DICLAZURIL

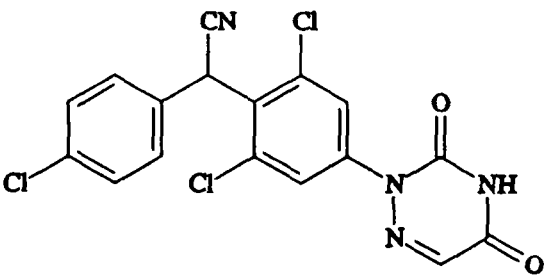
First draft prepared by
Dr. R.L. Ellis
Food safety and Inspection Service
U.S. Department of Agriculture
Washington, D.C., USA

IDENTITY

Chemical name: (±)-2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H-yl)benzeneacetonitrile

Synonyms: CAS number 101831-37-2; Janssen Research Code R064433

Structural Formula:



Diclazuril

Molecular Formula: $C_{17}H_9N_4O_2Cl_3$

Molecular Weight: 407.6

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure Active Ingredient: Diclazuril

Appearance: Slightly yellow to beige colour

Melting Point: 292-297°C (dec)

Solubility: Very low aqueous solubility (< 1 mg/l), 10 mg/l in 0.01N NaOH.
Low solubility in most organic solvents (except dimethyl sulfoxide, N,N-dimethylformamide, and tetrahydrofuran).
Octanol-water partition coefficient (log P) = 4.43 (pH = 3.0), 4.01 (pH = 4.98), 4.41 (pH = 7.03), and 4.48 (pH = 8.0).
pKa = 5.92

UV_{max}: 275 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITION OF USE

Diclazuril is a new anticoccidial drug intended for use in major poultry species including broiler chickens, replacement pullets and turkeys. It is also used in minor bird species, rabbits and lambs. For birds and rabbits, diclazuril is administered as a medicated feed premix at a recommended dose of 1 gram diclazuril per tonne (1 ppm). It is not intended for use in laying hens. It may be used in broiler chickens throughout the growing cycle but also may be used in a restricted manner designed to avoid the induction of resistance during the grow-out cycle as well. Diclazuril is used worldwide in poultry.

Lambs are treated with a 0.25% oral suspension at a 1 mg/kg bw dose when animals are 3-4 weeks old. Treatments may be repeated 2-3 weeks later. Registered use of diclazuril in lambs is currently limited but growing. Registered use in turkeys and rabbits is being developed.

PHARMACOKINETICS

Laboratory Species

A number of radiolabel drug studies were reported using ^{14}C-diclazuril in rats. In two early studies (5 animals each) with a 10 mg/kg bw oral dose in aqueous suspension, diclazuril residues were excreted rapidly (Meuldermans, W., et al., 1989a; Mannens, G., et al., 1992c). More than 90% of the radiolabel was excreted in faeces within 24 hours and approximately 97% in 96 hours. Most of the ^{14}C was identified as unchanged drug. Several minor metabolites were detected, however, the most prevalent metabolite accounted for less than 1% of the dose, indicating only minor metabolism in rats.

A ^{14}C-study on absorption and distribution was carried out in male rats using a 10 mg/kg bw aqueous oral suspension dose. Residue detection involved a gas chromatographic procedure with a limit of quantification of 0.05-0.2 mg/L in plasma (Van Beijsterveldt, L., et al., 1992d). Results indicated limited absorption as most of the radiolabel was eliminated by way of the gastrointestinal tract within 24 hours. Maximum plasma concentration of total residues and unchanged drug occurred at 8 h post dosing. The depletion was monophasic with a half-life of 53 h for total residues and 36 h for unchanged drug. On day 1, total residues in plasma were almost exclusively unchanged drug. Distribution to systemic tissues was rapid but limited. Maximum plasma concentrations of total residue and unchanged drug were approximately 1 mg/L at 8 hours post dosing. The $AUC_{0\text{-infinity}}$ (area under the curve) was 86.0 μg. h/L for total residues and 68.5 μg. h/L for unchanged parent drug. Results of the ^{14}C-radiolabel studies are summarized in Tables 1 and 2.

Table 1[1]. Total Residues (TR) and Unchanged Diclazuril (UD)(μg-eq/L) in Rat Plasma and Blood

Time (h)	Number of Animals	Blood (TR)	Plasma (TR)	Plasma (UD)
1	4	69 ± 7	104 ± 9	120 ± 13
2	4	200 ± 53	295 ± 68	323 ± 89
4	4	500 ± 87	710 ± 107	843 ± 113
8	4	723 ± 126	1035 ± 222	1213 ± 198
24	4	544 ± 59	822 ± 71	806 ± 75
48	4	450 ± 29	673 ± 61	578 ± 31
96	4	218 ± 67	332±110	215 ± 101

[1] = Data in all tables include mean value ± standard deviation unless noted otherwise.

Table 2. Total Residues and Unchanged Diclazuril (mg-eq/kg) in Rat Tissues

Time (h)		1	2	4	8	24	48	96
Brain	TR	ND	ND	0.027	0.058	0.061	0.045	0.045
	UD	ND	0.017	0.037	0.052	0.043	0.029	ND
Heart	TR	ND	0.065	0.157	0.229	0.173	0.158	0.137
	UD	0.016	0.058	0.132	0.178	0.141	0.100	0.036
Lung	TR	0.024	0.066	0.191	0.252	0.263	0.183	0.170
	UD	0.038	0.083	0.178	0.229	0.227	0.125	0.056
Liver	TR	0.069	0.149	0.366	0.557	0.514	0.464	0.268
	UD	0.065	0.149	0.344	0.522	0.395	0.286	0.097
Kidney	TR	0.037	0.111	0.257	0.363	0.318	0.310	0.183
	UD	0.039	0.100	0.226	0.319	0.232	0.189	0.071
Muscle	TR	ND	ND	0.058	0.101	0.080	0.061	0.053
	UD	ND	0.018	0.053	0.073	0.057	0.042	0.013

ND = not detected.

A whole body autoradiography study in rats was reported using an oral dose of 10 mg/kg bw of ^{14}C-diclazuril. Results from this study were consistent with the data in Tables 1 and 2. Residues were highest in liver and lowest in muscle and fat.

Toxicokinetic studies in 160 Wistar rats (Monbaliu, J., et al., 1991b) and 160 Albino Swiss mice (Monbaliu, J., et al., 1991a) were carried out in support of 3-month subchronic oral toxicity studies using 1000, 2000 and 3000 ppm diclazuril in feed. Residues were measured in rat serum and mice plasma. Because of the growth of the rats and mice during the study, the estimated dose per kg b.w. decreased approximately 50% during the 3-month period. The average serum and plasma concentrations are summarized in Table 3.

Table 3. Average Serum Concentration of Diclazurilin Rats and Plasma in Mice (mg/L)

Dose (PPM)	Number of animals		Rats		Mice	
			male	female	male	female
1000	20M	20F	7.18	18.8	6.64	5.24
2000	20M	20F	13.1	30.1	8.85	8.92
3000	20M	20F	11.3	24.4	10.3	9.69

Although the plasma concentrations in male and female mice were similar, the serum concentrations were 2.2-2.6 times higher in female than in male rats. In both species, the systemic availability of diclazuril increased less than proportionally with the dose. The lack of linearity was attributed to absorption saturation at the high dose levels.

Rabbits

Two absorption, distribution, metabolism and excretion studies were conducted using 3 and 12 rabbits, respectively, (Meuldermans, W., et al., 1988c; Michiels, M., et al., 1988d) using a single oral dose in gelatin capsules with ^{14}C-diclazuril at 1 mg/kg bw. The excretion of radioactivity was fairly rapid. Within 48 h, 70% was recovered in the faeces and 3% in the urine. Within 10 days, more than 98% of the radioactivity was excreted (estimated at 91.3% in faeces and urine and 6.7% expired in air). Gall bladder contained only very

low amounts of radioactivity, indicating that biliary excretion was a very minor pathway. Various metabolites were detected, although none accounted for more than 2% of the radioactivity. The two main metabolites were a glucuronide and a sulfate conjugate, based on comparison of radio-HPLC chromatograms from samples without enzymatic hydrolysis and with combination β-glucuronidase/arylsulfatase or arylsulfatase treatments. In the 12 animal study, radioactivity in plasma reached a plateau between 6 and 48 hours of 1.03-1.15 mg-eq/L and declined to 0.041 mg-eq/L after 240 h. The unchanged parent drug represented almost all the radioactivity up to 120 h after dosing. The elimination from plasma had an apparent half-life of 2-2.5 days. The distribution to tissues was limited with liver containing the highest concentrations. The half-life in liver was approximately 3 days. In muscle tissue, residues did not exceed 0.01 mg-eq/kg. Liver radioactivity was approximately 89-96% extractable. Tissue results from the 12 animal study using radio assay quantification procedures are summarized in Table 4.

Table 4. Total Residues (mg-eq/kg) in Rabbits Dosed Orally With 1 mg/kg b.w. of Diclazuril

Time (hours)	Liver	Kidney	Fat	Muscle
6	2.03 ± 0.23	0.88 ± 0.20	0.03 ± 0.01	0.01 ± 0.01
48	2.05 ± 0.77	1.12 ± 0.34	0.03 ± 0.03	0.05 ± 0.07
120	0.83 ± 0.24	0.29 ± 0.10	0.006 ± 0.005	0.005 ± 0.005
240	0.26 ± 0.07	0.05 ± 0.01	0.006 ± 0.005	ND

ND = not detected

Two tissue depletion studies using 16 rabbits (Michiels, M., et al., 1988a) and 48 rabbits (Van Beijsterveldt, L., et al., 1992f), respectively, also provide data on plasma kinetics. Each group of rabbits in these studies (equal numbers of male and females) received diclazuril medicated feed at a concentration of 1 mg/kg in feed for 14 days, using the commercial premix formulation. Taking account of the body weights and feed consumption of the rabbits, this gave an average intake of 0.067 mg/kg bw in the 16 rabbit study. Residues were determined using an UV-HPLC method. Steady state plasma concentrations (0.9-1.0 mg/L) were attained within 10 days of continuous dosing. The concentrations remained at this same level during the 24 h fast after removal of the medicated feed. Thereafter, diclazuril residues depleted with a half-life of about 2 days. Similar results were obtained in the 48 rabbit study. Plasma concentration 24 h post dosing was 0.67 ± 0.30 mg/L with a depletion half-life of 2.5 days.

Chickens

Three ^{14}C-diclazuril studies were reported in chickens. In the single dose study using a 1 mg/kg bw oral dose, maximum diclazuril concentrations of 1.5-2.0 mg-eq/L were observed in plasma at 6 h after administration, with an elimination half-life of about 50 h (Meuldermans, W., et al., 1988e). Plasma radioactivity was completely recovered in the supernatants after removal of proteins with acetonitrile. Radioactivity originated almost exclusively in the unchanged drug for up to 72 h. Equilibrium between plasma and tissues was rapid, with tissue concentrations 2-10 times lower than the plasma concentration. The half-life in tissues was about 50 h. Extraction of liver radioactivity in methanol/formic acid (100/0.5; v/v) was virtually quantitative. Diclazuril accounted for more than 90% of the residues in the 24 h liver samples with metabolites being less than 4% of the residues. After 10 days the cumulative excretion was greater than 95%. An excreta degradation product accounting for 5.3% of the residues was attributed to a derivative of 4-amino-2,6-a-(4-chlorophenyl)-benzeneacetonitrile formed by cleavage and further degradation of the 1,2,4-triazine-dione ring. Other metabolites accounted for less than 2% each of residues and were not identified. Results are summarized in Table 5.

Table 5. Total ^{14}C Diclazuril Residues (mg-eq/L or mg-eq/kg) in Chickens Receiving a Single Oral 1 mg/kg BW Dose

Time (hours)	Plasma	Liver	Kidney	Muscle (PM)	Muscle (FM)	Skin/Fat
6	1.7±0.24	1.26±0.18	1.07±0.17	0.15±0.03	0.17±0.04	0.14±0.02
24	1.30±0.23	0.92±0.12	0.73±0.12	0.12±0.02	0.11±0.04	0.11±0.03
48	1.10±0.10	0.79±0.05	0.63±0.05	0.10±0.01	0.06±0.04	0.09±0.02
72	0.74±0.16	0.42±0.06	0.36±0.07	0.05±0.03	0.06±0.02	0.06±0.01
120	0.45±0.12	0.28±0.10	0.23±0.05	0.05±0.02	0.05±0.02	0.05±0.02
168	0.21±0.12	0.09±0.05	0.12±0.06	0.02±0.01	0.02±0.00	0.03±0.03
240	0.08±0.04	0.03±0.00	0.05±0.02	0.01±0.00	0.01±0.01	0.01±0.00

PM = pectoral muscle; FM = femoral muscle.

One multiple dosing study was conducted in chickens and described in two separate reports (Michiels, M., et al., 1987; Meuldermans, W., et al., 1989b). In addition to drug absorption, excretion and residues in tissues following multiple dosing to chickens, the study reported on the mass balance of unchanged drug and metabolites in plasma, tissues and excreta. ^{14}C-diclazuril was given to broiler chickens for 14 days (from day 28 to 41 days of age) with an average daily dose of 0.090 mg/kg bw, divided into 2 treatments per day orally in a gelatin capsule. Total radioactivity depleted at similar rates from plasma and tissues with a half-life of about 2.5 days. The highest steady state concentration in plasma was 589 ± 49 µg-eq/L. The residues were determined using the gas chromatography-electron capture detector (GC-ECD) method. The extraction procedure recovered 88% of residues from plasma and more than 80% from tissue. Metabolic profiling studies using radio-HPLC confirmed that all plasma radioactivity was from parent drug. In plasma, muscle and skin/fat the total residue (TR) was unchanged drug (UD); in liver UD was also the main radiolabel compound. Mass balance degradation product studies after single and multiple dosing indicate the main metabolite in the excreta accounted for 5.6-8.3% of the dose. Results are summarized in Tables 6 and 7.

Table 6. Plasma Residues in Chickens (µg-eq/L) from 0.045 mg/kg bw b.i.d. 14 Day Treatment with Diclazuril in a Capsule Formulation eq. to 1 ppm in Feed

Time (hours)	6	24	72	120	168	240
TR	589±49	316±54	226±31	138±53	64±31	34±16
UD	608±51	321±57	224±23	53±23	65±31	34±14

A whole-body autoradiography study with five birds dosed at 0.5 mg/kg bw by gavage into the crop gave similar results to those listed in Table 7. Birds were sacrificed at 6, 24, 48, 72 and 120 h post dosing. In tissues, the highest radioactivity was observed in the liver, kidney, lung, connective tissue and skin; the lowest concentrations were in muscle, brain and fat. There was a similar distribution and concentration decrease as a function of time for blood and tissues (Michiels, M., et al., 1987b).

Table 7. **Tissue Residues in Chickens (μg-eq/kg) from 0.045 mg/kg bw b.i.d. 14 Day Treatment with Diclazuril in a Capsule Formulation eq. to 1 ppm in Feed**

Time (h)/ Tissue		6	24	72	120	168	240
Liver	TR	386±69	240±57	187±31	107±17	63±12	36±5
	UD	370±52	202±53	138±21	85±29	42±16	20±7
Muscle (PM)	TR	58±5	31±6	23±2	13±4	7±3	< 5
	UD	52±5	27±5	19±2	13[1]	< 10	< 10
Muscle (FM)	TR	87±7	44±5	32±5	19±6	10[2]±5	6±2
	UD	72±8	37±5	25±3	16[1]	< 10	< 10
Skin/Fat	TR	193±17	110±16	83±11	49±10	29[1]±12	17±8
	UD	158±22	85±13	59±5	41±13	22[1]±8	< 10[2]
Kidney	TR	324±38	199±38	133±20	79±21	41±14	23±8
	UD	NR	NR	NR	NR	NR	NR

PM = pectoral muscle; FM = femoral muscle; [1]n = 7 birds (all other groups 8 birds); [2]= median value; NR = not reported

Turkey

Two radiolabel studies were reported in turkeys using ^{14}C-diclazuril. In the single oral dose study (1 mg/kg bw) using 28 birds (Meuldermans, W., et al., 1990a), the highest residues were found in plasma 6 h post dosing with subsequent elimination proceeding with a half-life of approximately 38 h. Results were similar to those in chickens. The plasma radioactivity could be fully recovered in the deproteinized supernatants and was almost exclusively due to unchanged drug for up to 168 h. There was a rapid but limited distribution between plasma and tissues. Depletion half-lives in tissues ranged from 34-46 h. In liver, extraction of radioactivity in methanol/formic acid (100/0.5; v/v) was nearly quantitative. Radiochromatograms of liver tissue indicated that unchanged drug accounted for 98% of sample radioactivity at 6 h and 85% after 48 and 72 h. No metabolites accounted for more than 10% of the radioactivity in liver. At least eight metabolites were detected in the excreta extracts. The triazine-dione ring cleavage product seen in broilers accounted for 6.3% of the dose in the 0-96 h excreta. A second unidentified metabolite accounted for 2.4% and all others were less than 2% each. The radio-assay method in this study had a quantification limit of 0.01 mg/kg or mg/L. Residue data are summarized in Table 8. Standard deviations are available but not recorded here.

In the 12 turkey multiple dosing study, ^{14}C-diclazuril was given orally to male and female turkey poults approximately 11 weeks old for 14 days (Byrd, J. and Lucht, K., 1992e). The diclazuril dose was approximately 0.05 mg/kg bw divided over two treatments per day in a gelatine capsule. All birds were sacrificed 6 h after the last dose. Extraction using ethyl acetate recovered 89.1% of the radioactivity in livers of male turkeys and 95.7% in female turkeys. Male and female turkey livers contained minor additional peaks, but none representing more than 4% of the total residues or concentrations exceeding 0.015 mg-eq/kg. In the male liver tissue, total residues were 0.36 mg-eq/kg with 0.26 mg-eq/kg being unchanged drug. Two metabolites accounted for 0.014 and 0.013 mg-eq/kg, respectively. Nonspecified extracted total residues (peaks in the radiochromatograms which were not seen consistently between replicates) accounted for 0.04 mg-eq/kg and 0.04 mg-eq/kg unextracted total residues. In females, the respective values are 0.58 mg-eq/kg total residues, with 0.48 mg-eq/kg being unchanged drug, one metabolite at 0.016 mg-eq/kg, nonspecified extracted total residues at 0.06 mg-eq/kg and unextracted total residues of 0.03 mg-eq/kg. Characterization of liver residues noted above employed a radio-HPLC method. Average tissue concentrations summarized in Table 9 were determined by a combustion-scintillation radio-assay.

Table 8. Residues of Diclazuril in Turkeys (mg-eq/kg or mg-eq/L) Receiving a Single 1 mg/kg bw Oral Dose

Time (h) Tissue		6	48	72	120	168	240
Plasma	TR	1.78	0.85	0.46	0.18	0.10	0.03
	UD	1.36	0.77	0.39	0.13	0.08	0.02
Liver	TR	1.40	0.71	0.36	0.16	0.12	0.04
	UD	1.35	0.55	0.25	0.09	0.05	0.01
Kidney	TR	1.09	0.45	0.24	0.09	0.05	0.01
	UD	0.88	0.44	0.21	0.07	0.05	ND
Muscle (PM)	TR	0.21	0.08	0.04	0.02	0.01	ND
	UD	0.16	0.07	0.04	0.02[1]	ND	ND
Skin/Fat	TR	0.57	0.21	0.15	0.04	0.02	0.01
	UD	0.21	0.11	0.07	0.04	0.02	0.01[1]

PM = pectoral muscle; ND = not detected; [1] = Median value reported.

Table 9. Average Tissue Concentrations (mg-eq/kg) in Turkeys Six Hours Following Last Dose in a 14 Day Oral Dosing Study (Byrd, J. and Lucht, K., 1992e)

Tissue	TR in mg-eq/kg		Average % of dose	
	Males	Females	Males	Females
Breast muscle	0.049	0.062	1.44	1.44
Thigh muscle	0.070	0.088	1.22	1.40
Abdominal fat	0.186	0.307	0.31	0.62
Kidney	0.304	0.439	0.22	0.22
Liver	0.407	0.610	1.29	0.21

Sheep

There are no *in vivo* radiolabeled studies in sheep, however, there was a report concerning the comparative *in vitro* metabolism. Two non-radiolabeled studies were reported. In the first, 3 sheep were treated with a 1 mg/kg bw oral drench as a 0.25% suspension as intended for registered use. Poor systemic absorption of diclazuril was noted (Monbaliu, J., et al., 1993). Maximum concentrations in plasma were 0.012-0.016 mg/L at 24 to 48 h after dosing, and were below the limit of quantification (0.01 ug/ml) at all other sampling times. Plasma kinetics were evaluated as part of a residue depletion study where six lambs per group were dosed orally at 1 mg/kg bw with a 0.25% oral suspension. One group was dosed at 4 weeks of age and a second group twice, once at 4 weeks and once at 7 weeks of age (Van Beijsterveldt, L., et al., 1994b). Bioavailability was higher in the younger animals used in the second study. In the second study, the maximum plasma concentration was observed at 24 h post treatment for the first dose (0.15 ± 0.10 mg/L) and 0.08 ± 0.02 mg/L following the second dose. The area under the curve (AUC) calculations were 10.5 ± 7.5 mg.h/L and 4.89 ± 1.51 mg.h/L for the single and double treatment groups of sheep, respectively. Half-lives were estimated at 30.6 ± 5.9 h for the first study group and 28.0 ± 8.6 h for the second group of sheep. Residue concentrations are summarized in Tables 10 and 11.

Table 10. Residue Concentrations in Four Week Old Sheep (mg-eq/kg) after a Single Oral 1 mg/kg bw Treatment

Days Post Treatment	Plasma	Liver	Kidney	Muscle	Fat
1	0.14±0.05	0.30±0.10	0.09±0.03	0.03±0.01	0.08±0.03
3	0.04±0.03	0.11±0.07	0.03±0.02	≤ 0.01	0.03±0.01
5	0.01±0.01	0.03±0.03	≤ 0.01	≤ 0.01	0.04±0.05
7	≤ 0.005	0.02±0.01	≤ 0.01	≤ 0.01	≤ 0.01

Table 11. Residue Concentrations in Seven Week Old Sheep (mg-eq/kg) after Two Oral 1 mg/kg bw Treatments

Days Post Treatment	Plasma	Liver	Kidney	Muscle	Fat
1	0.07±0.04	0.28±0.19	0.04±0.02	0.01±0.01	0.04±0.02
3	0.02±0.03	0.06±0.02	0.01±0.01	≤ 0.01	0.01±0.00
5	≤ 0.005	0.02±0.03	≤ 0.01	≤ 0.01	≤ 0.01
7	≤ 0.005	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01

Depletion half-lives in tissue and plasma were similar.

TISSUE RESIDUE DEPLETION STUDIES

Residue depletion studies of diclazuril from the edible tissues of rabbits, broiler chickens, turkeys and lambs were conducted using conditions simulating normal treatment and husbandry practices.

Rabbits

Two studies were reported in rabbits using diclazuril at 1 mg/kg in their feed for 14 days (Michiels, M., et al., 1988a; Van Beijsterveldt, L., et al., 1992f). Concentrations of diclazuril were determined using an HPLC-UV method with a limit of quantification (LOQ) of 0.05 mg/L in plasma and 0.1 mg/kg in tissue. Steady state concentrations were reached within 10 days of continuous feeding. The plasma concentration at steady state conditions was 0.89±0.17 mg/L. In both studies the depletion half-life was 2-2.5 days for plasma and kidney. For liver the half-life was 3.9 days. Results are summarized from both studies in Table 12.

Table 12. Tissue Residues in Rabbits (mg-eq/kg) Following Fourteen Days Multiple Dosing

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1[1]	1.59±0.26	0.64±0.26	< 0.10	<0.21±0.18	1.02
7[1]	0.71±0.26	<0.16±0.07	< 0.10	< 0.10	0.23±0.15
1	1.45±0.30	0.44±0.10	0.05±0.03	ND	0.67±0.30
3	0.84±0.34	0.22±0.15	ND	ND	0.30±0.22
5	0.70±0.28	0.13±0.08	ND	ND	0.17±0.11
7	0.51±0.26	0.08±0.08	ND	ND	0.10±0.09
10	0.27±0.15	ND	ND	ND	0.05±0.07

[1] = Non-GLP study; ND = not detected.

Chickens

Two medicated feeding studies were reported with birds receiving diclazuril at 1 mg/kg medicated feed for a complete grow out (46 days). In the first study (Van Leemput, L., et al., 1989c) diclazuril was administered using the European type premix containing 0.5% of active ingredient. Residues in this study were determined using an HPLC-UV method with a LOQ of 50 μg/kg for tissues and 50 ng/ml for plasma, except skin/fat which had a LOQ of 100 μg/kg. In the second study (Van Leemput, L., et al., 1990b), the U.S. premix containing 0.2% of active ingredient was used with residues determined using a GC-ECD method with a LOQ of 10 μg/kg and 10 ng/ml for tissues and plasma, respectively. Sampling for residue analysis was based on selection of 10 birds from each treatment group (of 50 birds each). The two lightest and heaviest birds were eliminated from each group of 10, and the remaining six birds analyzed individually. Half-lives in tissues using the European premix were 34 h in kidney, 44 h in liver, 50 h in muscle and 52 h in plasma. Using the U.S. premix, half-lives were 61 h in kidney, 58 h in liver, 59 h in muscle, 65 h in plasma and 65 h in skin/fat. Results are summarized in Tables 13 and 14.

Table 13. Residues in Broiler Chickens (μg-eq/kg) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the European Premix Containing 0.5 Percent Diclazuril (46 Day Treatment Period)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	419±26	517±40	91±27	ND	951±123
72	154±19	186±94	ND	ND	430±100
120	92±17	64±52	ND	ND	259±79
168	ND	ND	ND	ND	129±40
216	ND	ND	ND	Nd	74±58

ND = not detected.

Table 14. **Residues in Broiler Chickens (μg-eq/kg) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the U.S. Premix Containing 0.2 Percent Diclazuril (46 Day Treatment Period)**

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	371±90	308±65	45±9	144±35	565±168
24	340±97	268±92	39±11	138±39	476±157
48	180±80	146±76	23±10	79±35	267±178
72	184±90	184±131	26±15	91±49	347±266
96	93±19	73±12	12±3	42±6	152±34
120	100±36	85±28	13±5	48±12	158±57
168	51±23	44±24	ND	23±11	95±57
216	33±9	30±8	ND	18±5	69±19

ND = not detected.

Turkey

For turkeys, two 16 week feeding studies were reported (320 birds each) using 1) the European premix (0.5%) at a 1 mg/kg final concentration in medicated feed, and 2) the U.S. premix (0.2%) at a 1 mg/kg final concentration in medicated feed (Van Beijsterveldt, L., et al., 1992a; Van Beijsterveldt, L., et al., 1992b). For residue analysis, turkeys were sampled as noted above for broiler chickens, using 160 turkeys as controls in both studies. Samples were frozen quickly after collection and analyzed within 2-3 months using a reverse-phase HPLC-UV procedure with a LOQ of 50 ng/ml for plasma, 50 μg/kg for liver, muscle and kidney, and 100 ug/kg for skin/fat for the 1990 study with the European premix and a GC-ECD procedure with a LOQ of 10 μg/L in plasma and 10 μg/kg in tissue in the 1992 study with the U.S. premix. Depletion half-lives were 3 days in plasma, liver, muscle and kidney. Results are summarized in Tables 15 and 16.

Table 15. **Residues in Turkeys (mg-eq/kg or mg-eq/L) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the European Premix Containing 0.5 Percent Diclazuril (16 Week Treatment)**

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	0.57±0.05	0.30±0.02	ND	0.16±0.05	0.80±0.12
24	0.37±0.025	0.18±0.02	ND	0.18±0.05	0.65±0.06
72	0.25±0.02	0.07±0.03	ND	0.11±0.06	0.41±0.05
120	0.17±0.02	ND	ND	ND	0.26±0.03
168	0.15±0.05	ND	ND	ND	0.16±0.03
216	0.09±0.03	ND	ND	ND	0.11±0.02

ND = not detected.

Table 16. Residues in Turkeys (mg-eq/kg or mg-eq/L) Ingesting Feed Containing 1 mg/kg of Diclazuril Formulated Using the U.S. Premix Containing 0.2 Percent Diclazuril (16 Week Treatment)

Hours Post Treatment	Liver	Kidney	Muscle	Skin/Fat	Plasma
6	0.40±0.04	0.29±0.01	0.05±0.01	0.15±0.02	0.57±0.02
24	0.30±0.03	0.27±0.02	0.04±0.05	0.13±0.02	0.52±0.03
48	0.26±0.02	0.22±0.02	0.03±0.00	0.12±0.01	0.44±0.05
72	0.19±0.01	0.16±0.01	0.02±0.00	0.10±0.01	0.33±0.05
120	0.12±0.01	0.10±0.01	0.02±0.00	0.08±0.02	0.18±0.02
168	0.08±0.02	0.06±0.01	ND	0.07±0.02	0.11±0.02
216	0.05±0.01	0.04±0.00	ND	0.05±0.01	0.08±0.00

ND = not detected

Sheep and Lamb

Two residue studies were reported in lambs using the recommended 0.25% suspension to provide a dose of 1 mg/kg bw. The first study was not done under GLP conditions. This study did not describe the HPLC method used for residue analysis except noting the LOQ in tissue (0.05 mg/kg for liver, kidney and muscle, and 0.10 mg/kg for fat). In all tissues at all post treatment times (24 h, 3 and 7 days) no residues were detected. In the second study (Van Beijsterveldt, L., 1994b), lambs were treated with a 0.25% suspension at 0.4 ml/kg (1 mg/kg bw) by gavage. Dosing was done once, at 4 weeks of age, or twice, at 4 and 7 weeks of age. Edible tissues were collected from 6 animals at each post treatment time. Residues were determined using a validated GC method with a LOQ of 5 μg/L for plasma and 0.01 mg/kg in tissue. Blood samples were collected also and the plasma analyzed for residues of diclazuril. Edible tissues were collected from 6 animals each at 1, 3, 5, and 7 days after treatment (for the single and repeat dosing groups). Maximum concentrations in plasma following a single dose was 0.15 ± 0.10 mg/L, the maximum concentration occurred at 21 ± 7 h, and the half-life was 30.6 ± 5.9 h. Results following the second dose treatment was 0.08 ± 0.02 mg/L, the maximum drug concentration occurred at 24 ± 0 h, and the half-life was 28.0 ± 8.6 h. Depletion half-lives in tissue and plasma were similar. Results are summarized in Tables 17 and 18.

Table 17. Residues in Lambs (mg/L or mg/kg) after a Single 1 mg/kg bw Treatment with a Diclazuril 0.25% Drench at the Age of 4 Weeks

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1	0.30±0.10	0.09±0.03	0.03±0.01	0.08±0.03	0.14±0.05
3	0.11±0.07	0.03±0.02	≤ 0.01	0.03±0.01	0.04±0.03
5	0.03±0.03	≤ 0.01	≤ 0.01	0.04±0.05	0.01±0.01
7	0.02±0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005

Table 18. Residues in Lambs (mg/L or mg/kg) after a Single 1 mg/kg bw Treatment with a Diclazuril 0.25% Drench at the Age of 4 and 7 Weeks

Days Post Treatment	Liver	Kidney	Muscle	Fat	Plasma
1	0.28±0.19	0.04±0.02	0.01±0.01	0.04±0.02	0.07±0.04
3	0.06±0.02	0.01±0.01	≤ 0.01	0.01±0.00	0.02±0.01
5	0.02±0.03	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005
7	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.005

Eggs

Two studies evaluated possible diclazuril residues in eggs. In the first study, 180 laying hens were given medicated feed at 1 or 5 mg/kg for 32 days to simulate a misfeeding or an overdosing in a non-target species. Eggs were collected during the dosing period and for 20 days following withdrawal of medicated feed. Residues were 3.7-3.9 times higher in egg yolk than in egg whites and 4.1-4.4 times higher after the 5 mg/kg than the 1 mg/kg dose (Van Leemput, L., et al., 1990e). Residues were determined using a GC method with a LOQ of 0.05 mg/kg. Steady state concentrations were noted in egg white after 11 treatment days and 14 days for egg yolk. The residue depletion half-life was 4-6 days. Average steady-state concentrations were 0.065 mg/kg in egg white and 0.24 mg/kg in egg yolk, equivalent to about 7.1 μg in a whole egg.

In the second study, 20 replacement pullets were maintained on 1 mg/kg diclazuril medicated feed until 16 weeks of age (Van Leemput, L., 1994a). The young hens were kept subsequently on a non-medicated feed and, on the onset of laying, all eggs from the first collection were sampled. Residue analysis using a GC method with a LOQ of 0.05 mg/kg indicated that none of the egg samples contained diclazuril at concentrations above the LOQ.

MARKER RESIDUE AND TARGET TISSUE

The radiolabel studies in laboratory and food producing animals measured total diclazuril related residues in edible tissues and characterized total residue depletion up to 240 hours post administration (depending on species). Diclazuril is metabolized to a very limited extent in all species tested. No study indicated more than 10% of total residues being diclazuril metabolic products. The studies also verified nearly quantitative extraction of diclazuril related residues, indicating minimal bound residues in edible tissue over all time periods studied. This is particularly evident in the 6-24 h residue data. These data support parent drug, diclazuril, as the appropriate marker residue.

The highest residue concentrations are consistently found in liver and kidney, with significantly lower residues in fat for red meat animals and skin/fat for poultry. Residues are lowest in muscle tissue for all species. For practical reasons, liver is the first tissue of choice for residue analysis. Concentrations of residues are higher in skin/fat or fat than in muscle tissue. Muscle tissue is, however, more convenient and more appropriate for residue analysis than skin/fat or fat tissue in international trade. A summary of residue data from studies using the recommended treatments is presented in Table 19.

Table 19. Mean and Maximum Observed Diclazuril Residues (mg/kg) in Food Animals Using Recommended Treatments[1]

Species		Liver	Kidney	Muscle	Skin/Fat	Fat
Rabbit	mean	1.59	0.64	< 0.10		< 0.21
	max.	1.93	0.90	< 0.10		0.38
Rabbit	mean	1.45	0.44	0.05		0.05
	max.	1.87	0.55	0.09		0.23
Chicken	mean	0.42	0.52	0.09	< 0.10	
	max.	0.45	0.59	0.13	0.14	
Chicken	mean	0.37	0.31	0.05	0.14	
	max.	0.50	0.39	0.06	0.19	
Turkey	mean	0.57	0.30	0.05	0.16	
	max.	0.64	0.34	0.08	0.26	
Turkey	mean	0.40	0.29	0.05	0.15	
	max.	0.44	0.29	0.05	0.17	
Lamb	mean	0.30	0.09	0.03		0.09
	max.	0.61	0.15	0.05		0.13

[1] Maximum residue concentration of diclazuril is at 6 h post treatment for chickens and turkeys, 24 h for rabbits and lambs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Three methods were reported for analysis of diclazuril residues - gas chromatography using a packed column with an electron capture detector (Meuldermans, W., et al., 1989b), gas chromatography using a capillary column with a thermionic specific detector (Corbin, T., 1990c; Corbin, T., 1990d), and a reverse-phase high performance liquid chromatography with UV detection (Woestenborghs, R., 1988b).

The capillary GC method was designed for use as a regulatory method for residues in chicken liver. The method involves extraction of 10 g of ground liver with ethyl acetate and clean-up with solid phase extraction over C-18 reversed-phase cartridges. Purified extracts are derivatized with pentafluorobenzylbromide. A specific thermionic detector is coupled with the chromatograph, using a 15 m x 0.25mm i.d. column coated with DB-1301. An internal standard (R062646) is used as an aid for quantification. Average recoveries were about 78% with analyte linearity from 25-250 μg/kg. The method has good specificity and a limit of detection of 20 μg/kg.

The GC-electron capture detection method is suitable for residues in plasma and edible tissue of broilers, turkeys and lambs as well as egg yolks and egg whites. The procedure requires homogenization of samples of muscle, liver or kidney. Aliquots of the homogenates are fortified with the internal standard noted above and residues are extracted with ethyl acetate. The extract is evaporated to dryness and redissolved in acetonitrile:water (1:1, v/v), washed with hexane and extracted again with ethyl acetate. The organic layers are evaporated to dryness. Samples of skin/fat or fat were ground and homogenized (1:9, v/v) in 0.1M citric acid. The homogenate is extracted with ethyl acetate, evaporated to dryness and redissolved in 80:20 (v/v) acetonitrile-water, washed three times with hexane and extracted with ethyl acetate and evaporated to dryness. The dry residues are dissolved in diazomethane solution, allowed to react for 5 minutes at room temperature, evaporated to dryness and redissolved into toluene for GC analysis. Chromatography uses a glass column (1 m x 4 mm i.d.) packed with 3% OV-17 on 80-100 mesh Supelcoport. Detection used a ^{63}Ni electron capture detector.

Performance characteristics for the GC-ECD method were reported. The limit of quantitation in animal tissues is 0.01 mg/kg and 0.025 mg/kg for egg whites and yolks. The linear range is 0.01-2.0 mg/kg with an accuracy

of 92-109% and precision of 0-7.8%. For eggs, linearity was demonstrated at 0.05-0.5 mg/kg. For egg yolk at 0.25 mg/kg, the accuracy was 78% with precision of 10.8%; for egg white at 0.1 mg/kg, the accuracy was 84% and precision was 14.1%. The method meets attributes suitable for regulatory purposes.

The HPLC method is applicable for plasma and edible tissues of broilers, turkeys and rabbits (Woestenborghs, R., 1988b). Tissues are homogenized in distilled water (1:4, v/v), and aliquots fortified with an internal standard (R062370). Residues are extracted with ethyl acetate, evaporated to dryness, redissolved in 50:50 (v/v) acetonitrile-water, washed with hexane and reextracted with ethyl acetate. The evaporated extract is reconstituted in HPLC elution solution and chromatographed on a reverse-phase 10 cm x 4.6 mm i.d. column packed with 5 μm particle size ODS-Hypersil using 0.1M ammonium acetate-tetrahydrofuran (69:31, v/v) and UV detection at 278 nm. The LOQ is 0.05 mg/kg in tissues. Some interferences can occur in fat and skin/fat samples and the method is not recommended for fatty samples with residues below 0.5 mg/kg. The linear range is 0.04-10 mg/kg in tissues and 0.08-20 mg/kg in fat. In these conditions, the accuracy is 78-117% with a precision of 1.4-16.3%. Overall, it is less suitable than the GC-ECD method because of inferior LOQ's and possible interferences in fatty samples.

APPRAISAL

Diclazuril is a relatively new anticoccidiostat drug. As such, almost all relevant studies have been performed under good laboratory practices. Published literature dates from 1985. Diclazuril pharmacokinetics have been studied in laboratory and food animal species. Residue and metabolism studies in edible tissues of broilers, turkeys, sheep and rabbits were conducted as recommended for the practical use of diclazuril.

Diclazuril is generally well absorbed in all species investigated, however, it may be somewhat less well absorbed in sheep than in lambs. There were no indications of significant metabolism of diclazuril. Residue based substances were eliminated mainly as unchanged drug in the excreta of bird species and the faeces of rats and rabbits. Radiolabel studies yield almost quantitative extraction of residues indicating no significant bound residues in any species. In edible tissues of food animals, the residues consisted almost exclusively of parent drug, particularly at short withdrawal times (6 h for poultry, 24 h for sheep and rabbits). Metabolites at these withdrawal times were less than 10% of total residues in all tissues. The lack of metabolism is a dominant feature in all species, particularly for short withdrawal times. The common identifiable metabolite among species indicates that there are no significant species differences in the metabolism of diclazuril. These data also support the selection of diclazuril as the marker residue compound for compliance purposes. Liver and muscle are the tissues of choice for residue analysis.

Diclazuril residues are dependent on the mode of administration. Poultry fed at 1 mg/kg bw in feed for prolonged periods (e.g., complete grow out) results in residues of approximately 0.6 mg/kg or less in liver and kidney tissues at 6 h withdrawal. The residues are lower in skin/fat and lowest in muscle tissue. In rabbits, residues are higher in liver and kidney than in poultry but exhibit the same general pattern, with residues lowest in muscle tissue. Treatment of lambs at 1 mg/kg bw oral dosing results in high liver, but relatively low residues in kidney, fat and muscle tissue. For broilers, turkeys and rabbits, the relative distribution of residues in edible tissues did not change with the time after last treatment. Depletion from all tissues proceeded with the same approximate half-life.

Analytical methods are capable of quantitating residues in all species and tissues at concentrations of interest. The GC-ECD procedure has a limit of quantitation of 0.010 mg/kg in tissues of broilers, turkeys and lambs (as well as egg white and yolk), whereas the HPLC method has a limit of quantitation of 0.04-0.05 mg/kg in edible tissues of broilers, turkeys and rabbits.

Maximum Residue Limits

Based on the temporary ADI of 0-20 μg per kg body weight established by the Committee using toxicological data, the permitted daily intake of diclazuril residues would be 1200 μg for a 60 kg person.

In recommending the temporary MRLs, the Committee took into consideration that two regulatory methods are available for residue analysis. The gas chromatography-electron capture detection method which has a limit of quantification of 0.01 mg/kg and a high performance liquid chromatography method using ultraviolet detection which has a limit of quantification of 0.05 mg/kg. The Committee also took into consideration the maximum residues observed in poultry, sheep and rabbits when diclazuril is used according to good veterinary practices.

The recommended temporary MRLs, expressed as parent drug, in poultry, sheep and rabbits, are shown in Table 20.

Table 20. Temporary MRLs (μg/kg) For Poultry, Sheep and Rabbit

Species	Tissue			
	Muscle	Liver	Kidney	Fat
Poultry	500	3000	2000	1000
Sheep	500	3000	2000	1000
Rabbit	500	3000	2000	1000

If these values are used for temporary MRLs, the theoretical intake of residues of diclazuril in all species listed is 600 μg, using a daily food intake of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat (fat/skin for poultry).

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