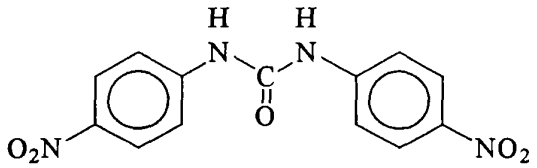
NICARBAZIN

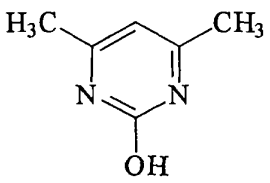
First draft prepared by
Dr. Robert J. Wells
Australian Government Analytical Laboratories
Pymble, Australia

IDENTITY

Chemical name: N,N'-Bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone (equimolar complex).
4,4'-Dinitrocarbanilide and 4,6-dimethyl-2-pyrimidinol (equimolar complex).

Chemical structure:





Molecular formula: $C_{13}H_{10}N_4O_5$ (phenyl urea portion of the molecular complex)
$C_{19}H_{18}N_6O_6$ (phenyl urea – dimethylpyrimidinone, 1:1 molecular complex)

Molecular weight: 292.25 (phenyl urea portion of the molecular complex)
426.38 (phenyl urea – dimethylpyrimidinone, 1:1 molecular complex)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Purity: nicarbazin consists of 1:1-molar mixture of N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone with a purity of not less than 96%

Appearance: pale yellow powder

Decomposion point: 265-275°C (dec.)

Solubility (g/L):

water	almost insoluble, complex dissociates slowly
alcohols, ether and chloroform	very slightly soluble, complex dissociates slowly
dilute acids	almost insoluble, complex dissociates faster
dimethylformamide	soluble (1:700), with dissociation of complex
dimethylsulfoxide	soluble, with dissociation of complex

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Nicarbazin is a coccidiostatic drug used for the prevention of caecal and intestinal coccidiosis in broiler chickens. This is its sole use in animal or poultry production. The complex between N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone which constitutes the commercial drug appears to be essential for the observed coccidiostatic

properties which are not duplicated by an equimolar mixture of the individual constituents, N,N'-bis(4-nitrophenyl)urea plus 4,6-dimethyl-2(1H)-pyrimidinone.

Dosage

Nicarbazin is fed continuously, mixed with feed, at a rate of 125 mg/kg (0.0125%).

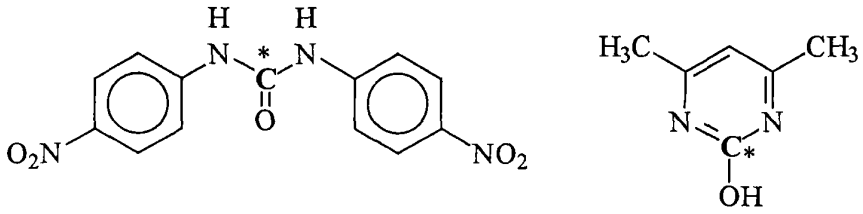
METABOLISM

Radiolabeled Nicarbazin

Various excretion and pharmacokinetic studies were conducted with 3 separate preparations of nicarbazin labeled with ^{14}C. Earlier studies utilisised two preparations where either the carbonyl of the bis-4-nitrophenyl urea was specifically ^{14}C-labeled or the 2 position of the pyrimidinone ring was specifically ^{14}C-labeled. The molecular labelling sites for these preparations are shown in Figure 1.

As nicarbazin passes into solution, the equimolar complex, comprising nitrophenylurea and pyrimidinone components, dissociates. Each of the two halves of the original complex is metabolised individually at separate rates (Porter and Gilfillan, 1955). The early labeling studies were therefore designed to determine the metabolic fate of both moieties comprising the original nicarbazin complex.

Figure 1 **Structures of ^{14}C-radiolabeled preparations of nicarbazin used in pharmacokinetic, metabolism and residue depletion studies**



Pharmacokinetics

Bioavailability and Excretion

Five studies in which chickens were dosed with ^{14}C-nicarbazin, radiolabeled either on the phenylurea or the pyrimidinone portion of the complex, are summarised in Tables 1 and 2. Birds were administered appropriately labeled ^{14}C-nicarbazin in the feed for 3 days followed by a withdrawal period of 4 days. During the whole period of the experiments, urine and faeces were collected and the radioactivity measured. Table 1 shows the recovery, during the course of the experiments, of ^{14}C from urine and faeces of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidinone portion of the drug for 3 days followed by a 4-day withdrawal period. Table 2 shows the results of other experiments where total radioactivity present in urine and faeces were measured over the 7-day period and compared with the administered doses (Nessel, 1977). Recovered radioactivity accounted for practically all of the administered ^{14}C-nicarbazin, averaging 94% in the chickens fed phenylurea-labeled nicarbazin and 104% in chickens fed pyrimidinone-labeled nicarbazin.

In separate experiments (Nessel, 1977), the distribution of ^{14}C nicarbazin in urine and faeces was measured in three different studies using the same labeled substances and the same feeding regimen as that used above (3 days on drug, 4 days withdrawal). After 3 days, an average of 50% of excreted radiolabeled phenylurea had been excreted in faeces and 5.6% in the urine. At the end of 4 days after withdrawal of drug, a further 41% was excreted in faeces and 3.3% in urine. By contrast, after 3 days, an average of 83% of excreted radiolabelled pyrimidinone had been excreted in urine and 7.4% in the faeces. At the end of 4 days after withdrawal of drug, a further 7% was excreted in urine and 2.6% in faeces.

The main excretion pathway for the pyrimidinone portion of the complex was in the urine (90%). This demonstrated that this moiety was well absorbed; it was also rapidly eliminated since by the third day, 83% of the dose had already

been eliminated. By contrast, the phenylurea portion of the nicarbazin complex was predominantly excreted through the faces (90%) at a slower rate than the pyrimidinone and the majority of the radioactivity was recovered in the first 3 days after withdrawal of medication. The observed urinary concentrations were only 5-10% of those of the pyrimidinone, indicating that the phenylurea portion was not rapidly eliminated by the kidney. Plasma levels of the phenylurea portion of nicarbazin were higher than those of the pyrimidinone portion and the plasma clearance value for the phenylurea portion was much lower than the pyrimidinone (Nessel, 1977).

Table 1. **Recovery of radioactivity from urine and faeces of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidinone portion of the drug for 3 days followed by a 4-day withdrawal period.**

Day	pyrimidinone ring ^{14}C-labeled				bis-4-nitrophenyl urea ^{14}C-labeled			
	drug dose (mg)	% total drug fed*	% drug excreted (urine)*	% drug excreted (faeces)*	drug dose (mg)	% total drug fed*	% drug excreted (urine)*	% drug excreted (faeces)*
1-fed with drug	27.5	37	21.7	1.3	17.5	22	0.4	4.6
2-fed with drug	28.8	75	53.8	4.1	34.4	65	5.9	23.1
3-fed with drug	17.5	100	83.9	6.7	28.1	100	7.5	44.5
1-no drug		100	90.4	8.2		100	8.4	63.8
2-no drug		100	90.7	9.0		100	8.8	79.4
3-no drug		100	90.7	9.3		100	9.0	83.7
4-no drug		100	90.8	9.8		100	9.0	85.4
Total pyrimidinone moiety excreted = 100.6%					Total urea moiety excreted = 94.4%			

* calculated as the % of the total drug administered over a 3 day period

Table 2. **Total recovery of ^{14}C, calculated as nicarbazin equivalents, from urine and faeces of chickens with artificial anus, fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled in either the phenylurea or pyrimidone moiety for 3 days followed by a 4-day withdrawal.**

Day	pyrimidinone ring ^{14}C-labeled				bis-(4-nitrophenyl)urea ^{14}C-labeled			
	drug dose (mg)	% ^{14}C excreted (urine)*	% ^{14}C excreted (faeces)*	% drug excreted (total)#	drug dose (mg)	% ^{14}C excreted (urine)*	% ^{14}C excreted (faeces)	% drug excreted (total)#
7 week chickens	53.1	89.8	10.2	110	50	10.1	89.9	95.4
14 week chickens Experiment 1	NM	NM	NM	NM	78.8	7.1	92.9	93.1
14 week chickens Experiment 2	73.8	90.3	9.7	100.5	80	9.5	90.5	94.4
Mean Recovery (%)		90	10	105.3		8.9	91.1	94.1

* calculated as the % of the combined total ^{14}C-drug excreted in urine and faeces
# calculated as the % of the administered ^{14}C-drug excreted in urine and faeces combined

Metabolism

The pyrimidinone portion of nicarbazin is shown, in ^{14}C-studies, to be rapidly eliminated, with no discernible residues evident 4 days after drug withdrawal. No metabolism studies have been conducted for this residue because of the very

low potential for detrimental residues with this molecule. By contrast, the phenylurea portion of nicarbazin is excreted much more slowly and leads to significant residues in liver and kidney.

Chickens were fed a diet containing 125 mg/kg of nicarbazin for 7 days and successively sacrificed between day 2 and day 7. Both urea and pyrimidinone moieties were ^{14}C-labeled as shown in Figure 1 and results of this study are summarised in Table 3. ^{14}C-Pyrimidinone-labeled concentrations peaked at 2.1 mg/kg in the plasma on day 2 whereas maximum ^{14}C-phenylurea-labeled plasma concentrations of 3.8 mg/kg occurred on day 4. Concentrations of the ^{14}C-labeled urea portion of the complex were much higher in liver and kidney than in plasma and muscle whereas, although ^{14}C-labeled pyrimidinone concentrations are highest in kidney, they are comparable in all four matrices. Liver and kidney concentrations of the pyrimidinone portion of the nicarbazin complex are about 10 times less than the concentrations of the phenylurea portion of the complex (Nessel, 1977).

Early Merck radiolabel studies conducted in the 1950s only positively identified or quantified, albeit colourimetrically, one metabolite, N,N'-bis(4-acetylaminophenyl)urea. From colourimetric analysis it was concluded that the ^{14}C-radiolabeled phenylurea was not extensively metabolised and was almost completely excreted 4 days after withdrawal of medication.

Table 3. Tissue profiles between days 2-7 in plasma, liver, kidney and muscle of 4-week old chickens fed for seven days a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled both in the phenylurea and pyrimidinone portions of the complex.

Day of Sacrifice	Concentration, calculated as nicarbazin, (mg/kg)*							
	Plasma		Liver		Kidney		Muscle	
	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr
Day 2	2.50	2.07	23.11	2.36	18.26	3.52	4.11	2.13
Day 3	2.54	1.84	26.48	2.15	19.26	3.09	3.86	2.03
Day 4	3.80	1.58	34.79	1.89	27.44	2.48	5.57	1.52
Day 5	2.75	1.07	29. 82	1.32	20.35	1.96	4.52	1.42
Day 7	3.33	1.79	33.78	2.08	26.74	2.95	5.98	1.63

^{14}C urea = carbonyl of the bis-(4-nitrophenyl)urea specifically ^{14}C-labeled
^{14}C pyr = 2-position of the pyrimidinone ring specifically ^{14}C-labeled; * mean of two replicates

A concern in early work based on radiolabeling studies lies in the placement of the ^{14}C-atom at the carbonyl group of the ^{14}C-radiolabeled phenylurea. This position would be expected to be labile and therefore the radiolabel is likely to be lost at an early stage of a possibly extensive metabolic degradation of the phenylurea portion of nicarbazin. A metabolite study using nicarbazin, generally ^{14}C-radiolabeled in the phenylurea portion, has been conducted (Manthey, 1986). Hubbard x White Mountain broiler chickens, approximately 6 weeks old, were fed 50 mg/kg ^{14}C-nicarbazin, alone or with ionophore, for 5 days and killed immediately at end of drug administration. The metabolic pattern was the same with or without accompanying ionophore. Parent nicarbazin accounted for about 79% of total liver radioactivity with about 10% of metabolite M-3 and 2% of metabolite M-1. Kidney radioactivity was 6% parent and 13% metabolite M-1 with the remainder as non-extractable polar activity. Metabolite M-2 was only found in excreta. The mean net radioactivity, calculated as mg/kg nicarbazin in tissues of chickens dosed with ^{14}C-nicarbazin in the above study is shown in Table 4.

Table 4. Drug-metabolite profiles in liver, kidney, muscle, skin and fat of chickens given 50 mg/kg of radiolabeled ^{14}C-nicarbazin for 5 days.

Study No.	No of Chickens	Tissue concentration, calculated as nicarbazin, (mg/kg)				
		Liver	Kidney	Muscle	Skin	Fat
1	6	10.84	7.17	1.47	1.52	1.77
2	8	11.64	7.57	1.35	1.62	2.00
3*	8	14.00	10.09	2.13	2.26	2.65

* = nicarbazin fed together with an ionophore

Metabolites of Nicarbazin

Metabolite Identification Code	Identity
M1	N,N'-bis(4-acetylaminophenyl)urea
M3	N,N'-4-acetylamino-4'-nitrodiphenylurea
M2	1,4-diacetylaminobenzene

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Tissue distribution and elimination studies carried out over a number of years by Merck & Co. and by Eli Lilly & Co. have been summarised as a consolidated document for submission to EU-SCAN for final compound evaluation (Merck and Lilly, 1986). Because of the non-availability of most of the source documents on which this summary was based, only the results presented in that summary paper are discussed here.

Results of studies in which chickens were fed a diet containing 125 mg/kg of nicarbazin for 7 days and successively sacrificed between day 2 and day 7 have been discussed earlier and are summarised in Table 3.

Residue depletion studies in which chickens were fed 125 mg/kg nicarbazin labeled in both moieties (see Figure 1) for 3 days are shown in Table 5 (Merck and Lilly, 1986). The birds were sacrificed successively, commencing at the withdrawal of medication (day 3), then after two days post-withdrawal (day 5) and then at three day intervals thereafter until day 14 and, finally, at day 21.

The data contained in Table 5 shows the rapid elimination of both drug and metabolites from the birds. Based on an assay sensitivity of 0.003-0.004 mg/kg, all tissues were essentially devoid of ^{14}C-residues from the pyrimidinone portion of nicarbazin by the fifth day after withdrawal. ^{14}C-Residues emanating from the phenylurea portion of nicarbazin were essentially only present in liver 5 days after withdrawal.

Table 5. Tissue profiles in plasma, liver, kidney and muscle of chickens fed a diet containing 125 mg/kg ^{14}C-nicarbazin, labeled both in the phenylurea and pyrimidinone portions of the complex for 3 days followed by withdrawal of medication.

Day of Sacrifice	Concentration, calculated as nicarbazin, (mg/kg)*							
	Plasma		Liver		Kidney		Muscle	
	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr	^{14}C urea	^{14}C pyr
Day 3*	4.48-5.32	1.50-1.79	41.48-51.5	1.80-2.38	36.58-40.05	2.63-3.73	8.15-9.30	1.78-2.00
Day 5#	<0.04	<0.04	0.2-0.34	0-0.216	0-0.085	<0.04	<0.04	0-0.18
Day 8#	<0.04	<0.04	0.105-0.228	<0.04	0-0.13	<0.04	<0.04	0-0.115
Day 11*	<0.04	<0.04	0.080-0.088	<0.04	<0.04	<0.04	<0.04	<0.04
Day 14*	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Day 21*	<0.04	<0.04	0.053-0.073	<0.04	<0.04	<0.04	<<0.04	<0.04

^{14}C urea = carbonyl of the bis(4-nitrophenyl)urea specifically ^{14}C-labeled
^{14}C pyr = 2-position of the pyrimidinone ring specifically ^{14}C-labeled
* range of values from two birds; # range of values from five birds

In studies conducted by Lilly (Merck and Lilly, 1986), chickens were fed either 50 or 60 mg/kg nicarbazin, ^{14}C-radiolabeled on either the urea or pyrimidinone portion of the molecular complex, in combination with ionophores. The chickens were dosed for 5 days and killed immediately after the final dose. Results, shown in Table 6, illustrate a tissue distribution pattern in line with other studies discussed earlier. The pyrimidinone portion of the complex contributes much lower residues, at the time of sacrifice, than do the dinitrophenylurea residues. As with all other studies, the dinitrophenylurea residues are highest in liver and kidney.

Table 6. **Drug residue profiles in liver, kidney, muscle, skin and fat of chickens administered radiolabeled ^{14}C-nicarbazin for 5 days and sacrificed immediately.**

Study No.	Labeled Portion and Dose	Tissue concentration, calculated as nicarbazin, (mg/kg)				
		Liver	Kidney	Muscle	Skin	Fat
1	^{14}C-urea, 60 mg/kg	14.86	11.46	2.36	2.59	2.43
1	^{14}C-pyrimidone, 60 mg/kg	0.28	0.34	0.31	0.18	-
2	^{14}C-urea, 50 mg/kg	11.15	7.24	1.18	1.81	1.93

A residue depletion study has also been conducted in which nicarbazin, ^{14}C-labeled in the phenylurea portion of the molecule, was fed to chickens in combination, with an ionophore, at 50 mg/kg for six days. Groups of four birds were sacrificed at 0, 1, 3, 5 and 7 days after withdrawal of drug. Total radioactivity was monitored and the concentration of the phenylurea portion of the drug was determined by HPLC. Results of these determinations are shown in Table 7. The results from radioactive and HPLC determinations were in good agreement for muscle , fat and skin, indicating that metabolites were not present in these tissues. At day 5 after drug withdrawal, liver was the only tissue with significant residues of parent drug.

Table 7. **Residue profiles in liver, kidney, muscle, skin and fat of chickens given 50 mg/kg BW of ^{14}C-nicarbazin for 6 days and sacrificed at various times after withdrawal of drug.**

Tissue		Sacrifice Day after withdrawal of Drug				
		0	1	3	5	7
Liver	**NC(mg/kg)**	10.24	4.82	0.50	0.10	ND
	TR (mg/kg)	16.81	7.88	1.19	0.22	0.06
	NC/TR ratio	0.61	0.61	0.42	0.45	-
Kidney	**NC(mg/kg)**	2.95	1.32	0.1	ND	NA
	TR (mg/kg)	12.09	5.38	0.8	0.14	0.03
	NC/TR ratio	0.24	0.25	0.13	-	-
Muscle	**NC(mg/kg)**	1.52	0.49	0.1	ND	NA
	TR (mg/kg)	2.19	0.76	0.11	0.02	ND
	NC/TR ratio	0.69	0.64	0.91	-	-
Skin	**NC(mg/kg)**	2.98	1.09	0.1	ND	NA
	TR (mg/kg)	2.44	0.85	0.13	0.03	0.01
	NC/TR ratio	1.22	1.28	0.77	-	-
Fat	**NC(mg/kg)**	2.67	0.78	0.12	ND	NA
	TR (mg/kg)	2.85	0.97	0.13	0.02	0.01
	NC/TR ratio	0.94	0.80	0.92	-	-

NC = N,N'-bis(4-nitrophenyl)urea; TR = Total residues; ND = not detected; NA = not analysed

The ratio of nicarbazin residues, determined by HPLC, to total residues, determined radiometrically for withdrawal days 0, 1, 3 and 5 were: in liver 0.61, 0.61, 0.42 and 0.45, respectively, in kidney 0.24, 0.25, 0.13 and not measurable, respectively, in muscle = 0.69, 0.64, 0.91 and not measurable, respectively, and in skin and fat, all values measured lay between 0.77 and 1.28.

Residue Depletion Studies Using Unlabeled Nicarbazin

A residue depletion study was conducted in which chickens were fed a diet containing 125 mg/kg nicarbazin from 3 days of age until suspension of medication at 44 days of age. Groups of 8 birds (4 male and 4 female) were sacrificed at

1, 3, 5, 7 and 9 days after the final dose. Edible tissues were analysed for the phenylurea portion of nicarbazin by a pulse polarographic method with a limit of quantification of 0.1 mg/kg and a limit of detection of 0.03 mg/kg (Wood and Dowling, 1980). The results of this study are shown in Table 8. These data indicated that the highest residue concentration occurred in liver at all withdrawal times, followed by kidney, skin/fat and muscle, respectively. Kidney, skin/fat and muscle residue values fell to < 0.1 mg/kg in four to six days and were about ten times lower than liver residue values at comparable withdrawal times after day 1. There was no evidence of drug recycling by chickens picking at the litter of the holding pen. This study also found that residues in frozen tissue were stable for at least five months at -20^{0}C. Analytical results are not corrected for recoveries, which exceeded 80%.

Table 8. Drug residues in liver, kidney, muscle and skin/fat of chickens given 125 mg/kg of nicarbazin for 42 days and sacrificed at various times after withdrawal of drug.

Day of sacrifice after withdrawal	Tissue concentration ranges* of nicarbazin, determined as phenylurea (mg/kg)			
	Liver	Kidney	Muscle	Skin/Fat
Day 1	14.4-21.0	2.8-5.4	1.4-2.2	1.6-3.0
Day 3	3.0-9.4	0.18-2.5	0.12-0.78	0.18-0.86
Day 5	0.40-2.7	<0.1-0.28	<0.1-0.1	<0.1-0.22
Day 7	0.14-0.59	<0.1	<0.1	<0.1-0.1
Day 9	<0.1-0.12	<0.1	<0.1	<0.1

LOQ = 0.1 mg/kg; LOD = 0.03 mg/kg; *8 birds sacrificed at each time point

In a more recent study (Kramer, 1990), chickens were dosed 125 mg/kg of nicarbazin in the feed for 49 days. After withdrawal of drug, groups of 4 birds (2 male, 2 female) were sacrificed at 24, 36, 48, 60 and 72 hours. N,N'-Bis-(4-nitrophenyl)urea residue concentrations in liver, muscle and skin/fat were determined by the HPLC method of Lewis (1989). The results, shown in Table 9, were in line with the earlier study but were not taken beyond 3 days withdrawal. At that time, muscle and skin/fat residues were at or below 0.2 mg/kg while the highest liver residue concentration measured was 3.39 mg/kg.

Table 9. Drug residues in liver, muscle and skin/fat of chickens given 125 mg/kg of nicarbazin for 49 days and sacrificed at various times after withdrawal of drug.

Hour of sacrifice after withdrawal	Tissue concentration ranges* of nicarbazin, determined as phenylurea (mg/kg)		
	Liver	Muscle	Skin/Fat
Hour 24	2.69-9.12	0.85-1.23	0.66-0.99
Hour 36	2.79-7.09	0.37-0.88	0.68-1.06
Hour 48	3.33-4.79	0.23-0.45	0.43-0.66
Hour 60	2.71-3.42	<0.1-0.233	0.14-0.51
Hour 72	0.90-3.39	<0.1-0.21	<0.1-0.28

* four birds sacrificed at each time point (2 male, 2 female)

METHODS OF ANALYSIS IN CHICKEN TISSUES AND EGGS

Earlier reported methods for the analysis of nicarbazin were based on either differential pulse polarography or colourimetry. These lack the necessary sensitivity or selectivity of a modern regulatory method but were used, none the less, to accumulate some of the residue data discussed above (eg. Michielli and Downing 1974). Residues in chicken tissues, down to the 1 mg/kg level generally required by regulatory agencies, has also been achieved by pulse polarography. The 4,4'-dinitrophenylurea portion of the complex was extracted with ethyl acetate. After removal of solvent, kidney and liver samples were cleaned up by a series of hexane washes of acetonitrile and acetonitrile/water solutions containing a small amount of dimethylsulfoxide (DMSO), followed by extraction into dichloromethane. After removal of dichloromethane, a pulse polarogram was obtained on a DMSO solution of the residue after washing with hexane/toluene. The resulting polarograms were essentially clean for tissues from untreated chickens, and recoveries of

fortified tissues at the 0.1-0.4 mg/kg level averaged 73%, 76%, 85% and 94% for liver, kidney, muscle and skin-fat, respectively (Wood and Downing, 1980). The limit of quantification in tissues was 0.1 mg/kg, but the estimated limit of detection was much lower at 0.03 mg/kg.

The first liquid chromatographic (HPLC) method for the determination of nicarbazin residues in chicken tissue appeared in 1983 (Takahashi and Yoshida) and was followed by a HPLC procedure for the determination of the phenylurea portion of nicarbazin in eggs, using UV-spectrophotometry as a confirmatory tool (Malisch, 1986).

A recent method for the analysis of the phenylurea portion of nicarbazin employed LC determination, with UV-detection, followed by LC-thermospray mass spectrometric confirmation of nicarbazin in chicken tissues (Lewis *et al.*, 1989). The dinitrophenylurea portion of nicarbazin was extracted from tissues with ethyl acetate. After filtration and evaporation, the extract was purified by liquid-liquid partitioning with acetonitrile-hexane followed by alumina chromatography. The dinitrophenylurea was separated and measured by reverse-phase LC on an octadecylsilyl column with UV-detection at 340 nm. The overall average recovery of the phenylurea from fortified tissues was 83.4±3.1% with coefficients of variation (CVs) below 10%. The lowest level validated in liver, kidney, muscle and fat tissues by this procedure was 0.10 mg/kg. The limit of detection was estimated to be 0.020 mg/kg. The identity of the analyte was confirmed by subjecting the purified extracts to LC with thermospray-mass spectrometric analysis using negative-ion detection and selective ion monitoring. Three ions at m/z 302 (M^-), 272 and 164 are characteristic of the analyte. A validation study of the method by the US-FDA has been reported using chicken liver and muscle at 2, 4 and 8 mg/kg using four laboratories (Leadbetter and Matusik, 1993). At the 4 mg/kg level, mean laboratory recoveries and CVs were 87.1% (10.9%) and 87.4% (7.5%) in muscle and liver, respectively (n=21). A separate set of validation data was generated for this method during a 1990 residue depletion study discussed earlier (Hazelton - Planalquimica, 1990). A similar LC method, based on a solid phase dispersion clean up has also been published (Schenck, 1992) but offers no obvious advantage over the Lewis method.

Although nicarbazin is not approved for use in laying hens, several methods are available that can monitor accidental residues in eggs, exemplified by a recent LC method (Kondo *et al.*, 1993). The recovery of nicarbazin added to eggs was 90.2% and the detection limit was 0.005 mg/kg. Nicarbazin was detected in 10% of eggs obtained by feeding chickens with a diet contaminated with nicarbazin within the range 0.07 to 1.39 mg/kg, but was not detected in eggs obtained commercially.

APPRAISAL

Nicarbazin, which has had a long history of use (four decades), is a coccidiostatic drug as an aid for the prevention of faecal and intestinal coccidiosis in broiler chickens. The complex between N,N'-bis(4-nitrophenyl)urea and 4,6-dimethyl-2(1H)-pyrimidinone which constitutes the commercial drug appears to be essential for the observed coccidiostatic properties. Nicarbazin is fed continuously, mixed in starter rations at a rate of 125 mg/kg (0.0125%).

Pharmacokinetics

An excretion study was performed in chickens using nicarbazin, [^{14}C]-radiolabeled in both phenylurea and pyrimidinone portions of the molecule. The main excretion pathway for the pyrimidinone portion of the complex was in the urine (>90%). This demonstrated that this moiety was well absorbed. It was also rapidly eliminated and by the third day after the last dose, 83% of the pyrimidinone had been eliminated. By contrast, the phenylurea portion of the nicarbazin complex was predominantly excreted (90%) through the faeces and at a slower rate than the pyrimidinone but the majority of the radioactivity was recovered in the first 3 days after withdrawal of medication. The observed urinary concentrations for the phenylurea portion were only 5-10% of those of the pyrimidinone indicating that kidney was not the major elimination pathway.

Metabolism

Broiler chickens were fed a diet containing 125 mg/kg of nicarbazin, [^{14}C]-labeled in both phenylurea and pyrimidinone, for 7 days and groups of birds were sacrificed between day 2 and day 7. Concentrations of the [^{14}C]-labeled phenylurea portion of the complex were much higher in liver and kidney than in plasma and muscle. [^{14}C]-labeled pyrimidinone concentrations were highest in kidney, they were not significantly lower in muscle, plasma and liver. Liver and kidney concentrations of the pyrimidinone portion of the nicarbazin complex are about 10 times less than the concentrations of the phenylurea portion of the complex. The rapid elimination of the pyrimidinone portion of nicarbazin and the non-detection of metabolites has led to an almost exclusive focus on the phenylurea portion of the complex in subsequent metabolism and residue depletion studies.

In another study, broiler chickens were fed 50 mg/kg [^{14}C]-nicarbazin, alone or with an ionophore, for 5 days and sacrificed immediately at end of drug administration. The metabolic pattern observed was the same with or without accompanying ionophore. The phenylurea portion of the parent nicarbazin accounted for about 79% of total liver radioactivity with about 10% of metabolite M-3 (N,N'-4-acetylamino-4'-nitrodiphenylurea) and 2% of metabolite M-1 [N,N'-bis(4-acetylaminophenyl)urea]. Kidney radioactivity comprised 6% of parent and 13% of metabolite M-1 with the remainder as non-extractable residues. From these data, N,N'-bis(4-nitrophenyl)urea was selected as the marker residue in all residue depletion studies.

Residue Depletion Studies

Residue depletion studies in which chickens were fed 125 mg/kg nicarbazin for 3 days with [^{14}C]-radiolabel in both moieties, showed the rapid elimination of both parent drug and metabolites from the birds. Based on an assay sensitivity of 0.003-0.004 mg/kg, all tissues were essentially devoid of radiolabeled residues from the pyrimidinone portion of nicarbazin by day five after withdrawal. [^{14}C] residues emanating from the phenylurea portion of nicarbazin were only present in liver five days after withdrawal.

In a second study, chickens were fed either 50 or 60 mg/kg nicarbazin, with a ^{14}C-radiolabel on either the phenylurea or pyrimidinone portion of the molecular complex, in combination with ionophores. The chickens were dosed for 5 days and sacrificed immediately after the final dose. The pyrimidinone portion of the complex contributed much lower residues than did the dinitrophenylurea residues. The ratios of phenylurea to pyrimidinone residues, at the time of sacrifice, were 53:1, 34:1, 8:1 and 14:1 in liver, kidney, muscle and fat, respectively. As with all other studies, the dinitrophenylurea residues were highest in liver and kidney.

Another residue depletion study was conducted in which nicarbazin was fed to chickens for six days at 50 mg/kg using ^{14}C-label in the phenylurea portion of the molecule, in combination with an ionophore. Total radioactivity was monitored and the concentration of the phenylurea portion of the drug was determined by HPLC. Table 10 shows the results of this study and also shows the ratios of N,N'-bis(4-nitrophenyl)urea, the marker residue, to the total residues.

Table 10. Residues of nicarbazin in chickens in mg/kg fed nicarbazin at 50 mg/kg BW for 6 days

Tissue	Portion of Nicarbazin	Sacrifice Day after withdrawal of Drug				
		0	1	3	5	7
Liver	NP	10.24	4.82	0.50	0.10	ND
	TR	16.81	7.88	1.19	0.22	0.06
	NP/TR	0.61	0.61	0.42	0.45	-
Kidney	NP	2.95	1.32	0.1	ND	NA
	TR	12.09	5.38	0.8	0.14	0.03
	NP/TR	0.24	0.25	0.13	-	-
Muscle	NP	1.52	0.49	0.1	ND	NA
	TR	2.19	0.76	0.11	0.03	ND
	NP/TR	0.69	0.64	0.91	-	-
Skin	NP	2.98	1.09	0.1	ND	NA
	TR	2.44	0.85	0.13	0.03	0.01
	NP/TR	1.22	1.28	0.77	-	-
Fat	NP	2.67	0.78	0.12	ND	NA
	TR	2.85	0.97	0.13	0.02	0.01
	NP/TR	0.94	0.80	0.92	-	-

NP = N,N'-bis(4-nitrophenyl)urea; TR = Total residues; ND = not detected; NA = not analyzed.

In one study, chickens were dosed 125 mg/kg of nicarbazin daily in the feed for 49 days. After withdrawal of drug, birds were sacrificed at 24, 36, 48, 60 and 72 hours. Residue concentrations in liver, muscle and skin/fat were determined by HPLC. At 72 hours after withdrawal, muscle and skin/fat residues were at or below 0.2 mg/kg. The

highest liver residue concentration measured was 7.09 mg/kg 36 hours following withdrawal. In an earlier, long term feeding study, young chicks were fed a diet containing 125 mg/kg nicarbazin daily from 3 days of age until 44 days of age. Groups of birds were sacrificed at 1, 3, 5, 7 and 9 days after the final dose. The highest residue concentration of N,N'-bis-(4-nitrophenyl)urea occurred in liver at all withdrawal times. Residues were lower in kidney, skin/fat and muscle, respectively. Kidney, skin/fat and muscle residue values declined to less than 0.2 mg/kg at five days and were about ten times lower than liver residue values at all withdrawal times after day 1. Marker residue concentrations in liver ranged from 14.4-21 mg/kg at day 1, 3.0-9.4 mg/kg at day 3, 0.4-2.7 mg/kg at day 5, 0.14-0.59 mg/kg at day 7, and <0.1-0.12 mg/kg at day 9.

Methods of Analysis

Several HPLC procedures for the determination of residues of the phenylurea portion of nicarbazin in chicken tissue are available. These methods, which employ UV-detection, appear to be suitable for the routine monitoring of nicarbazin residues. A limit of detection down to 0.02 mg/kg can be acheived. A recent method for the analysis of the phenylurea portion of nicarbazin employed HPLC determination, with UV-detection, followed by LC-thermospray mass spectrometric confirmation of nicarbazin in chicken tissues. The overall average recovery of the phenylurea from fortified tissues was 83% with coefficients of variation below 10%. An analytical method validated by six laboratories in a trial, organised by the US-FDA, has a limit of quantification of 0.1 mg/kg and is suitable for routine monitoring of an MRL of 0.2 mg/kg in all tissues. Although nicarbazin is not approved for use in laying birds, suitable methods are available which can monitor residues in eggs derived from accidental contamination with a detection limit of 0.005 mg/kg.

Maximum Residue Limits

Based on the ADI of 0-400 μg/kg established by the Committee, the permitted daily intake of parent drug and/or its equivalents is 24000 μg for a 60-kg person. In recommending MRLs for nicarbazin in broiler chickens, the Committee took the following factors into consideration:

- The limit of quantification of the analytical method is 0.1 mg/kg for all tissues.
- Nicarbazin is for use in broiler chickens only during the first 28 days post hatching.
- The marker residue is N,N'-bis-(4-nitrophenyl)urea.
- Mean ratios of marker residue to total residues in liver, kidney, muscle and skin/fat are approximately 0.45, 0.25, 0.65, and 0.90, respectively.
- The recommended MRLs are consistent with good practice in the use of veterinary drugs.

The Committee recommends MRLs of 200 μg/kg for muscle, liver, kidney and fat/skin in broiler chickens as N,N'-bis-(4-nitrophenyl)urea. Using these MRLs and food consumption factors of 300 g muscle, 100 g liver, 50 g kidney and 50 g fat, the theoretical maximum daily intake of residues as nicarbazin equivalents is 187 μg.

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