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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SUMMARY REPORT

CYMIAZOLE

1. Cymiazole is an iminophenyl thiazolidine derivative, active against acari (mites and ticks).

   In veterinary medicine, cymiazole is recommended for the control of varroatosis in honey bees. The intended pharmaceutical formulation, a 17.5 % granulate of cymiazole hydrochloride, is dissolved in water to obtain a 0.35 % aqueous solution. The freshly prepared solution is applied either topically as a drip-on onto the bee-ways or orally mixed into the winter feed of the bees. Doses of twice 100-350 mg cymiazole hydrochloride per hive (25-35 mg/occupied bee-way) at a 7 day interval are recommended for topical administration. In the winter feed approximately 500 - 700 mg cymiazole hydrochloride are administered per hive (50-70 mg/occupied bee-way).

   Some of the submitted studies were carried out with cymiazole hydrochloride, the substance concerned by this application, others with the free base cymiazole. The latter must be expected to be present to a large extent in honey and in the different body compartments of mammals.

2. Pharmacological studies were carried out with cymiazole hydrochloride disolved in distilled water and applied by gavage in mice, rats, dogs and rabbits (in the latter two species also the intravenous route was studied) as well as in a number of in vitro systems.

   In mice, oral administration of cymiazole hydrochloride prolonged the barbiturate sleeping time by 500 % at the lowest dose tested (600 mg/kg bw). The substance delayed pentetrazol and strychnine induced convulsions and decreased motor activity in mice, both at the lowest doses tested (1200 mg/kg bw). Gastric secretion of rats and intestinal motility of mice were decreased at 600 and 1200 mg cymiazole hydrochloride/kg bw, respectively, lower doses not being tested.

   The body temperature of rats decreased significantly after an oral dose of 1200 mg/kg bw of cymiazole hydrochloride but not following a dose of 600 mg/kg bw.

   Studies in dogs and rabbits indicated that cymiazole could exert haemodynamic and cardiovascular effects.

   These effects were investigated in dogs after intravenous administration in two studies, only one of which was submitted with the MRL-application. Doses of 0.01-4.8 mg cymiazole hydrochloride/kg bw resulted in similar changes (arterial blood flow, fall of blood pressure, bradycardia, also increase of the diastolic blood pressure in the right ventricle). High intravenous doses (10-20 mg/kg bw) caused a more pronounced decrease in blood pressure in the left ventricle. At these doses the bradycardia reversed into tachycardia, possibly as a compensatory reaction to the decreased cardiac output. First significant effects were seen following intravenous doses of 0.1 mg cymiazole hydrochloride/kg bw (increase of diastolic blood pressure in the right ventricle) in the study not submitted and 0.6 mg/kg bw (decrease of heart rate, arterial pressure and arterial blood flow) in the submitted study. Corresponding trends were apparent at doses of 0.01-0.2 mg/kg bw. The NOEL of 0.2 mg/kg bw proposed for the submitted study is doubtful because some of the recorded parameters were changed at the lowest dose tested, 0.01 mg/kg bw (e.g. arterial blood flow), and a dose of 0.1 mg/kg bw was found effective in the other study in a parameter (diastolic pressure in the right ventricle) not investigated in the submitted study.

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3. Data on bioavailability of cymiazole and cymiazole hydrochloride from the different vehicles employed in the submitted pharmacological and toxicological studies or from vehicles relevant to consumer safety are lacking.

4. Absorption of single doses of radiolabelled cymiazole administered by gavage (doses tested : 0.7 to 25 mg/kg bw, vehicle; aqueous solution) from the digestive tract of rats of both sexes was rapid and reached nearly 95 %. Tissue concentrations of cymiazole and/or metabolites in the brain 6 days after treatment were 23 µg/kg and 300 µg/kg following the low and the high dose, respectively. Other tissues contained tenfold lower concentrations.

   Dermal application of a single dose of 2.72 mg ^{14}C-cymiazole per kg bw to a lactating cow resulted in absorption of 50 % of the dose. Possible oral intake cannot be excluded. Of the absorbed dose 93 % was found in urine, 4.8 % in faeces within 48 hours, nearly all of which represented cymiazole metabolites, and 0.4 % of the dose were excreted via milk.

   Maximum concentrations of cymiazole in the blood of the cow were reached 6 hours after administration. The absorption phase was protracted. A fast and a slow elimination phase with $t_{½}$ of ca. 20 and ca. 60 hours respectively could be distinguished. Possibly, these values represent absorption half-lives. Tissue residues 9 days following administration in liver, thyroid and lung amounted to 100-200 µg/kg. Residues in all other tissues were tenfold lower. The highest radioactivity in milk was detected in the first two milkings equivalent to a maximum concentration of 104 µg/kg cymiazole and/or metabolites.

5. The major metabolic pathways proposed for rats and cattle involve oxidation of the 4-methyl group leading to the corresponding benzoic acid derivatives, which in the cow are also conjugated with glycine. Oxidation of the thiazoline ring leads to unstable products. After ring opening dihydroxy imidazole derivatives are formed, in which the nitrogen of the former aniline ring becomes incorporated into the imidazole ring structure, while the sulfur atom switches to an exo-position, forming a thiono-group. Opening of the former thiazoline ring eventually leads to formation of thiourea derivatives. These are oxidised to their corresponding urea metabolites. In all structurally identified metabolites (a total of 4, all from urine) the original bridge between the phenyl- and former thiazoline rings remains unopened.

   Identified metabolites in urine accounted for 49 % and 28 % of the absorbed doses in the cow and rats, respectively.

6. In rats, the oral toxicity of cymiazole depended on the vehicle. LD_{50}values of 725 mg/kg bw for both sexes when dissolved in polyethylene glycol, and 2235 and 1242 mg/kg bw for males and females, respectively, when dissolved in carboxymethylcellulose were reported. The oral LD_{50} of cymiazole hydrochloride in rats was 1155 mg/kg bw in males and 914 mg/kg bw in females. Since the vehicle in which it was tested is not identical to the two above, a comparison is not possible. In an acute dermal toxicity study on cymiazole in polyethylene glycol no rats died at doses up to 3170 mg/kg bw applied under an occlusive dressing for 24 h. The acute inhalation toxicity of cymiazole in rats (head/nose exposure, 240 minutes) was characterised by an LC 50 >2800 mg/m^3 (analytical concentration), the only concentration tested. 3 each of 9 males and 9 females died.

   Clinical signs of acute oral toxicity of cymiazole and its hydrochloride in rats were sedation, dyspnoea, ruffled fur and curved or ventral posture, with tremors at 500 mg/kg bw and convulsions at 2000 mg/kg bw.

   In mice and chinese hamsters the LD_{50} values of cymiazole following oral administration in polyethylene glycol were 911 and 7100 mg/kg bw, respectively.

7. Cymiazole was given to rats per gavage for 28 days in a vehicle of 0.5% aqueous carboxymethylcellulose at doses of 0, 20, 100 or 500 mg/kg bw. Because 7 of 20 animals in the high dose group died, the dose was altered to 250 mg/kg bw. Leucocyte counts decreased in both sexes at the high dose and in females at the medium dose. Significant reduction of bodyweight was seen in both sexes receiving the high dose with corresponding trends at the lower doses. Total protein concentration of blood was elevated in rats at the high dose, total globulin concentration only in females at the two high doses, and cholesterol levels in both sexes of these two dose groups. Absolute and relative liver weights increased in both sexes at the high dose and in males at the medium dose, accompanied by hypertrophy of hepatocytes.

Thymus weights decreased in males and females of all groups, as a trend at the low dose, significant with manifest atrophy at the two high doses.

Rats received cymiazole in the feed for 13 weeks at concentrations corresponding to 0, 2, 10 or 50 mg/kg bw. Two rats per sex of the control and of the high dose group were kept for a 3-week recovery period. No compound related deaths were observed. Bodyweight gain was reduced in the high dose group. Leucocyte counts were low in both sexes at the high dose and in females at the medium dose. This parameter did not return to normal during the recovery period in females of the high dose group. Serum glucose levels were elevated in males of all dose groups, but returned to normal within the recovery period. Also elevated were serum gamma and alpha-2-globulines in males at the low dose and total protein concentrations in females at the median and high dose. Decreased urine volumes with corresponding higher gravity were recorded for males of all treatment groups. Males receiving the high dose of cymiazole also had decreased thymus, liver, spleen and adrenal weights. In view of the changes detected at all doses the proposed NOEL of 2 mg/kg bw is questionable.

Two feeding studies with cymiazole were carried out in dogs. In the first study doses of 0, 100, 1000 and 10 000 mg/kg feed and, in a supplemental group, of 300 mg/kg feed were administered for four weeks, in the second study concentrations in the feed were 40, 80, 150 or 300 mg/kg for 13 weeks. The 1000 mg/kg feed level was not tolerated, 3 of 4 animals died. The tenfold higher concentration of 10 000 mg/kg feed only led to reduced health status (food consumption 5 to 50% of control values, weight loss of 17-31%, depression, bone marrow hypoplasia and thymus atrophy). Dogs receiving 300 mg/kg feed in both studies showed no other signs of impaired health than depression, ataxia and occasional vomiting. Cardiovascular and neurologic parameters were not examined in the two studies. No explanation was given for the deaths at the 1000 mg/kg feed level (2 dogs died on day 2, one on day 7 of the study, compound intake of the single surviving animal ca. 15-45 mg/kg bw). With these reservations, NOELs for cymiazole of 100 mg/kg feed corresponding to 3.8 mg/kg bw in the first and 150 mg/kg feed corresponding to 5 mg/kg bw in the second study can be retained.

8. Long-term toxicity of cymiazole was studied in rats and mice.

   Rats received dietary cymiazole concentrations corresponding to approximate doses of 0, 0.55, 1.5 and 5.31 mg/kg bw for 24 months. Blood and urine analysis were carried out at weeks 26, 52, 78, and 104. Changes related to the prothrombin time and to the concentrations of the aminoaspartate transferase, of bilirubin and of alpha-2 globulin fractions. Additionally, a dose independent increase of the liver/brain weight ratio and adrenal weights were recorded. The concurrent histological examinations demonstrated negative results. Referring to the before mentioned parameters a NOEL of 0.55 mg/kg bw is established.

   Mice were fed cymiazole in the diet for 24 months at concentrations corresponding to dose levels of 0, 1.21, 3.42 or 11.9 mg/kg bw, Increased water consumption was recorded in males at the high dose, a trend towards elevated sodium and potassium levels in blood noted in males of all dose groups and increases in absolute and relative liver, adrenals, thymus, thyroid and pituitary weights with significant dose related trends were observed. No histological deviations from controls were seen. A NOEL of >12 mg/kg bw is proposed. Considering the detected changes it is debatable as to whether a NOEL has been demonstrated in this study.

9. No information on tolerance in the target species has been submitted.

10. No teratogenic effects, even at maternotoxic doses, were seen following oral application (gavage) in a 0.5% aqueous carboxymethylcellulose solution in rats at doses of 0, 20, 60 or 120 mg/kg bw cymiazole (reduced weight gain at all doses) and in rabbits at doses of 0, 25, 75 or 150 mg/kg bw. Food consumption and weight gain of rabbit does and the average weight of the foetuses were decreased at the two high doses. Delayed ossification was noted in both species at the respective high dose levels.

11. A multigeneration study was carried out in rats at dietary dose levels corresponding to 0, 0.5, 1.5 and 5 mg cymiazole/kg bw. No reproduction toxicity was observed. A slight reduction in weight gain during gestation, marginally smaller litter sizes and weights in the high dose groups of both generations were the only observed changes. A NOEL of 1.5 mg/kg bw was identified.

12. Studies investigating the pre- and perinatal influence of cymiazole have not been submitted.

13. In one of the two Salmonella/mammalian microsome assays performed, cymiazole was mutagenic in strain TA 100 without and with metabolic activation (S9-Mix). Positive or equivocal responses to cymiazole were noticed in a host mediated assay in *Salmonella typhimurium*. A gene and chromosome mutation assay in *Saccharomyces cerevisiae* gave equivocal results for cymiazole. Cymiazole and its hydrochloride caused unscheduled DNA synthesis in rat hepatocytes in an *in vitro* UDS test. In contrast, an *in vivo*/*in vitro* UDS test of cymiazole in rat hepatocytes gave negative results.

    The significance of the positive result for cymiazole in strain TA 100 in one of two Salmonella/mammalian microsome assays is not clear. It may be due to an impurity, which later was quantitatively decreased in the production process. The negative outcome of the *in vivo*/*in vitro* UDS test of cymiazole cannot refute the positive results of the *in vitro* UDS tests for both cymiazole and its hydrochloride.

    Cymiazole hydrochloride was not mutagenic in the CHO/HGPRT mutation assay and not clastogenic in a *in vitro* cytogenetic study on human lymphocytes. No mutagenic effects were observed *in vivo* in a micronucleus test in chinese hamsters and a dominant lethal test in mice either.

14. No indication of tumorigenic or tumor promoting effects of cymiazole has been observed in the 24 month dietary studies in rats and mice at dose levels up to 5 and 12 mg/kg bw, respectively. As the highest dose tested in mice had no pronounced toxic effects, the study is of limited value with respect to the detection of carcinogenic properties of cymiazole.

15. Cymiazole was found to be mildly irritating to the eyes and to the skin of rabbits.

16. No studies investigating the influence of cymiazole on immunological parameters or neurotoxic effects of cymiazole have been submitted.

17. Regarding environmental toxicity of cymiazole and its hydrochloride, acute toxicity of the substances was tested in several species of birds and fish and found to be low.

18. There was no data for observations in humans.

19. A full ADI for cymiazole hydrochloride cannot be derived at present.

    The effects for which a NOEL must be established before a final ADI can be calculated are changes of haemodynamic parameters. These effects have not been investigated after repeated oral application in a sensitive animal species. Further explanations of the pharmacological findings in dogs are required before an final ADI can be set for cymiazole hydrochloride.

    Based on the NOEL of 0.55 mg/kg bw observed in the 2-year feeding study in the rat and employing a safety factor 500 to compensate for the inadequacies of the available data a provisional ADI of 0.001 mg/kg bw, i.e. 0.06 mg/person can be established

20. Residue studies in which cymiazole was administered to bee colonies using the drip-on method (where the product is applied with a syringe directly onto the bees in the beeways) or feeding method (where the product is applied to the bees in a medicated feed) at the recommended total dose of 50 - 70 mg/beeway or at higher doses were carried out between 1985 and 1990. Honey, honeycombs and beeswax were analysed for cymiazole residues by gas chromatography with thermionic phosphorus-nitrogen detector.

21. Following the drip-on treatment at the recommended dose cymiazole residues were in the range of 0.01 to 1.1 mg/kg in honey, 0.2 to 9.3 mg/kg in honeycomb and 0.37 to 1.25 mg/kg in beeswax. A maximum cymiazole concentration of 1.1 mg/kg in honey was found in one hive of the 4-year accumulation study (2nd year), while in samples of all other hives honey residues were below 1 mg/kg.

    The influence of accumulation of residues, higher doses and/or increased frequency of application, as well as harvest time, honey yields, honey stores and brood on cymiazole residues in honey and honeycomb was studied. No significant differences were detected in honey residue concentrations.

The design of the studies is often not comparable and instead of detailed protocols, the documentation consists only of short summaries, making a scientific comparison of the study results impossible.

22. The feeding method resulted in lower cymiazole concentrations in the range of 0.01 to 0.34 in honey, 0.33 to 2.4 mg/kg in honeycomb and 0.3 to 1.02 mg/kg in beeswax. A maximum cymiazole concentration of 0.34 mg/kg in honey was found in one hive of the 4-year accumulation study (2nd year), but the study is not sufficiently documented to give an explanation for this finding.

23. The metabolite 2,4-dimethylaniline (free and bound) was not detected in honey at a limit of detection of 0.01 mg/kg.

24. A method for the extraction of cymiazole from honeycomb was presented. Following a hot acid treatment of honeycombs cymiazole residues in the wax were below the limit of detection of the analytical method of 0.03 mg/kg.

25. An analytical method using gas chromatography with thermionic detection (specific for nitrogen and phosphorus) for the determination of cymiazole in honey is available but the method is not sufficiently described, validated and supported by raw data.

26. The following provisional MRL for cymiazole in honey has been set:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Cymiazole	Cymiazole	Bees	1000 µg/kg	Honey	Provisional MRL expires on 01.07.1999

This MRL would account for around a third of the ADI. There are many deficiencies in the residue documentation, but the data available indicate that the residue concentrations in honey are below the ADI after the recommended treatment.

LIST OF QUESTIONS

Safety file

1. Data on the physico-chemical properties of cymiazole (free base) and on photochemical stability of cymiazole and cymiazole hydrochloride should be submitted.

2. The applicant should comment on toxicity, mutagenicity and residues in honey of relevant impurities, e.g. 3-(2', 4'-dimethylphenyl)-l-methyl-thiourea or 2,4-aniline derivates, or break down products or metabolites of cymiazole hydrochloride.

3. The influence of different vehicles on the bioavailability and acute toxicity of cymiazole and cymiazole hydrochloride should be discussed.

4. The Reports No. CRA-76/12, 76/22, 76/41, 76/41, 76/63, 76/66, 77/28, 77/29, 77/30, 77/52, 77/56 on pharmacologic properties of cymiazole hydrochloride, which formed part of an application for a national marketing authorisation, should be submitted.

5. The pharmacodynamic mechanism of the effects of cymiazole hydrochloride in acari and mammals should be discussed. The company should likewise comment on possible threshold doses for the pharmacodynamic properties of cymiazole in mammals.

   Specifically, the applicant should discuss the possible pharmacological effects of cymiazole hydrochloride in dogs induced by the lowest intravenous dose of 0.01 mg/kg bw tested in the submitted study. The results of study CRA 77/U (not submitted), in which a significant effect level of an intravenous dose of 0.1 mg/kg bw was observed in dogs, should be included in the discussion.

6. The applicant should substantiate the pertinence of the intravenously derived NOEL in dogs of 0.2 mg/kg bw to cardiovascular effects in dogs following oral administration. It should be excluded that pharmacodynamic effects are caused in dogs following oral administration of 0.55 mg/kg bw, the NOEL derived from a chronic toxicity study in rats and proposed by the sponsor as base for an ADI.

7. All reports on mutagenicity of cymiazole or cymiazole hydrochloride should be submitted in the original form. The applicant should discuss the results and elaborate in particular on the significance of the positive outcome of the UDS *in vitro* study and the first Ames test in strain TA 100.

8. For the life time toxicity study in the mouse it should be stated whether mean dose levels (mg/kg bw) have been calculated with the respective corrected values from diet analysis. Also, the statistically significant findings should be commented upon with respect to the proposed NOELs.

9. Information on immunosuppressive and neurotoxic properties of cymiazole should be submitted.

Residue file

10. Detailed information has to be provided for each study in line with the principles laid down in Volume VI of the Rules Governing Medicinal Products in the European Community. Especially information on the total number of honeycombs in the individual hive, the location of the honeycombs in relation to the treated area and a detailed documentation on the selection of honeycombs for the extraction of honey and on sampling of honey for analysis is required. Raw data of all test results, methods of calculation and the summary of data in tabular form including the correction for recoveries should be presented.

11. The applicant should provide detailed information on the experimental design and on the performance of the analytical method for all studies.

12. All results should be discussed in conjunction with the other available studies, e.g. the applicant should comment on the influence of the different formulations of the administered drug and on the remarkably high cymiazole concentrations in the second year of the 4-year trials (drip-on and feeding method).

13. The routine analytical method for the determination of cymiazole has to be described and validated and supported by experimental data on specificity (e.g. against other substances used for treatment of bee diseases), accuracy, precision, limit of quantification, limit of detection and susceptibility to interference in line with the principles laid down in Volume VI.

    The analytical method has to be described using a standard internationally-recognised format (e.g. ISO 78/2).