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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FUROSEMIDE

SUMMARY REPORT

1. Furosemide (4-chloro-N-(2-furyl methyl)-5-sulphamoyl-anthranilic acid) is a loop diuretic which is used in both human and veterinary medicine. In veterinary medicine, it has been administered to cattle (0.5 to 1.0 mg/kg bw by the intramuscular or intravenous route, pigs (5 mg/kg bw by the intramuscular or intravenous route) and horses (0.5 to 1.0 mg/animal by the intramuscular or intravenous route, intervals of 6 to 8 hours) for oedema, accumulation of fluid in body cavities, to support forced diuresis in intoxications, renal failure with oliguria and diagnostic procedures. Treatment is normally repeated until the desired therapeutic effect is achieved.

   Furosemide is used in human medicine for the treatment of conditions such as oedema associated with heart failure, hypertension, oliguria and asthma. The usual oral dose is 20 to 80 mg/day but severe cases may require doses up to 600 mg/day. Furosemide may also be given intravenously at 40 to 80 mg/day.

2. The diuretic mode of action of furosemide is not fully understood but it appears to act primarily by inhibiting active reabsorption of chloride ions on the ascending limb of the loop of Henle. Urinary excretion of sodium, chloride, potassium, calcium, magnesium, ammonium and bicarbonate ions are increased; the chloride excretion exceeds that of sodium and there is an enhanced exchange of sodium for potassium. The resulting low osmolality of the medulla inhibits reabsorption of water by the kidney. The diuretic effect of furosemide is dose-dependent and is rapid in onset after oral or parenteral administration to most species. It usually reaches a maximum within 30 to 60 minutes of administration. Thereafter the diuretic effect rapidly declines and normally terminates 2 to 5 hours after dosing.

   Furosemide also possesses renal and extra-renal vascular effects, both of which depend on prostaglandin synthesis. In microdissected nephron segments, furosemide induced a dose-related increase in PGE2 production in cortical and medullary ascending limbs of the loop of Henle. The extra-renal vascular effects of furosemide, such as the relief of pulmonary oedema, depend on prostaglandin production in the kidney. The venodilating effect of furosemide is not seen in anephric patients or those treated with an inhibitor of prostaglandin synthetase such as indomethacin. Furosemide can also antagonise vasoconstrictor responses as well as acting as a vasodilator. In anaesthetised rats, the furosemide-induced inhibition of sympathetic vasoconstrictor responses in the *in situ* blood-perfused mesenteric artery was dependent on the presence of the kidneys and was also prevented by pre-treatment with indomethacin.

   Minimum effective diuretic doses have been established in a variety of species by oral and parenteral routes of administration. Overall, these results suggest that the dog is the most sensitive species. An oral dose of 0.25 mg/kg bw did not increase urine output, or sodium, potassium or chloride excretion, compared to controls.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

3. After single oral administration of ^{35}S-furosemide to rats at doses of 1 to 10 mg/kg bw, maximal plasma concentrations were measured at 20 minutes. Within 24 hours 16 to 34% and 44 to 54% of the oral dose was excreted in the urine or faeces, respectively. Tissue levels in rats after oral administration of ^{35}S-furosemide, were highest in liver and kidney and not significantly different after single or repeated administration. Radiolabel concentration in tissues did not significantly increase after daily oral dosing for up to 10 days. Biliary excretion in rats was only about 0.4% of the dose. However, in Swiss mice, biliary excretion as high as 30% was reported after oral doses. Thirty minutes after oral administration of 20 mg/kg bw furosemide to dogs, a C_{max} value of 22.73 µg/kg was observed. The oral bioavailability was estimated to be approximately 77%. The volume of distribution was low (less than 0.7 l/kg) suggesting limited distribution to the tissues. Furosemide was extensively bound to plasma protein (91%). The half-life for elimination was 1.42 hours after oral dosing. Excretion was rapid and almost complete (98 to 99%) within 3 days. In Rhesus monkeys, absorption was rapid, but incomplete with maximum plasma levels within 1 hour of dosing after an oral dose of 5 mg/kg bw. Plasma profiles did not change on repeated dosing. Maximum levels of radiolabel were found in the liver and kidney. There was a single disposition phase (half-life 10 hours). There was no evidence of accumulation from comparing tissue levels after a single or 20 daily doses.

   Cattle were given an intramuscular injection of 1 mg furosemide/kg bw/day on 2 consecutive days and the concentrations of furosemide in plasma determined by HPLC with fluorescence detection. Animals were killed at 4, 8 and 12 day time points after dosing. Plasma samples collected 15 minutes after the first and second dose administrations contained between 891 to 2314 µg/ml and 1350 to 2482 µg/mg, respectively. Samples collected 60 minutes after the first and second doses contained between 508 to 1251 µg/ml and 538 to 985 µg/mg respectively, all other plasma samples containing furosemide concentrations below the limit of quantification of 54.5 µg/kg.

   Two lactating cattle, given a single intramuscular dose of 1 mg ^{14}C-furosemide/kg bw/day, were killed at 24 and 48 hours, respectively, after dosing. The maximum plasma residue concentrations (1.35 and 0.86 µg equivalents/g) were attained in 0.25 hours. The total urinary elimination of residue accounted for 89.1 and 81.5% of the administered dose after 24 and 48 hours respectively. The total faecal elimination of residue accounted for 5.4 and 11.9% of the administered dose after 24 and 48 hours, respectively.

   In humans, furosemide is fairly rapidy absorbed from the gastrointestinal tract with peak plasma concentrations attained 1 to 2 hours after oral dosing. Oral bioavailability is erratic and subject to large inter- and intra-individual variation. Urinary excretion amounted to 42 to 70% after oral dosing, with 29 to 63% in faeces. After oral dosing elimination was 90% within 12 hours, 95% in 24 hours and 99% in 48 hours After oral dosing 24 hour urinary recovery was 26 to 54%, and faecal recovery about 2%. Oral doses of 80 mg furosemide were taken during fasting or after a meal. During fasting, furosemide was detected in serum within 10 minutes, peaked at 60 to 70 minutes and was almost undetectable after 3 to 4 hours. Taken after a meal, appearance in serum was delayed and peak levels lower with only a slight decrease at 4 hours. The total amount absorbed was similar with or without food (about 60%). The 24-hour excretion was, about 30 to 50%. For adults, the half-life for plasma elimination ranges from 30 minutes to over 24 hours in patients with renal disease. For infants, the half-life is longer due to immature renal function and ranges from 4.5 hours to 46 hours; repeated dosing can result in accumulation. Furosemide clearance is influenced by age, disease state and drug interactions.

4. Rat urine contained 40 to 50% furosemide, 30% 4-chloro-5-sulfamoyl-anthranilic acid, around 20% of an unknown metabolite and a further 3 metabolites which together accounted for around 10% of the administered radioactivity. In contrast, dog and monkey urine contained 85% of unmetabolised furosemide, 7% of 4-chloro-5-sulfamoyl-anthranilic acid and 6% unidentified metabolites.

In humans, a glucuronide metabolite is produced in varying amounts. There is debate over a potential metabolite, 4-chloro-5-sulfamoyl-anthranilic acid, which may be an artefact of the extraction process. 4-Chloro-5-sulfamoyl-anthranilic acid was reported to account for up to 13% and 10% of the radioactivity in serum from adult volunteers given oral and intravenous doses, respectively, of furosemide. The peak of an unidentified metabolite is also present in chromatograms of human serum. 4-Chlorosulpamoylanthranilic acid was identified as the only metabolite in serum, urine and faeces of healthy volunteers following oral or intravenous administration of ^{35}S-furosemide. After oral administration of ^{35}S-furosemide to adults, about 71% of the dose absorbed was recovered as unchanged furosemide, 9% as 4-chlorosulpamoylanthranilic acid and 13% as an unknown metabolite After oral or intravenous administration of ^{35}S-furosemide to healthy subjects, approximately 2/3 of the activity recovered in urine (about 56% of the oral and 83% of the intravenous doses) was unchanged furosemide, with probably a glucuronide conjugate as the major metabolite) 4-chlorosulpamoylanthranilic acid was not detected. Another study using non-labelled furosemide and a metabolite-specific HPLC analysis confirmed the absence of the acid metabolite, with only the glucuronide conjugate identified. In young adult males, about 14% of an 80 mg dose of furosemide was recovered from urine as the glucuronide conjugate within 24 hours. In older men, the glucuronide accounted for only 7% of the dose.

5. The acute oral LD_{50} values were 1050 to 1850 mg/kg bw (mice), 2700 to 7537 mg/kg bw (rats), 243 mg/kg bw (guinea pig) and 720 mg/kg bw (rabbit). The acute oral LD_{50} in dogs was greater than 1000 mg/kg bw. The acute oral LD_{50} in newborn rats was 380 mg/kg bw. Signs of toxicity included weight loss diuresis, hypothermia, diarrhoea and vomiting.

6. B6C3F1 mice were fed diets containing 0, 570, 1700, 5100, 15 300 or 46 000 mg furosemide/kg feed for 14 days. All 5 males and one female given 46 000 mg/kg feed died. There was a dose-related reduction in body weight gain. Nephrosis was observed in mice given 15 300 and 46 000 mg/kg feed.

   In a 13-week study, male mice were fed diets containing 0, 938, 1875, 3750, 7500 or 15 000 mg furosemide/kg feed and females were fed diets containing 0, 625, 1250, 2500, 5000, 10 000 or 20 000 mg/kg feed. There was a dose-related reduction in body weight gain. Liver to body weight ratios were higher in males which received 15 000 mg/kg feed and in females which received 5000 mg/kg feed and above. Nephrosis was observed in males given 7500 mg/kg feed and above and in females given 10 000 mg/kg feed and above. In addition, proteinaceous tubular casts were observed in males given 3750 mg/kg feed and above and in females given 5000 mg/kg feed and above. No NOELs could be identified from these studies as they did not include haematological, clinical chemistry or urinalysis investigations.

   In a rat study, furosemide was administered at concentrations of 0, 570, 1700, 5100, 15 300 or 46 000 mg/kg feed for 14 days. Increased diuresis was observed in all studies (dose levels not quoted). Nephrosis was observed at 15 300 and 46 000 mg/kg feed. In a 13 week rat study, male rats were fed diets containing 0, 625, 1250, 2500, 5000 or 10 000 mg/kg feed and females received 0, 938, 1875, 3750, 7500 or 15 000 mg/kg feed. Nephrosis was seen at 5000 and 10 000 mg/kg feed in males and 7500 and 15 000 mg/kg feed in females. No NOELs could be identified from these studies as they did not include haematological, clinical chemistry or urinalysis investigations.

   A 12-month study in rats (using dietary concentrations of 100, 320 or 1000 mg furosemide/kg feed) was also carried out. In addition, rats were given furosemide by gavage for 12 months at doses of 0, 50, 100, 200 or 400 mg/kg bw/day (5 days a week). Increased diuresis was seen in both studies at all doses. In the gavage study there were clinical signs and effects on body weight and food consumption at doses of 200 mg/kg bw or more, and treatment-related effects on mortality at 100 mg/kg bw or more. No NOEL could be identified in the dietary study due to inadequate reporting of results, or in the gavage study due to effects on kidney and adrenal weights at the lowest dose.

Groups of 2 Beagle dogs per sex were given oral doses of 10, 30, 100 or 350 (reduced to 250 after 2 weeks) mg/kg bw/day, 5 days a week, for 6 months. The dose of 350 mg/kg bw caused anorexia, hypotonia, decreased serum sodium and potassium concentrations and the deaths of 2 dogs. Serum urea nitrogen and creatinine concentrations were increased at 100 and 250 mg/kg bw. At necropsy, scarring of the renal parenchyma was observed in all treated groups. No NOEL could be identified due to the renal pathology observed in all groups and inadequacies in the reporting of the study. In a one-year study groups of beagle dogs were given daily oral doses of 0, 4, 8, 16 and 32 mg/kg bw 5 days a week, as a divided dose 7 hours apart. Dose-dependent effects on haematology were observed at doses of 8 mg/kg bw or more and renal pathology at doses of 16 mg/kg or more. A NOEL of 4 mg/kg bw (2 mg/kg bw twice a day) was established, but should be treated with caution due to the dosing regimen (divided dose at longer than the elimination half-life, only 5 days of treatment a week) and extensive electrolyte therapy received by treated animals.

Several studies were carried out in which monkeys were given oral doses of furosemide for periods ranging from 3 days up to 12 months. The doses used were 400 to 1600 mg/kg bw/day (for 3 days), 4.4, 12, 27 mg/kg bw (for 30 days), 0.8 to 30 mg/kg bw (for 3 months) 6 to 48 mg/kg bw/day (for 6 months and 12 months). Deaths occurred at 600 mg/kg bw and above in the 3-day study. Deaths, loss of body weight, anorexia, and diuresis were observed at 13.5 mg/kg bw and above in the 3-month studies. Effects on serum chemistry were observed at 2.2 mg/kg bw and above in the 3-month studies. Statistically significant increases in kidney and adrenal weights were observed at doses of 0.8 mg/kg bw and above in one 3-month study, which appeared to be independent of effects on bodyweight. Statistically significant increases in kidney weights were also observed at the lowest dose at 12 months, also without significant effects on bodyweight. The histological findings in animals which died included renal tubule dilatation with casts, bone marrow hypoplasia and skeletal muscle fibre atrophy. No overall NOEL could be identified for repeated dose toxicity in the monkey due to the effects on organ weights at the lowest doses in both the 3 and 12-month studies.

7. Oral and intramuscular administration to cattle at twice the therapeutic dose was well tolerated with diuresis for 4 hours after dosing and reduced serum potassium for up to 48 hours. In tolerance studies in the rabbit single and repeated parenteral administration resulted in transient localised irritation and inflammation after subcutaneous and intramuscular dosing.

8. According to brief summaries, groups of 30 male and female NMRI mice and Wistar rats were exposed to dietary doses equivalent to about 19, 60 and 200 mg/kg bw (mice), and 9, 28 and 90 mg/kg bw (rats) for at least 60 days prior to, and during mating. Exposure of females continued through pregnancy and lactation. No adverse effects on health, fertility, pregnancy and postnatal development were reported in either parent group or F_1 generation were reported. No NOELs could be identified due to the limited information available.

   Male and female rats were given 100 mg/kg bw/day, 5 days a week, for 3 weeks prior to mating. Treatment of the females continued through pregnancy and lactation. Treatment of the F_1 pups commenced after weaning and continued throughout mating, gestation and lactation. There was no effect on fertility or viability of the offspring. No NOEL could be identified from this study, the duration of dosing prior to mating was too short, only one dose level was used, and only a limited number of pups were examined.

9. Pregnant mice were given daily oral doses of 50 mg furosemide/kg bw from days 6 to 8, days 9 to 11 or days 12 to 14 of gestation followed by caesarian section on day 18. Increased incidences of hydronephrosis and/or skeletal irregularities were observed in the treated groups. No evidence of foetotoxicity or teratogenicity was reported in a second study in which oral doses of 25, 50 or 100 mg furosemide/kg bw were administered to pregnant mice. Maternotoxicity was observed at all doses, no skeletal examinations were conducted and only a small number of foetuses were available in the treated groups due to poor pregnancy rates.

   Daily oral administration of 50, 150 or 300 mg/kg bw to pregnant rats, throughout gestation, did not result in embryotoxicity or teratogenicity, according to the brief summary provided. Maternotoxicity was observed at all doses, no skeletal examinations were conducted and only a small number of foetuses were available in the treated groups due to poor pregnancy rates.

No evidence of teratogenicity was observed in foetuses from groups of pregnant rats orally treated with twice daily doses of 0, 37.5, 75, 150 and 300 mg/kg bw on gestation days 6 to 17. Maternal deaths occurred at 150 and 300 mg/kg bw. Maternal bodyweight gain was reduced at all but the lowest dose. Foetotoxicity (increased resorptions and reduced live weights) was observed at the two highest doses. Dose-related increases in wavy ribs were observed at all dose levels and 5 foetuses in the 150 mg/kg bw groups had scapular malformations.

Groups of NZW rabbits received furosemide by oral gavage at doses of 0, 50 and 75 mg/kg bw/day on days 6 to 18 of gestation. Survivors were sacrificed on day 29 of gestation. At 75 mg/kg bw marked toxicity was observed with 68.7% mortality. Significant reductions in bodyweights and food consumptions, and changes in haematocrit and chloride levels were observed in the treated groups. At 75 mg/kg bw pathological changes on the kidney were seen. No compound related effects on foetuses were reported. Groups of New Zealand White rabbits received oral doses of 0, 25, 50 and 100 mg/kg bw from days 8 to 15 of gestation. Bodyweight loss occurred at 50 and 100 mg/kg bw, with reductions in weight gain at 25 mg/kg bw. Increased incidence of abortions and maternal deaths, and resorbed or dead foetuses occured in all treated groups. No NOEL for materno- or foetotoxicity could be identified. Groups of New Zealand White rabbits were given daily oral doses of 50 mg/kg bw on days 6 to 8, 9 to 11, 12 to 14 or 15 to 17 of gestation. All treated groups lost body weight during treatment. Increased incidence of abortion and maternal death were observed in the groups treated from day 12 onwards. Foetotoxicity (increased late resorptions, foetal death and increased incidence of hydronephrosis) was observed in the treated groups.

Groups of pregnant yellow-silver rabbits received daily oral gavage doses of 0, 12.5, 25, 50 or 100 mg furosemide/kg bw from day 8 to 15 of gestation. Maternal toxicity was evident at all doses in a dose-related fashion. There was evidence of foetotoxicity in the form of decreased numbers of viable foetuses and decreased foetal weight at 25 and 50 mg/kg bw, and a slight decrease in viability at 12.5 mg/kg bw. There was no significant evidence of teratogenicity. Groups of mated female rabbits were dosed orally throughout gestation with daily doses of 0, 25, 50 and 150 mg/kg bw. All rabbits at the highest dose and 12 out of 21 at 50 mg/kg bw died during the study. Treatment-related increases in dead foetuses and resorptions were observed. There were no reported malformations and foetal weight was not affected. Groups of pregnant yellow-silver rabbits received furosemide at oral gavage doses of 0, 25 and 50 mg/kg bw/day on days 7 to 19 of gestation. Maternal deaths occurred in both treated groups. Significant reductions in bodyweights and food consumptions were observed in the treated groups. No compound related effects on foetuses were reported.

Domestic cats were given daily oral doses of 1.25, 2.5 or 5 mg furosemide/kg bw from days 13 to 27 of gestation and the foetuses were delivered on day 53. Cats given 5 mg/kg bw showed increased diuresis, reduced body weight gain and drank more water. There were no effects on litter size, placental or foetal weight, the numbers of dead/immature foetuses, and no evidence of teratogenicity. However, the highest dose used in the study was below the recommended therapeutic dose for cats.

Overall, despite the fact that none of the reported studies meet current standards, there was no evidence of overt teratogenicity from furosemide in any species. Adverse effects on the foetus or offspring were only observed in association with maternal toxicity. However, it is not possible to identify NOELs for fertility, maternotoxicity or foetotoxicity.

10. In 3 bacterial reverse mutation studies using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, and *Escherichia coli* WP2uvrA, furosemide at concentrations of up to 10 000 μg/ml did not increase incidence of mutations with or without metabolic activation. In an *in vitro* HPRT gene mutation test using cultured V79 cells, furosemide at concentrations of 200 to 2000 μg/ml did not increase incidence of mutations with or without metabolic activation. No increases in ^{3}H-thymidine incorporation were reported in an unscheduled DNA synthesis test using A549 human cell cultures treated with concentrations of 0.1 to 1000 μg furosemide/ml, with or without metabolic activation.

An *in vitro* mouse lymphoma assay was conducted using L5178Y cells treated with furosemide (62.5 to 1500 μg/ml without metabolic activation and 250 to 1800 μg/ml with metabolic activation). An equivocal response was observed without metabolic activation, with a marginal response at 1500 μg/ml in the first trial and a weaker response at 1000 μg/ml only in the second. With metabolic activation, positive responses occurred in both trials at 1800 μg/ml. Overall the test was considered positive, although the effect may have been due in part to an acidic pH shift. Reproduceable and dose-related increases in sister chromatid exchanges (SCEs) and chromosome aberrations were observed in an *in vitro* cytogenetics study using cultured Chinese hamster ovary (CHO) cells. Increases in sister chromatid exchanges were observed at concentrations of 500 μg/ml or more with or without metabolic activation. Increases in chromosome aberrations were observed at doses of 1257 μg/ml or more without metabolic activation, and 2500 μg/ml or more with or without metabolic activation. Increased incidences of chromosome aberrations were reported in cultured human leukocytes exposed to concentrations of 0.2 to 0.8 mg furosemide/ml for periods of 24 to 72 hours. In 3 brief reports, furosemide was also reported to induce chromosome aberrations in cultured Chinese hamster lung fibroblasts at concentrations of 0.5 to 2.0 mg/ml.

In a non-standard *in vivo* chromosomal study, furosemide was reported to cause chromosome aberrations (translocations and autosomal and sex chromosomal univalents) in testicular meiotic cells of male mice treated with single intraperitoneal doses doses of 0.31 to 50 mg furosemide/kg bw and sacrificed over a 5-week period, corresponding to the spermatogenic cycle. Statistically significant differences were sporadic and there was no clear dose relationship. Furosemide was negative in an *in vivo* bone-marrow micronucleus study in mice treated with two oral doses of 0, 4, 40 or 200 mg/kg bw, at 24-hour intervals. However, the study was not considered valid as the highest dose used was only 13% of the oral acute LD_{50} value in the mouse, there was no evidence of cytotoxicity in the target tissue, the single sampling time was only 6 hours after the second dose, and no positive control group was used. Furosemide was also negative in an *in vivo* bone marrow chromosome aberration study in Chinese hamsters given a single oral dose of 800 mg/kg bw and killed at 12, 24 or 48 hours. Although the dose used resulted in clinical effects lasting for 24 to 48 hours, there was little evidence of cytotoxicity in the target tissue.

Furosemide does not cause gene mutations in bacterial cells or mammalian cells *in vitro*. Furosemide was positive for clastogenicity in a number of *in vitro* studies including human leucocytes. The available *in vivo* studies were inadequate to conclude on the mutagenicity of furosemide. It should also be noted that the parent compound, sulphanilamide, has also been reported to be clastogenic and a spindle poison. No overall conclusions can therefore be reached on the mutagenicity of furosemide.

11. Furosemide was tested for carcinogenicity in rat and mouse bioassays carried out for the National Toxicology Program of the United States.

    $B6C3F_1$ mice received dietary doses equivalent to mean doses of 0, 91 and 191 mg/kg bw in males and 0, 99 and 214 mg/kg bw in females. A dose-related increase, statistically significant in the high dose, was observed in malignant mammary tumours in females. The incidence in the high dose was reported to be about five times the historical rate for this neoplasm. There was an increase in malignant lymphomas in treated females that showed a significant positive trend. There was a significant positive trend in the incidence of hepatocellular carcinomas in treated males. Mucosal epithelial hyperplasia and submucosal chronic focal inflammation of the urinary bladder was increased in male mice. Increased incidences and severity of nephropathy were observed in treated males and females. Increased incidences of nonneoplastic lesions of the forestomach, lung, brain, ovary or uterus and prostate were also observed in treated animals.

Groups of 50 male and female F344 rats received diets equivalent to mean doses of 0, 14 and 29 mg/kg bw in males; and 0, 16 and 31 mg/kg bw in females. Malignant meningiomas of the brain were observed in 3 low dose males and in none of the other groups. The historical control incidence of menigiomas in F344/N rats is 2 out of 1928 (0.1%). Renal tubular cell adenomas were observed in 3 control males, 4 low dose males and 5 high dose males. Renal tubular carcinomas were observed in one male in the low dose and one in the high dose. Combined incidences of both tumours were 6%, 10% and 12%; the highest previously recorded incidence in untreated controls was 6%. In females there was an increased incidence of C-cell adenomas with a significant positive trend (control 8%, low 12%, high 22%), but no increase in C-cell hyperplasia. Incidence of nephropathy was similar in all groups, but severity was greater in the high dose males.

Overall, it would appear that furosemide is carcinogenic in female mice and probably in males. The data available on the rat are inadequate as the doses used did not achieve the maximum tolerated dose, survival in males was inadequate, and the pathological examination of low dose females was insufficient.

12. Studies were carried out to investigate the nephrotoxic potential of furosemide in neonatal rats. Pregnant rats were treated with furosemide at various stages of gestation and the kidneys of the offspring were examined. The offspring of control rats had more glomeruli per kidney than the offspring of treated rats. The offspring of treated rats had larger glomeruli than the controls. The significance of these findings is unclear.

    Studies were carried out to investigate the ototoxicity of furosemide. There was a temporary loss of hearing when 60 mg/kg bw was given intravenously to guinea pigs but a single oral dose of 90 mg/kg bw had no effect on hearing. Oral doses of 45 mg/kg bw/day for 14 days had no effect on the hearing of guinea pigs. Pre-treatment with gentamycin did not increase the effect, but pretreatment with kanamycin slightly increased both incidence and severity of hearing loss. A higher incidence of hearing loss was also noted in furosemide-treated animals with mercuric chloride-induced renal damage. These studies indicate that furosemide is ototoxic, but only at high doses.

13. Furosemide was devoid of antimicrobial activity against 99 strains of bacteria of human or bovine gut origin.

14. In humans the rarely reported adverse effects include fluid and electrolyte imbalance, gastro-intestinal disturbances, skin rashes and photosensitivity reactions, bone marrow depression, pancreatitis and ototoxicity and deafness. These are generally associated with high-dose parenteral treatment. Furosemide has a number of serious interactions with other drugs. It may intensify the nephrotoxicity of some cephalosporin antibiotics and enhance the ototoxicity of aminoglycoside antibiotics. Furosemide enhances the hypotensive action of other antihypertensive agents. Co-administration of furosemide with angiotensin-converting enzyme inhibitors can result in a marked reduction in blood pressure.

15. A toxicological ADI could not be established because no overall NOELs could be identified from the repeated-dose toxicity and reproductive toxicity studies, no overall conclusion could be drawn regarding genotoxicity and there was evidence of carcinogenicity in mice. However a pharmacological ADI of 2.5 µg/kg bw (150 µg/person) was established by applying a safety factor of 100 to the oral NOEL of 0.25 mg/kg bw for diuresis in the dog.

16. Two lactating cattle, given a single intramuscular dose of 1 mg ^{14}C-furosemide/kg bw/day, were killed at 24 and 48 hours, respectively, after treatment. The total residue concentrations in the first 4 post-dosing milk samples (collected at 12-hour intervals with the first dose collected 12 hours after dosing) were 330, 50, 16 and 6 µg equivalents/kg respectively. At 24 and 48 hours after dosing the total residue concentrations were 152 and 96 µg equivalents/kg in kidney, 6 880 and 599 µg equivalents/kg in injection site skin, 65 000 and 25 000 µg equivalents/kg in injection site muscle and less than 40 µg equivalents/kg in all other tissues.

17. Three studies were reported aimed to clarify whether 4-chloro-5-sulfamoyl-anthranilic acid is a metabolite of furosemide or merely an artefact generated during the analytical procedures. From the new studies is was concluded that furosemide was resolved from 4-chloro-5-sulfamoyl-anthranilic acid by the proposed routine analytical method based on HPLC, that no 4-chloro-5-sulfamoyl-anthranilic acid is produced when using the proposed extraction procedure and that 4-chloro-5-sulfamoyl-anthranilic acid formation correlates to the length of time incurred tissue extracts are exposed to acidic conditions.

18. Cattle were given an intramuscular injection of 1 mg furosemide/kg bw/day on 2 consecutive days and the concentrations of furosemide in tissues and plasma determined by HPLC with fluorescence detection. Animals were killed at 4, 8 and 12 day time points after dosing. The concentrations of furosemide in all injection site tissues were below the limit of quantification of 54.5 µg/kg. With the exception of 1 fat sample (containing 54.5 µg/kg 4 days after the second dose) all other tissue samples contained residue concentrations below the limit of quantification of the analytical method (51.1 µg/kg).

19. Eight lactating cows (4 in early and 4 in late lactation) were given an intramuscular injection of 1 mg furosemide/kg bw/day on 2 consecutive days and the concentrations of furosemide in milk and plasma were determined by HPLC with fluorescence detection. The concentrations of furosemide in milk samples after the second dose ranged from 191 to 492 µg/kg at 12 hours, 8 to 55 µg/kg at 24 hours, less than 5 to 22 µg/kg at 36 hours and less than 5 µg/kg in all samples at 48 hours. Plasma samples collected immediately prior to, 15 and 60 minutes after the first dose contained less than 5 µg/ml, between 1.2 to 2.7 µg/ml and 0.8 to 1.2 µg/ml respectively. Samples collected immediately prior to, 15 and 60 minutes after the second dose contained less than 5µg/ml, between 1.1 to 2.3 µg/ml and 0.6 to 1.6 µg/ml respectively.

20. Comparison of the radiolabelled and non-radiolabelled residue depletion data for milk suggests that furosemide accounts for practically all of the total residue present. The likely consumption of residue from tissues should not present a risk to consumers. Furosemide concentrations in milk had depleted to below 5 µg/kg in 2 days (i.e. by the forth milking).

21. No residue data were provided for pigs and horses.

22. A routine analytical method for furosemide based on HPLC with fluorescence detection was available. The limits of quantification of the routine analytical method were 50 µg/kg for bovine liver, muscle, kidney and fat and 5 µg/kg for bovine milk.

Conclusions and recommendation

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

- a pharmacological ADI of 2.5 µg/kg bw (150 µg/person) was established for furosemide
- the substance is used in a small number of individual animals for infrequent and non-regular treatments,
- the animals are unlikely to be sent for slaughter during, or immediately after treatment,
- the substance is rapidly and extensively metabolised and excreted,
- intramuscular administration resulted in high residues at the injection site and consequently the use should be restricted to intravenous treatment only,
- no residue data were provided for pigs,
- the minor species guideline for the establishment of MRLs concerning the use in horses;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for furosemide 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
Furosemide	Bovine, equidae	For intravenous administration only

Member States should consider setting a withdrawal period for milk, taking into account the pharmacological ADI which had been established for furosemide.