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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CYROMAZINE

SUMMARY REPORT (1)

1. Cyromazine, a triazine derivative, is an insect growth regulator that is used in veterinary medicine for the protection of animals against insects**.** Cyromazine is used externally as a pour-on for sheep at a dose of 0.9 to 3.6 g/sheep (approximately 60 to 85 mg cyromazine/kg bw) every 8 to 10 weeks for the prevention of blowfly strike (*Lucilia sericata*). Cyromazine is not to be used on sheep producing milk for human consumption.

   Cyromazine is also used in plant protection. It is not used in humans.

2. Cyromazine is an insecticide that interferes with the first dipteran larval moult and possibly with metamorphosis. Larvae and pupae undergo typical morphological transformations before they die. Secondary pharmacological effects were investigated in mice, rats, guinea pigs and rabbits, and comprised effects on the central nervous system, the autonomic nervous system, the cardiovascular system and the gastrointestinal tract. The *in vivo* studies used very high doses, ranging from 500 to 3500 mg/kg bw. Therefore, a pharmacological NOEL could not be identified.

3. Pharmacokinetic studies were performed in rats, sheep, goats, monkeys, and chickens after oral administration. No substantial differences between these species were found. Cyromazine administered orally to rats, monkeys, sheep or goats was rapidly absorbed and rapidly excreted, predominantly in urine (approximately 85 to 95% within 24 hours), with low levels present in faeces (3 to 7%). Absorption and excretion were also rapid in the hen. In general, tissue levels after oral administration were low, the liver and kidney being the organs containing the highest residual amounts. Small amounts of cyromazine were also found in milk and eggs. The majority of the material excreted in the urine of rats, monkeys, sheep and goats and in the excreta of hens was unchanged cyromazine (more than 70%). Melamine was determined to be the major metabolite of cyromazine in all these species (less than 15%), showing the general degradation step of dealkylation. Other metabolites were hydroxy-cyromazine and methyl-cyromazine (9% and 2% in rats, respectively). Following dermal application on the back skin of rats 80 to 85% of the dose was not absorbed within 24 hours. The dose absorbed into the skin acted as a depot from which sustained release of cyromazine occurred. No pharmacokinetic data after dermal application to sheep were presented.

4. Cyromazine is of low acute oral toxicity. Oral LD_{50} values were 2029 mg/kg bw for mice, 3387 mg/kg bw for rats, and 1467 mg/kg bw for rabbits.

5. One repeated dose oral toxicity study was performed in rats (0, 30, 300, 1000 and 3000 mg/kg feed for 90 days) and two in dogs (0, 30, 300, 1000 and 3000 mg/kg feed for 90 days, or 0, 30, 300 and 3000 mg/kg feed for 26 weeks, both studies with a 4-week recovery period). A decreased food consumption, partly associated with reduced weight gain, was noted in both species at 1000 and 3000 mg/kg feed. At the highest dose slight anaemia was observed in dogs of both sexes and elevated plasma alanine aminotransferase activity was observed throughout the treatment period, returning to normal during the recovery period. No treatment related histopathological lesions were observed in both sexes. From these repeated dose studies, a lowest NOEL of 300 mg/kg feed (equivalent to 9.1 mg/kg bw/day) could be derived from the 26-week dog study.

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6. In a 12-months dietary toxicity study, Beagle dogs received cyromazine in their diets at concentrations of 0, 50, 200, 800 or 3500 mg/kg feed, equal to 0, 1.37, 5.74, 22.8, and 93.7 mg/kg bw/day for males and 0, 1.47, 6.03, 24.9 and 110 mg/kg bw/day for females. A number of haematological changes were observed in males at 800 and in males and females at 3500 mg/kg feed, comprising decreases in haemoglobin, haematocrit, and red blood cell counts, and increases in total protein. At 3500 mg/kg feed, also lower values were found for mean corpuscular cell volume, mean corpuscular haemoglobin, triglyceride levels, basophil counts, and plasma creatine kinase activity. In addition, increased plasma chloride levels were found in high dose females. Heart and liver weights were increased in females at 800 and in both sexes at 3500 mg/kg feed, and kidney weights were increased only in high dose females. High dose animals showed myocarditis, and one male also had foci of cartilaginous metaplasia in the affected heart muscle. Hypercellularity of bone marrow and tubular lesions in the kidneys were found in the high dose animals. At 50 mg/kg feed no effects were observed. At 200 mg/kg feed, only a very slight increase in the total plasma protein content was found in the male dogs only. Although this increase was statistically significant at some time points, the increase was only slight (maximally 10%), not consistent in time, and without any other findings apart from an increase in plasma globulin. The increases in globulin concentrations were somewhat higher (maximally 20%), but this was a calculated value (total protein minus albumin), and it was noted that the albumin concentrations were at the same level in each group and remained constant in time (using the non-specific bromcresol green method), which is in contrast with the total protein values that increased over time in each group (including the controls). It was concluded that these findings are unlikely to be biologically significant and are therefore not considered as adverse. Consequently, an NOAEL of 200 mg/kg feed, equivalent to 5.74 mg/kg bw/day, was established from this study, based on haematological changes and increased relative heart and liver weights in females.

7. The tolerance of sheep to dermal application with cyromazine was investigated in two studies. After jetting with 30 g cyromazine/animal no compound-related changes were noted in body weight, haematocrit, plasma glutamate dehydrogenase or alanine aminotransferase activity. Jetting with 12 g cyromazine/animal did not show any effect on foetal development or birth rate.

8. In a 2-generation reproduction study in rats (0, 30, 1000 and 3000 mg/kg feed) cyromazine did not affect fertility, but at maternally toxic doses there was increased perinatal mortality and reduced pup weight. The NOEL was 30 mg/kg feed, equivalent to 2 mg/kg bw/day, based on body weight effects in the parental generations.

9. In an oral teratogenicity study in rats (0, 100, 300 and 600 mg/kg bw/day) cyromazine did not demonstrate a teratogenic effect. Several rabbit teratogenicity studies, in which oral doses of 5 to 75 mg cyromazine/kg bw/day were given, showed low incidences of variable malformations in all groups, including controls, without dose-effect relations. It is therefore concluded that there is no evidence for teratogenicity of cyromazine in rabbits.

10. The mutagenic potential of cyromazine was evaluated in a range of *in vitro* studies (*Salmonella*-microsomal assay with *Salmonella typhimurium*, gene mutation tests with mouse L5178Y cells (TK-locus) and V79 Chinese hamster cells (HPRT-locus), a yeast assay with *Saccharomyces cerevisiae*, a chromosome aberration test with human lymphocytes, and unscheduled DNA synthesis (UDS) tests with rat and mouse hepatocytes and *in vivo* studies (an intraperitoneal mammalian spot test in mice, an oral dominant lethal test in mice, an oral micronucleus test in mice and an oral nucleus anomaly test in hamsters). Apart from an inconclusive mouse spot test, all tests were negative. Cyromazine is considered a non-genotoxic compound.

11. A long-term toxicity/carcinogenicity study with cyromazine was conducted in rats (0, 30, 300 and 3000 mg/kg feed), as well as a carcinogenicity study in mice (0, 50, 1000 and 3000 mg/kg feed). No tumour incidence appeared to be affected by treatment with cyromazine, although some tumour incidences were on the borderline of significance in the highest dose group in both species. It was also noted that no systemic toxicity was found at these highest doses in both rats and mice, apart from the decrease in bodyweight which could be largely attributed to a decrease in food consumption. It would have been desirable that higher doses than 3000 mg/kg feed had been given, but this is not practicable given the decreased food consumption at 3000 mg/kg feed. It was concluded that cyromazine is not carcinogenic in rats and mice. The NOEL of the study in rats was 30 mg/kg feed, equivalent to 1.8 mg/kg bw/day, based on the bodyweight changes in females. The NOEL of the study in mice was 15 mg/kg feed, equivalent to 6.5 mg/kg bw/day, based on the bodyweight effects in the males.

12. The European Chemical Industry Ecotoxicology and Toxicology Centre (ECETOC) evaluated the toxicity of melamine, the main metabolite, and concluded that melamine is of low acute toxicity, and is neither teratogenic nor genotoxic. Some of these studies were also available to the Committee for Veterinary Medicinal Products (CVMP), but these were either inadequate or only presented as short summaries. Thus, the CVMP was not in a position to endorse the conclusions of ECETOC.

13. In carcinogenicity studies with melamine in mice and rats, no tumorigenic effects were observed in mice and female rats. In high dose male rats (4500 mg/kg feed) an increased incidence of transitional cell neoplasms of the bladder was observed, accompanied by the finding of bladder stones, consisting mainly of melamine. It was, however, concluded that melamine is only indirectly responsible for the transitional cell neoplasms in that stones occurred in the bladder only at high melamine doses and the tumoragenic principle are the stones rather than melamine. Therefore, melamine should not be regarded as a carcinogenic compound.

14. Based on the overall NOEL of 1.8 mg/kg bw/day in the long-term toxicity/carcinogenicity study in rats and a safety factor of 100, an ADI of 0.02 mg/kg bw (i.e. 1.2 mg/person) was established. This figure has been rounded to correspond to the ADI established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) is based on the same toxicity data.

15. The fate of cyromazine was investigated in a radiometric study in sheep. The animals were treated dermally with a pour-on formulation at a dose of 82 mg ring-labelled ^{14}C-cyromazine/kg bw. The animals were slaughtered 2, 6 or 10 days after dosing. A run-off from the dose site of an average 28% of the total dose was observed. Peak plasma levels of radioactivity were observed 24 hours after treatment, and declined biphasically thereafter. Approximately 3 to 4 percent of the total dose was excreted within 10 days after dosing, 1% in urine and 2.5% in faeces . Very high levels of radioactivity remained in the wool, up to 33 000 mg cyromazine equivalents/kg at the treatment area and up to 2500 mg/kg at the ventral area, consisting of cyromazine only.

    The mean levels of radioactivity in the tissues were highest (1.15 mg cyromazine equivalents/kg) in fat underneath the treatment area at 6 days after dosing, declining to 1000 µg/kg after 10 days. Highest mean radioactivity of 0.84 mg/kg in muscle samples was observed at 6 days post dose, declining to 0.24 mg/kg after 10 days. The mean levels of radioactivity in liver and kidney were highest at 2 days post dose, i.e. 230 µg cyromazine equivalents/kg in liver, declining to 220 and 150 µg/kg after 6 and 10 days, respectively, and 170 µg/kg in kidney, declining to 10 and 20 µg/kg after 6 and 10 days, respectively.

The parent compound cyromazine was the main residue present in muscle (86%), fat (95%), kidney (77%), urine (96%) and faeces (95%). The residue composition in these matrices was constant in time. N-methyl cyromazine was found in kidney at day 2 (6% of radioactive residues in kidney) and in urine (15% of the radioactivity in urine). Melamine was found in some samples of muscle, fat and urine (up to 1% of radioactive residues in these samples). The residue pattern in liver differed from that in the other tissues. N-methyl cyromazine was the main metabolite in liver, increasing from 63% of the radioactivity in liver at 2 days to 84% at 10 days. The parent cyromazine was also found in liver and decreased from 23% of the radioactivity in liver at 2 days to 4% at 10 days. The overall picture of the residue profile in the edible tissues of sheep show that the parent cyromazine is the most appropriate marker residue.

16. Residues in sheep were determined after a pour-on application at approximately 100 mg cyromazine/kg bw, which is about 1.2 to 1.7 times the recommended dose. Highest cyromazine residues were found in omental fat, and averaged 260 µg/kg at a withdrawal time of 3 days, 160 µg/kg at 7 days, 250 µg/kg at 14 days and finally declined to 100 µg/kg at 21 days. Cyromazine residues in muscle, liver and kidney were in the same range, and averaged 40 µg/kg at 3, 7, 14 and 21 days withdrawal.

17. Four limited reports of cold residue studies in sheep were provided. Sheep received cyromazine at single doses of either 100 mg/kg bw by pour-on or 56 or 112 ml/sheep by spray-on of the medicinal products containing approximately 60 g cyromazine/l. The results of these studies, obtained with either gas liquid chromatography (GLC) or high performance liquid chromatography (HPLC), were inconsistent in the distribution and in the levels of cyromazine. These differences may be attributed to contamination of samples. At 3 days the concentrations of cyromazine in liver ranged from less than 50 to 110 µg/kg, declining to 20 to 70 µg/kg at 7 days, and less than 10 to 20 µg/kg at 14 days. In kidney the concentrations of cyromazine ranged from less than 50 to 300 µg/kg at 3 days, declining to less than 50 to 240 µg/kg at 7 days, and less than 10 to 80 µg/kg at 14 days. The concentrations of cyromazine in all muscle samples were less than 50 µg/kg at 3 days, but levels in muscle at 7 days ranged from less than 50 to 150 µg/kg, and from 20 to 40 µg/kg at 14 days. In fat, the concentrations ranged from less than 50 to 90 µg/kg at 3 days, from less than 50 to 150 µg/kg at 7 days, and from 50 to 290 µg/kg at 14 days.

18. The effect of the fleece length on the residue profile was investigated in 2 groups of 9 sheep with an average wool length of 10 mm (4 weeks of shear) or 53 mm (16 weeks of sheer), which is representative for the moment at which animals will be treated in the field situation. All animals were treated once with a pour-on containing cyromazine at the highest recommended dose of 90 mg/kg bw. Cyromazine levels were determined by HPLC in 3 animals per group at 3, 7 and 14 days after treatment. There was no apparent difference in the residue levels in the tissues of sheep with different fleece length. The highest residue levels were found in the kidney, ranging from less than 20 to 90 µg/kg and showing no apparent decline. Residues in other tissues were in most cases below the limit of detection (20 µg/kg in muscle and fat, 40 µg/kg in liver) at all time points.

19. Cyromazine residues were investigated 1 and 7 days after dipping in a cyromazine solution of 1000 mg/l. The results showed high variation in residual amounts of cyromazine: muscle 170 to 1800 µg/kg at day 1, declining to 30 to 230 µg/kg at day 7, kidney 140 to 6000 µg/kg declining to 80 to 900 µg/kg, liver 120 to 2200 µg/kg, declining to 50 to 350 µg/kg, and fat 270 to 720 µg/kg, declining to 150 to 310 µg/kg. Muscle was also analysed for melamine, but this metabolite was not detectable (less than 50 µg/kg).

20. A routine analytical method for the determination of the marker residue cyromazine in the edible tissues of sheep was proposed, based on HPLC with UV detection, followed by confirmation with HPLC-UV. The limits of quantification for cyromazine for the former method are 20 µg/kg in kidney, muscle and fat and 40 µg/kg in liver. The HPLC stages were well described according to ISO 78/2, but were not fully validated (lacking data in linearity and stability of samples). With respect to the specificity, the possible interference of other veterinary drugs has not been investigated. The proposed routine analytical method combined two basically similar methods (HPLC-UV) each validated for specific parameters only. Confirmation using two methods based on the same technique can only be accepted provisionally and a single fully validated method is required.

Conclusions and recommendation

Having considered that:

- an ADI of 0.02 mg/kg bw (i.e. 1.2 mg/person) has been established for cyromazine,
- cyromazine was identified as marker residue and represents 96% of the total residue in fat, 88% in muscle, 76% in kidney and 6% in liver at 6 to 10 days after treatment,
- inconsistent residue distribution was observed, therefore the same numerical values are set for all edible tissues,
- an analytical method for monitoring residues is available but is not yet fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community;

the Committee for Veterinary Medicinal Products recommends the inclusion of cyromazine in 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
Cyromazine	Cyromazine	Ovine	300 µg/kg 300 µg/kg 300 µg/kg 300 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption Provisional MRLs expire on 1.7.2001

Based on these MRLs values, the daily intake will represent about 53% of the ADI. This will leave scope for possible residues resulting from the use of cyromazine as a pesticide.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of cyromazine in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. An HPLC method for the routine determination of cyromazine in edible tissues of sheep was proposed. Nearly the same method was proposed for confirmatory purposes. None of both methods has been fully validated. The applicant should propose a single method fully validated in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community, and provide evidence that the method is specific.