European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

FLUBENDAZOLE (extrapolation to poultry)

SUMMARY REPORT (4)

1. Flubendazole is a benzimidazole anthelmintic. It is the fluoro- analogue of mebendazole and has many similar properties. Is administered orally to pigs, chickens and game birds.

   Flubendazole is currently entered into Annex I of Council Regulation (EEC) No. 2377/90 for turkey, chicken, game birds and porcine species as follows:

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

2. A request was submitted to the EMEA for the extrapolation of the existing entry in Annex I of Council Regulation (EEC) No. 2377/90 for turkey, chicken and game birds. The scientific justification for this extension was assessed taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL). Based on the approach explained in this guideline the CVMP considered whether the extrapolation to poultry would be possible.

3. In setting the ADI in the original assessment of flubendazole, the data summarised in the paragraphs below were considered:

4. 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.

5. 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.

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6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

15. Flubendazole had no significant antimicrobial activity.

16. 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 to 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 Cmax 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.

17. 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.

18. For the extrapolation to include all poultry species in Annex I of Council Regulation No 2377/90 the information summarised in the paragraphs bellow was taken into account.

19. The biotransformation of flubendazole was extensive and followed similar metabolic pathways in pigs, chickens and turkeys. Ketoreduction to methyl[5-[(fluorophenyl)hydroxymethyl]-1H-benzimidazol-2-yl]carbamate (also known as R038758 or M8) was the major metabolic pathway in chickens and turkeys. Carbamate hydrolysis to (2-amino-1H-benzimidazole-5-yl)(4-fluorophenyl)methanone (also know as R034575 or M7) was the major metabolic pathway in pigs. Both these metabolites were later converted to 2-amino-α-(4-fluorophenyl)-1H-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.

20. Data on the pharmacokinetics and metabolism of flubendazole were provided for laying hens. Following oral administration of ^{14}C-flubendazole at a dose equivalent to 60 mg/kg feed for 7 consecutive days, absorption was rapid. A C_{max} value of 0.24 µg equivalents/ml was obtained 4 hours after the initial dose. A slightly higher C_{max} value of 0.28 µg equivalents/ml was obtained approximately 5 hours after the 7th dose. There was no evidence of bioaccumulation with around 90% of the administered dose eliminated in excreta each day. Seventy nine to eighty six percent 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. Twenty-four hours after the last dose, approximately 60% of the residues in omental fat and 35% of the residues in skin + fat consisted of unmetabolised flubendazole.

However, flubendazole accounted for less than 3% of the total residues in liver and kidney and these organs contained residues of the metabolites (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone (7.9% and 5.8% of the residues in liver and kidney) and methyl[5-(fluorophenyl)hydroxymethyl]-1*H*-benzimidazole-2-yl]carbamate (5.3% and 1.4% of the residues in liver and kidney). An *in vitro* metabolism study using chicken and turkey hepatocytes confirmed that ketoreduction to methyl[5-(fluorophenyl)hydroxymethyl]-1*H*-benzimidazol-2-yl]carbamate was the major metabolic pathway in both species. 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 ^{14}C-labelled flubendazole was administered at a rate equivalent to 30 mg/kg feed and residues were shown to be most persistent in liver.

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 (2-amino-1H-benzimidazole-5-yl) (4-fluorophenyl)-methanone and methyl[5-[(fluorophenyl)hydroxymethyl]-1H-benzimidazol-2-yl]carbamate 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. Turkeys were fed diets containing flubendazole at 30 mg/kg feed for 7 days. Three male birds and three female birds were killed at 6 hours, one day, 3 days, 5 days, 7 days and 9 days after the end of treatment. Residues of flubendazole and its metabolites in tissues were determined using HPLC. The limits of quantification were: for flubendazole: 10 µg/kg for all tissues; for (2-amino-1H-benzimidazole-5-yl)(4-fluorophenyl)methanone and methyl[5-(fluorophenyl)hydroxymethyl]-1H-benzimidazol-2-yl]carbamate: 25 µg/kg for liver and 10 µg/kg for other tissues; and for 2-amino-alpha-(4-fluorophenyl)-1H-benzimidazole-5-methanol: 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 methyl[5-(fluorophenyl)hydroxymethyl]-1H-benzimidazol-2-yl]carbamate in these tissues were 200, 80, 42 and 32 µg/kg respectively. At the same time point, low residues of (2-amino-1H-benzimidazole-5-yl) (4-fluorophenyl)methanone were found in liver (29 µg/kg) and kidney (11 µg/kg) but not in muscle or skin + fat. At six hours low residues of 2-amino-alpha-(4-fluorophenyl)-1H-benzimidazole-5-methanol were found in kidney (10 µg/kg) and in liver but an interfering peak prevented the quantification of the residues in liver; residues of (2-amino-α-(4-fluorophenyl)-1H-benzimidazole-5-methanol were undetectable in muscle and skin + fat. One day after the end of treatment, detectable residues of flubendazole were found only in a sample of skin + fat from one bird (11 µg/kg) and detectable residues of methyl[5-(fluorophenyl) hydroxymethyl]-1H-benzimidazol-2-yl]carbamate were found only in the kidney of one bird (18 µg/kg); residues in other tissues and residues at all later time-points were below the respective 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. However, the CVMP considered that, the information on metabolism and residues depletion in the target species indicated that flubendazole was not the appropriate marker residue because it comprised only a relatively small proportion of the residues in the tissues of pigs and poultry.

    For pigs, the CVMP agreed that the marker residue should be redefined as the sum of flubendazole plus the metabolite (2-amino-1*H*-benzimidazole-5-yl) (4-fluorophenyl)-methanone (R035475 (M7)) because the metabolite was the main component of the residues in pig tissues. Although (2-amino-1*H*-benzimidazole-5-yl) (4-fluorophenyl)-methanone was only a minor component of the residues in the tissues of birds it was considered desirable to have the same marker residue for tissues of chicken and pigs. Flubendazole was retained as the marker residue for chicken eggs considering that approximately 40% of the residues in chicken eggs were flubendazole for up to 9 days after the end of the administration of the substance.

25. In accordance with the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) the sum of flubendazole and (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone was retained as the marker residue for poultry tissues. According to the same guideline, the chicken marker residue (flubendazole) and MRL values were considered appropriate for poultry eggs.

26. A validated analytical method for the determination of residues of flubendazole and the metabolite (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone in the edible tissues of chicken and turkeys and chicken eggs was provided. The method, presented in the ISO format 78/2, was based on HPLC with UV detection. The limit of quantification was 10 µg/kg for (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone for muscle and skin + fat and 25 µg/kg for liver and kidney of chickens. The limit of quantification for flubendazole was 10 µg/kg for all tissues of chicken and for eggs. For turkey, the limit of quantification was 100 µg/kg liver, 75 µg/kg for kidney and 10 µg/kg for muscle and skin + fat for both metabolites. The specificity of the method was acceptable and residues arising from the internal standard, mebendazole and fenbendazole were separated from those of flubendazole and (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone on the chromatograms. This method should be applicable to other poultry species and therefore from this aspect extrapolation to the tissues and eggs of other poultry species is possible.

Conclusions and recommendation

Having considered that:

- an ADI of 12 µg/kg bw (i.e. 720 µg/person) was previously established for flubendazole,
- the sum of flubendazole and (2-amino-1*H*-benzimidazole-5-yl)(4-fluorophenyl)-methanone was retained as the marker residue for poultry tissues,
- flubendazole was retained as the marker residue for eggs,
- MRLs were previously established in chicken, turkey and game birds; these MRLs are identical,
- an analytical method HPLC based, which had been validated for the edible tissues of chicken and turkey and chicken eggs was available which should be applicable to all poultry species;

the Committee for Veterinary Medicinal Products recommends the modification of the current entry for flubendazole for chicken, turkey and game birds 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
Flubendazole	Sum of flubendazole and (2-amino-1*H*-benzimidazole-5-yl) (4-fluorophenyl)-methanone	Poultry	50 µg/kg 50 µg/kg 400 µg/kg 300 µg/kg	Muscle Skin + fat Liver Kidney	
	Flubendazole	Poultry	400 µg/kg	Eggs	

Based on the above MRLs, the daily intake of total residues from edible tissues of poultry and eggs will represent approximately 80% of the ADI.

MRLs for flubendazole are also established for pigs, which are however, not part of this extrapolation and remain therefore unchanged.