



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### XYLAZINE HYDROCHLORIDE

#### SUMMARY REPORT (1)

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. In wild animals the recommended dose is 8 mg/kg bw.
2. Xylazine is a potent alpha-2 adrenergic agonist and is structurally related to clonidine, an antihypertensive agent recommended for human therapy. Its spectrum of effects is therefore, similar to that of clonidine. The primary pharmacodynamic effects following intravenous or intramuscular administration in mice, rats, rabbits, dogs and cats as well as in cattle, horses, sheep, goats and pigs were sedation, analgesia and muscle relaxation. There were marked species differences with cattle being the most sensitive species requiring approximately 1/10 of the dose used to induce an equivalent sedative state in horses, dogs and cats.

At recommended dose rates xylazine has considerable and variable secondary pharmacodynamic effects. A single intravenous or intramuscular administration produced a short-lived increase of the arterial pressure followed by a longer period of hypotension and bradycardia (in horses arrhythmias, atrioventricular blocks). Mydriasis, impaired thermoregulation, respiratory depression (cattle, horses, cats, dogs), hyperglycaemia, hypoinsulinaemia, polyuria, prolonged intestinal transit time, inhibition of reticulorumen contractions, salivation (sheep and cattle only) and emesis (dogs and cats only) were also observed. In horses, effects of xylazine persisted for 2 to 4 hours whereas in ruminants some effects (hyperthermia, hyperglycaemia, ruminal atony, prostration) persisted as long as 12 to 24 hours or up to 36 hours. The presented studies contained insufficient data to derive a pharmacological NOEL.

3. 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 <sup>14</sup>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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<sup>1</sup> Corrigendum dated November 2002

4. 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 <sup>14</sup>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 a GLP-compliant study, the qualitative and quantitative metabolism of xylazine was investigated in cattle urine and tissues using a single intramuscular dose of <sup>14</sup>C-xylazine at 0.3 mg/kg bw (<sup>14</sup>C-phenyl ring label). Metabolite profiles in β-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 hydroxylated 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.

5. Metabolic profiles in cattle tissues were investigated by HPLC and thin layer chromatography at 4 hours (all edible tissues) and 24 hours post dose (liver only, radioactivity was too low for analysis in the remaining tissues). Metabolites were identified by co-chromatography with β-glucuronidase treated urine samples, and authentic standards of parent xylazine and 2,6-xylidine. The pattern of metabolites in tissues 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 only 4% of the liver radioactivity after 24 hours. The critical substance 2,6-xylidine was not detected in tissues neither qualitatively in thin layer chromatography systems nor by quantitative LC-MS/MS (limit of detection: 5 µg/kg). These new data for 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. The metabolic pattern of xylazine in cattle milk was not investigated.
6. Acute toxicity data were presented for mice, rats, dogs and cats (non-GLP-compliant). LD<sub>50</sub> values were of the same order of magnitude with respect to the route of administration (LD<sub>50</sub> intravenous: 22 to 43 mg/kg; LD<sub>50</sub> intramuscular: 47 mg/kg; LD<sub>50</sub> subcutaneous: 100 to 121 mg/kg; LD<sub>50</sub> oral: 130 to 240 mg/kg). Clinical symptoms comprised lethargy, convulsions, salivation and vomiting. In horses, a minimum lethal dose of about 15 to 27 mg/kg bw after intravenous and 60 to 70 mg/kg bw after intramuscular injection was reported. Convulsions were the predominant symptom. In addition, some unexplained deaths in horses were reported after intravenous injection of xylazine at much lower dose rates (0.5 to 2.8 mg/kg bw). In cattle, the most sensitive species, the minimal lethal dose was approximately three times the maximum recommended dose level, i.e. 0.9 mg/kg bw.

7. Repeated dose toxicity studies in rats and dogs were performed before implementation of GLP and do not meet current scientific standards.

A 13-week study was conducted in Beagle dogs (2 animals per sex and dose) treated with daily doses of 0, 10, 30 or 100 mg/kg feed corresponding to estimated doses of 0, 0.3, 0.9 or 3 mg/kg bw. Comprehensive clinical investigations including ophthalmological, neurological, and cardiovascular functions, complete haematology and blood chemistry and post mortem examinations were carried out. No treatment related toxicological or pharmacological effects were recorded in any dose group. As xylazine was not analysed in feed for confirmation of content, homogeneity and stability, as the recommended therapeutic parenteral dose in dogs is 1 to 3 mg/kg bw, as no pharmacological effects were noted up to the highest administered oral dose of 3 mg/kg bw, the actual tested dosages are not clear. As the validity of the test result was not fully demonstrated, and in the absence of pharmacological or toxicological effects at the highest dose tested, a definite NOEL was not derived from this study. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has retained a NOEL of 3 mg/kg bw from this study.

In a 16-week study mongrel dogs were treated with doses of 25, 50 or 100 mg/kg bw in gelatine capsules 5 days per week (1 male, 1 female in the low dose group, 2 males in the mid-dose group, 2 males and 2 females in the high dose group). The study was inadequate due to the lack of a control group, small animal numbers in the dosed groups, limited blood chemistry and histopathological examination (liver and kidney only). A NOEL could not be derived since dose-related temporary clinical signs of apathy and muscular debility were observed immediately post-dose in all groups, which were attributed to the pharmacological effects of xylazine. Some degenerative alterations in the liver and kidneys of the highest dose group were considered to be possibly related to treatment.

An oral study was conducted in rats (32 weeks, 10 animals per sex and group) with doses of 0, 50, 100, 250 or 500 mg/kg feed, corresponding to estimated doses of 0, 4, 7, 20 or 43 mg/kg bw. No blood parameters were included in the study and histopathological examinations were limited. A significant growth retardation of the females in the highest dose group and, in all dose groups, a fatty degeneration of the epithelium of the renal tubuli was reported, and the presence of infections in all groups. Animals of all dose groups suffered from severe lung infections. Due to insufficient study design no NOEL could be established.

8. Tolerance studies were performed in dogs, cattle and horses after repeated intravenous or intramuscular administration of xylazine at the recommended dose rates (cattle) or up to 2 to 3 times the recommended maximum dose levels (dogs, horses) at regular intervals for 1 to 6 weeks (dogs up to 2 years). The studies were inadequate with respect to study design, animal number, dose rates, test parameters and reporting. Apart from transitory sedation and analgesia, the only effects reported were transitory mild epileptiform seizures in dogs, which were attributed to the combined application of xylazine and ketamine, reduced feed intake and ruminal activity in cattle and increased blood clotting time in horses.
9. A GLP-compliant study on embryotoxicity/teratogenicity, was reported for rats (22 animals per group). Following daily oral doses of 0, 1, 4, 16 mg/kg bw (per gavage) on gestation days 6 to 15 no teratogenic effects were recorded. Maternal effects (partial closing of the eyelids, hypoactivity, occasional ataxia, marginal decrease in body weight) and reduction of mean foetal weight were recorded in the highest dose group only. The NOEL for maternal toxicity and embryo/foetotoxicity was 4 mg/kg bw.
10. GLP-compliant studies on mutagenicity included *Salmonella*-microsomal assay with and without metabolic activation (0.4 to 12 mg/plate, with and without metabolic activation), mammalian forward point mutation assay (HPRT locus) in Chinese hamster lung cells (V79) (2 to 40 µg/ml, with metabolic activation and 62 to 1250 µg/ml, without metabolic activation) and micronucleus test (50 mg/kg bw, intraperitoneally) in mice. The compound was considered to be devoid of mutagenic potential.
11. The carcinogenic potential of xylazine was not specifically evaluated. With regard to the negative results of the mutagenicity studies and the absence of structural alerts this was not considered necessary.

12. No specific studies on immunological properties of xylazine were presented. In the oral repeated dose studies in dogs, no signs of immunotoxic effects (e.g. lymphoid atrophy in the thymus) were observed following doses up to 43 mg/kg bw. In horses, serum gamma globulin levels were not altered after an intravenous dose of 1.1 mg/kg bw. However, in dogs, an unexplained degranulation of subcutaneous mast cells was observed following intramuscular doses in the range of 0.1 to 0.3 mg/kg bw.

The sensitising potential of xylazine was not evaluated.

13. Xylazine is not authorised for use in human medicine. In some earlier experiments however, it was demonstrated to be effective in lowering blood pressure and heart rate in 6 patients with essential hypertension after single oral doses of 10 to 20 mg/person, equivalent to 0.17 to 0.3 mg/kg bw. In healthy volunteers, xylazine induced sedation, muscle relaxation and analgesia after single intravenous doses of 0.27 or 0.68 mg/kg bw or a single oral dose of 0.54 mg/kg bw. At all dose levels xylazine produced a significant depression in blood pressure and heart rate for several hours.
14. Available toxicity studies were not complete enough to establish definite NOELs for repeated-dose toxicity and a toxicological ADI could therefore not be derived. Available data were also too limited to establish a pharmacological ADI. As regards the pharmacological activity of xylazine, it may be noted that pharmacological effects in the most sensitive species (cattle) occur at parenteral doses as low as 16 µg/kg bw. In humans, oral doses of 170 µg/kg bw have been reported to produce first pharmacological effects while doses known to exert acute toxic effects start at 700 µg/kg.
15. JECFA has considered xylazine at the 47th meeting. At that time, based on the unresolved issue whether the genotoxic and carcinogenic compound 2,6-xylydine is formed as metabolite in cattle tissues, JECFA was unable to conclude the assessment of xylazine. The new metabolism data described here, indicating that 2,6-xylydine is not formed in cattle tissues, were not available to JECFA for their evaluation.
16. In a GLP-compliant residue study 3 calves and 1 cow were each injected with <sup>14</sup>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.
17. In a GLP-compliant total residue study using an adequate number of 16 cattle were given an intramuscular dose of <sup>14</sup>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).

18. Potential consumer intake of xylazine derived residues in the standard food package from cattle tissues at 24 hours post dose was as low as 14.1 µg for the total residues and 0.6 µg for the parent compound (ratio parent compound to total residues of approximately 4% at 24 hours post dose). This amount of parent compound (equivalent to 0.010 µg/kg bw) was approximately 17 000 fold lower than the oral doses needed to exert pharmacological effects in humans. In view of this margin of safety of several orders of magnitude and taking into consideration the rapid elimination of xylazine derived residues in cattle tissues a recommendation of xylazine for use in non-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 is very rapidly excreted,
- depletion of total xylazine residue in cattle tissues was very rapid with total residues and parent xylazine residues in the standard edible tissues portion after 24 hours post dose being as low as 14 µg and 0.6 µg, respectively,
- 2,6-xylidine is not found in cattle urine and tissues and no metabolites derived from cleavage of the thiazine and the phenyl ring or decomposition of the thiazine ring are present in cattle tissues,
- inadequate data on residue depletion in milk were available and in particular that the composition of residues in milk is unknown,
- pharmacokinetic data are available in horses and taking into account the CVMP note for guidance on the establishment of maximum residue limits for minor animal species;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for xylazine and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

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