DICYCLANIL

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ADDENDUM

To the dicyclanil residue monograph prepared by the 54th meeting of the Committee and published in FAO Food and Nutrition Paper 41/13 in Rome, 2000

IDENTITY

Chemical name: International Non-Proprietary Name (INN): DICYCLANIL

International Union of Pure and Applied Chemistry (IUPAC) name:

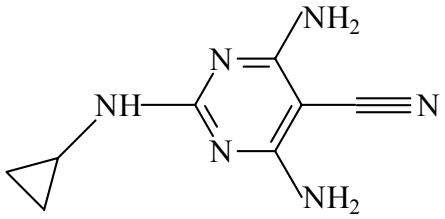
4,6-diamino-2-cyclopropylaminopyrimidine-5-carbonitrile

Chemical Abstract Service (CAS) name:

4,6- diamino-2-(cyclopropylamino)-5-pyrimidinecarbonitrile

Synonyms: A-9568 B, CGA 183893

Structural formula:



CAS number: 112636-83-6

Molecular formula: $C_8H_{10}N_6$

Molecular weight: 190.2

Dicyclanil was first reviewed by the Joint Expert Committee on Food Additives (JECFA) at the 54th meeting of the Committee, which considered its use as an insect growth regulator in sheep. MRLs for muscle, liver, kidney and fat tissues were set.

These MRLs were discussed by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) at its 13th Meeting. The Committee noted that the use of dicyclanil itself as a marker could result in an estimated total maximum daily intake above the ADI and therefore, recommended that JECFA consider this issue (ALINORM 03/31).

The present re-evaluation of dicyclanil residues was undertaken to address the questions of MRLs relative to ADI, MRL allocation to various tissues, the appropriate marker residue and suitable analytical procedures.

The Committee reviewed all studies that the sponsor had performed in order to investigate the influence of a variety of factors such as breed, type and length of the wool, time of off-shears and the applied dose on the concentrations of dicyclanil related residues in edible tissues. Although Merino sheep were used in most studies, statistical analysis of the results indicated that there were differences among the various breeds in terms of residue kinetics. The other variables among and within the studies were also analyzed and they were considered when indicated. For the purpose of statistical evaluations data obtained for different tissues (e.g. for different types of fat tissues as well as for the muscle tissue from various parts of the body) were pooled when appropriate. The Committee also noted the inconsistency in the metabolic profile established using radiolabel dicyclanil and the observed residues in the subsequent residue depletion studies using non-radiolabel dicyclanil.

RESIDUES IN FOOD AND THEIR EVALUATION

Metabolism

Rats

The previous evaluation by JECFA addressed the studies using ^{14}C-labeled dicyclanil (5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine) in rats by Hassler (1994) and Thanei (1996). These studies showed that orally administered dicyclanil equivalent radioactivity was practically completely recovered in urine and feces. The remaining tissue and carcass residues represented approximately 1% or less of the total administered radioactivity. The following compounds were identified using ^{1}H-NMR, IR and mass spectrometry:

N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U),
5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893 = dicyclanil),
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)
Only MET 2U and MET 4U could be unequivocally characterized by mass spectrometry.

Biotransformation was initiated by oxidative cyclopropyl-ring opening at various positions followed by further oxidation. Biotransformation was limited to the cyclopropyl-ring while the cyano-group was metabolically stable. The most significant route was the conversion of the dicyclanil to MET 1U corresponding to 50% of the administered dose. CGA 297107 represented 11% of the excreted metabolites.

Sheep

Altogether 6 studies concerning administration of ^{14}C-labeled dicyclanil to sheep were conducted. The studies of Gifford and Dunsire (1994), McLean and Dunsire (1996) and Anderson and Speirs (1998) concerned the absorption, distribution and excretion of ^{14}C-labeled dicyclanil (Here after, Study 1R, Study 2R and Study 3R, respectively). The studies of Thanei (1996a), Phillips (1996) and Loeffler (1998) were aimed at determination of the nature of the residues (hereafter, Study 4R, Study 5R and Study 6R, respectively). The major findings of the radiolabel-studies are presented in Tables 1 and 2.

Table 1. Mean concentrations of ^{14}C- dicyclanil related radioactivity in tissues and excretion

	Study 1R Gifford & Dunsire	**Study 2R MacLean & Dunsire**	**Study 3R Anderson & Speirs**	
Dose run-off	1.25 g by jetting 37-59%	1.5 g by pour-on 2-10%	100 mg/kg pour-on 22%	
No. of animals	4	4	4	4
Days post-adm	1	7	7	21
Mean Concentrations (±SD)* in				
Liver	0.289 ±0.092	0.513 ±0.101	2.646 ±0.755	1.475 ±0.255
Kidney	0.071 ±0.020	0.077 ±0.013	0.762 ±0.480	0.230 ±0.085
Muscle tenderl.	0.027 ±0.008	0.069 ±0.083	1.013 ±0.370	0.880 ±0.790
Muscle fore	0.034 ±0.020	0.128 ±0.083	0.896 ±0.438	0.503 ±0.202
Muscle hind	0.057 ±0.018	0.165 ±0.145	2.955 ±1.445	0.506 ±0.175
Fat omental	0.052 ±0.031	0.020 ±0.005	0.431 ±0.262	0.208 ±0.077
Fat perirenal	0.038 ±0.005	0.028 ±0.005	0.633 ±0.403	0.068 ±0.050
Fat dorsal sc.	0.262 ±0.199	0.395 ±0.237	19.908 ±19.014	13.844 ±12.181
Fat ventral sc.	0.206 ±0.098	0.482 ±0.336	1.164 ±0.991	1.123 ±0.826
Recovered by 168 hours				
In urine	0.83%	1.58%	1.66%	
In feces	1.05%	2.26%	1.47%	

*mg/kg CGA 183893 equivalents

Only the study 3R used the highest recommended dose (the applied tested dose was twice the commercial dose recommended for the body weight of the animals in the study) and the method that is recommended for application in the field. This was also consistent with the higher radioactivity counts found in the various tissues compared to study 1R and 2R. According to the results about 2-4% of the dose was absorbed during the first 168 hours and 7% by 21 days post-administration. No clear correlation between the administered dose and the counts of radioactivity was seen among the three studies. Dicyclanil related radioactivity was excreted almost equally via urine and feces. The concentration measured in the dorsal subcutaneous fat of the

study 3R was exceptionally high compared with to other tissues and to this tissue in other studies. The concentration of dicyclanil related radioactivity was highest in the liver in study 1R and 2R, but in study 3R the concentrations in the dorsal subcutaneous fat and in hindquarter muscle were higher than the concentration measured in the liver. In study 3R only the concentration in omental fat was below 0.5 mg/kg dicyclanil equivalents 7 days post-administration and in omental fat, perirenal fat and kidney concentration 21 days post-administration. In study 1R, except for the lowest dose, also the "run-off" was highest.

The characterization of the ^{14}C-labeled dicyclanil related radioactivity in tissues is presented in Table 2. Some of the described extraction procedures included a Soxhlet extraction step, which was omitted in the following extractability calculations because the analytical method used in the residue depletion studies with non-radioactive dicyclanil did not use that procedure.

Table 2. Nature of radioactive residues after topical administration of 14C-labeled dicyclanil

	Study 4R Thanei (1996a)	**Study 5R Phillips (1996)**	**Study 6R Loeffler (1998)**	
Source of sample	Gifford & Dunsire (R1)	MacLean & Dunsire (R2)	Anderson & Speirs (R3)	
Number of animals	4	4	4	4
Days post-administration	3	21	7	21
LIVER				
Microwave extraction	YES	NO	NO	NO
Solvents	Acetonitrile/ hexane	Methanol/ hexane	Acetonitrile/ H_2O/SPE	Methanol/ H_2O/SPE
Extractability	50.3%	20%	64.2%	40.5%
MET 1U	N.D.	N.D.	2.9%	7.9%
MET 2U	(2.7%*)	N.D	18.7%	11.5%
MET 3U	N.D.	N.D.	N.D.	N.D
MET 4U	13.9%	N.D.	15.7%	19.0%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	33.7%	20%	26.9%	41.7%
KIDNEY				
Microwave extraction	YES	NO	NO	NO
Solvents	Methanol/ hexane	Methanol/ hexane	Acetonitrile/ H_2O	Methanol/H_2 O
Extractability	77.2%	58%	91.5%	68.6%
MET 1U	N.D.	N.D.	3.1%	13.4%
MET 2U	(20.1%*)	N.D.	24.4%	22.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	17.4%	N.D.	21.2%	9.5%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	62.5%	42%	51.3%	55.1%
MUSCLE				
Microwave extraction	YES	NO	NO	NO
Extraction solvents	Acetonitrile/ methanol/hexane	Methanol/ hexane	Acetonitrile/ methanol/ H_2O	Acetonitrile/ methanol/ H_2O
Extractability	90.6%	94%	99.7%	98.9%
MET 1U	5.8%	N.D.	3.5%	0.7%
MET 2U	61.9%	67.8%	83.7%	86.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	6.3%	N.D.	4.3%	2.9%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	23.3%	26.3%	8.5%	10.4%
FAT				
Microwave extraction	YES	YES	NO	NO
Solvents	Acetonitrile/	Acetonitrile/hex	Methanol	

	Study 4R Thanei (1996a)	Study 5R Phillips (1996)	Study 6R Loeffler (1998)	
	hexane	ane		
Extractability	95.9%	89%	96.9%	98.0%
MET 1U	N.D.	N.D.	0.8%	0.6%
MET 2U	90.2%	83.6%	90.7%	86.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	2.7%	N.D.	1.3%	0.8%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	3.0%	5.5%	5.9%	12.6%
URINE				
Microwave extraction	NO	NO	N.I.	N.I.
Extraction solvents	None	None	N.I.	N.I.
Extractability	100%			
MET 1U	21.0%	N.D.	N.I.	N.I.
MET 2U	32.2%	69.4%	N.I.	N.I.
MET 3U	N.D.	N.D.	N.I.	N.I.
MET 4U	13.2%	9.3%	N.I.	N.I.
MET 5U	5.7%	N.D.	N.I.	N.I.
Unresolved and/or unidentified	24.6%	21.3%	N.I.	N.I.
FECES				
Microwave extraction	NO	NO	N.I.	N.I.
Extraction solvents		Methanol, Methanol:water	N.I.	N.I.
Extractability	90.7%	91%		
MET 1U	2.9%	N.D.	N.I.	N.I.
MET 2U	75.2%	85.4%	N.I.	N.I.
MET 3U	N.D.	N.D.	N.I.	N.I.
MET 4U	7.7%	N.D.	N.I.	N.I.
MET 5U	N.D.	N.D.	N.I.	N.I.
Unresolved and/or unidentified	4.9%	5.6%	N.I.	

N.D. = Not detected
N.I. = Not investigated
SPE = Solid Phase Extraction
* = no definitive separation from MET 1U

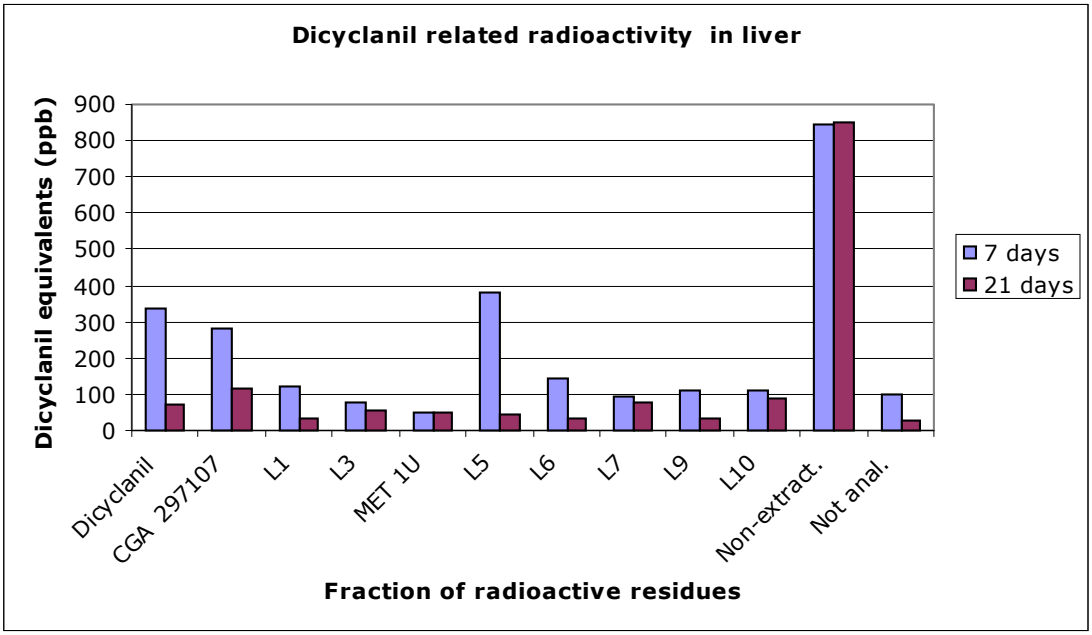
The study 4R used microwave assisted extraction procedure, which is not part of either of the two analytical methods described here. It appears, therefore, unlikely that the results of this study would accurately characterize the extractability or the ratio of the identified metabolites found in the residue depletion studies. Moreover, according to Thanei (1996a), there appears to be a clear trend of other (more unstable) metabolites to break down to CGA 297107 under harsh extraction conditions (microwave and Soxhlet treatment). As indicated above, the excreted amount represented the amount absorbed (2-4% of the dose retained on the animal). Therefore, the amount absorbed in study 1R would not exceed 16 mg while in study 2R the amount absorbed would be up to 57mg.

Of the excreted amount, 56-80% represented the parent compound, dicyclanil, while only 10.2% in study 1R/4R and 4.9% in study 2R/5R consisted of the CGA 297107 metabolite. Therefore, dicyclanil was not extensively metabolized in sheep and CGA 297107 appeared to be a minor metabolic product in excreta. Results of the nature of the residues in urine and feces of study 3R/6R were not available. However, the two earlier studies appeared sufficient to clarify this aspect. Study 3R/6R is the radiolabel study that appeared to best address the characterization of dicyclanil related metabolism in sheep. Furthermore, the application mode was identical and the analytical extraction procedure comparable to the described analytical methods used in the non-radiolabel studies.

In study 3R/6R, dicyclanil represented 18.7% and CGA 297107 15.7% of the extractable residues in the liver 7 days post-administration. The respective concentrations 21 days post-administration were 11.5% and 19%. Another 2.9% of the extractable residues were characterized as MET 1U 7 days and 7.9% 21 days post-administration. Over 60% of the extractable radioactive residues in liver could not be identified. In the study 1R/4R dicyclanil was not unequivocally identified in the liver (possibly 4.4%) and the only metabolite that could be characterized in that study was the CGA 297107. In the study 2R/5R

only dicyclanil was identified while CGA 297107 was not detected. Figure 1 shows the concentration of the various components as a function of time in study 3R/6R in liver tissue. The non-extractable residue concentration appeared to remain practically constant over time. The same trend was seen also in studies 1R/4R and 2R/5R. Dicyclanil concentration declined somewhat faster than CGA 297107 concentration. However, some of the unidentified fractions declined slower than CGA 297107. The changing ratio of the extractable and non-extractable residue concentration may bias the calculation of total residues when factors are used.

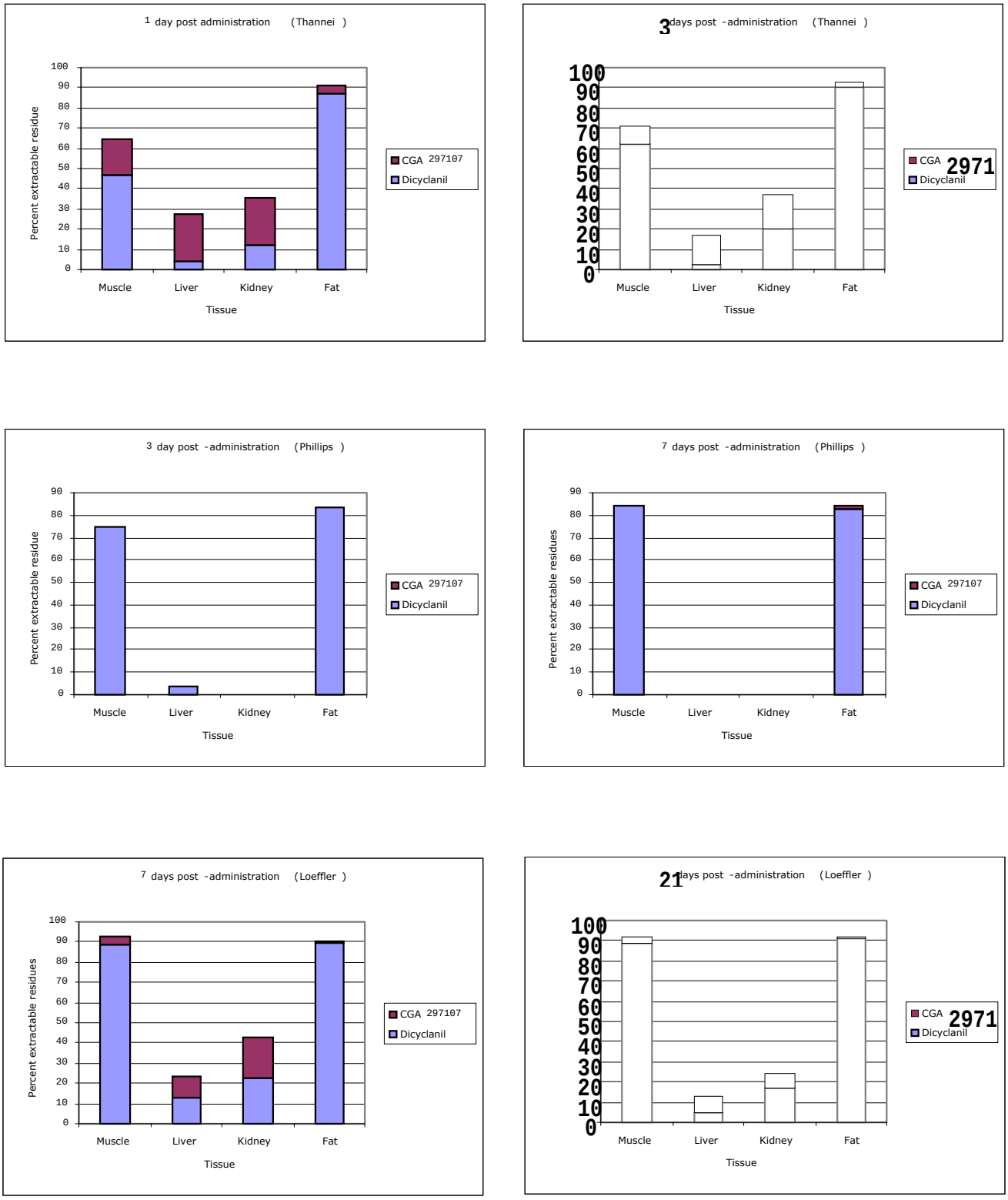
Figure 1. Concentration and identification of dicyclanil related radioactivity in liver tissue as a function of time after pour-on administration of dicyclanil at 100 mg/kg (Loeffler, 1998)



In the kidney, dicyclanil related total radioactivity was low at 7 days post-administration (0.723 mg/kg dicyclanil equivalents). The extractable residues consisted of 24.4% dicyclanil and 21.2% CGA 297107 in HPLC analysis. The respective values at 21 days post-administration were 22.0% and 9.5% but the extractability decreased. As in liver also in kidney the concentration of non-extractable residues did not change as a function of time. At 21 days post-administration total dicyclanil related residues were 0.230 mg/kg dicyclanil equivalents. In the kidney the MET 1U represented 3.4% of the extractable residues. As in the liver, a high proportion of the extractable radioactive residues could not be identified in kidney either. In the Study 1R/4R the kidney metabolite profile was almost identical to that in the liver. If the metabolite unresolved from MET 1U would be dicyclanil, the profile would be similar to that in the subsequent studies.

As indicated in the previous evaluation, the major component in muscle and fat tissues was dicyclanil (Figure 2). The CGA 297107 contributed only nominally (less than 5%) to the total extractable radioactive residues. In the study 1R the extractability was lower than in the two other studies while the CGA 297107 also appeared in higher proportion in that study. However, only studies 2R and especially 3R included time points of sampling that were relevant to the subsequent residue depletion studies. Unfortunately none of the studies provided information beyond 21 days post-administration.

Figure 2. Percentage of extractable dicyclanil and its metabolite CGA 297107 residues in muscle, liver, kidney and fat tissues after topical administration of dicyclanil at 1.25 g (Thanei, 1996a), 1.5 g (Phillips, 1996) and 100 mg/kg (Loeffler, 1998)



As can be seen from Figure 2, in all studies performed using radioactive dicyclanil, the extractable muscle and fat tissue residues were almost exclusively dicyclanil.

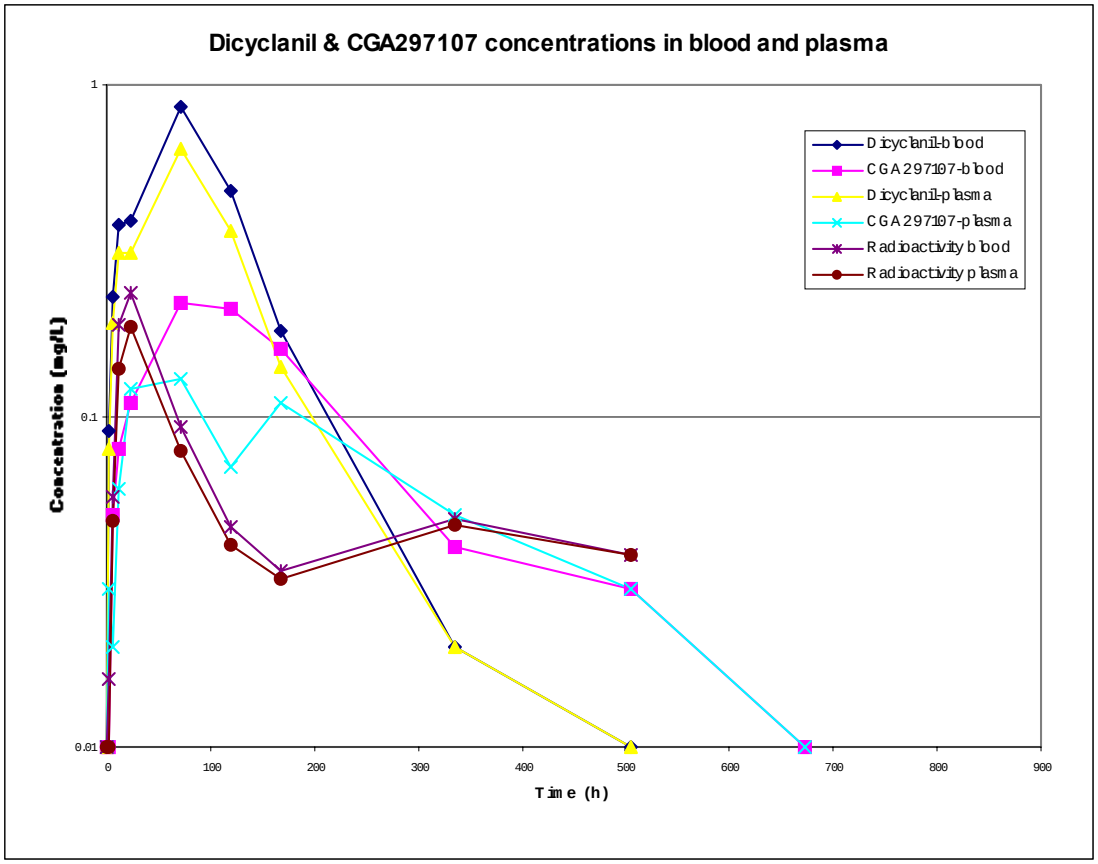
TISSUE RESIDUE DEPLETION STUDIES

Residue Depletion Studies (with Unlabelled Drug)

The Committee reviewed eight studies which were performed in sheep. Three of these studies were GLP non-compliant and the five other studies were done in accordance to the current GLP standards. Different parameters were investigated including formulation, dose, application method, age, breed and wool length. Furthermore, the studies were not identical in terms of compounds (parent and metabolites) analyzed. These studies were described in detail in the previous evaluation. For the present evaluation, the tissue residue depletion data were analyzed statistically using the logarithms of the concentrations in the various tissues and linear regression. For this purpose data obtained for different tissues, e.g., different types of fat tissues as well as the muscle tissues from various parts of the body, were pooled when appropriate. Thereafter, statistical tolerance limits were calculated as the one-sided upper 95% confidence limit over the 95th percentile of the population.

Figure 3 demonstrates the blood and plasma dicyclanil and CGA 297107 concentrations from the study of Hotz (1999) compared to the dicyclanil related radioactivity expressed as dicyclanil equivalents in blood and plasma by Anderson and Speirs (1998).

Figure 3. Concentrations of dicyclanil and CGA 297107 in blood and plasma after topical administration of non-radiolabelled dicyclanil (Hotz, 1999) compared to the respective total dicyclanil related radioactivity after topical administration of radiolabelled dicyclanil (Loeffler, 1998)



The blood and plasma concentrations appeared much higher in the non-radiolabel study. The dicyclanil concentration was also considerably higher than the CGA 297107 concentration. The half-life of dicyclanil seemed shorter than the half-life of the CGA 297107.

All of the residue depletion studies were considered. Extensive statistical analysis showed that the studies of Peterson and George (1997) in Merino sheep and Hotz (1999) in White Alp sheep adequately represented the depletion of dicyclanil related residues in sheep. The highest residue concentrations in muscle, liver and kidney tissues were recorded in Merino sheep and, therefore, considered representative for the depletion pattern. For the same reason the fat tissue of the White Alp sheep depletion data were used. Variations in the ratio of dicyclanil and CGA 297107 were seen in fat tissues collected from various parts of the animals.

For the present evaluation concerning the dicyclanil and CGA 297107 residues the major features of the study of Peterson and George are presented in Table 3. Only one dose is presented here. The full description of this study was provided in the previous evaluation. The results of the study by Hotz are presented in Tables 4 and 5.

Table 3. Mean (±standard deviation) residues of dicyclanil and CGA 297107 in tissues of Merino sheep treated with dicyclanil at 4mL/kg 0 day off-shears (Trial 97/4/1559 by Peterson and George, 1997)

	Mean ±SD concentrations (mg/kg)*				
Post treatment	**7 days**	**14 days**	**21 days**	**26 days**	**56 days**
Fat Renal					
Dicyclanil	0.16 ±0.03	0.06 ±0.02	0.03 N.C.**	0.03 N.C.	<0.01 N.C.
CGA 297107	0.04 ±0.02	0.01 N.C.	0.01 N.C.	0.01 N.C.	<0.01 N.C.
Subcut.					
Dicyclanil	0.24 ±0.09	0.89 ±1.60	0.05 ±0.04	0.04 ±0.04	0.02 N.C.
CGA 297107	0.05 ±0.03	0.03 ±0.01	0.03 ±0.01	0.02 N.C.	<0.01 N.C.
Muscle					
Dicyclanil	0.80 ±0.43	0.34 ±23	0.20 ±0.20	0.14 ±0.17	0.02 N.C.
CGA 297107	0.48 ±0.07	0.11 ±0.02	0.12 ±0.06	0.10 ±0.09	0.03 ±0.01
Kidney					
Dicyclanil	0.94 ±0.54	0.33 ±0.32	0.22 ±0.23	0.18 ±0.23	0.02 N.C.
CGA 297107	0.41 ±0.19	0.24 ±0.15	0.34 ±0.14	0.26 ±0.16	0.06 ±0.02
Liver					
Dicyclanil	1.21 ±0.69	0.46 ±0.39	0.32 ±0.35	0.22 ±0.26	0.02 N.C.
CGA 297107	0.49 ±0.11	0.23 ±0.05	0.37 ±0.14	0.18 ±0.23	0.08 ±0.01

* Each concentration represents the data of 4 animals

**N.C. = not calculated

Table 4. Mean residues of dicyclanil (CGA 183893) and CGA 297107 in tissues of White Alp sheep treated with dicyclanil at 2mL/kg 1 day and 7 weeks off-shears (Trial 99/17 by Hotz, 1999)

	Mean ±SD concentrations (mg/kg)*							
Post treatment	**7 days**		**14 days**		**21 days**		**35 days**	
Time off-shears	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**
Fat								
Oment. Dicyclanil	0.415	0.365	0.185	0.280	0.138	0.128	0.055	0.067
CGA 297107	0.008	0.018	0.012	0.005	0.005	0.005	0.005	0.005
Renal								
Dicyclanil	0.042	0.033	0.019	0.020	0.033	0.008	0.008	0.005
297107	0.019	0.024	0.005	0.008	0.005	0.009	0.005	0.005
Subcut. Dicyclanil	0.363	0.248	0.223	0.298	0.157	0.090	0.045	0.068
Remote 297107	0.022	0.021	0.008	0.008	0.006	0.005	0.005	0.005
Subcut. Dicyclanil	0.040	0.030	0.017	0.008	0.011	0.011	0.008	0.008
Adm. 297107	0.017	0.013	0.006	0.007	0.005	0.008	0.005	0.005
Muscle								
Hind- Dicyclanil	0.080	0.083	0.026	0.012	0.021	0.008	0.005	0.005
CGA 297107	0.065	0.0072	0.032	0.027	0.075	0.017	0.007	0.011
Fore- Dicyclanil	0.087	0.085	0.026	0.013	0.023	0.008	0.006	0.005
CGA 297107	0.068	0.080	0.042	0.028	0.031	0.020	0.008	0.009
Tender- Dicyclanil	0.087	0.087	0.026	0.013	0.020	0.008	0.006	0.005
CGA 297107	0.067	0.081	0.038	0.028	0.028	0.017	0.008	0.011
Kidney								
Dicyclanil	0.077	0.081	0.023	0.012	0.022	0.011	0.005	0.005
CGA 297107	0.165	0.0195	0.067	0.053	0.057	0.062	0.018	0.028
Liver								
Dicyclanil	0.127	0.127	0.038	0.025	0.033	0.019	0.007	0.005
CGA 297107	0.257	0.237	0.103	0.087	0.077	0.062	0.025	0.032

* Each concentration represents the mean of 6 animals

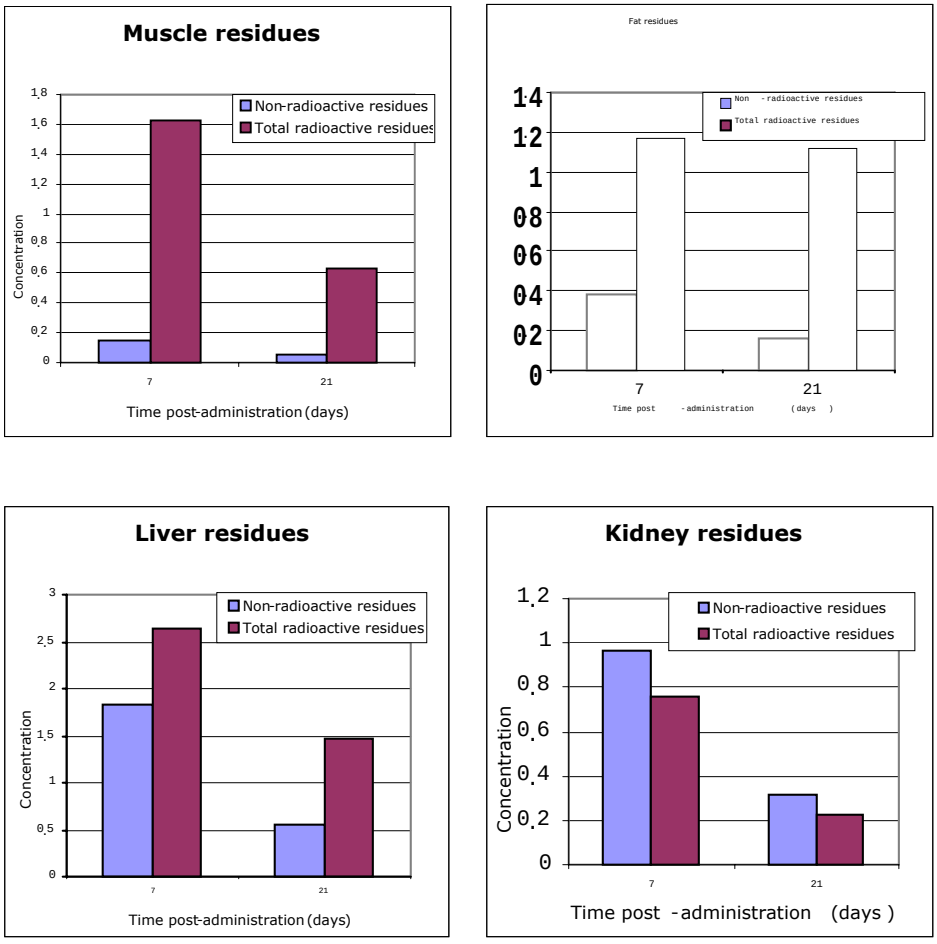
Table 5. The ratio of the mean residues of dicyclanil and the sum of dicyclanil and CGA 297107 ([dicyclanil]/[dicyclanil + CGA 297107]) in tissues of White Alp sheep treated with dicyclanil at 2mL/kg 1 day and 7 weeks off-shears (calculated from Trial 99/17)

	Ratio dicyclanil/(dicyclanil + CGA 297107)							
Post treatment	**7 days**		**14 days**		**21 days**		**35 days**	
Time off-shears	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**
Fat Oment.	0.98	0.95	0.94	0.98	0.97	0.96	0.92	0.93
Renal	0.69	0.58	0.79	0.71	0.87	0.47	0.62	0.50
Subcut. remote	0.94	0.92	0.97	0.97	0.96	0.95	0.90	0.93
Subcut. Adm.	0.70	0.70	0.74	0.53	0.69	0.58	0.62	0.62
Muscle Hind-	0.55	0.92	0.41	0.31	0.22	0.32	0.42	0.31
Fore-	0.56	0.52	0.38	0.32	0.43	0.29	0.43	0.36
Tender-	0.56	0.52	0.41	0.32	0.42	0.32	0.43	0.31
Kidney	0.32	0.81	0.26	0.18	0.28	0.15	0.22	0.15
Liver	0.33	0.35	0.27	0.22	0.30	0.23	0.22	0.14

The residue concentrations in the study of Peterson and George were generally higher than those reported in the study of Hotz. According to the values given in Table 4, the combined concentration of dicyclanil and CGA 297107 exceeds the MRL suggested by the Committee for dicyclanil only in fat tissue 14 day or more post-administration. The concentrations of both compounds in all tissues appeared to decrease as a function of time. No effect concerning time off-shears could be identified. The ratio of dicyclanil to the sum of dicyclanil and CGA 297107 decreased as a function of time as shown in Table 5.

There appeared to be some inconsistencies between the results obtained in the non-radiolabel and in the radiolabel studies (particularly 3R/6R). All the studies performed with non-radioactive dicyclanil, and in which the CGA 297107 concentration was also determined, appeared to produce similar results. In the study 6R only 0.7-1.5% of the residues in fat was CGA 297107, while the study by Hotz found CGA 297107 up to 53% in perirenal fat and up to 47% in the administration site subcutaneous fat. The ratio in omental and subcutaneous remote site fat, however, appeared to agree with Study 6R. In the muscle tissue 7 days post administration, the concentration of CGA 297107 represented almost 50% of the radioactive residue while in study 6R only 4.3% of the radioactive residue could be attributed to CGA 297107. In the study by Hotz the proportion of CGA 297107 increased with time. In the kidney tissue, except for one value, the concentration of CGA 297107 represented more than 68% of the total residues while the study 6R

Figure 3. Comparison of tissue dicyclanil residues after administration of radiolabel dicyclanil (Loeffler, 1998) and non-radiolabel dicyclanil (Hotz, 1999) to sheep at 100 mg/kg pour-on. The non-radiolabel residues consist of dicyclanil and CGA 297107. Correction factors of 0.15 and 0.25 (suggested by the sponsor) were applied for liver and kidney for the combined dicyclanil and CGA 297107 concentrations



suggested practically equal concentrations of the two compounds. In the liver tissue the study by Hotz found 35% or less of dicyclanil while the study 6R indicated that the concentration of dicyclanil was higher than the CGA 297107. According to the results of Hotz, the tissue of concern would be fat because dicyclanil concentrations exceeding the proposed MRLs can be found only in fat tissue in the observation period exceeding 7 days. The dicyclanil concentrations were high only in the specific fat tissues where the residue appeared to be dicyclanil only. The tissue concentrations in study 3R were higher compared to the results of Hotz, except for kidney tissue, regardless of the tissue when compared to the present study (Figure 3). Based on the available data, it is not possible to explain the discrepancy.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The method was described in the previous evaluation. The document, Summary Report EMEA/MRL/573/99-Rev.1 (1999), of which only parts could be included in the previous evaluation due to time limitations, incorporate important data concerning the analytical method. The submitted data also contained chromatograms obtained by use of the recommended analytical method. The analytical method was validated according to all the requirements of Volume VI of the rules governing medicinal products in the EU for all sheep tissues with both substances.

Dicyclanil and CGA 297107 are analyzed by a method that uses two different HPLC procedures for compound separation and detection. The extraction procedure is identical, using the same sample, until the final elution where dicyclanil is eluted using 1% isopropyl alcohol in dichloromethane while the CGA 297107 is eluted using 25% isopropyl alcohol in dichloromethane. These compounds are eluted from a strong anion exchange solid phase extraction (SPE) cartridge using organic solvents. The compounds here are cations and elution from such cartridges are generally performed with aqueous solutions using changes in the pH as the eluting factor. Therefore, the cartridges are used here as in direct phase separation. The two fractions are injected separately to two separate HPLC systems. The method description contains a warning indicating that the two fractions contain compounds that may interfere with the signal of dicyclanil or vice versa. Although validated for sheep tissues, significant modifications, including extraction, elution and mobile phase comnposition, were made in the most recent residue depletion study by Hotz (1999).

The determinative method applies strong cation exchange column while the described confirmatory method utilizes C18 column for dicyclanil and NH_2 column (with CN guard column) for CGA 297107 analysis. The confirmatory method does not fulfill the criteria of specificity compared to techniques such as mass spectrometry. There was a good agreement between the determinative and confirmatory method concerning dicyclanil while the agreement was poor for CGA 297107 in muscle and fat tissue samples. The limit of quantification for CGA 297107 was set at 100 µg/kg because of a large interfering peak in the confirmatory method. Based on the documentation the confirmatory method for dicyclanil appeared to exhibit best chromatographic performance.

An attempt was made to compare the analytical method and the concentration determined earlier by radioactivity detection (Smal, 1999). It was not clear from the submitted data how the expected ratio and concentrations of dicyclanil and CGA 297107 in the samples were determined. Dicyclanil was not detected in liver and kidney samples that contained 300 and 90 µg/kg dicyclanil related radioactivity. CGA 297107 was not detected in muscle and fat samples containing 80 and 180 µg/kg dicyclanil related radioactivity, respectively.

The use of two components, dicyclanil and its metabolite CGA 297107, as marker residue was suggested by the sponsor. Such an approach is not free of problems. Because the ratio between the components in this case is not constant, a significantly lower limit of quantification (LOQ) of the analytical method is required for both components. The recovery of both components should be independent of the concentration and similar in all tissue types. There should be no interference between the two analytes. Analytical reference material should be readily available for both compounds. The quality assurance procedures must control simultaneously both analytical processes. The cumulative effect of two analytes on precision and accuracy of the total residues must be calculated. The need to use two separate HPLC systems for the analysis of the two compounds was also considered a disadvantage. Based on the radiolabel studies, there seems to be no justification for use of the two components as marker residue for fat and muscle tissue. The proposed HPLC residue control method, however, indicated presence of CGA 297107 in muscle and fat tissues that cannot be explained based on the radiolabel dicyclanil studies. The use of sum of two components as marker residue could be appropriate in the case of liver and kidney. The problem in this case is that the sum of these two components does not form even 50% of the total residue in these tissues. Therefore, it is questionable whether the use of the sum of these two components provides additional accuracy to the determination of the total residues. Because CGA 297107 is a minor metabolite, physiological/pathological fluctuations in its concentration may cause misinterpretation in the total residue concentration. It appears, therefore, that the use of dicyclanil as the only marker residue should be preferred.

APPRAISAL

Dicyclanil was reviewed by the Committee at its 54th Meeting. Data were provided on the use of dicyclanil applied as a pour-on to sheep. Most of the studies were conducted according to current GLP standards. In its previous review, the Committee suggested that dicyclanil should be used as the marker residue. Concern was expressed that the consequence would be that the MRLs would exceed the TMDI. JECFA was requested to clarify its recommendation to use dicyclanil as the marker residue instead of the sum of dicyclanil and its metabolite CGA 297107.

Dicyclanil appeared to be considerably less metabolized in sheep than in laboratory animals. Radiolabel studies indicated that CGA 297107 was a minor dicyclanil metabolite consisting of not more than 5-10% of the excreted dicyclanil related radioactivity, which was consistent with the dicyclanil metabolism reported in laboratory animals.

According to the radiolabel studies dicyclanil is the major residue in muscle and fat tissues. Dicyclanil and CGA 297107 could be found in liver and kidney in almost equal concentrations but not exceeding 50% of the total residues.

The depletion of dicyclanil related residues from tissues was studied extensively and determined as a function of application technique, dose, wool length, sex, breed and age differences. The results of these studies were summarised in the documents produced by the 54th Meeting of the Committee. Most of the studies were conducted using higher than the recommended label doses. Although Merino sheep were used in most studies, statistical analysis in the present evaluation indicated that there were differences among the various breeds in terms of residue kinetics. The other variables among and within the studies were also analyzed and they were considered when indicated. The highest residue concentrations in muscle, liver and kidney tissues were recorded in Merino sheep and, therefore, considered representative for the depletion pattern. For the same reason the fat tissue of the White Alp sheep depletion data were used.

For the present evaluation, the tissue residue depletion data were analyzed statistically using the logarithms of the concentrations in the various tissues and linear regression. For this purpose data obtained for different tissues, e.g., different types of fat tissues as well as the muscle tissues from various parts of the body, were pooled when appropriate. Thereafter, statistical tolerance limits were calculated as the one-sided upper 95% confidence limit over the 95th percentile of the population.

The only available study that could be used for the determination of the ratio between total residue and marker residues was done using Dorset sheep included data until 21 days post-administration. The statistical analysis of the data examined the predictability of total residues when dicyclanil was used alone or together with CGA 297107 as the marker residue. The results showed that for time periods up to 21 days post-administration the variability of the data was only slightly smaller when the sum of the two compounds was used. However, the estimates of theoretical maximum daily intakes using either approach were similar. The Committee considered the use of a single compound approach to be preferred for several reasons. The most important was the need to analyze dicyclanil and CGA 297107 in two separate HPLC runs. This would place an unnecessary burden for a residue control program.

An estimate of a TMDI on the basis of the ratio of marker to total residue was only possible for the time period up to 21 days due to limited data available from the radiolabel study. However, the Committee has attempted to propose MRLs that further limit the exposure of consumers to residues, and therefore, MRLs were reduced to concentrations of dicyclanil that were consistent with good practice in the use of veterinary drugs and which could be determined with practical analytical methods. Estimates of TMDI could not be given for these MRLs since the corresponding concentrations of residues would be reached after approximately 28-32 days following the application of the dose. However, the Committee assumed that at such a late time after the treatment of the animals the parent drug and the metabolite CGA 297107 were the only residue of concern. Sufficient data were available to estimate the concentrations of these two compounds at 28-32 days following treatment with reasonable statistical certainty. Therefore a TMDI could be estimated using the statistical tolerance limits calculated for the sum of dicyclanil and CGA 297107.

Two analytical methods were described. They allowed separate detection of dicyclanil and its CGA 297107 metabolite. Significant amendments had to be made in the determinative method in the most recent residue depletion study. The Committee considered the second method, described as the confirmatory method, for dicyclanil best suited for monitoring in a routine residue control program.Two analytical methods were described. They allowed separate detection of dicyclanil and its CGA 297107 metabolite. Significant amendments had to be made in the determinative method in the most recent residue depletion study. The Committee considered the second method, described as the confirmatory method, for dicyclanil best suited for monitoring in a routine residue control program.

The following points were considered in setting the MRL:

- An ADI of 0 - 0.007 mg/kg of body weight, based on a toxicological endpoint, was recommended which resulted in a maximum daily intake of 0.42 mg for a 60 kg person.
- The marker residue is the parent dicyclanil.
- The total residue of concern at time points beyond 28 days after treatment of the animals is the sum of dicyclanil and its metabolite CGA 297107.
- Dicyclanil residues can be detected using liquid chromatography (HPLC) at the limit of quantification of 0.01 mg/kg.

Estimates of residue intake are tabulated as follows:

Food commodity	MRL (µg/kg)	Concentration of total residue of concern[1)] (µg/kg)	Consumption (g/person/day)	Intake (µg/person/day)
Liver	125	340	100	34
Kidney	125	340	50	17
Muscle	150	230	300	69
Fat	200	200	50	10
Sum				130

[1)] The the upper limit of the 95% confidence interval for the 95th percentile of the sum of the concentrations of Dicyclanil and metabolite CGA297107, expressed as equivalents of dicyclanil

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues in sheep, expressed as the parent drug:

Muscle - 0.15 mg/kg

Liver - 0.125 mg/kg

Kidney - 0.125 mg/kg

Fat - 0.20 mg/kg

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat, the theoretical maximum daily intake of dicyclanil residues from veterinary use 130 µg/person/. The Committee did not consider dicyclanil use in lactating sheep.

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