The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AMPROLIUM

SUMMARY REPORT (1)

1. Amprolium hydrochloride is 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinum chloride. It is a coccidiostat which is used for the treatment and prevention of coccidiosis in chickens, including laying hens, and turkeys. For treatment, it is administered in the drinking water at a concentration of 120 to 240 mg/l, or in the feed at a concentration of 125 mg/kg feed, for 5 to 7 days. For prevention of re-infection, it is administered in the drinking water at a concentration of 60 mg/l for 1 to 2 weeks. The maximum doses employed depend on age, and range from 25 to 73 mg/kg bw/day in chickens and 14 to 60 mg/kg bw/day in turkeys. Amprolium is authorised as feed additive according to Council Directive 70/524/EEC; for poultry at concentrations in the range of 62.5 to 125 mg/kg of complete feed, and in combination with ethopabate (25 parts : 1.6 parts) for chickens, turkeys and guinea fowl at concentrations in the range of 66.5 to 133 mg/kg of complete feed; the authorisation prohibits the use of the substance from laying age onwards and for at least 3 days before slaughter.

2. Amprolium is a thiamine (vitamin B1) analogue and is a competitive antagonist of thiamine transport mechanisms. The affinity constant for the uptake mechanism (Ki_{app}) for amprolium hydrochloride was 1150 µg/l in chick intestinal cells. The affinity for the blood-brain uptake system in rats is similar to that of thiamine. No classical pharmacological studies in laboratory animals were provided. At sub-optimal dietary intakes of thiamine, amprolium can cause decreases in bodyweight gain and tissue thiamine concentrations, suggesting a selective inhibition of thiamine uptake. These effects were observed in rats receiving daily doses of 2 or 8 mg amprolium and 10 or 20 µg thiamine, respectively (the normal thiamine intake being 40 µg). No pharmacological NOEL can be derived from these data.

3. A GLP-compliant absorption, distribution, metabolism and excretion study was conducted in rats dosed orally with 20 mg ^{14}C-labelled amprolium/kg bw for 7 days. Peak plasma radioactivity was observed 4 hours after the first dose (males, 537 µg equivalents/kg, females 469 µg equivalents/kg), and declined to 57 and 36 µg equivalents/kg, respectively by 24 hours. Mean concentrations slightly increased with time over days 2 to 7 but were essentially at steady state in both sexes (males, 69 µg equivalents/kg, females 79 µg equivalents/kg at 24 hours after the 6th dose). Faeces were the major route of excretion with mean values of 82.2% in males and 82.5% in females. Urinary excretion accounted for 8.5% and 9.6% of the total dose in males and females, respectively. Total excretion amounted to 91.6% in males and 93.4% in females.

   At this time, tissue concentrations in liver amounted to 0.13% and 0.05% in males and females, with 0.08% and 0.1% in the gut, tissue concentrations in other organs amounted to a non-significant amount of the administered dose. Tissue concentrations at 4 hours after the final dose ranged from 86 µg/kg in fat to 6700 µg /kg in liver, by 24 hours levels had depleted 6 µg/kg in brain and 3785 µg/kg in liver. Eight unidentified metabolites were detected in liver. Five were common to both sexes at both 4 and 24 hours after the final dose, amprolium was only detected in 4 hour female livers at 0.7% of the total residues. Up to 16 metabolites were detected in kidneys, the major component had a retention time of 25 minutes. Muscle extracts from the 4 hour males

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and females appeared to contain the same major metabolite at 55.8 and 57.7%, respectively. Up to 17 metabolites were detected in urine at levels ranging from 0.4 to 35.5% from 0 to 24 hours and 0.9 to 20.6% from 144 to 168 hours. The major component appeared the same. Parent amprolium was detected in all faecal samples, at levels of 45 to 64% in males and around 52% in females. A metabolite with a retention time of 5 to 6 minutes was detected in all faecal samples, probably the same polar component as in urine.

A whole body autoradiography study was conducted in CD-1 mice given two daily oral doses of 2000 mg ^{14}C-amprolium hydrochloride/kg bw. One animal per sex was sacrificed at 6, 24 and 48 hours after the second dose. At 6 hours, highest levels of radioactivity (around 70 µg equivalents/g) were observed in liver, salivary gland and kidney, with lesser amounts in adrenals, fat, bone marrow, lung, muscle and testes. By 24 hours only liver, kidney and adrenal contained levels of activity above background levels. By 48 hours radioactivity was only detectable in liver and kidney, at levels similar to those at 24 hours.

4. Acute oral LD_{50} values of 4000 and 4890 mg/kg bw were established in male and female Wistar rats. The acute oral LD_{50} in female CF1 mice was 3980 mg/kg bw. Deaths occurred within 36 hours of dosing. Overt signs of toxicity included lacrimation, reduced activity, ptosis of the eyelids, decreased respiratory rate, ataxia, tremors and clonic convulsions. Dogs survived an oral dose of 300 mg/kg bw but 2 out of 3 males and both females given 500 mg/kg bw died. Signs of toxicity in dogs included emesis, dyspnoea, head-drooping, ataxia, loss of righting reflex and tremors.

5. In a 6-week study, amprolium was administered in the diet to groups of 10 animals per sex and dose Sprague-Dawley rats at concentrations of 0, 5, 10 or 25% (equivalent to approximately 0, 5000, 10000 or 25000 mg/kg bw/day. Inappetance and malnutrition were observed at all dose levels and no NOEL was established. In a poorly conducted and reported 12-week study, groups of 5 per sex and dose Sprague-Dawley rats were given daily oral doses of 20, 60, 200, 600 or 2000 mg/kg bw/day amprolium. Two rats given 2000 mg/kg bw died. Rats given 600 and 2000 mg/kg bw were flaccid during the first 4 weeks. No effect on body weight was observed. Histopathology was carried out only on the 8 surviving rats given 2000 mg/kg bw and on the controls; no substance-related effects were found. Due to the deficiencies of the study, no conclusion could be drawn regarding a NOEL.

6. Groups of 10 animals per sex and dose Sprague-Dawley rats were given daily oral doses of 0, 200, 600 or 2000 mg/kg bw/day for up to 56 weeks. Parallel groups of rats were given the same treatment and fed 0.05% thiamine hydrochloride in the diet, equivalent to 50 mg/kg bw/day. The group given 2000 mg/kg bw was terminated after 49 weeks because 11 of the rats had died. Signs of toxicity included flaccidity, ocular and nasal porphyrin discharge, polyuria and diarrhoea. Tremors and convulsions were observed in the rats which died. No signs of toxicity were observed in the rats given 200 or 600 mg/kg bw though mortality was increased in these groups compared with the controls. Mortality was also higher in the amprolium-treated groups given thiamine supplementation, compared with the controls. The high incidence of respiratory disease (53%) may have compromised the results of this study and no conclusions regarding a NOEL could be drawn.

7. In a 2-year study, 35 rats per sex and dose were fed diets containing amprolium at doses equivalent to 0, 20, 200 or 1000 mg/kg bw/day. There were no signs of toxicity and no substance-related effects on haematology, blood thiamine concentrations or organ weights at termination. Histopathology was carried out on decedents and 10 surviving animals per sex in the control and high dose groups; there were no substance-related effects. No clinical chemistry parameters were monitored. In males, at weeks 96 and 104, bodyweights of the mid and high dose groups were significantly lower than controls. With females, high dose bodyweights were significantly lower than controls at weeks 36, 48, 60, 84 and 96. A NOEL for effects on bodyweight of 20 mg/kg bw was identified.

8. Amprolium was administered orally in gelatin capsules to Beagle dogs at doses of 0 (1 male and 1 female), 50 (1 male + 1 female), 400 (1 male + 1 female) or 1000 mg/kg bw/day (3 males + 2 females) for 12 weeks. Each dog received thiamine supplementation. Diarrhoea and emesis were observed at 1000 mg/kg bw and all the dogs in this group died. The study was inadequately conducted and reported and no conclusions regarding a NOEL could be drawn. In a 59-week study carried out during the early 1960s, amprolium was administered orally in gelatin capsules to groups of 2 animals per sex and dose Beagle dogs at doses of 0, 100, 300 or 800/600 mg/kg bw/day. Dosing was carried out 5 days per week. An additional group of 4 dogs was administered amprolium at 300 mg/kg bw per day together with thiamine supplementation (50 mg/kg bw/day). One male given 800 mg/kg bw died after 2 days and this dose was reduced to 600 mg/kg bw/day for the remainder of the study. One female given 600 mg/kg bw also died; the signs of toxicity included head drooping, ataxia, loss of righting reflex, tremors and seizures. The second female given 600 mg/kg bw showed no signs of toxicity but was found dead after 81 doses. One dog given 300 mg/kg bw with thiamine supplementation was killed after exhibiting convulsions and coma. There were no substance-related effects on ophthalmoscopy, cardiovascular parameters, or the limited haematology, clinical chemistry and urinalysis values which were monitored. Histopathological changes were observed only in the dogs which died and were consistent with secondary changes in animals in extremis. A NOEL of 100 mg/kg bw was identified.

9. In a 2-year study, groups of 3 animals per sex and dose Beagle dogs were given daily oral doses of 0, 10, 100 or 500 mg/kg bw amprolium in gelatin capsules. A group of 3 animals per sex and dose Mongrel dogs was administered 400 mg amprolium/kg bw/day for 12 days; 3 died and the dose was reduced to 300 mg/kg bw for the remainder of the study. An additional group of mongrel dogs (3 per sex) was administered 300 mg amprolium/kg/day throughout the study. Dosing was carried out 5 days per week. The dogs given 500 mg/kg bw died and 3 additional dogs given 300 mg/kg bw also died. Signs of toxicity included pupillary dilatation, paralysis and collapse. There were no treatment-related effects on the limited range of haematology parameters examined. A NOEL of 100 mg/kg bw was identified.

10. In a published study, amprolium was orally administered to 3 healthy calves. The first calf was given 321 mg/kg bw/day for 58 days, the second was given 418 mg/kg bw/day for 27 days and the third, 335 mg/kg bw/day for 34 days. Treatment of the calves continued until the animals showed central nervous signs (inability to stand). All 3 calves showed central nervous signs characterised by ataxic gait, clonic spasm and opisthotonus. Two calves were necropsied; a large necrotic lesion was found in the cerebral cortex and tissue thiamine levels were significantly decreased, especially in the cerebrum and cerebellum. The calf given 418 mg/kg bw/day amprolium was injected with 25 mg thiamine tetrahydrofurfuryl disulfide 2 hours after the onset of central nervous signs; 36 hours later the animal had recovered. In all 3 calves, bradycardia was observed for 3 weeks before the appearance of neurological signs but changed into tachycardia after the onset of these signs. No NOEL was established in this study.

11. Amprolium was added to the diet of White Leghorn and broiler-type chicks from 3 days of age to 3 weeks. No adverse effects were observed at concentrations up to 500 mg/kg feed. At 800 mg/kg feed resulted in reduced weight gain. At 1000 mg/kg feed caused polyneuritis and death. The adverse effects were reversed by adding thiamine at 100 or 1000 mg/kg feed to the diet. A second study in chicks from one day of age to 8 weeks showed no adverse effects up to 700 mg/kg feed; at higher levels, polyneuritis and mortalities were observed. In White Cross cockerels given amprolium at 100 mg/kg feed from 3 days to 9 weeks of age, no histopathological changes were found in any organs/tissues. Administration of up to 250 mg/kg feed of amprolium to 68-week old White Leghorn hens had no effect on egg production or hatchability; hatchability was slightly decreased at 500 mg/kg feed.

12. A brief summary of a 5-generation study in mice was provided. In each generation, 20 males were paired with 20 females when they were 8 weeks of age. Amprolium was administered in the diet at 1000 or 5000 mg/kg feed or at 25000 mg/kg feed plus 1000 mg/kg thiamine. The doses were equivalent to approximately 200, 1000 or 5000 mg/kg bw/day of amprolium (200 mg thiamine/kg bw), respectively. A full copy of the report of this study was not available. At 1000 mg/kg bw, maternal mortality and decreased pregnancy rates were reported. There was also evidence of decreased litter sizes in the group fed 5000 mg/kg bw amprolium and 200 mg/kg bw thiamine. However, no adverse maternal effects, or effects on fertility were reported at 200 mg/kg bw.

13. According to a brief summary, groups of 24 female Sprague-Dawley rats were given daily oral doses of 0, 120, 600 or 3000 mg amprolium/kg bw/day from days 7 to 17 of gestation. Maternal toxicity was observed at 600 mg/kg bw and above. There was no evidence of teratogenicity. At 3000 mg/kg bw there was an increased incidence of foetuses with delayed ossification of the coccygeal bones. Delayed ossification of the cervical corpus vertebrae was apparently observed at all dose levels though this was not clear from the brief details provided.

    Groups of 15 Japanese White rabbits were given daily oral doses of 0, 12.5, 50 or 200 mg/kg bw/day of amprolium from days 6 to 18 of gestation. There was no evidence of maternal toxicity, teratogenicity, or foetotoxicity at any dose level.

14. Although detailed information was not available for the reproduction study in mice, or the teratogenicity study in the rat, the rabbit study was conducted in accordance with current guidelines and GLP. No adverse maternal or foetal effects were observed up to 200 mg/kg bw, which can be regarded as the overall NOEL for reproductive effects. It can be concluded that amprolium does not exert any significant effects on fertility or foetal development at sub-maternotoxic doses.

15. In a GLP-compliant study, there was no increase in the incidence of revertants in an *in vitro* gene mutation assay in *Salmonella typhimurium* TA1535, TA97a, TA98 and TA100 and in *Escherichia coli* WP2, WP2uvrA and WP2uvrA pKM101, at concentrations up to 10 000 µg/plate. In a published study, a positive result was reported in strain TA100 at a the top concentration of 20000 µg/plate, but only in the presence of metabolic activation; negative results were obtained all the other strains (TA98, TA1535, TA1537 and TA1538). Negative results were also reported in a recombinant assay with *Bacillus subtilis* H17Rec+/M45Rec-, but no details were provided of the concentrations tested.

    A GLP-compliant *in vitro* chromosome aberration study was conducted using Chinese hamster ovary (CHO) cells. Cultures were treated with amprolium at concentrations of 1250, 2500 or 5000 µg/ml for 6 hours with metabolic activation or 22 hours without metabolic activation. In the presence of metabolic activation, amprolium gave positive results at 5000 µg/ml. A GLP-compliant *in vitro* mouse micronucleus assay was conducted using concentrations of up to 5000 µg/ml with and without metabolic activation with exposure periods of 4 hours, and without metabolic activation for 24 hours. Amprolium gave negative results at 4 hours of exposure, but was positive without metabolic activation after 24 hours exposure at concentrations of 2500 µg/ml or greater.

    According to a brief published report of an *in vivo* mouse micronucleus test, no increase in micronucleated polychromatic erythrocytes (PCEs) was reported following a single intraperitoneal injection of 21 mg amprolium/kg bw (shown to be one fifth of the LD_{50} value); no details were provided concerning sampling time or possible effects on the bone marrow and so it is not clear whether the study was adequately conducted. A GLP-compliant *in vivo* mouse bone marrow micronuleus test was conducted in animals dosed orally with 2000 mg amprolium/kg bw at 0 and 24 hours, and sampled 24 hours after the second dose. No increases in micronuclei were observed. A GLP-complaint *in vivo* rat liver unscheduled DNA synthesis study using single oral doses of 0, 500, 1000 or 2000 mg amprolium/kg bw and harvest times of 2 to 4 or 12 to 16 hours, was also negative.

Although there was some evidence of mutagenicity *in vitro*, these findings were inconsistent. Amprolium was positive only with one strain with metabolic activation in the *Salmonella*-microsomal assay, and positive in a Chinese hamster ovary (CHO) cells assay with metabolic activation, both only at the highest concentration tested. It was only positive in the mouse lymphoma assay without metabolic activation and only at an extended treatment period. On the other hand, amprolium was clearly negative in *in vivo* in two mouse bone marrow micronucleus tests and one rat liver unscheduled DNA synthesis (UDS) test. Overall, it can be concluded that amprolium is devoid of mutagenic activity *in vivo*.

16. Despite inadequacies in the conduct of the 2-year dietary study in the rat, there was no evidence of increased incidence of tumours in the treated animals. This, combined with the negative *in vivo* mutagenicity studies, indicates no overall concerns regarding the carcinogenicity of amprolium.

17. No effects indicative of an effect on the immune system were observed in the repeated dose toxicity studies. There was no information regarding sensitisation potential.

18. Amprolium is not considered to have antimicrobial activity other than its action on coccidia. The bacterial mutagenicity assays confirmed that amprolium did not possess any significant antibacterial activity. It was agreed that further information on antimicrobial activity were not required.

19. Amprolium has been used in a limited number of AIDS patients with coccidiosis. In one patient, polyneuropathy occurred after 7 days of treatment with 90 mg/kg bw/day but no further effects were reported on reducing the dose to 30 to 45 mg/kg bw/day for 8 days. In another patient, heart failure and neurological symptoms attributable to the treatment were observed after 4 weeks dosing with 200 mg/kg bw/day.

20. A toxicological ADI was established, based on the NOEL of 20 mg/kg bw for effects on bodyweight gain observed in a 2-year dietary study in the rat. To take into account the methodological weaknesses in this study an uncertainty factor of 200 was used. This gives an ADI of 100 µg/kg bw, or 6 mg/person for a 60 kg adult.

    No pharmacological ADI could be established from the data available. However, as the pharmacodynamic effect of amprolium is competitive inhibition of thiamine uptake, the potential effect of extreme amprolium ingestion on typical dietary thiamine intakes were compared with the recommended dietary intake of thiamine. Although the available data indicates that the affinities of amprolium and thiamine for the thiamine uptake system are similar, a 10-fold greater affinity for amprolium was assumed for additional reassurance. It was assumed that the entire animal-derived proportion of the average daily thiamine intake (15% of total dietary intake) was provided entirely by chicken tissues and eggs containing amprolium at 2 times the limit of quantification. Using this approach, the residual thiamine intake would be about 117% in pre-school children and 107 to 140% in adults of the minimum recommended daily intakes of thiamine. This indicates that ingestion of amprolium at these levels will have no adverse effects on thiamine intake.

21. In a published study, chickens (6 animals per dose level) were dosed orally with 10 or 20 mg amprolium/kg bw. The maximum blood concentrations (C_{max})were found at 4 hours (t_{max}) after dosing and were 9.5 and 26 mg/ml for the 10 and 20 mg/kg bw dose groups respectively. Other kinetic parameters were given for the 20 mg/kg dose only and were 0.5 l/kg for the apparent volume of distribution, 1.98 ml/kg/min for the clearance (Cl_B) and 168 µg h/ml for the area under the blood concentration curve. Eight hours after dosing the residue concentrations in liver, kidney, and muscle samples from the 10 mg amprolium/kg bw dosed birds ranged from 18 mg/kg in the kidney to 46 mg/kg in the caecum. In birds treated with 20 mg amprolium/kg bw, the residues in these two tissues were 36 mg/kg in the kidney to 74 mg/kg in the caecum. Few conclusions could be drawn from the results of this study because the details provided were minima.

22. In a mass balance study, pairs of 4-week old chickens were orally dosed with approximately 3 or 30 mg ^{14}C-amprolium. Excreta collected over 24 hours after dosing contained 74 to 94% of the radioactivity in the administered dose, this had risen to 81 to 104 % after 3 days. No radioactivity was detected in expired carbon dioxide. The excretory recovery of amprolium, measured fluorimetrically was lower (48 to 68% of the dose) than the recovery of radioactivity but the identity and ratio of the metabolites present in excreta were not investigated. Labelling amprolium in the picoline or pyrimidine ring appeared to have no influence on the distribution of residues detected.

23. In a non-GLP depletion study, chickens were fed a diet supplemented with 125 mg ^{14}C-amprolium/kg for periods ranging from 12 hours to 8 days. The concentrations of residues determined in muscle, liver and kidney samples from treated chickens were variable. Tissues contained 0.03 to 1.93 mg amprolium equivalents/kg during medication. Following withdrawal of treatment, residues were detected only in muscle samples taken after 2 days withdrawal. There was no evidence of bioaccumulation, preferential tissue partitioning or differences due to labelling positions used (picoline or pyrimidine ring). Because total and marker residues were not determined in the same birds and a variety of dosing regimes were used in the radiometric studies and the studies using unlabelled amprolium, it was not possible to estimate the ratio of marker to total residues.

24. A GLP-compliant study was carried out in laying hens and broilers but only a draft report with unaudited results was available. The birds were given daily oral gavage doses of ^{14}C-amprolium for 21 days. The treatment was designed to mimic administration of the commercial formulation in the drinking water at a rate of 240 mg/l for 7 days followed by a lower dose of 60 mg/l for 14 days. Groups of 6 laying hens and 6 broilers were killed at 6, 12, 24 and 36 hours and 7 days after the end of dosing. In laying hens, the mean total residues were 701, 1160, 63 and 94 µg equivalents/kg in liver, kidney, muscle and skin with fat, 6 hours after the end of dosing. At 12 hours after the end of dosing, the mean total residues in these tissues were 406, 401, 38 and 90 µg equivalents/kg respectively. By 24 hours after dosing, residues in all muscle samples and in one (out of 6 samples) of skin with fat were lower than twice the background limit of detection (28 and 38 µg/kg for muscle and skin + fat, respectively). In liver, the mean residues at 24 hours were 173 µg equivalents/kg and depleted to 116 µg equivalents/kg at 36 hours. In kidney, the mean residues at 24 hours were 139 µg equivalents/kg and depleted to 85 µg equivalents/kg at 36 hours. The total residues in tissues of broilers depleted in a similar pattern. Six hours after the end of dosing, mean total residues in liver, kidney, muscle and skin + fat were 519, 765, 46 and 137 µg equivalents/kg respectively. 24 hours after the end of dosing, mean total residues in liver, kidney and skin + fat were 191, 120 and 45 mg equivalents/kg, respectively and total residues in all muscle samples were less than twice the background limit of detection (28 µg/kg for muscle).

25. In the same study, eggs were collected from laying hens. Some of the eggs were composited on a daily basis by group; others were analysed separately. Highest residues were found in eggs collected on days 7 and 8 of the treatment period, corresponding to the end of the period of the high dose administration. For eggs collected on day 7 of treatment, residues in individual eggs ranged from 390 to 1530 µg equivalents/kg with a mean value of 733 µg equivalents/kg. For eggs collected on the last day of treatment (day 21), residues in individual eggs ranged from 107 to 434 µg equivalents/kg with a mean value of 271 µg equivalents/kg. Six days after withdrawal of treatment, residues in all eggs were less than twice the background limit of detection (34 µg/kg).

26. A GLP-compliant study was carried out in turkeys but only a draft report with unaudited results was available. The birds were given daily oral gavage doses of ^{14}C-amprolium for 21 days. The treatment was designed to mimic administration of the commercial formulation in the drinking water at a rate of 240 mg/l for 7 days followed by a lower dose of 60 mg/l for 14 days. Groups of 3 male and 3 female turkeys were killed at 6, 12, 24 and 36 hours and 7 days after the end of dosing. Mean total residues in liver depleted from 544 µg equivalents/kg at 6 hours to 260 µg equivalents/kg at 12 hours to 188 µg equivalents/kg at 24 hours. Over the same time period, mean total residues in kidney depleted from 496 µg equivalents/kg to 219 µg equivalents/kg to 118 µg equivalents/kg. The results for the analyses of the muscle and skin + fat samples were not yet available.

27. In a non-GLP study, chicks (10 animals per time point) were fed a diet containing 150 or 250 mg amprolium/kg from 1 day to 8 weeks of age. At sacrifice the residue concentrations in samples of muscle, kidney, skin + fat (limit of detection: 10 µg/kg) and liver (limit of detection: 20 µg/kg) were determined with a recovery correction of 73 to 79% by a fluorimetric thiochrome method for thiamine. At zero and 2-day withdrawal periods respectively after the 250 mg/kg feed dose the residues concentrations were: 90µg/kg and less than 10µg/kg in muscle, 410 µg/kg and less than 20 µg/kg in liver, 380 µg/kg and 40 µg/kg in kidney and 420 µg/kg and 20 µg/kg in skin + fat. At the same time points after a 150 mg/kg feed dose the tissue residue concentrations were determined as follows: 90 µg/kg and less than 10 µg/kg in muscle, 420 µg/kg and less than 20 µg/kg in liver, 350µg/kg and less than 10 µg/kg in kidney and 160 µg/kg and less than 10 µg/kg in skin + fat. The validity of these determinations is questionable due to the control blank samples having been found to contain small concentrations of amprolium and the results having been calculated in such a way so as to prevent an assessment of their precision.

28. A published study was presented in which adult laying hens were fed supplemented diet containing 125 and 250 mg amprolium/kg feed. Residues were determined in eggs (2 per hen) from 4 hens and in edible tissues from 3 or 5 hens using a gas chromatography method. The expert report indicated that the residue concentrations were 30 to 60 µg/kg in liver and less than 10 µg/kg in all other tissues, 3 days after withdrawal of the treated feed but no details of the residues in eggs were provided. This study did not meet the requirements of Volume VI of the Rules Governing Medicinal Products in the European Union, due of the small numbers of samples analysed.

29. Two non-GLP studies were reported in which White Cross cockerel chicks (5 animals per group) received an oral dose of amprolium via their drinking water (0.024, 0.048, 0.096 and 0.192% w/v) from the ages of 2 days to 3 weeks, or diet (0, 50, 100 and 200 mg/kg) from the ages of 3 weeks to 8 weeks. Birds receiving the highest dose via their drinking water were also supplemented with thiamine via the same route and individual birds were also given thiamine by injection when signs of polyneuritis were observed. The treated birds were sacrificed after 22 days and 8 weeks of treatment in the first and second studies respectively; in both cases with a zero withdrawal period. Tissue samples were analysed by a fluorimetric thiochrome method using silver to eliminate non-specific signals due to the presence of thiamine. The concentrations of amprolium found in tissues were proportional to the oral dose administered irrespective of the diet or water being the vehicle. These studies were not conducted in accordance with Volume VI.

30. A GLP-compliant study was carried out in broilers but only a draft report with unaudited results was available. The birds were administered amprolium via the drinking water at a rate of 240 mg/l for 7 days followed by a lower dose of 60 mg/l for 14 days. Groups of 6 broilers were killed at 0, 1, 2, 4 and 7 days after the end of treatment. Residues in tissues were determined using the proposed routine analytical method based on HPLC with UV detection. For liver, the limits of quantification and detection were 100 and 40 µg/kg respectively. In the first group of birds killed immediately after the end of treatment, residues of amprolium in liver were in the range 178 to 330 µg/kg (mean 250 µg/kg). Residues in most samples of liver at later time points were below the limit of detection. The results for the analyses of the muscle, kidney and skin with fat samples were not yet available.

31. In a company study also referred to in a published paper, chickens fed a diet containing 0.6 to 2000 mg/kg amprolium in a variety of experimental designs for 21 days or 20 weeks. The results indicated a linear relationship between the amprolium in the feed and its concentration in egg yolk. Administration of feed containing 250 mg amprolium/kg was estimated to result in a concentration of 400 µg/kg in egg yolk at zero withdrawal. At the lower concentration of 125 µg/kg feed, the concentration in yolk was estimated to be 200 µg/kg at zero withdrawal. The values were calculated from a regression analysis of dietary concentration against egg yolk concentrations for a total of 89 samples.

32. In an unpublished report of 2 studies, hens (4 animals per dose) were fed a diet containing 5, 25, 125 and 250 mg amprolium/kg for 14 days or 5 and 250 mg amprolium for 21 days. Amprolium content was measured by an unvalidated HPLC method. Concentrations of amprolium in the yolks from hens treated at 5 and 250 mg/kg amprolium for 21 days plateaued at 200 µg/kg and 2000 µg/kg respectively. Concentrations in the egg whites from these 2 groups were 7 and 50 µg/kg respectively. Following withdrawal of medication, there was a linear decline in the concentration of amprolium in the yolks and the concentration was below the claimed limit of detection (5 µg/kg) around 10 days after cessation of treatment. These studies did not comply with the requirements of Volume VI.

33. A GLP-compliant study was carried out in 16 laying hens but only a draft report with unaudited results was available. The birds were administered amprolium via the drinking water at a rate of 240 mg/l for 7 days followed by a lower dose of 60 mg/l for 14 days. The eggs were collected from all birds and all birds were killed at 7 days after the end of treatment. Residues in eggs and tissues were determined using the proposed routine analytical method based on HPLC with UV detection. Residues of amprolium in all samples of kidney were below the limit of quantification (200 µg/kg) and in most samples the residues were below the limit of detection (40 µg/kg). Residues of amprolium in liver ranged from below the limit of detection (40 µg/kg) to 135 µg/kg. The highest residues in eggs were found on day 7 of the treatment period, corresponding to the end of the period of the high dose administration. For eggs collected on day 7 of treatment, residues of amprolium ranged from below the limit of quantification (500 µg/kg) to 854 µg/kg. Residues in all eggs taken on day 21 (the last day of treatment) were below the limit of quantification.

34. The proposed routine analytical method was described in the ISO 78/2 format and was based on HPLC with UV detection. The limits of quantification were stated to be 100 µg/kg for chicken and turkey muscle, skin + fat and liver, 200 µg/kg for chicken and turkey kidney and 500 µg/kg for hens' eggs. However, the supporting raw data were not provided and there was no information regarding specificity and susceptibility to interference from residues of similar substances.

Conclusions and recommendation

Having considered that:

- an ADI of 100 µg/kg bw, (i.e. 6 mg/person) was established for amprolium,
- amprolium was retained as the marker residue, and taking a conservative value, represented approximately 50% of the total residues in chicken liver and the major portion of the residues in eggs,
- residues in the edible tissues of chicken and turkeys and in hens eggs were rapidly depleted and so MRLs could be set at twice the limit of quantification,
- an analytical method for the determination of residues of amprolium in chicken and turkey tissues and the eggs of hens was available but was not fully validated;

The Committee considered that amprolium should be included into Annex III of Council Regulation (EEC) No 2377/90, in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Amprolium	Amprolium	Chicken, turkeys	200 µg/kg 200 µg/kg 200 µg/kg 400 µg/kg 1000 µg/kg	Muscle Skin + fat Liver Kidney Eggs	Provisional MRLs expire on 1.1.2002

Based on these MRLs values, the daily intake will represent about 5% of the ADI.

Before the Committee can consider the inclusion of amprolium into Annex I or II of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The Applicant should provide the final, audited, reports of the total residues depletion studies and the marker residue depletion studies in chickens, turkeys and eggs.

2. Should amprolium be a candidate for Annex I, the Applicant should validate the proposed routine analytical method in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community and re-present the method in an internationally recognised format (e.g. ISO 78/2), accompanied by full raw data.