



COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

FLUAZURON

SUMMARY REPORT

1. Fluzauron is an insect growth regulator belonging to the class of benzoylphenyl urea derivatives, a class of chitin synthesis inhibitors. Fluzauron specifically interferes with chitin formation in ticks during engorgement, moult and hatching. The substance is intended for tick control in beef cattle applied topically as a pour-on for use at single dose levels of 1.5 and 2.5 mg/kg bw with a possible additional treatment after 3 to 6 months.
2. After oral administration of (radiolabelled) fluzauron to rats, the extent of absorption was high (60 % within 24 hours after administration) and elimination was primarily via faeces (59% within 1 week after administration, compared to 3% via urine), indicating biliary excretion of fluzauron and its metabolites. The major portion of the absorbed dose was retained in the adipose tissues as unchanged fluzauron, with significantly lower levels in liver, kidney, lung, muscle and brain. Fluzauron was released from adipose tissues by a passive diffusion controlled process that followed first order kinetics with a half-life of about 13 days. About one third of the dose was eliminated as unchanged fluzauron with the faeces; the remaining two thirds was slowly released from adipose tissues and ultimately metabolised. Metabolism consisted of cleavage of the urea moiety between the benzoyl carbon and the urea nitrogen, followed by hydroxylation of the remaining urea.
3. Orally administered fluzauron was found to have low acute toxicity, with an LD₅₀ value of more than 5000 mg/kg bw in rats.
4. In a 28 day study, rats received fluzauron by gavage at dose levels of 0, 10, 100 or 2000 mg/kg bw/day. Increases in prothrombin time and liver weight, and decreases in platelet counts and thymus weight were mainly observed in mid and high dose animals, particularly in males. The NOEL in this study was 10 mg/kg feed, equal to 3.2 mg/kg bw/day.
5. Male rats were also more sensitive to the effects of fluzauron in a 13 week feeding study at dose levels of 100, 600, 3500 or 20000 mg/kg feed (equivalent to 0, 6.4, 39, 220 and 1300 mg/kg bw/day for males and 0, 6.6, 41, 240 and 1400 mg/kg bw/day in females). At 3500 and 20000 mg/kg feed male rats showed increases in prothrombin time, platelet counts, lymphocytes, and in absolute and relative liver weight, as well as thyroid follicular hypertrophy, pituitary hypertrophy, and hepatocellular hypertrophy. The increases in absolute and relative liver weight were also observed in males at 600 mg/kg feed. Females at 3500 and 20000 mg/kg feed showed hepatocellular hypertrophy. The NOEL for rats was established at 100 mg/kg feed, equal to 6.4 mg/kg bw/day.

6. In a 52-week feeding study with interim sacrifice at 13 weeks, dogs received fluazuron at dose levels of 200, 3000 or 50000 mg/kg feed (equivalent to 0, 7.5, 110 and 1900 mg/kg bw/day in males and 0, 7.1, 120 and 2000 mg/kg bw/day in females). Fluazuron treatment mainly affected high dose males (transient decreases in food consumption and body weight loss, increased activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, and minimal multifocal haemorrhage with slight multifocal chronic inflammation of the liver). A slight increase in alkaline phosphatase activity was also seen in males at 3000 mg/kg feed and in females at 50000 mg/kg feed. The NOEL in this study was 200 mg/kg feed, equal to 7.5 mg/kg bw/day.
7. In a two-generation reproductive toxicity study with rats at dose levels of 100, 1500 or 20000 mg fluazuron/kg feed (equivalent to 5 to 1000 mg/kg bw/day), fluazuron had no adverse effects on the reproductive function. The only effects observed were a slightly retarded pup growth at 1500 and 20000 mg/kg feed, and a marginally increased neonatal pup mortality at 20000 mg/kg feed. The NOEL in this study was 100 mg/kg feed, equivalent to 5 mg/kg bw/day.
8. Fluazuron was not maternotoxic and did not cause embryo-/fetotoxicity or teratogenicity in rats and rabbits at oral doses up to 1000 mg/kg bw/day.
9. Fluazuron has been tested in several *in vitro* mutagenicity tests (covering a variety of genetic endpoints) and in an *in vivo* nucleus anomaly assay with Chinese hamsters. The results of the *in vivo* test were inconclusive because it was not clear whether or not the bone marrow was exposed. All the *in vitro* tests gave negative results. It was concluded that fluazuron has no genotoxic potential.
10. One carcinogenicity study with mice and one combined long-term toxicity/carcinogenicity study with rats were available. Mice received a diet containing 40, 400, 4000 or 9000 mg/kg feed for two years (equivalent to 0, 4.5, 45, 450 and 990 mg/kg bw/day for males and 0, 4.3, 43, 430 and 970 mg/kg bw/day for females). Females at 4000 and 9000 mg/kg feed showed increased water consumption, cataracts, and several uterine changes (inflammatory polyps, luminal dilatation and, at 9000 mg/kg feed only, haematomas and dilatation of blood vessels associated with thrombosis). Increased water consumption and inflammatory uterine polyps were also seen in females at 400 mg/kg feed. Male mice at 4000 and 9000 mg/kg feed showed cataracts, and a trend to an increase in diffuse hyperplasia of prostatic glandular tissue was observed. The NOEL in this study was 40 mg/kg feed, equivalent to 4.3 mg/kg bw/day.
11. Rats were given 50, 500, 10000 or 20000 mg fluazuron/kg feed for 2 years (equivalent to 0, 1.9, 18 380 and 780 mg/kg bw/day for males and 0, 2.1, 21, 440 and 920 mg/kg bw/day for females). Only in the top dose group some effects were observed at interim sacrifice after one year of treatment: decreased body weight development and relative liver and kidney weights in females, and minimal hypertrophy of hepatocytes in males. None of these effects were observed at termination of the study. The NOEL in this study was 20000 mg/kg feed, equal to 730 mg/kg bw/day.

It was concluded that fluazuron was not carcinogenic in these studies.

12. Benzylphenyl urea derivatives have demonstrated anti-fungal activity in some published studies. The proposed mode of action is likely to involve inhibition of chitin synthesis in the fungal cell wall. Apart from these anti-fungal properties fluazuron is not expected to have significant antimicrobial activity. The current MRL application is not intended for animals producing milk for human consumption. Therefore, no further information is required to determine whether residues may affect technological processes used in food processing.
13. On the basis of the lowest overall NOEL of 4.3 mg/kg bw/day for pathological changes in the uterus in the 2-year study in mice and a 100-fold safety factor, the ADI is established at 0.043 mg/kg bw (i.e. 2580 µg per day for a 60 kg person).

14. Subcutaneous administration of radiolabelled fluazuron to steer formed a depot at the injection site. Fluazuron was slowly released into the circulation, with a maximum in plasma reached after 48 hours, and an elimination half-life in plasma of 78 days. After release, fluazuron was mainly taken up by the adipose tissues and to a lesser extent by other tissues. Depletion of fluazuron from the tissues, which consisted mainly of unchanged fluazuron, was slow. Ultimately, fluazuron was partially (for about one third) metabolised into more polar metabolites. Sixteen weeks after administration 16 % of the administered dose was eliminated as unchanged fluazuron and 8% as its degradation products. The major route of elimination was the faeces (23% of the dose after 16 weeks), including bile, while renal excretion (1% of the dose after 16 weeks) was of minor importance. Although the fate of fluazuron in cattle was very similar to that in rats, the extent of metabolism appeared to be higher in rats compared to cattle.
15. When radiolabelled fluazuron was administered topically to cattle, it was slowly absorbed, either percutaneously, orally (by licking), or both. A steady state between absorption and elimination was observed for three to four weeks after treatment. The absorbed radiolabel was taken up mainly by adipose tissues and to a lesser extent by other tissues. Depletion of fluazuron from plasma and edible tissues was slow, with half-lives of elimination of 10.5 and 4.5 to 5.5 weeks, respectively. The major route of elimination was the faeces (62% of the dose after 16 weeks), while renal excretion was of minor importance (1% of the dose after 16 weeks). There was some indication of biliary excretion. Fluazuron was not extensively metabolized, as unchanged fluazuron generally accounted for more than 90% of the total residues in tissues and faeces. At the first time point (2 weeks), fluazuron accounted for 90 % of the total residues in liver, 99% in kidney, 97% in muscle, and 100% in fat. Comparing topical administration to subcutaneous administration, the pattern of metabolites excreted in faeces was somewhat more complex after subcutaneous administration (with about one-third of the fluazuron metabolised into more polar metabolites). Although the fate of fluazuron in rats was similar to that in cattle, they metabolised fluazuron to a greater extent than cattle.
16. Several residue studies with fluazuron on cattle were performed. In addition, some residue data were available from field trials. In all studies, the method of administration was in accordance with the recommended therapeutic use (1.5 to 2.5 mg/kg bw). In some studies higher doses were used, up to 4 mg/kg bw. In several studies, treatment was repeated after 12 weeks, the minimal recommended interval, or already after 9 weeks. Also the transfer of residues from treated dams to their calves via the milk was studied.

Four weeks after a single topical dose of 2 mg/kg bw, fluazuron concentrations were highest in fat (2.4 mg/kg). Lower residue concentrations were found in liver (0.10 mg/kg), kidney (0.07 mg/kg) and muscle (0.07 mg/kg). The residue concentrations in fat declined slowly to 0.5 mg/kg at 16 weeks after treatment. This residue pattern and depletion were confirmed by the other single dose studies. In general, the residue concentrations in fat were approximately ten times higher than in other tissues. There was no difference in the residue concentrations in subcutaneous fat from the application site and in fat from other locations (subcutaneous, renal or omental).

Six weeks after topical doses of 3 mg/kg bw given twice at an interval of 9 weeks, fluazuron concentrations were highest in fat (1.18 mg/kg). Lower residue concentrations were found in liver (0.09 mg/kg), kidney (0.04 mg/kg) and muscle (less than 0.04 mg/kg). The residue concentrations in fat declined slowly to 1.31 mg/kg at 16 weeks after treatment. This residue pattern and depletion were confirmed by the other repeated dose studies. In general, the residue concentrations in fat were several times higher than in other tissues. There was no difference in the residue concentrations in subcutaneous fat from the application site and in fat from other locations (subcutaneous or renal).

Six weeks after topical doses of 4 mg/kg bw given three times at intervals of 12 weeks, fluazuron concentrations in fat samples taken by biopsy were 2.1 to 3.0 mg/kg. The residue concentrations in plasma and in fat were less following the second treatment and less again following the third treatment.

Fluazuron was excreted via cows milk to calves, finally resulting in higher plasma and fat residue levels in calves than in the cows. Multiple treatments with 12-weeks intervals did not lead to accumulation of residues, although the residues following treatment in spring tended to be higher, may be due to grooming of the winter coats.

17. An HPLC-UV method was proposed for the routine monitoring of fluazuron in edible tissues of bovine species. The method was not described according to ISO 78/2 or other international format and was insufficiently validated with respect to specificity (including interference), accuracy and precision, limit of detection, linearity and stability. The limit of quantification was established provisionally at 20 µg/kg for muscle, liver and kidney and 10 µg/kg for fat. Despite of the deficiencies identified, the method can be considered suitable for monitoring of residues provisionally.
18. Fluazuron was evaluated by the 48th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The JECFA established the same ADI but rounded it to one significant figure according to their usual practice: 0-40 µg/kg bw. The following Codex MRLs were established for cattle: 7000 µg/kg in fat, 200 µg/kg in muscle, and 500 µg/kg in liver and kidney.

Conclusions and recommendations:

Having considered that:

- an ADI of 0.043 mg/kg bw, i.e. 2580 µg/person, was established for fluazuron,
- fluazuron is hardly metabolised, and was therefore selected as the marker residue,
- The ratio of marker to total residues in cattle tissues represent 97% in muscle, 100% in fat, 90% in liver, and 99% in kidney,
- residue concentrations of the marker residue were generally 10 times higher in fat than in other tissues,
- a routine analytical method for the determination of the marker residue in edible tissues of cattle is available that can provisionally be used for monitoring purposes until the outstanding issues relating to validation have been completed;

The Committee for Medicinal Products for Veterinary Use recommends the inclusion of fluazuron in Annex III of Council Regulation (EC) No. 2377/90, in accordance with the following table:

Pharmacologically active substance	Marker residue	Target animals	MRLs	Target tissues	Other provisions
Fluazuron	Fluazuron	Bovine	200 µg/kg 7000 µg/kg 500 µg/kg 500 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption Provisional MRLs expire on 1.1.2007

Based on these MRL values, the daily intake will represent about 19% of the ADI.

The recommended MRLs are the same as those established by Codex Alimentarius.

Before the Committee can consider the inclusion of these MRLs into Annex I of Council Regulation (EC) No. 2377/90, the issues included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should further validate the proposed routine analytical method in accordance with Volume 8 of the Rules Governing Medicinal Products in the European Union. The following aspects should be addressed:
 - specificity of the method in relation to metabolites and analogues of fluazuron and susceptibility to interference from use of other veterinary medicinal products,
 - substantiated data of accuracy and precision at half the MRL, the MRL and twice the MRL,
 - a substantiated limit of detection and quantification,
 - data about linearity,
 - data about stability of the method with regard to duration and circumstances of keeping samples of analyte,
 - full information on the description of the method in accordance with ISO 78/2 or other internationally recognised format.