The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

VETRABUTINE HYDROCHLORIDE

SUMMARY REPORT

1. Vetrabutine hydrochloride [1-(3,4-dimethoxyphenyl)-1-dimethylamino-4-phenylbutane-hydro-chloride] is a spasmolytic agent. In veterinary medicine it is administered to sows to facilitate parturition, as a single intramuscular injection at the dose of 1.67 mg/kg bw.

2. As a musculotropic spasmolytic agent vetrabutine hydrochloride was reported to act directly on the smooth muscle fibres and to have no neurotropic activity. In pigs, its activity was specific to the uterine body and cervical musculature where it acted on the myometrial cells, sealing off the membrane against the passage of potassium ions, thereby increasing membrane potential and lowering tonus. Following a single intravenous injection of 10 mg/kg bw, the spasmolytic activity of vetrabutine hydrochloride on the rat uterus *in situ* was comparable to that of the same dose of papaverine hydrochloride; both substances were inactive when injected intravenously at a dose of 3 mg/kg bw. In a series of studies, vetrabutine hydrochloride was shown to have the same spasmolytic as papaverine against barium chloride induced spasm in isolated guinea pig or rat intestine and to cause a similar fall in blood pressure in anaesthetised cats and dogs (mode not stated). Injection of 2 or 5 mg/kg bw vetrabutine hydrochloride had no effect on calcium chloride-induced cardiac arrhythmia in rats and 2.5 or 10 mg/kg bw had no effect on vagal nerve action potentials in guinea pigs. There was no information to demonstrate a NOEL for uterine spasmolysis in the target species.

3. In an non-GLP compliant poorly-reported study, 2 female rats were given a single intramuscular dose of 50 mg/kg bw of ^{14}C-vetrabutine hydrochloride. Peak blood concentrations of around 0.4 µg equivalents/ml were obtained 2 hours after dosing. The terminal half-life was 5.7 hours. Over a period of 72 hours, around 50% of the dose was excreted in the faeces and 30 to 40% in the urine. Autoradiography studies in pregnant rats and mice showed that there was almost ubiquitous distribution of the radioactivity in the tissues of maternal animals after intravenous dosing with 15 mg/kg bw ^{14}C-vetrabutine hydrochloride but that the amount crossing the rodent placenta was very small. In a GLP compliant study, male and female rats were given an oral dose of 5 mg/kg bw ^{14}C-vetrabutine hydrochloride. Within 24 hours of dosing, around 60% of the administered dose was excreted in faeces and around 18% in the urine. The rats were killed 48 hours after dosing; residues in liver, kidney and whole blood were 220 to 370 µg equivalents/kg, 40 to 150 µg equivalents/kg and 10 to 20 µg equivalents/kg, respectively. No unchanged vetrabutine was detected in rat urine. A major urinary metabolite was 1-(3,4-dimethoxyphenyl)-1-diethylamino-4-(4-hydroxyphenyl)butane. From these experiments, it was estimated that the oral bioavailability in rats was around 50%.

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4. In a GLP compliant study, 3 sows (bodyweight 110 to 130 kg) were given a single intramuscular injection of 5 mg/kg bw of ^{14}C-vetrabutine hydrochloride (approximately 3 times the recommended dose). The substance was formulated as the proprietary product. Mean peak plasma concentrations of 1 µg equivalents/ml were achieved 2.3 hours after dosing. Plasma concentrations then declined mono-exponentially with a mean terminal half-life of approximately 22 hours. Mean plasma protein binding up to 48 hours was 33.5 to 77.4%. More than 59% of the radioactivity was excreted within 48 hours of dosing; at least 52% in the urine and the remainder in the faeces. Urinary metabolite patterns were investigated using thin-layer chromatography. The major component was a glucuronide conjugate of 1-(3,4-dimethoxyphenyl)-1-diethylamino-4-(4-hydroxyphenyl)butane, which accounted for 60% of the urinary radioactivity. No unchanged vetrabutine was detected in urine. Significant amounts of radioactivity were found in bile (48 000 µg equivalents/kg, 120 000 µg equivalents/kg and 24 000 µg equivalents/kg, at 12, 24 and 48 hours after dosing, respectively).

5. In a second GLP compliant study, 17 sows were given a single intramuscular injection of 5 mg/kg bw ^{14}C-vetrabutine hydrochloride (approximately 3 times the recommended dose). Mean peak plasma concentrations of 1.25 µg equivalents/ml were achieved 3.3 hours after dosing. The mean area under the blood-concentration-time curve (AUC) value was 72.6 µg equivalents·hours/ml and the apparent volume of distribution was 20 l/kg. Plasma concentrations declined with a mean terminal rate constant of 0.0027 hour^{-1}, corresponding to a mean terminal half-life of around 11 days. One sow was killed 12 hours after dosing and the remaining sows were killed (4 per time point) 3, 4, 6 and 28 days after dosing. Urinary excretion was the major route of elimination in the pigs killed 6 days after dosing (81.9 to 86.6% of the dose over 144 hours). Faecal excretion accounted for 7.3 to 11.2% of the dose for the same animals. No unmetabolised vetrabutine was detected in urine. The major urinary metabolite was a glucuronide conjugate of 1-(3,4-dimethoxyphenyl)-1-diethylamino-4-(4-hydroxyphenyl)butane, accounting for 22.2 to 48.7% of the dose in the urine.

6. Vetrabutine hydrochloride was of moderate acute oral toxicity. Acute oral LD_{50} values of 500 and 910 mg/kg bw were reported for rats and of 206.5 mg/kg bw for mice. The acute subcutaneous LD_{50} values were 320 and 260 mg/kg bw in rats, and 63 mg/kg bw in mice. These studies were poorly reported with no details of clinical signs or pathological findings.

7. In an non-GLP compliant, poorly-conducted and reported 6-month study, groups of 12 female rats per dose were fed diets containing vetrabutine hydrochloride at concentrations intended to give doses of 0, 10, 50 or 100 mg/kg bw. Two rats given 50 mg/kg bw and 3 given 100 mg/kg bw died but the cause of death was not established. The lymphocyte/neutrophil ratio was significantly increased at 100 mg/kg bw. No biochemical urinalysis or organ weight analyses were carried out. Histopathology was restricted to lungs, liver, heart, spleen, kidneys, stomach and intestines from 4 rats per group; there were no treatment-related effects. No conclusions regarding a NOEL could be drawn because of the poor quality of the study.

8. In a preliminary dose range-finding study, groups of Sprague-Dawley derived rats (5 animals/sex/dose) were given daily oral doses of 0, 5, 20, 80 or 240 mg/kg bw/day of vetrabutine hydrochloride for 4 weeks. Females given 240 mg/kg bw were terminated after the first dose due to severe toxicity and replaced by a group given 160 mg/kg bw. Subsequently, 2 females given 160 mg/kg bw and one given 80 mg/kg bw died. Collapsed posture, unsteady gait, trembling, laboured respiration, post-dosing salivation and partially closed eyelids were observed in some females given 20 mg/kg bw and above. Salivation was also observed in males given 80 and 240 mg/kg bw. Increased adrenal weights were observed in females given 5 mg/kg bw though the increase was statistically significant only at 160 mg/kg bw. Histopathological examinations were limited to the adrenals and showed increased width of the adrenal cortex in 1, 2, 5 and 4 females given 5, 20, 80 and 160 mg/kg bw, respectively. However, it was noted that the study was conducted using a diluted injectable formulation product that contained 0.5% phenol, which could have been responsible for the excessive salivation.

9. In the main GLP compliant study, groups of Sprague-Dawley derived rats (10 animals/sex/dose) were given daily oral doses of 0, 1.5 or 20 mg/kg bw/day of vetrabutine hydrochloride for 13 weeks. A further group of 10 males was given 240 mg/kg bw/day and a group of 10 females was given 80 mg/kg bw/day. Four males given 240 mg/kg bw died. Salivation (and resulting wet coat) were observed in males given 240 mg/kg bw, in females given 80 mg/kg bw and in a few animals given 20 mg/kg bw. Also at 240 mg/kg bw, bodyweight gain was adversely affected, water intake was significantly increased, plasma alkaline phosphatase, potassium and phosphorus concentrations were increased and spleen and kidney weights were increased. Thyroid weights were increased in females given 80 mg/kg bw. Histopathology revealed increased height of the thyroid follicular epithelium with decreased colloid in 3 males given 240 mg/kg bw. There were no substance-related effects on the adrenals. The NOEL was 1.5 mg/kg bw/day, based on the observation of salivation at the next dose level of 20 mg/kg bw. However, it was noted that the study was conducted using a diluted injectable formulation product that contained 0.5% phenol, which could have been responsible for the excessive salivation.

10. In a study carried out in accordance with Good Clinical Practice, groups of 4 non-pregnant sows were administered intramuscular doses of 0, 1.67, 5 or 8.3 mg/kg bw/day or 1.67 mg/kg bw twice daily with 1 hour between injections, for 3 days. Blood samples were collected daily for measurement of haematology and clinical chemistry values. There were no effects on behaviour and no substance-related changes in body temperature, respiratory or heart rate or haematology values. Significant differences from the control values were observed in bilirubin and total protein concentrations in the 8.3 mg/kg bw group and in creatinine kinase and aspartate aminotransferase activity in the 5 and 8.3 mg/kg bw groups. The pigs were not killed for pathological examination. The NOEL for the study was 1.67 mg/kg bw/day.

11. In a controlled study, the effects on parturition of a single intramuscular injection of 1.67 mg/kg bw vetrabutine hydrochloride and/or oxytocin were investigated in a total of 55 gilts and 69 sows. There were no significant effects on the sow, the birth process, the puerperium or on the piglets. Compared with the control group, expulsion time per piglet was reduced by 30% (7.6 minutes). In another study the effects of a single intramuscular dose of 1.67 mg/kg bw/day of vetrabutine hydrochloride in sows at the time of birth of the first piglet were examined in a randomised placebo-controlled trial. Farrowing was induced by prostaglandin injection. There were 115 and 116 sows in the vetrabutine hydrochloride and placebo groups, respectively. Litter size and the duration of farrowing were similar in both groups. The mean number of piglets born dead was reduced by 40% in the group treated with vetrabutine hydrochloride.

12. No multigeneration studies were provided. In an non-GLP compliant, poorly-designed and reported study, groups of 6 female rats were given vetrabutine hydrochloride in the diet at doses equivalent to 0, 20 or 100 mg/kg bw/day for 10 oestrus cycles (40 to 50 days). Vaginal smears were taken daily. No changes were observed in the oestrus cycle, behaviour, bodyweight gain or food consumption.

13. In a GLP compliant study, groups of 25 mated female Sprague-Dawley derived rats were given daily oral doses of 0, 1.5, 20 or 80 mg/kg bw/day of vetrabutine hydrochloride from days 6 to 15 of gestation. The dose levels were selected on the basis of results from a preliminary study in which doses of 20 and 80 mg/kg bw caused post-dosing salivation, reduced maternal bodyweight gain and at the top dose, increased water consumption, but no evidence of foetotoxicity or teratogenicity. In the main study, post-dosing salivation was observed in the dams given 20 and 80 mg/kg bw. Water consumption was increased throughout the treatment period in the dams given 80 mg/kg bw. There were no substance-related effects on the numbers of embryonic deaths, implantation losses or live young. Litter and foetal weights were unaffected by treatment. There was no evidence of foetotoxicity or teratogenicity at any dose level. The NOEL for maternal toxicity was 1.5 mg/kg bw/day.

14. It was considered that the absence of teratogenicity data was justified by the lack of structural relationship to known teratogens, the limited use of the substance in sows at parturition and the record of safe use of the substance in pigs and humans. However, no studies have been conducted in laboratory species to investigate effects of vetrabutine on pregnant animals during the perinatal period.

15. There was no increase in revertants in an *in vitro* bacterial assay for gene mutation using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and concentrations of up to 5000 µg/plate of vetrabutine hydrochloride, both in the presence and absence of metabolic activation. In an *in vitro* cytogenetics assay in cultured human lymphocytes, reproducible evidence of clastogenicity was observed in the presence of S-9 metabolic activation. An 18-hour harvest time was used throughout. A significant increase in the numbers of aberrant cells were observed at a concentration of 156.3 µg/ml in the first assay, (though not at the lower doses of 19.5 and 78.1 µg/ml) and a dose-related increase in the numbers of aberrant cells was observed at the concentrations of 150 and 200 µg/ml in the second assay (though not at the lowest concentration of 75 µg/ml); the relative mitotic index at 150 and 200 µg/ml was 54% and 32%, respectively.

    In the absence of S9, no evidence of clastogenicity was observed at concentrations of 9.8, 39.1 and 78.1 µg/ml in the first test but a significant increase in aberrant cells was observed at a concentration of 75 µg/ml in the second test (though not at the lower concentrations of 6.25 and 25 µg/ml).

    In an *in vivo* micronucleus test, groups of NMRI mice (5 animals/sex/sampling time) were given intravenous doses of 0 or 10 mg vetrabutine hydrochloride/kg bw and bone marrow cells were harvested 24 hours or 48 hours later. A dose level of 20 mg/kg bw killed the test animals. Further groups of 5 male mice were given 2.5 or 5 mg/kg bw and killed 24 hours later. There was no increase in micronuclei in any of the treated groups. Although ratios of polychromatic to normochromatic erythrocytes were unaffected by treatment, the route of administration and dose levels employed suggested that it was reasonable to assume that the test substance had reached the bone marrow. In a limited *in vivo* unscheduled DNA synthesis study, groups of 1 to 3 male rats were exposed to subcutaneous doses of 37.5, 75 or 150 mg vetrabutine hydrochloride/kg bw and killed at 4 or 16 hours after dosing, primary hepatocyte cultures were prepared and analysed for repair induction. No increases in unscheduled DNA synthesis were observed at any dose group.

    In conclusion, negative results were obtained with vetrabutine hydrochloride in an *in vitro* gene mutation study and two *in vivo* studies, these results outweigh inconsistent positive findings of an *in vitro* cytogenetics study in cultured human lymphocytes and indicate that vetrabutine hydrochloride is unlikely to present a genotoxic hazard.

16. No data on carcinogenicity were provided.

17. No data on sensitisation potential were provided. There were no effects in the toxicity studies which were indicative of an effect on the immune system.

18. No data on potential microbiological effects were provided. It was agreed that such data were not necessary for this class of substance.

19. Vetrabutine hydrochloride has been used in human medicine as a spasmolytic agent in obstetrics. In the studies provided, a total of 1582 births were reported. The dose administered to the mothers was usually 50 mg by intramuscular injection or 100 mg as a suppository. Multiple doses were used in a small number of cases, 300 mg being the largest total dose quoted. The substance regularised the contractions and reduced the duration of parturition. Reported beneficial effects included reduced infant ataxia cases, reduced incidence of forceps birth and reduced pain. No adverse effects were reported in either the mothers or offspring. In an old study which was not well reported, an intramuscular injection of 25 mg vetrabutine hydrochloride to women (equivalent to approximately 0.4 mg/kg bw) had no effect on oxytocin-induced spasms.

20. For vetrabutine hydrochloride a pharmacological ADI of 0.04 mg/kg bw was calculated by applying a safety factor of 10 to the NOEL of 0.4 mg/kg bw for spasmolysis in humans. A toxicological ADI of 0.015 mg/kg bw was calculated by applying a safety factor of 100 to the NOEL of 1.5 mg/kg bw/day which was established in a 13-week study in rats, based on salivation, and in a reproductive toxicity study in rats, based on maternal toxicity. Taking into account the limited human data, the lower, toxicological ADI was adopted as the definitive ADI.

21. In the study described in paragraph 4, in which 3 sows were given a single intramuscular injection of 5 mg/kg bw ^{14}C-vetrabutine hydrochloride, the sows were killed (1 per time-point), 12, 24 and 48 hours after treatment. Residues were highest at the injection site and were 357 000 µg equivalents/kg, 314 000 µg equivalents/kg and 76 000 µg equivalents/kg, 12, 24 and 48 hours after dosing, respectively. Residues in kidney were 4 800 µg equivalents/kg 12 hours after dosing, 7 030 µg equivalents/kg 24 hours after dosing and 2 680 µg equivalents/kg 48 hours after dosing. The corresponding values for liver were 2 720, 2 970 and 1 700 µg equivalents/kg respectively. Residues in muscle and fat were 290 µg equivalents/kg and 1 120 µg equivalents/kg at 12 hours and 80 µg equivalents/kg and 260 µg equivalents/kg, 48 hours after dosing.

22. In the study described in paragraph 5, in which 17 sows were given a single intramuscular injection of 5 mg/kg bw ^{14}C-vetrabutine hydrochloride, Total residues in the sow killed 12 hours after dosing were 2 900 µg equivalents/kg in liver, 5 440 µg equivalents/kg in kidney, 300 µg equivalents/kg in muscle (remote from the injection site), 580 µg equivalents/kg in skin and 1 140 µg equivalents/kg in perirenal fat. At all later time points (3, 6 and 28 days after dosing, 4 sows/time-point), residues in all samples of muscle (remote from the injection site), skin and fat were undetectable. Mean residues in kidney samples were 970 µg equivalents/kg, 550 µg equivalents/kg, 410 µg equivalents/kg and 80 µg equivalents/kg, 3, 4, 6 and 28 days after treatment, respectively. Mean residues in liver samples were 710 µg equivalents/kg, 460 µg equivalents/kg, 360 µg equivalents/kg and 60 µg equivalents/kg, 3, 4, 6 and 28 days after treatment, respectively. Residues were highest in injection site muscle and were 598 000 µg equivalents/kg 12 hours after treatment. For the pigs killed 3, 4 and 6 days after treatment, there was no general decline in concentrations of radioactivity at the injection site; mean residues were 68 380 µg equivalents/kg, 55 060 µg equivalents/kg and 63 220 µg equivalents/kg respectively. Twenty-eight days after treatment mean residues in injection site muscle had declined to 1 030 µg equivalents/kg (range 460 to 1700 µg equivalents/kg).

23. Analysis of both protease-treated and protease/β-glucuronidase treated extracts of liver and kidneys from the sow killed 12 hours after dosing showed that around 55% of the radioactivity was 1-(3,4-dimethoxyphenyl)-1-diethylamino-4-(4-hydroxyphenyl)butane. At least 5 other radioactive com-ponents were also present. In the liver and kidney samples taken from the pigs killed 3 days after treatment, this metabolite accounted for around 21% of the extracted radioactivity. 4 days after treatment, the metabolite accounted for around 13% of the radioactivity in liver and kidney samples. Analysis of the injection site samples showed that these consisted of 2 major components: unmetabolised vetrabutine and the keto-metabolite, 1-(3,4-dimethoxyphenyl)-4-phenyl-butan-1-one. This keto-metabolite was not found in any of the liver or kidney samples. In the sow killed 12 hours after dosing, unchanged vetrabutine accounted for 90% of the total residues and the keto-metabolite 6.5%. The relative percentages of the 2 substances were investigated in injection site samples from single animals killed 3 and 4 days after dosing. 3 days after dosing, vetrabutine and the keto-metabolite accounted for 74.7% and 19.7% of the injection site residues. By day 4, the percentage of vetrabutine had declined to 53% and the percentage of the keto-metabolite had increased to 42%. In injection site samples from the 4 pigs killed 28 days after dosing, 2 contained higher proportions of vetrabutine relative to the keto-metabolite and 2 contained higher proportions of the keto-metabolite. Due to the low concentrations present, no attempt was made to characterise the nature of the residues in fat and muscle remote from the injection site. The studies used a combination of TLC-radioscanning, Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy, to identify the substances present in tissues.

24. There was no validated analytical method for the determination of residues of vetrabutine or its metabolites in tissues.

Conclusions and recommendation

Having considered that:

- an ADI of 0.015 mg/kg bw (900 µg/person) was established for vetrabutine hydrochloride,
- without considering the injection site, consumer intake of total residues represents approximately 75% of the ADI, 12 hours after the end of treatment,
- vetrabutine hydrochloride is used only in pregnant sows, either immediately prior to parturition or during labour,
- treated sows will not be sent for slaughter immediately after treatment;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for vetrabutine hydrochloride 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
Vetrabutine hydrochloride	Porcine	

In order to ensure that residues at the injection site would deplete to concentrations resulting in a consumer intake not exceeding the ADI, Member States should consider establishing withdrawal periods.