DICYCLANIL

First draft prepared by
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IDENTITY

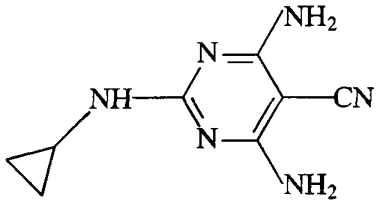
Chemical name: 4,6- diamino-2-(cyclopropylamino)-5-pyrimidinecarbonitrile
Chemical Abstract Service (CAS): CAS number:112636-83-6

4,6-diamino-2-cyclopropylaminopyrimidine-5-carbonitrile
International Union of Pure and Applied Chemistry (IUPAC) name:

International Non-Proprietary Name (INN): DICYCLANIL

Synonyms: Clik, A-9568 B, CGA 183893

Structural formula:



Molecular formula: $C_8H_{10}N_6$
Molecular weight: 190.2

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Dicyclanil
Appearance: White crystalline powder.
Melting point: 250.5-252.4 °C with thermal decomposition
Thermal stability: >150 °C
Solubility: 4900 mg/L in methanol, 1400 mg/L in ethyl acetate, 1200 mg/L in acetone, 320 mg/L in octanol, 610 mg/L in buffer solution at pH 5.0 and 350 mg/L water at pH 7.0. Poorly soluble in hexane, toluene and dichloromethane.
Dissociation constant: pKa = 4.58 (basic)
Ultraviolet maxima: Not reported.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use
Dicyclanil is an ectoparasiticide of the aminopyrimidine group used for the control of myiasis and fly-strike in sheep. Dicyclanil is sold as a pour-on formulation containing 5% (w/v) of the compound. Data were provided for use in sheep only.

Dosage

Dicyclanil is applied as a single, seasonal spray-on treatment using an applicator gun provided with the drug. The recommended use is approximately 1-2 ml of the formulated product (5g dicyclanil/100 ml) per kg body weight according to the following guide:

	Body weight of the sheep	
(kg)	ml	ml/kg
10-20	20	2.0-1.0
21-30	25	1.2-0.8
31-50	30	1.0-0.6
>50	35	0.7

According to these instructions the maximal administered amount of the active compound is 1.75 g/animal while the maximal dose is 0.1 g dicyclanil/kg body weight.

METABOLISM

Laboratory animals

Rats

Dicyclanil metabolism was investigated in 2 studies in which ^{14}C-labeled dicyclanil (5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine) was administered orally to rats (Hassler, 1994) and (Thanei, 1996). The [2-^{14}C]pyrimidyl labeled CGA 183893 was given to the rats orally by a stomach tube at two different doses (20 mg/kg and 0.5 mg/kg) for 7 consecutive days. The rats weighed 200 g at the initiation of the study. Four study groups were used consisting of 6 animals (3 males and 3 females) each. More than 80% of the administered dose was absorbed and 24 hours after the last of the 7 doses, radioactivity was recorded in all tissues. The radioactivity was recovered mainly in urine and feces representing 79-85% and 6-13%, respectively. Consequently, 24 hours after the last of the 7 administrations 93-96% of the total radioactivity was recovered. By use of thin layer TLC) and high performance liquid chromatography (HPLC) a total of 12 metabolite fractions were separated from urine, feces and tissues. No sex or dose dependency was observed. One of the urinary fractions represented 50% of the given dose and was the major metabolic product of CGA 183893.

In the second study (Thanei, 1996) identification of the metabolites was attempted. Approximately 20 mg/kg was given to the test animals and samples were obtained from 4 rats (2 male and 2 female). Urine samples were collected from 4 rats over a period of 168 h and pooled for analysis. Fecal samples from the 2 male rats and the 2 female rats were also pooled for analysis. The rat liver tissue 24 hours after the last dose were obtained for analysis. For the identification of the metabolites, ^{1}H-NMR, IR and mass spectrometry were used. The following compounds were characterized:
N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U),
5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893),
2-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-3-hydroxy-propionic acid (MET 3U),
2-4,6-triamino-pyrimidine-5-carbonitrile (MET 4U = CGA 297107),
3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid (MET 5U)

Biotransformation was initiated by oxidative cyclopropyl-ring opening followed by further oxidation. Biotransformation was limited to the cyclopropyl-ring while the cyano group is metabolically stable. The mode of action of compounds containing cyclopropyl group has been studied at length, indicating interaction with P450 enzymes as a possible mode of action. The reaction mechanism most frequently involves cyclopropyl ring opening resulting in free radicals. Inhibition of redox reactions of enzyme systems has been associated to these groups. The most significant route was the conversion of the CGA 183893 to MET U1 consisting of 50% of the administered dose.

Food producing animals

Sheep

In a study by Phillips (1996), specimens from male and female sheep, treated with a pour-on formulation containing radiolabeled [^{14}C-pyrimidine] dicyclanil, from an earlier study were used. The urine, feces, bile, wool, fat, muscle, liver, and kidney samples were pooled. Thin layer chromatography (TLC) was used for characterization of the metabolites.

One and two-dimensional TLC were performed. The portion of the dose remaining in the wool was investigated at 3 and 21 days after treatment. The wool contained mainly unchanged parent compound (CGA 183893) but low levels of CGA 297107 and an unknown component were also detected.

In urine, collected for 48 hours after administration and representing <1% of the total dose, CGA 183893 and CGA 297107 accounted for 63 and 6% of the urine radioactivity, respectively. Six additional unidentified components were also detected of which 3 accounted for <1% of radioactivity and the 3 others for 10%, 7% and 1%. Less than 1% of the total radioactivity was detected in the pooled feces from samples collected during 48 hours post-administration. Exhaustive extraction recovered 91% of the fecal radioactivity. Unchanged CGA 183893 accounted for 72% and CGA 297107 for 2% of the combined extracts. The residuals consisted of polar material and unresolved radioactivity. The bile contained polar components and unresolved components.

Extractable radiolabelled compounds from fat and muscle tissue was high (over 90%), containing mainly the parent compound in the subcutaneous fat and the parent compound and unresolved matter in the peritoneal fat and muscle. The extractability from liver was 20-31% and subsequent soxhlet extraction released an additional 17-24%. The TLC analysis of the extractable substance was inconclusive due to the extremely low levels of radioactivity. From kidney 58-72% was extracted with additional 14-20% following soxhlet extraction. As with the liver, the TLC analysis was inconclusive due to low radioactivity present.

The extractable radiolabelled residues were high at all time points from muscle and fat while the extractability from liver and kidney was less. The major residue in fat and muscle tissues was the parent compound (CGA 183893). In liver and kidney most of the extractable residues were characterized as unresolved CGA183893 and an unknown compound. CGA 297107 was identified in a kidney extract.

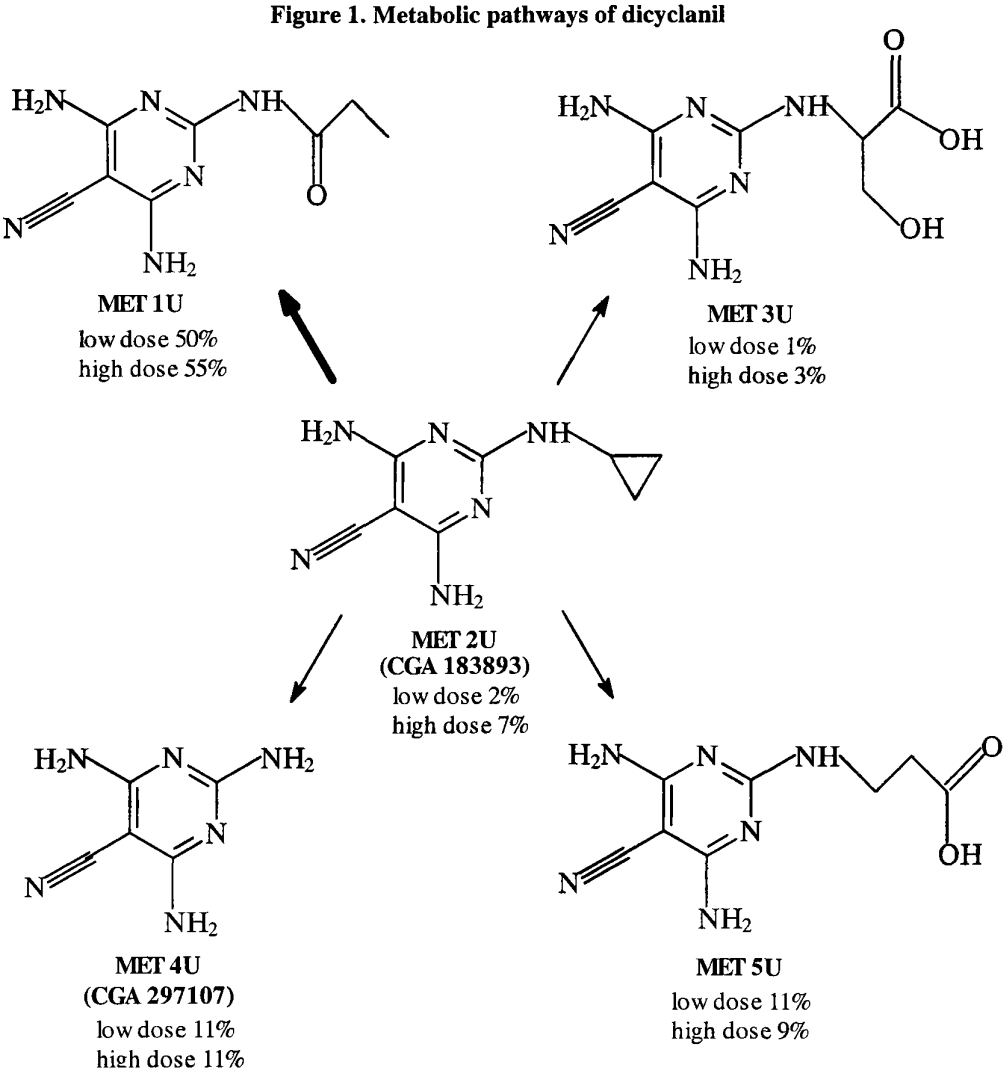
Thanei (1996a) studied the metabolic pattern of dicyclanil in sheep utilizing TLC and HPLC techniques. Also in this study, urine, feces, bile, dose run-off, wool, fat, muscle, liver and kidney samples obtained from another study were utilized. About 40-60% of the administered dose was found in the run-off during the first hour after administration. The run-off contained essentially only CGA 183893 except for traces of CGA 287107. The same substances in the same proportions were found in the wool 1 and 14 days post-administration. Less than 2% of the dose retained on the skin was recovered in urine and faeces. In urine 5 fractions could be distinguished: N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U), 5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893), 2-4,6-triamino-pyrimidine-5-carbonitrile (MET 4U = CGA 297107), 3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid (MET 5U) and a polar unidentified fraction that released CGA 297107 following microwave treatment.

The extractability of radiolabelled products was about 90% from feces and consisted almost entirely of CGA 183893. In bile the majority of radioactivity appeared to be associated with very polar metabolites. However, unchanged CGA 183893 and CGA 297197 were also found.

Extractability of the radioactivity from adipose and muscle tissue was almost 100% and the major component in these tissues was the unchanged CGA 183893. CGA 287107 was present in low amounts in these tissues as well as the MET 1U in muscle tissue. In kidney and liver tissues, extractability decreased as a function of time. In the kidney the initial extractability was 90% but decreased to 50% in 14 days. In the liver 40-60% of the radioactivity was extractable and an additional 20% could be extracted under harsh conditions. The extractable metabolites had a half-life of about 1 day. The concentration of non-extractable residues was 0.006 and 0.002 mg/kg in kidney and liver, respectively.

The metabolic pathways in sheep were essentially the same as found in rats. The metabolic conversion going through cyclopropyl-ring opening and oxidation of the α-carbon to a secondary propionic acid amide (MET 1U) or cyclopropyl-ring opening and oxidation to a β-alanine-derivative (MET 5U) and dealkylation to descyclopropyl CGA 183893 (MET 4U = CGA 297107). The proposed dicyclanil metabolic pathways are presented in Figure 1.

Figure 1. Metabolic pathways of dicyclanil



TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies in sheep

Three sheep studies were reported with target animals using a radiolabeled dicyclanil formulation typical of those used in current veterinary applications (Gifford and Dunsire, 1994, Thanei, 1996a and Anderson and Speirs, 1998).

In one study (Gifford and Dunsire, 1994) the ^{14}C-dicyclanil was administered by use of the jetting technique (the other studies used a pour-on formulation). With the jetting technique, 35 mg dicyclanil/kg was applied as a single dose to Oxford Down sheep. Approximately 37-59% of the total dose remained on the animals while the rest could be recovered as "run-off". Dermal absorption was estimated as 2% based on the radioactivity retained on the animal. The absorption was rapid and the maximum whole blood concentration appeared 4-6 hours after the drug administration. During 7 days following the drug administration, 0.83% and 1.05% of the retained dose was recovered in urine and feces, respectively. The ^{14}C-label compounds recovered in the urine were the parent compound and 4 metabolites of which each fraction consisted of less than 0.2%. The fecal excretion was predominantly the parent compound. Considerable radioactivity could be recovered from the wool. The radioactivity in the wool did not decrease notably with time (Gifford and Dunsire, 1994 and Thanei 1996a)

The second study was carried out by administering the ^{14}C-dicyclanil as a pour-on to Grayface sheep at 35 mg dicyclanil/kg (MacLean and Dunsire, 1996). In this study the maximum blood concentration was reached at 12-48 hours and the absorption was slower than in the previous study. The absorption of the administered dose over a period of 7

days was 4%, twice as much after the pour-on administration when compared to the administration by jetting. This study also indicated continuous dermal absorption, something not observed in the previous study. In this study the radioactivity was recovered in urine and feces. The metabolic pattern generally agreed with the previous study although the ratio of the metabolites differed from the earlier studies (McLean and Dunsire, 1996 and Phillips, 1996).

The third study was carried out by administering the ^{14}C-dicyclanil as a pour-on to Dorset sheep at 100 mg dicyclanil/kg (Anderson and Speirs, 1998). Moderate run-off was noticed. Radioactivity was detected in the systemic circulation at 2 hours and the peak concentrations at 24 after administration. The absorption over 21 days was about 7% of the administered dose. Biphasic excretion of the radioactivity was observed. The decline of radioactivity could be characterized by a half-life of approximately 2 days during the period of 24-120 hours post-administration. Radioactivity was widely distributed to the body and the highest radioactivity count was recorded in the subcutaneous fat under the application area. From day 7 to day 21 the radioactivity decreased most in blood and muscle and least in omental and subcutaneous fat.

Other residue depletion studies in sheep

All together eight studies were performed in sheep. Three of these studies were GLP non-compliant and the remaining five studies were done in accordance to the GLP standards. Different parameters were investigated including formulation, dose, application method and wool length. Furthermore, the studies were not identical in terms of compounds (parent and metabolites) analyzed.

Dicyclanil was applied by jetting the formulation in 3 liters per animal (1.5g) at 29 to 44 mg/kg body weight onto 8 Merino sheep with wool length of 2-3 cm (Strong and Kearney, 1992) Tissue from two animals was collected at 2, 7, 14 and 28 days after application. Muscle, fat, liver and kidney tissues were analyzed for the presence of the parent compound. No residues exceeding 10 µg/kg were detected in muscle, liver or kidney tissues (20 µg/kg in kidney). A dicyclanil residue of 20 µg/kg was found in one fat tissue sample at 7 days and another at 14 days post application. Deposits of 1200-2500 mg/kg of dicyclanil were measured in the wool. Results are tabulated in Table 1.

Table 1. Dicyclanil residues (mg/kg) in sheep tissues after 1.5 g topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
2	<0.01	<0.01	<0.02	<0.01
7	<0.01	<0.01	<0.02	<0.02
14	<0.01	<0.01	<0.02	<0.02
28	<0.01	<0.01	<0.02	<0.01

The second study involved 15 Merino wethers, shorn 2 weeks before treatment (Bull, 1995). The sheep were divided to 5 groups of 3 animals each. The sheep (mean ±SD) in the groups were from 55 ±6 to 60 ±7 kg. A 2.5-3 times the label dose of 90 mg/kg body weight was given as a topical application of 1.8 ml/kg of a pour-on formulation. Animals per time point were sacrificed at 3, 7, 14 and 21 days after application. Three untreated control animals were sacrificed before the first group of treated animals. Hind leg muscle, kidney fat, liver and kidney tissue samples were analyzed for the presence of the parent compound. The highest residue concentrations were found in muscle (0.93 mg/kg), in liver and in kidney (1.4 mg/kg) 7 days after the treatment; in fat tissue, 1.89 mg/kg was observed at 3 days after treatment. The dicyclanil residues decreased to ≤0.30 mg/kg by day 14 but apparently increased again by day 21 (Table 2). No explanation was provided for the day 14 versus day 21 results.

Table 2. Mean dicyclanil residues (mg/kg) in sheep after 90 mg/kg topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
3	0.75	0.98	0.66	0.92
7	0.71	0.90	0.82	0.36
14	0.12	0.13	0.18	0.18
21	0.58	0.68	0.57	0.20

The third study involved 23 Merino wethers, shorn 2 weeks before treatment (Smal and Adams, 1995). The animals were divided to 6 groups of 3 sheep. The mean body weight in the groups ranged from 53.0 to 54.0 kg. The two

sheep with the lowest body weight (mean = 39.4kg) were allocated as the untreated control animals. A dose of 45 mg/kg body weight was given as a topical application of 0.9 ml/kg of a pour-on formulation. Three animals per time point were sacrificed at 3, 7, 14, 21, 28, 35 and 42 days after application. Three control animals were sacrificed before the first group of the treated animals. Hind leg muscle, kidney fat, liver and kidney tissue samples were analyzed for dicyclanil . The highest residue concentrations were found in muscle (0.16 mg/kg), in liver (0.33 mg/kg) and in kidney (0.21 mg/kg) 21 days after the treatment. The highest concentration in fat tissue (0.67 mg/kg) was also observed at 21 days after treatment. The residues of dicyclanil decreased to <0.0.1 mg/kg by day 42 after treatment in muscle and liver tissue (Table 3).

Table 3. Mean dicyclanil residues (mg/kg) in sheep with 45 mg/kg topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
7	0.09	0.14	0.09	0.17
14	0.06	0.08	0.06	0.26
21	0.10	0.14	0.12	0.64
28	0.09	0.10	0.08	0.05
35	0.04	<0.03	<0.04	0.09
42	<0.01	<0.01	<0.04	0.07

In the forth study 78 lambs (2nd cross: 25% Merino, 25% Border Leicester and 50% Dorset), containing equal number of wethers and ewes, were used (Smal *et al.*, 1996). The animals were ranked by sex and weight and divided to light, medium and heavy groups. From each sub-group, animals were randomly assigned to 2 treatment groups of 3 animals to include one lamb from each weight group. The mean body weight in the groups ranged from 40.7 to 41.7 kg. The control sheep, containing 3 males and 3 females, had a mean body weight of 44.2 kg. A dose of 2 ml/kg or 4 ml/kg, was given as a topical pour-on formulation using an applicator gun. Three animals per time point from each treatment group were sacrificed at 3, 7, 14, 21, 28 and 35 days after application. Three control animals were sacrificed before the first slaughter of the treated animals. Equal mix of tenderloin and hindquarter muscle, kidney and associated fat, liver and subcutaneous fat (from the application area) were analyzed for the parent compound and the major metabolite. Results are tabulated in Table 4a-4d. The highest residue concentrations of parent and metabolite, respectively, were: in muscle (0.18 and 0.08 mg/kg), liver (0.29 and 0.26 mg/kg), kidney (0.25 and 0.37 mg/kg) and adjoining (0.03 and <0.01 mg/kg) and subcutaneous (0.05 and 0.01 mg/kg) fat samples 14 days after the treatment with the lower dose. The highest residue concentrations, after administration of the higher dose, of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.17 and 0.09 mg/kg), liver (0.28 and 0.24 mg/kg), kidney (0.26 and 0.20 mg/kg) and adjoining (0.04 and 0.02 mg/kg) and subcutaneous (0.06 and <0.01 mg/kg) fat 3 days after the treatment. The highest concentration of the major metabolite in muscle tissue (0.10 mg/kg) was found at 21 days after treatment.

Table 4a. Mean dicyclanil concentrations (mg/kg) in sheep after topical administration at 45 mg/kg.

Withdrawal time, (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.01	<0.01	<0.01	<0.01	<0.01
7	<0.01	<0.02	<0.02	<0.01	<0.01
14	<0.04	<0.06	<0.05	<0.02	<0.01
21	<0.01	<0.01	<0.01	<0.01	<0.01
28	<0.01	<0.01	<0.01	<0.01	<0.01
35	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4b. Mean dicyclanil concentrations (mg/kg) in sheep after topical administration at 90 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.05	<0.07	<0.07	<0.03	<0.02
7	<0.01	<0.02	<0.02	<0.01	<0.01
14	<0.02	<0.01	<0.01	<0.01	<0.01
21	<0.03	<0.03	<0.03	<0.01	<0.01
28	<0.03	<0.03	<0.03	<0.01	<0.01
35	<0.01	<0.02	<0.02	<0.01	<0.01

Table 4c. Mean CGA 287107 concentrations in sheep (mg/kg) after dicyclanil topical administration at 45 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.02	0.04	0.04	<0.01	<0.01
7	<0.02	0.05	0.06	<0.01	<0.01
14	<0.03	0.07	0.11	<0.01	<0.01
21	<0.01	0.03	0.04	<0.01	<0.01
28	<0.01	<0.03	0.04	<0.01	<0.01
35	<0.01	<0.02	<0.02	<0.01	<0.01

Table 4d. Mean CGA 287107 concentrations in sheep (mg/kg) after topical administration of dicyclanil at 90 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.04	0.12	0.10	<0.01	<0.01
7	0.03	0.08	0.08	<0.01	<0.01
14	0.02	0.05	0.06	<0.01	<0.01
21	0.04	0.07	0.07	<0.01	<0.01
28	<0.02	0.08	0.08	<0.01	<0.01
35	<0.01	0.05	0.05	<0.01	<0.01

The fifth study used 43 Merino wethers and 41 Merino ewes (Peterson and George, 1997). The animals were assigned by weight to 4 groups. Two groups were treated immediately after shearing with normal and high doses and 2 other groups were dosed six weeks after shearing using similar treatments. Five slaughter groups for each of the 4 treatment groups and consisting of 2 males and 2 females each were generated. The mean body weight was 43-47 kg, 45-46 kg, 51-54 kg and 53-55 kg in the four treatment groups, respectively. A dose of 2 ml/kg and 4 ml/kg, was given as a topical application over the backline as a pour-on formulation using an applicator gun. Three animals per time point from each treatment group were sacrificed at 7, 14, 21, 28 and 58 days post treatment. Four untreated control animals representing the 3 heaviest males and the heaviest female were sacrificed before the first slaughter of the treated animals. Tenderloin and hindquarter muscle, kidney, renal fat, liver and subcutaneous fat (from the application area) were analyzed for the presence of the parent compound and the major metabolite. Results are summarized in Tables 5a-5h.

In the group receiving the lower dose immediately after shearing the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.76 and 0.19 mg/kg), liver (1.13 and 0.36 mg/kg), kidney (0.20 and 0.50 mg/kg), renal fat (0.13 and 0.03) and subcutaneous fat (0.28 and 0.06 mg/kg) samples 7 days after the treatment except for diclycnil residues in subcutaneous fat and the metabolite residues in renal fat at 14 days post-administration.

In the group receiving twice the lower dose immediately after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (1.18 and 0.56 mg/kg), liver (1.83 and 2.59 mg/kg), kidney (1.58 and 0.63 mg/kg), renal fat (0.20 and 0.06) and subcutaneous fat (3.29 and 0.07 mg/kg) samples 7 days after the treatment except for the diclycnil residues in subcutaneous fat in a sample obtained 14 days post-administration.

In the group receiving the lower dose 6 weeks after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.32 and 0.13 mg/kg), liver (0.45 and 0.24 mg/kg), kidney (0.36 and 0.30 mg/kg), renal fat (0.08 and 0.01) and subcutaneous fat (0.62 and 0.02 mg/kg) samples 14 days after the treatment except for the metabolite residues in muscle and kidney at 7 days post-administration and in the subcutaneous fat in a sample obtained 28 days post-administration.

In the group receiving the higher dose 6 weeks after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.95 and 0.41 mg/kg), liver (1.38 and 0.68 mg/kg), kidney (1.22 and 0.98 mg/kg), renal fat (0.11 and 0.07) and subcutaneous fat (3.86 and 0.08 mg/kg) in samples

7 days after the treatment except for the metabolite residues in kidney, in renal fat and in the subcutaneous fat measured in samples at 14 days post-administration.

Table 5a. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 2 ml/kg topical dose immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.32	0.42	0.35	0.08	0.04
14	0.12	0.12	0.08	0.10	0.02
21	0.03	0.04	0.02	0.01	0.01
28	0.02	0.02	0.01	0.01	<0.01
56	0.05	0.08	0.04	0.04	0.01

Table 5b. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 4 ml/kg topical dose immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous Fat	Renal fat
7	0.80	1.21	0.94	0.24	0.16
14	0.34	0.46	0.33	0.89	0.06
21	0.20	0.32	0.22	0.05	0.03
28	0.14	0.22	0.18	0.04	0.03
56	0.02	0.02	0.02	0.02	<0.01

Table 5c. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous Fat	Renal fat
7	0.18	0.24	0.20	0.04	0.02
14	0.13	0.18	0.14	0.21	0.03
21	0.05	0.07	0.05	0.03	0.01
28	0.05	0.05	0.04	0.12	0.01
56	0.02	0.02	0.04	<0.01	<0.01

Table 5d. Mean dicyclanil concentrations in sheep (mg/kg) treated with a 4 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.58	0.81	0.73	0.20	0.08
14	0.40	0.59	0.43	1.46	0.09
21	0.24	0.39	0.33	0.08	0.05
28	0.18	0.22	0.16	0.03	0.03
56	0.10	0.20	0.02	0.03	0.02

Table 5e. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 2 ml/kg topical dose of dicyclanil immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.12	0.24	0.39	0.03	0.01
14	0.07	0.14	0.34	0.02	0.01
21	0.04	0.11	0.11	0.01	0.01
28	0.02	0.08	0.10	0.01	<0.01
56	0.03	0.06	0.06	0.01	<0.01

Table 5f. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 4 ml/kg topical dose of dicyclanil immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.48	0.49	0.41	0.05	0.04
14	0.11	0.23	0.24	0.03	0.01
21	0.12	0.37	0.34	0.03	0.01
28	0.10	0.18	0.26	0.02	0.01
56	0.03	0.08	0.07	<0.01	<0.01

Table 5g. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.10	0.15	0.23	0.01	<0.01
14	0.07	0.15	0.16	0.01	<0.01
21	0.04	0.09	0.13	0.01	0.01
28	0.03	0.08	0.08	0.01	<0.01
56	0.01	0.03	0.05	<0.01	<0.01

Table 5h. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 4 ml/kg dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.25	0.44	0.46	0.03	0.01
14	0.20	0.37	0.48	0.04	0.03
21	0.08	0.28	0.30	0.02	0.01
28	0.08	0.20	0.14	0.01	0.01
56	0.03	0.09	0.06	0.01	0.01

In the sixth study, 20 Merino and 20 2nd cross lambs were used (Peterson and George, 1997a). The two lightest and heaviest animals of both breeds were selected as the untreated control group. The remaining animals were randomly assigned to 3 slaughter groups to contain 6 animals of both breeds. The mean body weight of the groups was about 35 kg. A dose of 2 ml/kg was given as a topical application 6 weeks after shearing over the backline as the "Clik" formulation using an applicator gun. Six animals per time point from each group were sacrificed at 11, 28 and 35 days after application. The four control animals were sacrificed before the first slaughter of the treated animals. Tenderloin muscle, liver, kidney and renal fat were analyzed for the presence of the parent compound and the major metabolite.

In the Merino group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (0.10 and 0.09 mg/kg), liver (0.11 and 0.10 mg/kg), kidney (0.14 and 0.28 mg/kg) and renal fat (0.03 and 0.02) in samples 11 days after the treatment. In the cross-breed group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (0.04 and 0.05 mg/kg), liver (0.07 and 0.11 mg/kg), kidney (0.06 and 0.11 mg/kg) and renal fat (0.03 and 0.02) samples 11 days after the treatment. Results are summarized in Tables 6a-d.

Table 6a. Mean dicyclanil concentrations (mg/kg) in cross breed lambs treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal times (days)	Muscle	Liver	Kidney	Renal fat
11	0.01	0.02	0.02	0.01
28	<0.01	<0.01	<0.01	0.01
35	<0.01	<0.01	0.01	<0.01

Table 6b. Mean dicyclanil concentrations (mg/kg) in merino sheep treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Renal fat
11	0.03	0.04	0.04	0.01
28	<0.01	0.01	<0.01	0.01
35	<0.01	<0.01	0.01	<0.01

Table 6c. Mean CGA 287197 concentrations (mg/kg) in cross breed lambs with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Days after	Muscle	Liver	Kidney	Renal fat
11	0.03	0.07	0.08	<0.01
28	<0.01	0.03	0.03	<0.01
35	<0.01	0.03	0.04	<0.01

Table 6d. Mean CGA 287197 concentrations (mg/kg) in merino sheep with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Renal fat
11	0.06	0.07	0.19	0.01
28	0.02	0.04	0.06	<0.01
35	0.01	0.03	0.07	<0.01

The seventh study contained 22 Merino sheep and 22 second cross lambs of both sexes aged 14 months to 6 years (Smal and George, 1997). The mean body weight of the Merino group was 42 kg and 26 kg in the cross breed group. The lightest and heaviest animal of both breeds was selected as the untreated control group. The remaining animals were randomly assigned to 5 slaughter groups with 4 animals of both breeds. The Merinos were given a topical application of 1 ml/kg and the cross-breeds 2 ml/kg using the "Clik" formulation over the backline with an applicator gun 1 day after shearing. Six animals per time point from both groups were sacrificed at 7, 28, 56, 84 days and 4 months after application. The four control animals were sacrificed before the first group of treated animals. Tenderloin muscle, liver, kidney, subcutaneous fat from the application area and renal fat were analyzed for the parent compound and the major metabolite.

In the Merino group, the highest residue concentrations of parent and metabolite found, respectively, were in muscle (0.02 and 0.03 mg/kg), in liver (0.03 and 0.05 mg/kg), in kidney (0.03 and 0.06 mg/kg), in renal fat (<0.01 and <0.01 mg/kg) and in subcutaneous fat (0.03 and <0.01 mg/kg) samples 56 days after treatment except for the subcutaneous fat sample where the highest residues were obtained at 84 days post-treatment. In the cross-breed group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (<0.01 and 0.01 mg/kg), liver (<0.01 and 0.03 mg/kg), kidney (0.02 and 0.04 mg/kg), renal fat (0.03 and 0.01 mg/kg) and subcutaneous fat (0.13 and 0.04 mg/kg) samples obtained 7 days post-treatment except for the kidney and renal samples which were obtained 28 days after treatment.

The eight study (Hotz, 1999) was performed in order to determine the effect of wool length at the time of treatment on the residues in tissues. Fifty-two White alp sheep of both sexes were used. The mean weight of the males and females, respectively, were 43 kg and 36 kg. The animals were divided to two groups to be treated 1 day off shears and 7 weeks after shearing. The sheep were given dicyclanil at 2 ml/kg using an applicator gun. Tissue samples were collected 7, 14, 21, and 35 days after dosing. Six animals were killed at each sampling time from both treatment groups. Two male control animals from both groups were sacrificed before dicyclanil administration. Dicyclanil and CGA 297107 concentrations were determined in hindquarter, forequarter and tenderloin muscle, omental, renal, subcutaneous and subcutaneous application site fat, liver and kidney tissues. At 7 days post administration the concentrations of dicyclanil in muscle tissue ranged from 0.02 to 0.19 mg/kg and CGA 297107 from <0.01 to 0.17 mg/kg. At 14 and 21 day post-administration neither concentration exceeded 0.06 mg/kg (except for 0.33 mg/kg CGA 297107 in one sample). At 35 days after administration the highest CGA 297107 concentration was 0.02 mg/kg while the dicyclanil concentration was below the limit quantification in all but 2 (0.01 mg/kg) of 36 samples. The highest dicyclanil concentration was found in the omental and subcutaneous fat. The highest concentration was 0.97 mg/kg at 7 days and 0.19 at 35 days after

administration. The CGA 297107 concentrations in fat were at or below the limit of quantification. The highest dicyclanil concentration in liver was 0.30 mg/kg and 0.18 mg/kg in the kidney 7 days after administration while the respective CGA 297107 concentrations were 0.50 and 0.48 mg/kg. Dicyclanil and CGA 297107 concentrations in liver and kidney declined rapidly and were at or below the limit of detection at 35 days post administration.

METHODS OF ANALYSIS

The analytical approach for determination of dicyclanil residues in muscle, liver, kidney and fat tissues is based on high performance liquid chromatography (HPLC). The developed method allows separation of dicyclanil (CGA 183893) from its metabolite (CGA 297107). The limit of quantification (LOQ) for both compounds was 0.01 mg/kg. Two sample clean-up procedures were described. The extraction procedures were coded 239A and 239A.01. Both methods use aqueous acetonitrile for the primary extraction followed by filtration and separation of the lipids by a C18 solid phase cartridge. Additional clean-up was achieved by Tox Elut® solid phase cartridges. Final cleanup was performed by use of a quaternary methyl amine (method 239A) or a strong anion exchange (method 238A.01). The CGA 183893 and CGA 297107 were eluted separately in different fractions.

The separation in the HPLC was obtained by use of a strong cation exchange column. The mobile phase consisted of acetonitrile:0.01M sodium perchlorate:perchloric acid (70:30:0.1). The CGA 183893 and CGA 297107 eluted at different times. In the method 239A, using C18 μBondapak column and acetonitrile:water (20:80), only CGA 183893 elutes. The use of dual amine column and acetonitrile:water (99.5:0.5) elutes only CGA 297107. The LOQ of the two last methods was 0.02 and 0.1 mg/kg, respectively.

The method 239A.01 has been fully validated according to the requirements of the European Union for setting maximum residue limits for medicinal products.

APPRAISAL

Dicyclanil has not previously been reviewed by the Committee. Data were provided on the use of dicyclanil applied as a pour-on to sheep only. Most of the studies were conducted according to current GLP standards. Dicyclanil metabolism was well characterised and residue depletion was studied extensively taking into account application technique, dose, wool length, sex, race and age differences. In considering all the residue depletion studies, the mean ratio of marker residue to total residue for liver is (MR/TR) 0.15 in liver and 0.25 in kidney. Under almost all conditions the amount of adsorption of drug was low; the preferred treatment being a pour-on application. Most studies were conducted using higher than the recommended label doses.

Two analytical methods were described which allowed separate detection of dicyclanil and its CGA 297107 metabolite. The methods had a limit of quantification of 0.01 mg/kg.

MAXIMUM RESIDUE LIMITS

The following factors were considered in recommending MRLs:

1. An ADI of 0 - 0.007 mg/kg of body weight based on a toxicological endpoint was established, permitting a maximum daily intake of 0.42 mg for a 60 kg person.

2. The appropriate marker residue is the parent dicyclanil.

3. Due to the limited extractability of dicyclanil residues from the liver and kidney tissue (about 50%) as well as the ratio between dicyclanil and its main metabolite in tissues (30:70 in liver and 50:50 in kidney), a correction factor expressed as MR/TR (marker residue per total residue) was applied. The ratio (correlation factor) for liver was 0.15 and for kidney 0.25.

4. Dicyclanil residues can be detected using liquid chromatography (HPLC) based methods with a limit of quantification of 0.01 mg/kg. The method is appropriate to meet regulatory needs. No confirmatory method was provided in the submitted information.

5. The Committee did not consider dicyclanil use in lactating sheep.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues in sheep, expressed as parent drug: muscle, 0.2 mg/kg; liver, 0.4 mg/kg; kidney, 0.4 mg/kg; and fat, 0.15 mg/kg

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat and using the factors for liver and kidney as given above, the theoretical maximum daily intake of dicyclanil residues from veterinary use is 0.42 mg/kg.

Table 7. Estimate of theoretical maximum daily intake of dicyclanil

Food Item	MRL (mg/kg)	Food Basket (kg)	mg	MR/TR[1]	TMDI (mg)
Muscle	0.2	0.3	0.06	1	0.06
Liver	0.4	0.1	0.04	0.15	0.27
Kidney	0.4	0.05	0.02	0.25	0.08
Fat	0.15	0.05	0.0075	1	0.01
Total:					**0.42**

[1] MR = marker residue (parent drug); TR = total residues

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