 1st and 2nd milking post dose was 95 µg and 28 µg for total residues and 24 µg and 7 µg for parent xylazine, respectively (a ratio parent compound to total residues of approximately 25 % was estimated at the 1st milking post dose). The combined potential daily intake from cattle tissues and milk was approximately 110 µg and 42 µg for total residues and 25 µg and 8 µg for the parent xylazine. From these data it was estimated that consumer exposure to parent xylazine in cattle tissues and milk at the first day post treatment (equivalent to 0.4 and 0.13 µg/kg bw) would already be up to 1300 fold (400 to 1300) lower than oral doses known to exert first pharmacological effects in humans (see Paragraph 1). This margin of safety would still be more than 200, even for total residue intake including pharmacological inactive residues. In view of this adequate margin of safety and taking into consideration the rapid elimination of xylazine derived residues in cattle tissues and milk a recommendation of xylazine into Annex II for use in lactating cattle is justified.

Conclusions and recommendation

Having considered 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,

the Committee for Veterinary Medicinal Products recommends the current entry for xylazine in Annex II of Council Regulation (EEC) No 2377/90 be modified in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Xylazine hydrochloride	Bovine, *Equidae*	