



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### CHLORHEXIDINE

#### SUMMARY REPORT

1. Chlorhexidine is a cationic bis-biguanide antiseptic and disinfectant which was discovered in 1950. It was first made available as the low solubility hydrochloride salt and subsequently the acetate salt. The freely-soluble gluconate salt was introduced in 1957. Most formulations currently available are based on the gluconate salt.
2. Aqueous solutions of chlorhexidine salts may decompose to produce trace amounts of 4-chloroaniline; decomposition is increased by heating and alkaline pH conditions. 4-chloroaniline gave equivocal evidence of carcinogenicity in bioassays carried out by the US NCI in Fischer 344 rats and B6C3F1 mice. The European Pharmacopoeia sets a limit of 500 ppm 4-chloroaniline for chlorhexidine salts. In the carcinogenicity assays summarised in paragraphs 13 and 14, concentrations of 4-chloroaniline were monitored in the feed and tissues of rodents. No residues of 4-chloroaniline were detected in kidneys from mice killed up to 52 weeks. However, measurable concentrations (up to 0.232 µg/g) were found in kidneys taken at 65 weeks from mice given 200 and 400 mg/kg bw. In the rat study, concentrations of 4-chloroaniline were small (0.01 µg/g in kidney at 12-15 months and 0.22 µg/g in the lung at 18 months). It was estimated that the top dosed rats and mice had received 0.36 mg/kg bw per day and 0.6 mg/kg bw per day of 4-chloroaniline respectively.
3. In veterinary medicine, chlorhexidine is used as a general purpose disinfectant for cleansing wounds, skin, instruments and equipment. These products normally contain around 1.5% w/v chlorhexidine gluconate and are diluted with water by a factor of 1 in 30 to 1 in 100 before use. Chlorhexidine is also used in teat dips and sprays, as an aid in the control of mastitis. These products are normally applied post-milking, to the whole herd, and are diluted to a concentration of 0.425% w/v chlorhexidine gluconate before direct application to the udder.
4. At concentrations greater than 100 µg/ml, chlorhexidine is bactericidal. Concentrations in the range 1-100 µg/ml are bacteriostatic. The mode of action involves rapid attraction to the bacterial cell, absorption to phosphate compounds on the bacterial surface, overcoming the cell wall exclusion mechanisms, attraction towards the cytoplasmic membrane, leakage of low molecular weight cytoplasmic components and precipitation of the cytoplasm by the formation of complexes with phosphated moieties.
5. Because of its cationic nature, chlorhexidine binds strongly to skin and mucosa. It is therefore poorly absorbed after oral or topical application. In oral dosing studies in rats (5 and 50 mg/kg bw), dogs (0.05 and 5 mg/kg bw), marmosets (6.6 and 7.3 mg/kg bw) and rhesus monkeys (5.5 mg/kg bw) using <sup>14</sup>C-labelled chlorhexidine, oral bioavailability was estimated to be less than 1%. In all these species, over 90% of the administered doses were recovered from faeces and only 0.2-1.3% from urine. In rats, biliary excretion was less than 2% of the dose. The one major component found in faeces was unmetabolised chlorhexidine. Human volunteers were given an oral dose of 0.07 mg/kg bw <sup>14</sup>C-labelled chlorhexidine in gelatine capsules. No radioactivity was detected in blood samples (the limit of detection (LOD) was 0.005 µg/ml). 0.3% of the dose was recovered from urine and 81.9% from faeces. No detectable residues of chlorhexidine were found in blood samples taken from 5 neonatal Rhesus monkeys bathed daily for 90 days in a cleanser containing 8% chlorhexidine gluconate (LOD was 0.011 µg/ml). Residues were found in samples of 2 (out of 5) samples of fat (15, 19 µg/kg), all 5 kidney samples (18-44 µg/kg) and 1 liver sample (17 µg/kg). Residues were appreciable in skin (70-200 µg/kg). No chlorhexidine was

detected in the blood of human infants washed in a 4% chlorhexidine solution (LOD : 0.1 µg/ml). Studies in adult volunteers failed to detect chlorhexidine in blood samples after a single topical application of a 5% solution of <sup>14</sup>C-labelled chlorhexidine to 50 cm<sup>2</sup> of skin (LOD 0.005 µg/ml) or repeated daily use over 6 months as a pre-operative "scrub" (LOD 0.01 µg/ml).

6. Chlorhexidine was of low acute toxicity. The acute oral LD<sub>50</sub> values for the gluconate salt were 2515 and 2547 mg/kg bw in male and female mice (Alderley Park strain), 2270 and 2000 mg/kg bw in male and female Wistar rats and greater than 3000 mg/kg bw in male and female Alderley Park rats. It was more toxic when administered parenterally with acute subcutaneous LD<sub>50</sub> values of 637 and 632 in male and female mice and greater than 1000 mg/kg bw (the maximum dose administered) in rats.
7. Groups of rats were given chlorhexidine gluconate in the drinking water at concentrations intended to provide 0, 5, 25 or 40 mg/kg bw per day (expressed as base) for up to 2 years. The intended top dose was 50 mg/kg bw but palatability problems caused reduced water consumption and the test substance intake was only 40 mg/kg bw. At 40 mg/kg bw, mortality was increased and dehydration, aggression, incontinence and staining of the fur were also observed. Body weight gain was reduced at 25 and 40 mg/kg bw. Packed cell volume was increased at 40 mg/kg bw, probably as a consequence of dehydration. There were no substance-related effects on ophthalmoscopy, clinical chemistry or urinalysis values. There were no significant gross pathological findings. Histopathology revealed histiocytosis of the mesenteric lymph nodes, in all treated groups. The effects were dose-related in severity and reversible on withdrawal of the treated diets. A second study was carried out in which rats received 0, 0.5, 1.0, 1.5, 2.5, 5, 10, 15, 25, 50 or 100 mg/kg bw chlorhexidine gluconate, orally for 50 days, and were examined only for histiocytosis; the NOEL was 0.5 mg/kg bw.
8. Combined 6-month and 12-month studies were carried out in dogs using oral doses of 0, 0.5, 5 and 40 mg/kg bw per day. The top dose level of 40 mg/kg bw had to be reduced to 25 mg/kg bw after 29 weeks due to loss of bodyweight. Two dogs given 40 mg/kg bw had bronchopneumonia and were euthanased; their condition was exacerbated by the chlorhexidine administration. Serum ALT concentrations were significantly increased at 40 mg/kg bw. Focal or irregular areas of liver necrosis with inflammation, fibrosis, bile duct hyperplasia and haemosiderosis were found in the 40 mg/kg bw group. Similar though less severe effects were observed in the livers from the animals in the 5 mg/kg bw group. The NOEL was 0.5 mg/kg bw per day.
9. Two separate studies were carried out to investigate effects on fertility and reproductive performance in female and male rats. The rats were given chlorhexidine in the drinking water at concentrations which resulted in intakes of 0, 4.9 or 44.4 mg/kg bw per day. These intakes were slightly less than the intended doses (5 and 50 mg/kg bw) because the test substance caused a dose-related reduction in water consumption. The females were gradually acclimatised to the treated water over 2 weeks and then received the intended doses for 14 days prior to mating with untreated males. 50% of the dams were killed on day 13 of pregnancy and the uterine contents examined; the numbers of viable foetuses were significantly reduced in the group given 44.4 mg/kg bw. The remaining dams were allowed to litter naturally and rear the offspring to weaning. Maternal bodyweight gain was reduced in the group given 44.4 mg/kg bw. There was no substance-related effect on mating performance, pregnancy rate or gestation period. Pup weights in the 50 mg/kg bw group were significantly lower than the controls on day 4 postpartum. There was no evidence of teratogenicity. The male rats were gradually acclimatised to the treated water over 2 weeks and then received the intended doses for 63 days prior to mating with untreated females. Again 50% of the dams were killed on day 13 of pregnancy and the uterine contents examined - the remaining dams were allowed to litter down. The treated males had greasy coats and a dose-related reduction in bodyweight gain. However there were no effects on mating performance, number of viable foetuses, pup bodyweight or survival. The overall NOEL was 4.9 mg/kg bw per day, based on reduced pup weights on day 4 postpartum.
10. In a peri- and postnatal development study, pregnant female rats were given daily oral doses of 0, 10 or 50 mg/kg bw per day from day 15 of gestation, through lactation, to day 21 postpartum. The dams given 50 mg/kg bw showed irritability during the first week of dosing but there were no other signs of maternal toxicity. Litter parameters were unaffected by treatment.

11. Teratology studies were carried out in which rats and rabbits were fed diets containing 0, 0.05%, 0.1%, 0.25% or 0.45% chlorhexidine throughout pregnancy. The rats were killed on day 20, the rabbits on day 28 and the foetuses were stained for examination of skeletal abnormalities. In rabbits, 0.45% chlorhexidine caused abortion and the litter size was reduced at 0.25%. Female rats given 0.45% chlorhexidine lost weight. There was no evidence of teratogenicity in either species. The studies were carried out during the 1960s and were flawed by small group sizes. In a more recent rat teratology study, oral doses of 0 (distilled water) 10, 25 or 50 mg/kg bw chlorhexidine (expressed as base) were administered from days 6-15 of gestation. Excitability was observed among dams given 50 mg/kg bw. There was no evidence of foetotoxicity or teratogenicity at any dose level.
12. According to a published summary, concentrations of 0.002-2% chlorhexidine in DMSO were not mutagenic in an *in vitro* bacterial mutation assay in either the presence or absence of metabolic activation. A published study employing a modified Ames test gave weak positive results but was flawed by the absence of positive controls.
13. In a carcinogenicity study, carried out in accordance with GLP, groups of mice were fed diets to provide 0, 100, 200, 400 or 800 mg/kg bw per day chlorhexidine for up to 78 weeks. Due to high mortality, surviving mice in the group given 800 mg/kg bw were killed after 4 weeks. There was a dose-related reduction in bodyweight gain in the groups given 200 and 400 mg/kg bw. There was no evidence of carcinogenicity.
14. In a carcinogenicity study carried out in accordance with GLP, chlorhexidine was administered to Wistar rats in the diet at doses which provided 0, 5, 25 and 50 mg/kg bw for 105 weeks. Bodyweight gain was reduced in males given 50 mg/kg bw and in females given 25 and 50 mg/kg bw. There was no evidence of carcinogenicity.
15. Chlorhexidine has been used in human medicine as an antiseptic and disinfectant for over 40 years. Most preparations are intended for topical use and may contain up to 4% chlorhexidine gluconate. An orally-administered throat lozenge contains 5 mg of chlorhexidine hydrochloride. One application of a dental gel contains approximately 10 mg of chlorhexidine gluconate. The incidence of adverse reactions is low and involves mostly irritation of the skin, eye and mucosa and hypersensitivity reactions. Tooth discoloration has been reported after the use of mouthwash preparations. No adverse effects were observed following the oral administration of 2000 mg/day of chlorhexidine hydrochloride to human volunteers for 7 consecutive days.
16. A toxicological ADI of 0-0.005 mg/kg bw per day (300 µg/day) may be calculated by applying a safety factor of 100 to the NOEL of 0.5 mg/kg bw per day established in the repeated-dose toxicity studies in rats and dogs.
17. *In vitro* MIC values were reported for a range of microorganisms including some species which were typical of the normal human gut flora. *E. coli* appeared to be the bacteria most sensitive to chlorhexidine with *in vitro* MIC values in the range 1.5-2 mg/l.
18. The depletion of residues of chlorhexidine was determined in milk from cows treated with chlorhexidine teat dips and sprays according to the indicated dosage regimes. In most studies, residues in all samples were below the limit of quantification (LOQ) of the analytical method employed (usually 50 µg/l). However in one study, residues of 4.4 µg/l were found in the milk from 1 out of 6 cows; the limit of detection in this assay was 1 µg/l. In another study, 5 cows had their teats dipped in a solution containing 1% chlorhexidine gluconate (235% of the indicated dose) after each milking, for a period of 20 weeks. Mean chlorhexidine concentrations of 43 (+4) µg/l at the morning milking and 78 (+8) µg/l at the afternoon milking were found during weeks 4-20 of the study.
19. An analytical method was developed for the determination of residues of chlorhexidine in milk. The method was based on HPLC with UV detection. The claimed limit of quantification was 50 µg/l. However the method was not validated in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community.

## Conclusions and recommendations

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:

- chlorhexidine was poorly absorbed after oral and topical administration,
- chlorhexidine was of low toxicity,
- residues in milk from use as a teat dip or spray have been demonstrated to be low,

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

Pharmacologically active substance(s)	Animal species	Other provisions
Chlorhexidine	All food producing species	For topical use only