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 (2)

1. Cyromazine, a triazine derivative, is an insect growth regulator that is used in veterinary medicine for the protection of animals against insects. This application concerns the external use of cyromazine for the prevention of blowfly strike (*Lucilia sericata*) on sheep and lambs, administered as a pour-on at a dosage of 0.9 to 3.6 g/sheep (approximately 60 to 5 mg cyromazine/kg bw) every 8 to 10 weeks. Cyromazine is not to be used on sheep producing milk for human consumption.

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

   Currently, cyromazine is entered in Annex III of Council Regulation (EC) 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<br>300 µg/kg<br>300 µg/kg<br>300 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | Not for use in animals from which milk is produced for human consumption.<br>Provisional MRLs expire on 1.7.2001 |

   Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for cyromazine.

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. This mode of action is different from most conventional insecticides, which have the nervous system as target organ. 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 can not be derived.

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), and with low levels 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 amount. 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 (greater than 70%). Melamine was determined to be the major metabolite

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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 of rats 80 to 85% of the dose was not absorbed after 24 hours. The dose absorbed in the skin acted as a depot from which sustained release of cyromazine occurred. The documentation did not contain pharmacokinetic data after dermal application to sheep.

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; 3000 mg/kg feed for 90 days) and two in dogs (0; 30; 300; 1000; 3000 mg/kg feed for 90 days, or 0; 30; 300; 3000 mg/kg feed for 26 weeks, both studies with a 4-week recovery period). A decreased food consumption, partly associated with retarded 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 (ALAT) 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 the repeated dose studies, a lowest NOEL of 300 mg/kg feed (equal to 9.1 mg/kg bw/day) could be derived from the 26-week dog study.

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 mg/kg feed and in males and females at 3500 mg/kg feed, comprising decreases in haemoglobin, hematocrit, 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, basophil counts, and plasma creatine kinase activities. 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 the 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 a-specific bromcresol green method), which is in contrast with the total protein values that increased over time in each group (including the controls). The Committee concluded that these findings were unlikely to be biologically significant and were therefore not considered as adverse. Consequently, an NOAEL of 200 mg/kg feed, equal to 5.74 mg/kg bw/day, was derived from this study, based on haematological changes and increased relative heart and liver weights in females. The tolerability 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, hematocrit, plasma glutamate dehydrogenase or ALAT activity. Jetting with 12 g cyromazine/animal did not show any effect on foetal development or birth rate.

7. In a two-generation reproduction study in rats (0; 30; 1000; 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, equal to 2 mg/kg bw/day, based on body weight effects in the parental generations.

8. In an oral teratogenicity study in rats (0; 100; 300; 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.

9. The mutagenic potential of cyromazine was evaluated in a range of *in vitro* studies (*Samonella*–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 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 with hamsters). Apart from an inconclusive mouse spot test, all tests were negative. Cyromazine is considered a non-genotoxic compound.

10. A long-term toxicity/carcinogenicity study with cyromazine was conducted in rats (0/30/300/3000 mg/kg feed), as well as a carcinogenicity study in mice (0; 50; 1000; 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 body weight which could be attributed for a substantial part 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 practically applicable given the decreased food consumption at 3000 mg/kg feed. It was concluded that cyromazine is not carcinogenic in rats and mice. The NOEL in rats was 30 mg/kg feed, equal to 1.8 mg/kg bw/day, based on the body weight changes in females. The NOEL in mice was 15 mg/kg feed, equal to 6.5 mg/kg bw/day, based on the body weight effects in the males.

11. 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 not teratogenic and not genotoxic. Some of these studies were also available to the Committee for Veterinary Medicinal Products (CVMP), but these were either inadequate or only short summaries. Hence, the conclusions of ECETOC could not be confirmed.

12. 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 it is the stones, not melamine, that is tumorigenic. Therefore, melamine should not be regarded as a carcinogenic compound.

13. 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 in accordance with the ADI established by the Joint WHO/FAO Meeting on Pesticide Residues (JMPR) in their 1990 meeting, based on the same toxicity data.

14. 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 per kg body weight. The animals were slaughtered at 2, 6 or 10 days after treatment. A run-off from the dose site of 28% of the total dose on average was observed. Peak plasma levels of radioactivity were observed at 24 hours after treatment, and declined biphasically thereafter. Approximately 3 to 4% of the total dose was excreted in urine (1%) and faeces (2.5%) within 10 days after treatment. Very high levels of radioactivity remained in the wool, up to 33000 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 from below the treatment area at 6 days after treatment, declining to 1000 µg/kg at 10 days. Highest mean radioactivity of 0.84 mg/kg in muscle samples was observed at 6 days after treatment, declining to 0.24 mg/kg at 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 at 6 and 10 days, respectively, and 170 µg/kg in kidney, declining to 10 and 20 µg/kg at 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.

15. 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.

16. Four limited reports of cold residue studies in sheep were provided. Sheep received cyromazine as a pour-on at single doses of 100 mg/kg bw or 56 or 112 ml of the formulation per sheep. The results of these studies, obtained with either GLC or HPLC, were inconsistent in the distribution and in the levels of cyromazine. Contamination of samples may have attributed to these differences. At three 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 was 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.

17. The effect of the fleece length on the residue profile was investigated in two groups of nine 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 three 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 quantification (20 µg/kg in muscle and fat, 40 µg/kg in liver) at all time points.

18. Cyromazine residues were investigated one and seven days after dipping in a 1000 mg/l cyromazine solution. 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).

19. An HPLC-UV method for the routine determination of the marker residue cyromazine in the edible tissues of sheep was proposed. The method is well described according to ISO 78/2, and fully validated according to Volume VI of the Rules Governing Medicinal Products in the European Union. The limits of quantification for cyromazine are 20 µg/kg in kidney, muscle and fat and 40 µg/kg in liver. The validation was performed at a number of concentrations ranging from the limit of quantification to 400 µg/kg. It was considered as acceptable that this highest concentration was 1.3 x the MRL instead of twice the MRL as required, because the good accuracy and precision at all levels and in particular at 400 µg/kg indicate that it would be very unlikely that at twice the MRL the performance of the method would not meet the criteria for validation.

**Conclusions and recommendation**

Having considered that:

- an ADI of 0.02 mg/kg bw (i.e. 1.2 mg/person) has been established,
- cyromazine was identified as marker residue and considering that the marker represents 96% of the total residue in fat, 88% of the total residue in muscle, 76% of the total residue in kidney and 6% of the total residue 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 of cyromazine in ovine tissues, fully validated in accordance with volume VI of the Rules Governing Medicinal Products in the European Community, is available;

the Committee for Veterinary Medicinal Products recommends the inclusion of cyromazine in Annex I 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<br>300 µg/kg<br>300 µg/kg<br>300 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | Not for use in animals from which milk is produced for human consumption |

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