



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CHLORMADINONE

SUMMARY REPORT

1. Chlormadinone acetate (6-chloro-17-hydroxypregna-4,6-diene-3,20-dione acetate, CAS Number 302-22-7) is a synthetic progesterone analogue. In cattle the compound is used for oestrus synchronisation at daily oral doses of 12 mg per animal for up to 20 days. Chlormadinone acetate is also used in sheep and goats for the same indication at daily oral doses of 2.5 mg per animal and in horses at daily oral doses of 12 mg per animal for up to 20 days.
2. The pharmacodynamic effect of chlormadinone acetate of interest in relation to oestrus synchronisation is the inhibition of release of gonadotrophin releasing hormone from the hypothalamus thereby blocking the output of gonadotrophins from the pituitary gland. Being a derivative of progesterone, chlormadinone exhibits typical progestogenic effects peripherally. However, considerable inter-species variation in progestational activity is observed, dogs being especially sensitive. The oral hormonal NOEL based on endometrial proliferation in the uterus of juvenile, oestrogen pre-treated rabbits was 0.007 mg/kg bw/day. This dose was administered over 5 days. Its antiandrogenic, antiestrogenic and glucocorticoid effects are reported to be relatively weak. The minimal observed effective level in humans was 50 µg/day, stated as 1 µg/kg bw/day in women showing changes in the endocervical mucus. In addition the study was carried out on a limited number of persons, the number of persons per group differed and no control group was used. Therefore, no pharmacological (hormonal) NOEL could be established in humans.
3. Chlormadinone acetate is readily absorbed in rats following oral administration with maximum plasma concentrations being reached within 30 to 60 minutes. The plasma half-life is stated as 16 hours in the rat and 30 hours in the dog.

Metabolism shows considerable inter-species variation. In rabbits the compound appears to be metabolised by two pathways: 2-hydroxylation and dechlorination at the C6-atom, and, to some extent, by hydrogenation of one or both of the double bonds in the steroid ring system. A small amount of a metabolite oxidised at the C21-atom was also detected. Incubation of chlormadinone acetate with rat and human liver microsomes produced the 3-hydroxy product as the major metabolite. When chlormadinone acetate was incubated with liver microsomes from phenobarbital-pretreated rats and rabbits, the major metabolite was the 2-hydroxy derivative. Thus the metabolism seems to be dependent on the induced state of hepatic monooxygenases. No information is available on the inductive potential of chlormadinone itself on liver oxidative enzymes. Significant enterohepatic circulation of metabolites appears to take place in some species, e.g. the rat, but not in other species, e.g. the baboon.

After oral administration to cattle chlormadinone acetate is absorbed rapidly and completely, reaching maximum plasma concentration (106.62 ng/ml) after approximately 5 hours. During the first liver passage there is intensive metabolism to metabolites, which are, to a large extent, excreted in the bile without systemic circulation. In plasma 2 metabolites were found at low concentrations compared to parent chlormadinone acetate (1 and 15% of the plasma concentration of chlormadinone acetate). Chlormadinone acetate is eliminated from systemic circulation with an elimination half life of about 14 hours. The main route of elimination is faeces, with about 60% of the administered dose recovered during a collection period of 36 hours after medication. Eight percent is parent compound and the rest metabolites. Less than 0.1% of the administered dose is detected in urine during a collection period of 36 hours after medication.

4. The substance is of low acute toxicity. In rats and mice the oral LD₅₀ is 6400 mg/kg bw.
5. Studies in male rats treated orally with chlormadinone acetate 50 mg/kg bw/day for 21 days revealed atrophy of adrenal cortex, prostate, and pituitary corticotropin-producing cells. Female rats treated orally for 30 days with a dose ranging from 10 to 1000 mg/kg bw/day developed significant decrease in uterine weights in the lowest dose group.

Female guinea pigs receiving 0.5 mg/kg bw/day for 2 to 6 months developed renal damage.

Male and female Beagle dogs were treated with chlormadinone acetate doses 0, 0.6 mg/kg bw/day and 0.06 mg/kg bw/day. Both sexes of dogs dosed with 0.6 mg/kg bw/day chlormadinone acetate up to 7 months developed severe toxicological effects such as polydipsia, hyperglycaemia, glomerulopathy and pyometra. The dose of 0.06 mg/kg/day for 5 months did not induce any toxicological symptoms. A 4-year study in female Beagles treated with 0.25 mg/kg bw/day revealed cystic endometrial hyperplasia, pyometra, gall bladder hyperplasia, atrophy of adrenal cortices and signs of diabetes. Apart from the hormonal effects on male and female reproductive organs, the major toxic effects might be due to development of diabetes mellitus. A toxicological NOEL of 0.06 mg/kg bw/day was established in the 5-month dog study.

6. In a published study no adverse effects were recorded in heifers treated with 12 mg chlormadinone acetate for 20 days.
7. In mice administration of chlormadinone acetate by gavage at doses of 1, 3, 10 and 50 mg/kg bw/day on days 8 to 15 or days 14 to 17 of gestation resulted in malformations in the offspring, cleft palate being most frequent. The highest doses (10 and 50 mg/kg bw/day on days 8 to 15) were associated with 33.1% foetal deaths and 68.9% resorptions.

In another study in which mice were given either 1 or 10 mg/kg bw/day orally, only the higher dose resulted in a significantly increased rate of malformations.

In rat studies oral doses up to 300 mg/kg bw/day given between day 7 and day 18 of gestation did not induce teratogenicity.

In rabbits treated on days 8 to 20 of gestation, doses of 1 or 3 mg/kg bw/day had no effects on reproduction, while 10 mg/kg bw/day was associated with 60% malformations in the offspring and 45% embryoletality.

The teratogenicity of chlormadinone acetate varies depending on dose and animal species. The oral threshold dose for teratogenicity is about 10 mg/kg bw/day in mice and 3 to 8 mg/kg bw/day in rabbits, while rats could be dosed up to 300 mg/kg bw/day without showing teratogenic effect.

The effect of chlormadinone acetate on reproduction is clearly dose-dependent. High oral doses in heifers (up to 8 times of the therapeutic dose) induce reversible sterility for up to 3 months. High daily oral doses in adult pigs (60 mg/animal for 14 to 18 days) and dogs (1 mg/animal for 21 days) reversibly reduced libido in both males and females.

Three generation studies in mice treated with daily doses of 7 to 14 mg/animal of chlormadinone acetate for 10 days before mating revealed a negative correlation between oestrus synchronisation and litter weight and size.

8. Chlormadinone acetate has been tested for genotoxicity in several assays. There was no indication of mutagenicity in a *Salmonella* microsomal assay, which used 5 strains of bacteria in presence and absence of metabolic activation. Assays for unscheduled DNA synthesis in human and rat hepatocytes *in vitro* and an *in vivo* cytogenesis assay in human lymphocytes also gave negative results. However, *in vitro* assays in human and rat liver showed that chlormadinone acetate could form DNA adducts. In an *in vivo* micronucleus assay in rat liver a single dose of chlormadinone acetate produced micronuclei in hepatocytes. The CVMP concluded that overall chlormadinone acetate, like other progestagens, can be considered as non-genotoxic.
9. Chlormadinone acetate has been tested for carcinogenicity in several experiments, which were evaluated by the International Agency on Research on Cancer (IARC) in 1979. Oral administration was tested in 3 mouse studies, 1 rat study and 2 studies in dogs.

MTV⁻-mice (mammary tumour virus not expressed) were fed daily doses of either 2 to 5 times the human contraceptive dose of chlormadinone acetate (0.02 to 0.05 mg/kg bw; a human dose assumed to be equivalent to 0.01 mg/kg bw/day), 50 to 150 times the human dose (0.5 to 1.5 mg/kg bw) or 200 to 400 times the human dose (2 to 4 mg/kg bw) for 80 weeks. No increase in the tumour incidence was noted. When the same doses were fed together with mestranol (a synthetic estrogen) in a ratio of 25:1 there was a 5 to 10-fold increase in the incidence of pituitary tumours, but no increase in tumours of other tissues.

MTV⁺-mice (mammary tumour virus expressed) were fed chlormadinone acetate at estimated levels of 0.06 to 0.08 mg/kg bw/day or 0.6 to 0.8 mg/kg bw/day. The lower dose was not associated with an increase in the (already high) incidence of mammary tumours in females or castrated males, although the development of post-castration adrenal adenomas was inhibited. The higher dose slightly increased the latent period for mammary tumour development in females but not in castrated males.

Mice were fed a combined preparation of chlormadinone acetate (97.5%) and ethinylestradiol (2.5%) at an estimated dose level of 20 to 30 µg/animal. In females neither mammary tumour incidence nor latency period was altered, while a significant increase in the tumour incidence and a significant decrease in the latency period was observed in both intact and castrated males.

Rats were fed estimated doses of either 0.02 to 0.05 mg/kg bw/day, 0.5 to 1.5 mg/kg bw/day or 2 to 4 mg/kg bw/day for 104 weeks. No differences in tumour incidence were observed.

Female Beagle dogs were fed chlormadinone acetate doses of 250 µg/kg bw/day, commencing at 26 to 52 weeks of age. After 104 weeks, 6 out of 20 animals had small nodules (less than 1 cm) in mammary tissue, and similar but transitory nodules were noted in 4 untreated controls. Histological examination of the latter revealed no abnormal mammary proliferation. One further dog had a somewhat larger nodule, which at autopsy was found to be a benign tumour composed of connective tissue and some epithelial elements with proliferation of myoepithelial tissue.

In a study of 22 mammary nodules which appeared in 14 dogs after 4 years of treatment with chlormadinone acetate (dose not specified), 12 were classified as nodular hyperplasia, 4 as benign, mixed tumours and 1 as an adenocarcinoma. By 7 years there had been an increase in the total number of neoplastic lesions of the mammary glands from 5 to 14 (benign, mixed tumours from 4 to 9; ductal papillomas from none to 3; and adenocarcinomas from 1 to 2). In the controls 1 benign, mixed tumour was observed.

IARC, upon evaluation of the substance in 1974 and again in 1979, found limited evidence of chlormadinone acetate being carcinogenic in the dog. High doses of chlormadinone acetate can induce tumors, but the effect of lower concentrations is unclear.

10. Between 1997 and 1999, new data became available on the genotoxicity and carcinogenicity of steroid hormones, although not including chlormadinone acetate. These data were also reviewed and discussed by the Joint FAO/WHO Committee on Food Additives (JECFA) in 1999, by the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) of the European Commission in 1999 and by IARC in 1999. Upon evaluation of these data, mainly concerning 17 β -oestradiol, the CVMP concluded that steroid hormones are devoid of genotoxic activity *in vivo* and that these compounds exert their carcinogenic action only after prolonged exposure and at levels considerably higher than those required for a physiological (hormonal) response.
11. No information on the immunotoxicity of chlormadinone acetate is available.
12. Until 1970 the substance was used in the treatment of various female reproductive disorders, e.g. dysmenorrhoea and endometriosis; daily doses up to 10 mg were recommended. It was also incorporated in oral contraceptives either alone or in sequential-type contraceptives (in combination with an estrogen) at a recommended daily dose of 0.5 mg. Blockage of the oviducts has been reported following 2 mg/day for 19 days.
13. The use of chlormadinone acetate in human medicine has been discontinued in the early 1970's. The substance was withdrawn after evidence had become available indicating that the incidence of mammary tumours in Beagle dogs treated with the substance was higher than in animals treated with other synthetic progestagens. However, according to the conclusions drawn in paragraphs 8 and 10 these tumourigenic effects are considered to be caused by gestagenic interaction with hormonal receptors in target tissues.
14. A pharmacological ADI of 0.07 $\mu\text{g}/\text{kg}$, i.e. 4.2 $\mu\text{g}/\text{person}$ was established based on the oral NOEL of 0.007 mg/kg bw/day, (7 $\mu\text{g}/\text{kg}$ bw/day) from the rabbit study, applying a safety factor of 100.
15. In a GLP compliant residue study, 12 lactating cows were orally dosed with 10 mg chlormadinone acetate/animal for 20 consecutive days. Treated animals (3 per time-point) were sacrificed on day 1, 4, 7 and 8 day after last treatment. The tissue residue concentrations of chlormadinone acetate were determined by a validated HPLC technique with a limit of quantification of 1 $\mu\text{g}/\text{kg}$ for edible tissues except fat (2 $\mu\text{g}/\text{kg}$). One day after the last treatment the residue concentrations were below limit of quantification in kidney and muscle and showed mean values of 17 $\mu\text{g}/\text{kg}$ for fat and 9 $\mu\text{g}/\text{kg}$ for liver. Four days after last treatment only one animal revealed 4 $\mu\text{g}/\text{kg}$ in the liver and two animals showed 3 and 10 $\mu\text{g}/\text{kg}$ fat. Day 7 after last treatment 2 $\mu\text{g}/\text{kg}$ fat was measured in one cow. No other cows at day 7 or at day 8 after last treatment revealed residue concentrations above limit of quantification.

In the same study, milk samples from 8 cows were taken daily during treatment and day 1, 2 and 7 after last treatment. The limit of quantification of the analytical method in milk was 0.25 $\mu\text{g}/\text{kg}$. On day 1 after last treatment, 5 of 8 cows showed residues above limit of quantification with a mean value of 2.1 $\mu\text{g}/\text{kg}$. Day 2 after last treatment 2 of 8 cows had milk residues above the limit of quantification with a mean value of 1 $\mu\text{g}/\text{kg}$. Seven days after last treatment only one cow had milk residues above the limit of quantification (2.1 $\mu\text{g}/\text{kg}$).

16. No radiolabelled studies have been submitted, however, results from studies on absorption, distribution, metabolism and excretion in cattle indicate that only 2 metabolites were present in plasma and in concentrations of 2% and 15% of the C_{max} for chlormadinone acetate. The t_{max} for chlormadinone acetate was 5 hours while t_{max} for the 2 metabolites was 8.8 and 10 hours. The elimination of the two metabolites followed the elimination of chlormadinone acetate. The metabolites excreted in milk were less than 2% of the chlormadinone acetate concentration in milk.

Considering that the ADI established is a pharmacological one, the CVMP considered that all the pharmacological activity is due to the parent compound and considered that the ratio marker residue/total pharmacologically active residues equal to 1. Therefore the parent compound can be considered as marker residue and radiolabelled studies are not required. This approach is in line with what has been done for other compounds of the same family.

17. A validated routine analytical method based on HPLC with UV detection described in the ISO 78/2 format was provided. The limits of quantification of the analytical method were 1 µg/kg for liver, kidney, muscle, 2 µg/kg for fat and 0.25 µg/kg for milk.

Conclusions and recommendation

Having considered that:

- a pharmacological ADI 0.07 µg/kg, i.e. 4.2 µg/person was established,
- only the parent compound is considered relevant for the pharmacological effect,
- residues in muscle and kidney were below the limit of quantification of the analytical method, no MRLs are necessary for these tissues,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- a validated analytical method for monitoring residues of chlormadinone is available;

the Committee for Veterinary Medicinal Products recommends the inclusion of chlormadinone 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
Chlormadinone	Chlormadinone	Bovine	4 µg/kg 2 µg/kg 2.5 µg/kg	Fat Liver Milk	For zootechnical purposes only

Based on these MRLs values, the daily intake will represent about 98% of the ADI.