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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BROMHEXINE

SUMMARY REPORT

1. Bromhexine (CAS No 3572-42-8) is a benzylamine with expectorant properties. It is used alone or in combination with antimicrobials for the treatment of respiratory diseases where there is impaired mucociliary clearance and production of viscid mucus. It is indicated for use in calves, pigs and poultry and administered by oral and intramuscular route at the dose level of 0.5 mg/kg bw/day for 5 consecutive days. Bromhexine is also used in humans as an expectorant.

2. Bromhexine increases the volume of bronchial secretion, modifies the pattern of mucosubstances produced by the secretory cells, reduces the viscosity of the mucus and increases the tracheobronchial ciliary activity. The effects on bronchial secretion are elicited in rabbits at 0.5 mg/kg bw. Dose levels of 10 to 20 mg/kg bw caused a bradychardia in dogs, cats and rabbits, in cats a short lived increase of blood pressure, but in some dogs a transient fall. After oral administration at 40 mg/kg bw, it induced in dogs, but not in cats, retching and vomiting, which is due to hyperacidity of gastric juice. Bromhexine, at an intravenous dose level of 50 mg/kg bw, induced in rabbits a resting pattern in an electroencephalogram. It did not affect the intestinal motility in mice dosed orally at 8 to 32 mg/kg bw. In high concentrations (1000 µg/ml) it increased vascular permeability. No pharmacological NOEL was identified in laboratory animals.

3. The pharmacokinetics of bromhexine were studied in several laboratory animal species and in humans, in most cases with the ^{14}C-labelled molecule. After oral administration to guinea pigs, dogs, baboons and humans, it was rapidly absorbed (t_{max}: 0.5 to 2 hours). In rats, the absorption was slower (t_{max}: 6 to 8 hours). In dogs and humans, in which studies with non-labelled bromhexine were also carried out, the oral bioavailability was low (5.8% and 18.2%, respectively). The results suggest a first pass effect rather than poor absorption. The half-life of radioactivity of plasma was 20 hours in rats after both oral and intravenous dosing, 4 hours in dogs after intravenous administration, 22 hours and 4.6 hours, respectively in guinea pigs and baboons after oral administration and 13 to 14 hours and 18 to 19 hours, respectively after intravenous and oral dosing in humans.

4. After a single intramuscular injection of ^{14}C-bromhexine to pigs (0.5 mg/kg bw) the total radioactivity reached the peak plasma level at 1.5 hours and was eliminated from plasma with a half-life of 7.3 hours. Bromhexine was cleared rapidly from the site of injection with only about 4% of the dose remaining at the site 24 hours after dosing. After repeated intramuscular administration (0.5 mg/kg bw/day for 5 consecutive days) trough plasma levels of radioactivity increased slightly over the treatment period. After repeated oral administration (0.25 mg/kg bw twice a day for 5 consecutive days) bromhexine was rapidly absorbed (t_{max}: 1 to 3 hours). Steady state was achieved 12 hours after the 2nd or 3rd dose. The apparent half-life of the elimination of total radioactivity from plasma after the last dose was 20 to 30 hours.

5. In calves given a single intramuscular dose of ^{14}C-bromhexine at 0.5 mg/kg bw, the radioactivity was cleared rapidly from the site of injection, with about 3% of the dose retained at the site 24 hours after dosing. Plasma levels of total radioactivity increased gradually during the first 10 hours after dosing and them remained constant over the following 14 hours. After repeated intramuscular administration of ^{14}C-bromhexine (0.5 mg/kg bw/day), trough plasma levels increased markedly over the 5 days-treatment period and a steady state could not be achieved. Also, after repeated oral administration (0.25 mg/kg bw twice a day for 5 consecutive days), a steady state could not be achieved. The apparent half-life of the elimination of total radioactivity from plasma after the last dose was about 40 to 50 hours.

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6. In broiler chickens and turkeys, peak plasma levels of total radioactivity occurred at 2 to 4 hours after oral dosing of ^{14}C-bromhexine at 1 mg/kg bw/day for 5 consecutive days. A steady state seems not to be achieved. The apparent half-life of the elimination of total radioactivity from plasma after the last dose was about 40 to 50 hours.

7. Bromhexine is extensively metabolised in laboratory animals and humans. Rabbits, guinea pigs and humans showed almost similar metabolic patterns with primary alkaline metabolites in urine (90%, 82.4% and 80% of the radioactivity, respectively). In rats, acidic metabolites were predominant (64% of the radioactivity), and dibromoanthranilic acid was found to be the major compound (60% and 15 to 20% of the total radioactivity in blood and 24 hours urine, respectively). The dog and mouse took an intermediate position with both alkaline and acidic metabolites in similar amounts. The pattern of excretion varied across the species. In the rat, the radioactivity was excreted in about the same proportion in both the urine and faeces (29 to 35% and 15 to 41%, respectively). The mouse, rabbit, guinea pig, baboon and humans excreted labelled material predominantly via the urine (57%, 91%, 68.7%, 81% and 60 to 70%, respectively). The dog excreted a higher proportion of the radioactivity administered in faeces than in urine (59% and 36%, respectively).

8. ^{14}C-Bromhexine was rapidly metabolised to mainly polar components in both calves and pigs. This was observed in the various biological extracts examined (urine, bile, plasma, liver, kidney). The majority of residues in liver and kidney were associated with highly polar components (in liver 73 to 91% and 100%, in kidney 94 to 100% and 76 to 94% in pigs and calves, respectively). A proportion of these appeared to be conjugated. Fat (and skin in pigs) consistently contained residues with a higher proportion of parent compound than was observed for liver and kidney (fat: 70 to 81% and 60 to 90% in pigs and calves, respectively; skin in pigs 66 to 93%).

9. After a single intramuscular injection of ^{14}C-bromhexine, over 60% of the administered radioactivity in pigs was excreted via the urine within 24 hours post dosing, with less than 1% being eliminated in the faeces. In calves, high levels of radioactivity were present in the bile. The recovery of total radioactivity in the urine within 24 hours after dosing was only 25%. These data suggest an enterohepatic circulation.

10. No metabolic studies were carried out in poultry. In these species, 80% of the oral administered radioactivity was excreted within 24 hours post dosing.

11. The acute toxicity of bromhexine is low. The oral LD_{50} in rats was 8 000 to 16 000 mg/kg bw, in male and female guinea pigs 2 200 and 4 200 mg/kg bw, respectively, in mice greater than 10 000 mg/kg bw, in dogs greater than 21 500 mg/kg bw and in female rabbits greater than 4 000 mg/kg bw. The subcutaneous LD_{50} in rats was greater than 27 mg/kg bw, the intraperitoneal LD_{50}s in rats and in mice greater than 134 and 400 mg/kg bw, respectively. Clinical signs of toxicity included sedation, short convulsive episodes, epistaxis, haemorrhage in the chambers of the eye and loss of hair, increased respiratory depth with increased rate, and in the dog emesis. At necropsy of mortalities, there was some evidence consistent with irritation and ulceration of the gastrointestinal tract.

12. In an oral sub-acute toxicity study, groups of mice received bromhexine at 0, 2, 200 or 2000 mg/kg bw/day for 5 weeks. Additional groups were allocated to the control and top dose group for a 6 weeks recovery period. There were extensive mortalities in animals receiving 2000 mg/kg bw/day. In this group the weight gain was reduced and food and water consumption were increased. Haematological changes suggested increased reticulocyte numbers in all groups receiving bromhexine, but there were no effect on erythrocytes, bone marrow and spleen to support this suggestion. Cholesterol values were increased in animals receiving 2000 mg/kg bw/day. Organ weight data suggested a possible increase in liver weight in males receiving 2000 mg/kg bw/day, but there were no histopathological findings to support this change. Based on the cholesterol level, the NOEL was 2 mg/kg bw.

13. No adverse effects were observed in dogs treated intramuscularly with 4 mg bromhexine twice daily for 6 weeks.

14. On the basis of an oral sub-acute toxicity dose ranging study with restricted observations (cage side observations and gross autopsy) in rats over 4 weeks, 500 mg/kg bw was chosen as the high dose level for chronic studies. A second study of 6 weeks duration in rats showed no effects of oral bromhexine treatment at dose levels of 20, 40 or 200 mg/kg bw/day.

15. Two oral toxicity studies were done in rats in which the effects of 0, 1, 25 and 500 (after 35 weeks 400) mg/kg bw/day bromhexine were examined. The substance was administered for 100 and 104 weeks, respectively. In the 100 weeks study an interim kill took place at 26 weeks. There was a good degree of consistency between the results. A dose level of 500 (after 35 weeks 400) mg/kg bw/day resulted in increased mortality and was associated with convulsions, salivation post dosing, lens opacities, alopecia, loss of vibrissae and sporadic depression of the erythrocytic values, as well as increased adrenal weights, and in one study increased weights of the lung, liver and thyroid gland and decreased spleen weights without any histopathological changes. None of these effects were identified at 1 or 25 mg/kg bw/day. Based on the clinical, mortality and organ weight data, the NOEL was 25 mg/kg bw/day.

16. Three oral toxicity studies were carried out in dogs. In one study, groups of 3 dogs were dosed with bromhexine at 0, 1, 25 and 100 mg/kg bw/day for 9 months. There was no evidence of toxicity attributable to the test substance, but the study design, conduct and reporting were not in accordance with current requirements. The other two studies were adequate. In these, groups of 5 male and 5 female dogs received bromhexine at 0, 1, 25 and 100 mg/kg bw/day for 104 weeks. In one study an interim sacrifice of 2 males and 2 females per group was carried out at 26 weeks. The most consistent effects were an increase of the alkaline phosphatase activity at 25 and 100 mg/kg bw/day, a reduction of bodyweight gain and increased liver weights at 100 mg/kg bw/day in both studies. One study showed also evidence of an increased incidence of convulsions at 100 and 25 mg/kg bw/day and on a few occasions at 1 mg/kg bw/day, and decreased weights of the prostate gland at 100 mg/kg bw/day, whereas in the other study a slight decrease in the red cell parameters at 25 and 100 mg/kg bw/day and an increase in the cholesterol levels (which were not monitored in the first study) at 100 mg/kg bw/day were seen. There were no correlating histological findings to the organ weight changes. A LOEL of 1 mg/kg bw was retained.

17. No tolerance studies in target species were provided.

18. Two fertility studies were done in rats. One study was GLP-compliant and followed the Japanese protocol. Rats received orally 0, 10, 50 or 300 mg/kg bw/day bromhexine from day 60 premating until mating (males) and from day 14 premating until day 7 of gestation (females). Results indicated reduction in weight gain and slight increase of water consumption in both sexes and a reduction in female food consumption at 300 mg/kg bw/day. There were no effects on reproductive performance or on foetuses that could be treatment related. In the other, not GLP-compliant study, rats received 0, 25 or 250 mg/kg bw/day bromhexine orally from day 63 premating until mating (males) and from day 14 premating until day 13 or 21 of gestation (females). There was evidence of reduced weight gain in parents and F1 pups as well as a slightly reduced number of pups per litter in the high dosage group. From both studies, a NOEL for maternotoxicity of 50 mg/kg bw and for fertility of 25 mg/kg bw can be derived.

19. A wide range of teratogenicity studies was conducted in rats, mice and rabbits. Most of the studies were not in compliance with GLP and briefly reported. In a GLP-compliant study in rats (0, 10, 50, 300 mg/kg bw/day bromhexine orally from day 7 to 17 of pregnancy) and rabbits (0, 10, 50, 200 mg/kg bw/day bromhexine orally from day 6 to 18 of pregnancy), maternal toxic effects such as increased water consumption, reduced food consumption and weight gain were seen in the high dosage groups. Substance-related effects on litter and foetuses were only seen in the rat study and included reduced litter and foetus weights at 50 and 300 mg/kg bw, different sex ratio as compared with control, reduced sites of implantations and number of ossified centres of metatarsophalanges and tarsus at 300 mg/kg bw/day. None of these studies gave any indication of teratogenic effect attributable to bromhexine. From the rat study, a NOEL for maternotoxicity of 50 mg/kg and for foetotoxicity of 10 mg/kg can be derived.

20. Two peri- and postnatal toxicity studies were performed in rats. One study was briefly reported and no behavioural and developmental tests were conducted. In the second GLP-compliant study, groups of rats received orally 0, 10, 25 or 150 mg/kg bw/day bromhexine from day 17 of gestation until day 21 of lactation. In the high dosage group, numbers of pups delivered were slightly reduced and the mortality of pups until weaning was increased. The weight gain of these pups was slightly decreased and some developmental tests indicated delays. Based on these effects, the NOEL for pup development is 25 mg/kg bw/day.

21. No evidence for mutagenic potential was found in the Ames test with *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) up to concentrations of 5000 µg/plate with and without metabolic activation, and in the micronucleus assay in mice receiving a single oral dose at 3000 mg/kg bw. Bromhexine is capable of nitrosation *in vitro* to N-methylnitrosocyclohexylamine which is a genotoxic carcinogen.

22. The two oral long-term toxicity studies in two different rat strains, with 0, 1, 25 and 500 (after 35 weeks 400) mg/kg bw/day, included also a macroscopic and microscopic evaluation of tissues for the presence of tumours. In the 100 weeks study, which used fewer animals than would currently be considered acceptable for an adequate evaluation of carcinogenic potential, the incidence of mammary tumours in terminal sacrifice female rats was 4/9, 9/10, 10/13 and 0/6 in the control, low, mid and high dosage groups, respectively. In premature decedents, the corresponding incidences were 5/12, 3/9, 4/11 and 0/13, respectively. From the 104 week study, in which many tissues were examined, there was no evidence of an increased incidence of tumours in any group of bromhexine-treated rats. Therefore bromhexine can be considered as not carcinogenic in rats.

23. Bromhexine exerts a non specific cytotoxic effect on leucocytes and inhibits phagocytosis at 10 µg/ml and above *in vitro*. No specific immunotoxicological investigations have been carried out.

24. Bromhexine had negligible antibacterial activity (minimum inhibitory concentration (MIC) for the most susceptible pathogen tested: 250 µg/ml) and failed to exert a significant effect on bacterial adherence *in vitro* or on the persistence of bacteriuria in rats experimentally infected with *Escherichia coli*.

25. The recommended oral dose level in order to obtain a pharmacological effect for adults is 24 mg bromhexine/person/day. In patients suffering from chronic bronchitis and receiving 4 mg bromhexine four times daily, no appreciable effects could be noted from the drug. Therefore the pharmacological NOEL in humans is 4 mg/person (corresponding to 0.07 mg/kg bw). Twenty-one out of 25 cases of intoxication with bromhexine remained symptom free. One adult started vomiting after the intake of 200 mg bromhexine and three small children showed vertigo, reduced consciousness and/or ataxia after the intake of 76 to 160 mg bromhexine. After injection of 4 to 16 mg bromhexine to patients after cerebral neurosurgery no side effects were observed. Therefore the neurotoxicological effects are deemed to be of no concern with the use of bromhexine in humans even at doses much higher than that clinically. In a long-term safety study over 3 months (48, 72 and 96 mg/day for one month, respectively) nausea and dyspepsia were encountered more frequently in the bromhexine-treated group, and at the two higher dose levels the serum asparatate aminotransferase, serum alanine aminotransferase and gamma-glutamyl transferase values were slightly but not significantly increased. The treatment had no effect on heart rate, blood pressure or haematological parameters. In another study (72 mg/day over 14 days), the treatment had no effect on the haemostasis.

26. On the basis of a LOEL of 1 mg/kg bw in a two years toxicity study in dogs and a safety factor of 200 an ADI of 0.005 mg/kg bw or 0.3 mg per person was calculated. From the human data a pharmacological ADI of 0.4 mg was established.

27. In pigs, after a single intramuscular dose of radiolabelled bromhexine of 0.5 mg/kg, the residues in muscle, liver and kidney began to decline at 3 hours post dosing. The residue levels in fat tissue increased over the 24 hour period. In the main depletion study 8 animals received intramuscular doses of ^{14}C-bromhexine at 0.5 mg/kg bw/day on 5 consecutive days and were slaughtered at 12 and 48 hours post last dose. The total radioactivity levels in liver, kidney and fat at 12 hours were similar (934, 1011 and 879 µg equivalents/kg, respectively), but those in skin and muscle were lower (387 and 63 µg equivalents/kg, respectively). The levels in liver, kidney and muscle declined faster than those in fat and skin, and at the 48 hours time point the levels in fat (863 µg equivalents/kg) were 2 to 3 fold higher than those in liver and kidney (303 and 228 µg equivalents/kg, respectively). The levels in the skin declined only slightly (to 336 µg equivalents/kg). At the injection sites, the radioactivity declined from 9789 µg equivalents/kg at 12 hours to 1848 µg equivalents/kg at 48 hours.

28. Two oral studies were conducted in pigs with doses of ^{14}C-bromhexine at 0.25 mg/kg bw twice a day for 5.5 or 5 consecutive days. At each time point (6, 12, 24 hours and 12, 48 hours post last dose, respectively), 4 animals were killed. In the first study, the residues of total radioactivity in fat declined from 516 µg equivalents/kg at 6 hours to 340 µg equivalents/kg at 24 hours, in liver from 320 to 94 µg equivalents/kg, in kidney from 343 to 71 µg equivalents/kg and in muscle from 35 to 17 µg equivalents/kg. In the second study, highest residue levels were also seen in the fat tissue. They declined from 752 at 12 hours to 381 µg equivalents/kg at 48 hours. In liver (from 250 to 60 µg equivalents/kg), kidney (from 259 to 89 µg equivalents/kg), skin from 260 to 147 µg equivalents/kg) and muscle (from 30 to 6 µg equivalents/kg), the decline was faster.

29. In calves, after a single intramuscular dose of ^{14}C-bromhexine of 0.5 mg/kg bw, the residues in muscle, liver and kidney began to decline at 12 hours post dosing. The residue levels of total radioactivity in fat tissue increased over the first 12 hours and remained constant until 24 hours post dose. In the main depletion study, 8 calves received intramuscularly 0.5 mg/kg bw/day ^{14}C-bromhexine for 5 consecutive days and were killed at 12 and 72 hours post last dose. The residue levels of total radioactivity in liver declined from 2275 µg equivalents/kg at 12 hours to 589 µg equivalents/kg at 72 hours, in kidney from 936 to 378 µg equivalents/kg, in fat from 1367 to 899 µg equivalents/kg, in muscle from 98 to 47 µg equivalents/kg, and at the injection site from 32046 to 1611 µg equivalents/kg.

30. In two oral studies, calves were dosed with radiolabelled bromhexine at 0.25 mg/kg bw twice a day for 5.5 or 5 consecutive days. At each time point (6, 12, 24 hours and 12, 72 hours post last dose, respectively) 4 animals were killed. In the first study, the radioactivity levels in fat, kidney and muscle increased from 6 to 24 hours post last dose, whereas those in liver decreased slightly during this period. In the second study, the residue levels of total radioactivity in all tissues decreased between 12 and 72 hours post last dose (in fat from 664 to 271 µg equivalents/kg, in liver from 2103 to 474 µg equivalents/kg, in kidney from 835 to 225 µg equivalents/kg and in muscle from 95 to 36 µg equivalents/kg).

31. Residue depletion studies have been done in broiler chickens and turkeys after oral administration of ^{14}C-bromhexine at 1 mg/kg bw/day for 5 consecutive days. The studies were conducted in 2 stages, a pilot study using 2 birds per species at each of 4 sacrifice times (8, 24, 48 and 96 hours post last dose) followed by a second study using 4 birds per species at each of 2 sacrifice times (8 and 24 hours post last dose). The total residue levels of radioactivity at 8 and 24 hours post last dose in broilers were in liver 464 and 290 µg equivalents/kg, respectively, in kidney 495 and 265 µg equivalents/kg, respectively, in skin + fat 183 and 128 µg equivalents/kg, respectively, and in muscle 88 and 59 µg equivalents/kg, respectively. There was a suggestion of a slight accumulation of total residues in the omental fat. For turkeys, the corresponding values were for liver 508 and 404 µg equivalents/kg, respectively, for kidney 279 and 178 µg equivalents/kg, respectively, for skin + fat 132 and 141 µg equivalents/kg, respectively, and for muscle 48 and 44 µg equivalents/kg, respectively.

32. No residue depletion studies in milk of lactating animals and eggs of laying hens were provided.

33. For broilers and turkeys 8 hours after oral treatment, the amount of residues susceptible to be ingested by humans represents about 30% of the toxicological ADI. For pigs, 12 hours after oral administration this amount represents about 30% of the toxicological ADI, whereas it is necessary to wait until 48 hours after intramuscular administration to reach this percentage (no intermediate data between 12 and 48 hours). In calves the depletion of residues is slower. Twelve hours after oral or intramuscular administration the amounts in edible tissues exceeded the toxicological ADI, whereas at 72 hours they represent 28% and 46% of the toxicological ADI, respectively (no intermediate data between 12 and 72 hours were available).

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:

- a toxicological ADI of 0.3 mg per person was established,
- bromhexine is extensively metabolised and eliminated so that the amounts of total residues in edible tissues of poultry, pigs and calves are below the toxicological ADI within 8, 12 and 72 hours after treatment, respectively,
- the animals are unlikely to be sent for slaughter immediately after treatment;
- no residues studies were provided for milk and eggs;

the Committee concludes that there is no need to establish an MRL for bromhexine 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
Bromhexine	Bovine	Not for use in animals from which milk is produced for human consumption
	Porcine	
	Poultry	Not for use in animals from which eggs are produced for human consumption