

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUBENDAZOLE

SUMMARY REPORT (2)

1. Flubendazole is a benzimidazole anthelmintic. It is the fluoro- analogue of mebendazole and has many similar properties. It is administered orally to pigs, chicken, turkeys and game birds in the form of a paste, tablet, pellet or premix for incorporation into the feed at the following doses: pigs: 5 mg/kg bw (single administration) and 30 mg/kg feed for 10 days, equivalent to 1.2 mg/kg bw per day; poultry: up to 60 mg/kg feed for 7 days, equivalent to 5 mg/kg bw per day for broilers and 3.6 mg/kg bw per day for laying hens; game birds: 60 mg/kg feed for 7 days, equivalent to 2.7 mg/kg bw per day for pheasants, 3 mg/kg bw per day for partridges and 4 mg/kg bw per day for quails. It is currently entered into Annex III of Council Regulation (EEC) 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Flubendazole	Flubendazole	Poultry, game birds	500 µg/kg 200 µg/kg 400 µg/kg	Liver Muscle Eggs	Provisional MRLs expire on 1.1.1998
		Porcine	10 µg/kg	Muscle, liver, fat, kidney	

2. Flubendazole had low oral bioavailability in the rat, dog and the target species. In rats, the half-life for plasma elimination was around 6 hours. In all species, more than 50% of the administered dose was excreted in the faeces as unchanged flubendazole. The absorbed portion of the drug was rapidly metabolised so that concentrations of the parent drug in the blood and urine were very low. The urine contained a mixture of metabolites. The main metabolic pathways were the same in all the species studied and involved reduction of the ketone functional group and hydrolysis of the carbamate moiety.
3. Flubendazole was of low acute oral and subcutaneous toxicity. The acute oral LD₅₀ values were greater than 5000 mg/kg bw in mice, rats and guinea pigs. Acute subcutaneous LD₅₀ values were greater than 5000 mg/kg bw in the rat and the mouse and 4679 and 4834 mg/kg bw in male and female guinea pigs, respectively. The substance was more toxic when administered intraperitoneally with an acute intraperitoneal LD₅₀ of 528 and 434 mg/kg bw in male and female rats, respectively.
4. Dogs were given oral doses of 0, 2.5, 10 or 40 mg/kg bw per day of flubendazole, in gelatin capsules, 6 days per week, for 3 months. There were no adverse effects on behaviour, bodyweight, electrocardiogram, blood pressure, haematology, clinical chemistry or urinalysis values. At necropsy, some slight histopathological changes, which were difficult to interpret, were seen in both the male and female genital tracts. The slides were re-examined by an independent expert. The changes in females were considered to be within age-range normal limits and not related to treatment. It was agreed that the changes in males (prostatic fibrosis) were probably not treatment-related. However as a precautionary measure, and due to the absence of conclusive evidence regarding the aetiology of the findings, it was agreed that the dose level of 2.5 mg/kg bw per day should be regarded as a NOEL for the study.

5. In a 3-month study, Wistar rats were fed diets which provided approximate intakes of 0, 8, 30 or 130 mg/kg bw per day in males and 0, 9, 40 or 150 mg/kg bw per day in females. There were no adverse effects on mortality, behaviour, bodyweight gain, food intake, haematology, clinical chemistry or urinalysis values. There were no treatment-related gross- or histo-pathological findings.
6. Flubendazole was well-tolerated by the target species. No effects on fertility and no evidence of teratogenicity were observed in studies in pigs. Dietary concentrations of up to 180 mg/kg feed did not affect reproductive performance or egg quality in hens. 60 mg/kg feed had no effect on fertility in pheasants.
7. In a Segment I fertility study in Wistar rats, flubendazole was administered in the diet at doses equivalent to 0, 2.5, 10 and 40 mg/kg bw per day. Females were treated for 14 days and males for 60 days prior to mating. Untreated animals were mated with treated ones. The dams were killed on day 22 post-mating. There were no treatment-related effects on male or female fertility or on pregnancy rate, which was 100% in almost all groups. No embryotoxic, foetotoxic or teratogenic effects were observed. In a fertility study in dogs, oral doses of 99 mg/kg bw per day produced no effects on male or female fertility or reproductive performance.
8. In a Segment III peri- and post-natal study in Wistar rats, flubendazole was administered in the diet at doses equivalent to 0, 2.5, 10 and 40 mg/kg bw per day from day 16 of gestation and throughout lactation. In the 40 mg/kg bw group, 2 dams died and maternal bodyweight gain was significantly reduced. There was an increased incidence of stillbirths at 40 mg/kg bw. There were no effects on pup weight at birth, weight gain during lactation or pup survival. No grossly-malformed pups were found. The NOEL was 10 mg/kg bw per day.
9. There was no evidence of teratogenicity in a study in which oral doses of 20, 40 or 60 mg/kg bw per day were administered to pregnant Bourgogne rabbits. There was no evidence of teratogenicity in a study in which New Zealand White rabbits were given oral doses of 0, 10 or 40 mg/kg bw per day from days 6-18 of gestation.
10. Several teratology studies of variable quality were carried out in rats. In a study in which Sprague-Dawley rats were given daily oral doses of 0, 31.3, 46.9, 62.6 or 125.3 mg/kg bw per day from days 8-15 of gestation, there was an increased incidence of resorptions and malformations at 46.9 mg/kg bw and above; the study was inadequate because only 4-8 dams/group were employed. In a second, poorly-reported study, Sprague-Dawley rats were given daily oral doses of 20, 40 or 60 mg/kg bw per day from days 6-14 of gestation, the incidence of abortions was increased at 40 and 60 mg/kg bw and 23 (out of 443) foetuses in the 60 mg/kg bw group were malformed. The NOEL was 20 mg/kg bw per day. In a 1987 published study using material extracted from a commercial formulation, oral doses of 0, 2.5, 10, 40 or 160 mg/kg bw per day (as an aqueous suspension) were administered to Sprague-Dawley rats. Foetal weights were significantly reduced at 40 and 160 mg/kg bw per day and the incidence of resorptions was significantly increased at 160 mg/kg bw. The incidence of malformations was significantly increased in a dose-related manner at 40 and 160 mg/kg bw. The malformations included tail defects, anophthalmia/microphthalmia and hydrocephalus. The NOEL for teratogenicity in this study was 10 mg/kg bw per day. In contrast, no evidence of embryotoxicity or teratogenicity was found in 2 identical teratology studies in Wistar rats using different batches of flubendazole and dietary concentrations of flubendazole equivalent to 0, 2.5, 10 and 40 mg/kg bw per day. There was no evidence of teratogenicity in 2 further rat teratology studies: one study used oral doses of 0, 2.5, 10 or 40 mg/kg bw per day flubendazole suspended in water with *Tween*; the other employed administration in the diet at concentrations equivalent to 0, 10, 40 and 160 mg/kg bw per day.
11. Negative results were obtained in several *in vitro* assays for gene mutation in bacteria and yeasts, an *in vitro* assay for DNA damage, a sex-linked recessive lethal assay in *Drosophila melanogaster*, a dominant lethal assay in mice and *in vivo* micronucleus tests in rats and mice.

12. A carcinogenicity study was carried out in which Wistar rats were fed diets which provided the equivalent of 0, 5, 10 or 20 mg/kg bw per day flubendazole for 24 months. Swiss albino mice were fed diets which provided the equivalent of 0, 7.5, 15 or 30 mg/kg bw per day flubendazole for 18 months. There was no evidence of carcinogenicity in either study though both studies were marred by poor survival.
13. Flubendazole had no significant antimicrobial activity.
14. Flubendazole is used as an anthelmintic in human medicine. The usual dosage is 100 mg once or twice a day for 3 consecutive days. Several pharmacokinetic studies have been carried out in human volunteers. Peak concentrations of 0.35 ng/ml and 0.74 ng/ml were attained 1-4 hours after oral administration of a dose of 100 mg and 2000 mg respectively. Absorption was enhanced when the substance was administered after a meal; resulting in a C_{max} of 4.06 ng/ml after an oral dose of 2000 mg/kg. In another study in which humans were given an oral dose of 100 mg; more than 80% of the orally administered dose was recovered from the faeces and less than 0.1% in the urine within 3 days of dosing. No adverse effects of flubendazole were reported in these studies.
15. The WHO/FAO Joint Expert Committee on Food Additives (JECFA) calculated an ADI of 0-12 µg/kg bw per day by applying a safety factor of 200 to the NOEL of 2.5 mg/kg bw per day which was established in the 3-month study in dogs. The safety factor of 200 was used to take account of the fact that the doses were administered only 6 days per week. The ADI provided an approximately 1000-fold safety margin over the NOEL of 10 mg/kg bw per day which was established for teratogenicity in a published study in Sprague-Dawley rats. The CVMP agreed to adopt the same ADI as JECFA.
16. The biotransformation of flubendazole was extensive and followed similar metabolic pathways in pigs, chickens and turkeys. Ketoreduction to methyl[5-[(fluorophenyl)hydroxymethyl]-1 *H*-benzimidazol-2-yl]carbamate (also known as R038758 or M8) was the major metabolic pathway in chickens and turkeys. Carbamate hydrolysis to (2-amino-1 *H*-benzimidazole-5-yl)(4-fluorophenyl)methanone (also known as R034575 or M7) was the major metabolic pathway in pigs. Both these metabolites were later converted to 2-amino- α -(4-fluorophenyl)-1 *H*-benzimidazole-5-methanol (also known as R045198 or M6). Conjugation of R038758 and R045198 also occurred. It was noted that the metabolites retained the benzimidazole structure and were likely to have toxicological properties similar to those of flubendazole.
17. The metabolism of ^{14}C -labelled flubendazole was studied in pigs. The dose regime was designed to simulate practical treatment at 30 mg/kg in the feed for 5 days. Six hours after the end of treatment, the bound portion of the residues was 29% in liver, 20% in kidney, 10% in muscle and 11% in fat. Five days after administration, the bound fraction in liver had increased to 52%. During the period 5-30 days after treatment, approximately 50% of the residues in liver were bound. A similar increase was observed in the percentage of bound residues in kidney. Unmetabolised flubendazole accounted for around 1% of the total ^{14}C -labelled residues in liver and 1.7-2.6% of the residues in kidney. Six hours after treatment, residues of flubendazole in muscle and fat corresponded to 11.5% and 29% respectively. The metabolite R035475 was the major component of the residues accounting for 47%, 93.5%, 94% and 31% of the total residues in liver, kidney, muscle and fat, 6 hours after treatment. Ten days after treatment the percentage of R035475 declined to 18% and 23% of the total residues in liver and kidney respectively. The metabolite R045198 also accounted for 12%, 8%, 8% and 5% of the total residues in liver, kidney, muscle and fat, respectively, 6 hours after treatment. Residues of R038758 were low. In this study, mean total residues in tissues depleted from 3865 and 2678 µg/kg in liver and kidney respectively, 6 hours after treatment, to 529 and 78 µg/kg, 10 days after treatment. Mean total residues in muscle and fat, 6 hours after treatment, were 262 and 212 µg/kg respectively.

18. Three piglets were given feed containing 30 mg flubendazole/kg feed for 5 days. The piglets were killed at 16, 30 and 54 hours after treatment respectively. Residues of flubendazole in tissues were determined by HPLC. The limit of quantification (LOQ) was 10 µg/kg. Residues in all edible tissues were below the limit of quantification. In the same study, residues in 5 sows given feed containing 30 mg flubendazole/kg feed for 10 days could not be determined due to interference. Several other residues depletion studies confirmed that residues in pigs were low and were rapidly depleted. In a study in which piglets were given a single oral treatment of 5 mg/kg bw flubendazole and slaughtered in groups of 5, residues were determined using a radioimmunoassay with a limit of quantification of 5 µg/kg. The mean residues depleted from 120, 120, 70 and 96 µg/kg in liver, kidney, muscle and fat respectively, 24 hours after treatment, to 28, 24, 22 and 69 µg/kg, 72 hours after treatment.

19. The metabolism of ¹⁴C-labelled flubendazole was studied in laying hens. The birds were dosed at a rate equivalent to 60 mg/kg in the feed for 7 days. 79-86% of the residues in muscle, skin and fat samples, 24 hours after treatment, were extractable. At the same time point, only 49% of the residues in kidney and 61% of the residues in liver were extractable. At later time points, only around 30% of the residues in liver and kidney were extractable. Around 60% of the residues in omental fat and around 35% of the residues in skin/fat consisted of unmetabolised flubendazole. However, flubendazole accounted for less than 3% of the total residues in kidney and liver samples; these organs contained significant amounts of the metabolites R035475 and R038758 as well as some unidentified metabolites. In this study, mean total residues declined from 1500, 610, 30 and 68 µg/kg in liver, kidney, muscle and skin/fat respectively, 24 hours after dosing, to 241, 29, 3 and 12 µg/kg, 10 days after dosing. The results of this study were in agreement with those of an earlier study in which ¹⁴C-labelled flubendazole was administered at a rate equivalent to 30 mg/kg feed and residues were shown to be most persistent in liver.

20. One day after treatment, more than 80% of the residues in eggs were extractable. Flubendazole was the major component of the residues in eggs, accounting for 40% of the total residues. Detectable residues of the metabolites R035475 and R038758 were also present in eggs one day after treatment. Subsequent analysis of further egg samples obtained up to 9 days after withdrawal of treatment confirmed that the percentage of residues present as flubendazole remained constant.

21. In a residues depletion study, laying hens were fed diets containing 60 mg flubendazole/kg feed for 7 days. Six birds were killed 0, 7 and 28 days after treatment and residues of flubendazole in tissues were determined using HPLC. The limit of quantification was 10 µg/kg for all tissues. Mean residues in liver, kidney and muscle immediately after the end of treatment were 198, 173 and 79 µg/kg respectively. Residues at later time points were below the limit of quantification. Mean residues of flubendazole in eggs declined from 230 µg/kg to 118 µg/kg 7 days after treatment, to 13 µg/kg 11 days after treatment. The study was poorly reported and residues of metabolites were not monitored.

22. Residues were rapidly depleted in turkeys. Turkeys were fed diets containing 30 mg/kg feed flubendazole for 7 days. Three males and 3 females were killed at various time points and the residues of flubendazole and metabolites in tissues were determined using HPLC. The limits of quantification were: for flubendazole 10 µg/kg for all tissues; for R035475 and R0387586 25 µg/kg for liver and 10 µg/kg for other tissues; and for R045198 50 µg/kg for skin+fat and 10 µg/kg for other tissues. Six hours after the end of treatment, mean residues of flubendazole in liver, kidney, muscle and skin/fat were 64, 67, 18 and 60 µg/kg respectively. Six hours after the end of treatment, mean residues of R038758 in these tissues were 200, 80, 42 and 32 µg/kg respectively. At the same time point, low residues of R035475 and R045198 were found in liver and kidney. R045198 was also found in 1 skin/fat sample. One day after treatment, residues of flubendazole were found only in the skin/fat of 1 bird (11 µg/kg) and residues of R038758 were found only in the kidney of 1 bird (18 µg/kg). Residues in other tissues and residues at later time points were below the limits of quantification.

23. In pheasants, residues of flubendazole were rapidly depleted in all tissues and were most persistent in skin/fat. Pheasants were given feed containing 60 mg/kg feed for 7 days. Five males and 5 females were killed at various times and residues of flubendazole were determined using HPLC. The limit of quantification was 10 µg/kg. Six hours after the end of treatment, mean residues in liver, kidney and muscle were 35, 57.5 and 18.5 µg/kg respectively. One day after the end of treatment, residues were found only in 1 sample of liver (60 µg/kg), 1 sample of kidney (114 µg/kg) and 1 sample of muscle; residues in all other samples were below the limit of quantification. Mean residues in skin/fat samples declined from 76 µg/kg, 6 hours after treatment, to 29 µg/kg, 1 day after treatment, to 12 µg/kg, 7 days after treatment. There was no information on residue concentrations of metabolites in pheasants.
24. The CVMP noted that JECFA had elaborated MRLs for pigs and poultry with flubendazole as the marker residue. The CVMP had previously adopted provisional MRLs which were very similar to those proposed by JECFA. However the new information on metabolism and residues depletion in the target species indicated that flubendazole was not an appropriate marker residue because it comprised only a relatively small proportion of the residues in the tissues of pigs and poultry. Moreover the existing MRLs for poultry and game birds would result in consumer intake of total flubendazole-derived residues which considerably exceeded the ADI.
25. It was agreed that the marker residue for pigs should be redefined as the sum of flubendazole + the metabolite R035475 (M7) because the metabolite R035475 (M7) was the main component of the residues in pig tissues. Although R035475 was only a minor component of the residues in the tissues of birds, for the sake of consistency it was desirable to adopt the same marker residue for poultry and game birds. Flubendazole should be retained as the marker residue for eggs because flubendazole was the major component of the residues in eggs obtained up to 9 days after withdrawal of treatment.
26. The proposed routine analytical method, presented in the ISO format, was based on HPLC with UV detection and was validated for the edible tissues of chicken and pigs and for hens eggs. The limit of quantification for flubendazole was 10 µg/kg for all tissues and for eggs. The limit of quantification was 10 µg/kg for R035475 for muscle and skin/fat of pigs and chickens, 50 µg/kg for liver and kidney of pigs and 25 µg/kg for liver and kidney of chickens. The specificity of the method was acceptable and residues arising from the internal standard, mebendazole and fenbendazole were separated from those of flubendazole and R035475 on the chromatograms.

Conclusions and recommendation

Considering that:

- an ADI of 12 µg/kg bw (i.e. 720 µg/person) had been established for flubendazole,
- the major metabolites of flubendazole retained the benzimidazole structure and would have to be taken into account in estimating consumer intake,
- in pigs, 17% and 39% of the residues in liver and kidney were presented as the marker residue (flubendazole + R035475); 5 days after withdrawal of the substance around 100% and 60% of the residues in muscle and skin + fat were present as the marker residue 6 hours after withdrawal of the substance; no data were available for the latter tissues at the 5-day time point,
- approximately 40% of the residues in hens eggs were flubendazole for up to 9 days after withdrawal of the substance,
- a validated analytical method is available for monitoring purposes;

the Committee recommends the inclusion of flubendazole in Annex I in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Flubendazole	Sum of flubendazole and (2-amino-1 <i>H</i> -benzimidazole-5-yl)(4-fluorophenyl)-methanone	Porcine, chicken, game birds	50 µg/kg 50 µg/kg 400 µg/kg 300 µg/kg	Muscle Skin + fat Liver Kidney	
	Flubendazole	Chicken	400 µg/kg	Eggs	

Based on the above MRLs, it was calculated that the consumer intake of total flubendazole-related residues from the consumption of eggs (100 µg) and pig meat (352 µg) per day was 452 µg, which represents 63% of the ADI.