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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

HEXETIDINE

SUMMARY REPORT

1. Hexetidine (5-amino-1,3-bis(2-ethylhexyl)hexahydro-5-methylpyrimidine) is a cationic antiseptic with a wide spectrum of actions against Gram-positive and Gram-negative bacteria, as well as some fungi and parasites. In veterinary medicine, it is indicated for use in horses as a shampoo, at low concentrations (0.1% solution), for topical application to the skin. The use of hexetidin as teat disinfectant, both as teat dip and as teat spray, is also known. In human medicine, is used as a 0,1% mouthwash for local infections and oral hygiene.

2. Hexetidine is bacteriostatic at concentrations in the range 5 to 15 mg/l and 100 to 150 mg/l against Gram-positive and Gram-negative organisms, respectively. At higher concentrations, hexetidine also has pronounced bactericidal activity. At the therapeutic concentration of 0.1% hexetidine kills organisms such as *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumoniae* in less than 1 minute. Pharmacological evidence indicates that the primary mode of action is effected by its interference with vital metabolic processes necessary for the growth of microorganisms. Due to the presence in the hexetidine of a pyrimidine nucleus it may exert a competitive action with thiamine. It is also known that hexetidine inhibits carbohydrate metabolism in microorganisms.

3. When used externally in a shampoo for horses, hexetidin is washed off after use thus it is unlikely that it will be absorbed through the skin in any significant amount.

4. Acute toxicity studies were performed in mice, rats, and dogs. The oral LD_{50} values for rats were higher than 1000 mg/kg bw, thus hexetidine was considered of low toxicity. The subcutaneous LD_{50} values for mice rats and dogs were 2150, 1430 and 1600 mg/kg bw, respectively. The intraperitoneal LD_{50} values for mice, and rats were 77.5, and 30 to 85 mg/kg bw, respectively.

5. In a well-conducted GLP-compliant short term repeated-dose toxicity study, groups of 10 male and 10 female Sprague Dawley rats were given hexetidine orally at concentrations of 0, 0.5, 2 or 8 mg/kg bw/day for up to 13 weeks. No adverse signs were observed in terms of appearance or behaviour, haematological indices, blood coagulation or urinalysis. No signs of toxicity related to hexetidine were observed. A NOEL of 0.5 mg/kg bw/day can be identified. In males given the higher dose of 8 mg/kg/day a slight decrease in mean values for platelet count and protrombin time was noted and a slight decrease in urea serum levels in the same sex of all treated animal groups was observed. No differences were observed between treated and untreated animals concerning the bodyweight, food intake, on *post-mortem* histological examination.

6. In a non-GLP-compliant long term repeated dose toxicity study, hexetidine was included in the diet of Albino rats at levels of 0.02% (corresponding to 20 mg/kg bw/day) and 0.05% (corresponding to 50 mg/kg bw/day) for a period of one year. Food consumption and bodyweight gain data did not reveal any signs of toxicity. Histopathological studies on representative rats from the chronic feeding study showed no pathological lesions attributable to hexetidine in any of the tissues examined. A NOEL of 20 mg/kg bw/day could be identified.

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Other non-GLP-compliant chronic toxicity studies were carried out. Groups of 4 Beagle dogs, each received 40 mg/kg bw, 80 mg/kg bw and 160 mg/kg bw in the daily diet for up to 6 months. Blood analysis consisting of haemoglobin determinations, erythrocyte and leukocyte counts, and differential counts at 30 days intervals were within normal limits. Blood glucose determinations, cephalin flocculation, bromsulfalein retention and phenolsulfonphthalein tests all gave normal findings at the end of the study. At necropsy, there were no significant gross or histological abnormalities. A NOEL of 40 mg/kg bw/day could be established.

7. No data on target species tolerance were provided.

8. A GLP-compliant study on effects on reproduction of hexetidine was carried out. Groups of 20 New Zealand White rabbits given hexetidine orally, during day 6 to 18 of gestation, at dose levels of 0, 5, 10 and 20 mg/kg bw/day. Hexetidine induced a toxic effect on animals at all doses employed. Two females dosed with 5 mg/kg bw/day, three with 10 mg/kg bw/day and eleven with 20 mg/kg bw/day died of toxic effects of hexetidine (gastric ulcers and intestinal hemorrhages). The doses of 10 and 20 mg/kg bw/day induced lower maternal body weight increases during pregnancy. At these high doses, hexetidine was considered to have an embryotoxic effect but never showed evidence of teratogenicity at any dose level. Under the experimental conditions, the only malformation was that found in the group treated with 10 mg/kg bw/day, and this was considered to be incidental.

9. In an *in vitro* assay for gene mutation in *Salmonella typhimurium*, TA 1535, TA1537, TA 1538, TA 98 and TA 100, in both in presence and absence of metabolic activation, hexetidine did not produce a significant increase in revertants up to concentrations of 10 µg/plate (this is an unusually low concentration to use in this test).

10. No specific studies for carcinogenicity have been performed with hexetidine.

11. Hexetidine has been used in human medicine as an antiseptic agent for over 40 years. Although, mild buccal irritacion has been reported on rare occasions, there is no evidence of any significant toxic effect from hexetidine in man (e.g. if swallowed when being used as a mouthwash). Therefore, the risk of a systemic toxicity should be entirely negligible. The possibility of voluntarily or accidental acute intoxication can be excluded.

    Because of its cationic nature, hexetidine is adsorbed to the mucous membranes and dental plaque after oral administration and is not easily removed. Studies in human beings with radiolabeled hexetidine have shown that it is retained on buccal tissues for 8 to 10 hours after a single oral rinse and it has been possible to detect the continued presence of it on the oral tissues for as long as 65 hours after application.

12. Primary skin irritation and sensitisation studies in humans were also conducted. Two hundred twenty human subjects were patch tested on the forearm with an ointment containing 1% hexetidine; 48 hours later 212 subjects were available for re-examination. None showed evidence of dermatitis or irritation at the contact site of the test material. Two weeks later 168 of these subjects were available for retesting and the original sites were again used for the repeated patch test. There was no evidence of primary irritation or sensitisation.

    A gingival irritation tests was also conducted by exposing the mucobuccal fold to one gram of a test dentrifice containing 0.1% hexetidine for three minutes. There was no apparent difference in oral mucous membrane irritating properties of the control and test samples.

13. No data on the effects of hexetidine on human gut flora were available. However, *in vitro* minimum inhibitory concentration (MIC) values were reported from a range of bacteria and fungi. The most sensitive bacteria to hexetidine were *Streptococcus faecalis* and *Pasteurella multocida* with MIC values in the range of 1 to 5 mg/l. *Escherichia coli* appeared to be one of the less sensitive with MIC values in the range of 25 to 100 mg/l.

14. No residue depletion studies in target species after administration of the recommended hexetidine dosage were provided. These studies are not considered necessary due to its poor cutaneous absorption. Hexetidine is marketed for therapeutic use as a 0.1% topical solution. Taking into account the low toxicity of hexetidine and also its poor oral absorption, it may be concluded that the use in the horse is unlikely to result in residues in edible tissues posing a risk to the health of the consumer.

15. No routine analytical method for the determination of hexetidine in tissues of target animals was provided.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II of Council Regulation (EEC) No. 2377/90 and in particular that:

- hexetidine was of low toxicity,
- hexetidine will only be used occasionally in a small number of individual animals, that are unlikely to be sent to slaughter soon after treatment,
- hexetidine is only used externally, and when used in a shampoo it is diluted and washed off after use,
- hexetidine has been used safely as an antiseptic (for skin and mucous) in human medicine for many years;

the Committee concludes that there is no need to establish an MRL for hexetidine 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
Hexetidine	Equidae	For topical use only