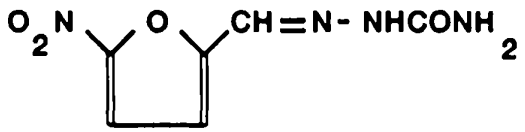
NITROFURAZONE

IDENTITY

Chemical name: 2-[(5-nitro-2-furanyl)methylene]-hydrazinecarboxamide or 5-nitro-2-furaldehyde semicarbazone

CAS number: 59-87-0

Structural formula:



Molecular formula: $C_6H_6N_4O_4$

Molecular weight: 198.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Nitrofurazone

Appearance: Yellow needles or crystalline powder

Melting point: 236-240°C (decomposes)

Solubility: Slightly soluble in water, c. 240 mg per L.
Slightly soluble in 95% ethanol, c.1.69 g per L
Soluble in dimethylformamide, 1 g in 15 mL
Soluble in alkaline solutions
Not soluble in chloroform, ether or benzene

UV maxima: 264 nm and 367 nm

Stability: Unstable in light

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

The nitrofurans are most commonly administered by the oral route in both animal and human medicine. Solutions, suspensions, capsules, tablets, powders for reconstitution and veterinary feed premixes are available. Topical ointments, aerosol powders, soluble dressings, urethral and vaginal suppositories, and ophthalmic, nasal and ear solutions have also been developed to accommodate other routes of administration.

Nitrofurazone is a broad spectrum antibiotic and also has some antiprotozoal activity. It is often used as a second line antibiotic particulary when bacteria are found to be resistant to other first line antibiotics. It is used a feed additive for pigs and poultry both therapeutically and as a prophylactic for gastrointestinal and respiratory disorders.

The length of administration of the drug varies between very short periods for some therapeutic uses and almost continuous administration as an in-feed additive.

METABOLISM

Pharmacokinetics

Pharmacokinetic studies were carried out in rats and calves. ^{14}C-Nitrofurazone (aldehyde-labelled) was administered as a single oral dose to male rats. The rats were either prefed or not fed "cold" Nitrofurazone. Rapid excretion of the radioactivity was exhibited by all the rats, with an average of 100.5% of the administered radioactivity appearing in the urine, bile, faeces and exhaled CO_2 within 48 hours. Tissue residues at 48 hours after dosing were <1 mg per kg. There were no differences in Nitrofurazone residues between rats from the two feeding regimens (Bowman, 1961).

^{14}C-Nitrofurazone was administered orally to rats. 88% of the radioactivity administered was absorbed from the gastrointestinal tract into the bile and urine. After 96 hours almost all of the radioactivity administered was excreted in the urine and faeces. Only a small amount (not stated) was excreted as parent drug (Tatsumi et al., 1971).

A single dose of nitrofurazone (14 mg per kg body weight) was administered to five preruminant calves. Peak plasma concentrations (mean 3.5 mg per L) were observed at approximately 3 hours after administration. The final elimination half-life was 5 hours. The renal clearance of the unbound drug was c. 0.42 ml/min/kg. Less than 2% of the administered dose was recovered as parent drug in the urine. (Nouws et al., 1986)

Metabolism in Food Animals

No detailed metabolism studies were available. As Nitrofurazone is a minor fraction of excreted residues in preruminant cattle and presumably other farm animals, it is certain that Nitrofurazone is extensively metabolised. One might assume that the 5-nitro group is reduced to the amine. However in the absence of radiometric studies or "cold" drug studies in food animals the only conclusion is that the parent drug is extensively metabolised to unknown metabolites.

Metabolism in Laboratory Animals

Tatsumi et al, (Sponsor Abstracts, Nº 455-459) studied the absorption, excretion and metabolism of Nitrofurazone in rats. Nitrofurazone is readily absorbed from the GI tract, extensively metabolised and rapidly excreted. In *in vivo* and *in vitro* studies the drug is metabolised in the mucosa wall of the small intestine. One identified route was the reduction of the 5-nitro group to the amine involving the enzyme, xanthine oxidase. The metabolites were less well absorbed than the parent compound.

The xanthine oxidase system in milk also reduces Nitrofurazone to the 5-amine. (Taylor et al.,1951).

After the incubation of ^{14}C-Nitrofurazone in the presence of 9,000g rat liver supernatant or xanthine oxidase-hypoxanthine, three unidentified metabolites were found by HPLC, one of which was thought to be a cysteine conjugate (Goodman et al.). Further evidence for both the aerobic and anaerobic reduction of the 5-nitro group by the xanthine oxidoreductase system in rat liver extracts is reported by Kutcher et al. (1984).

Paul et al., (1960) sugggested that Nitrofurazone is metabolised in mammalian tissues both by 5-nitro reduction and cleavage of the -CH=N- linkage and that none of the end products posessed antibacterial properties.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No studies are available for food animals. In rats no residues were identified at the 1 mg per kg level (Bowman, 1961).

Residue Depletion Studies with Unlabeled Drug

Four pigs were fed Nitrofurazone at 0.011% inclusion in the feed for ? days. The pigs were killed at zero withdrawal time. The tissues analysed for Nitrofurazone by extracting the Nitrofurazone and measuring the UV spectra between 400-460 nm. Quantitation was made by iteration against standards at 430 nm and

subtracting the blank value for untreated pigs. The residues in liver, kidney, fat, ham and loin were all <0.1 mg per kg. (SKB submission from study done 1967).

One-day-old chicks were raised to maturity on a diet fortified with 0.0055% Nitrofurazone. At 42 days of age nine chickens were sacrificed, the tissues from pairs of birds (and on single bird) were blended and residues measured by the method of Parks (1989). The results are shown in table 1. A further nine birds were sacrificed following a two day withdrawal of the drug. No residues (<0.5 μg per kg) were detected in these birds. (Parks and Kubena, 1992).

Comment: The tissues were sampled quickly and immediately frozen to temperatures <-50°C, sometimes using liquid nitrogen. Nevertheless there is no appraisal of the stability of the nitrofurazone under these conditions of storage. Laurensen and Nouws (1989) stabilised the Nitrofurazone by immediately homogenising the samples in buffer before freezing (see below).

Table 1. Residues of Nitrofurazone (μg per kg) in Chickens fed Nitrofurazone

Bird numbers	Liver	Thigh	Breast
1024, 1028	146	2.22	2.64
1033, 1040	120	2.20	1.39
1047, 1053	87	1.17	0.69
1058, 1065	148	2.30	2.72
1067	63	9.11	5.36
All (mean ± SD)	113 ± 37	3.40 ± 3.23	2.56 ± 1.78

Chickens were fed Nitrofurazone at 150 g per ton of feed for 14 days and the residues measured at 3, 4 and 5 days after drug withdrawal (Mertz, 1971). The results are shown in table 2.

Table 2. Residues Nitrofurazone (μg per kg) in chickens

Tissue	3 days WT	4 days WT	5 days WT
Liver		<2 (7)	<2 (5)
Muscle		<2 (4)	<2 (4)
Skin with fat		<2 (4)	<2 (4)
Kidney	<2 (3), 2 (1)	<2 (2)	<2 (1), 2 (1)

The number of chickens are given in parentheses.

Bound Residues/Bioavailability

No evidence was available.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Summary: As indicated above, Nitrofurazone was shown to be less than 2% of the total residues of the dosed animals. It is assumed that Nitrofurazone almost certainly forms a very minor proportion of the total residues in the edible tissues. Nevertheless there are numerous validated methods for measuring residues of the parent drug. Four of the methods are discussed.

A TLC method for Nitrofurazone in tissues of chickens was one of the earlier developed methods. After homogenisation, exhaustive solvent-solvent partition and chromatography on a celite:silica gel column an aliquot of the final extract was run on a TLC plate. The Nitrofurazone spot was measured at 360 nm. This method is semi-quantitative and claims a lower limit of detection of 2 μg per kg Nitrofurazone in chicken kidney. (Heotis et al, 1971).

A gas chromatography - electron capture detection (GC-ECD) method for determing Nitrofurazone in chicken tissues at the 2 μg per kg level is provided by the sponsors (Hobson, 1976). Nitrofurazone was extracted into ethyl acatate. The extract was reduced in volume and transferred with benzene and hexane. The organic solution was extracted with water. The water extract was acidified and the Nitrofurazone hydrolysed at 75°C to form the 5-nitro-2-furaldehyde (NFA). The extract was placed on a Florisil column, washed with benzene, and the NFA eluted with benzene/ethyl acetate. An aliquot was placed onto a 6 foot x 4 mm column packed with 3% DEGA on Chromosorb W at 150°C. Standards were run and the amount of NFA (as Nitrofurazone) determined by iteration.

The lower limit of detection was 2 μg per kg. The average recovery of Nitrofurazone as spikes at 2 μg per kg from tissues and the CVs for the method are shown in table 3.

Table 3. Recovery of Nitrofurazone from chicken tissues

Tissue	% Recovery of 2 μg per kg spike (n = 5)	CV (%)
skin	75	5.7
muscle	76	5.2
liver	75	4.4
kidney	74	5.6

Two of the HPLC methods are detailed below.

Laurensen and Nouws (1989) describe a method which prevents degradation of the Nitrofurazone in organic tissue and measures the residue at a 1 ug per kg level by an HPLC procedure. The fresh samples of urine, plasma and edible tissues were blended or homogenised with 1.5*M* KH_2PO_4 containing 0.2% sodium azide. They were immediately frozen and stored in the dark until assayed. During extraction the samples were protected from light. The samples were extracted with dichloromethane-ethyl acetate. The extract was evaporated to dryness and taken up in a mixture of n-hexane and phospate buffer, pH 5.0. An aliquot of the aqueous phase was injected into an HPLC system. The columns were a guard column and a separation column (Zorbax CN). The eluent was sodium acetate buffer (pH 5.0) and methanol; flow rate 1.5 ml per min at 20°C. Detection was by UV at 365 nm. Quantification was done by iteration with calibration graphs using the addition of 1 to 100 ug per kg spikes. The mean recovery values and CVs are shown in table 4.

Table 4. Recovery and Reproducibility of 1-100 μg per kg Nitrofurazone in bovine tissues

Tissue	Recovery (%)	CV (%)	Linearity
plasma	69.7	3.0	0.9999
meat	60.7	2.2	0.9998
liver	60.7	2.0	0.9998

An HPLC-ECD method for measuring residues of Nitrofurazone in chicken tissues was published by Parks (1989). The method includes extraction of tissues with chloroform-ethyl acetate-dimethyl sulfoxide (50:50:8), adsorption onto neutral alumina and subsequent elution of the residues with pH 6.0 phosphate buffer-methanol. An aliquot was injected into an HPLC system with a Supelcosil LC-18 column. The mobile phase was pH 6.0 phosphate buffer-methanol and 0.001*M* EDTA running at 1 ml per min. Quantification was done by iteration with calibration graphs using the addition of 6 to 200 ug per kg spikes at 5 to 6 concentrations. The linearity was 0.9995 and the mean recovery values and CVs are shown in table 5.

Table 5. Recovery and Reproducibility of 6-200 μg per kg Nitrofurazone in chicken tissues

Tissue	Mean Recovery (%)	CV (%)
Liver	74.0	7.7
Thigh	78.7	6.1
Breast	76.8	8.1

This method has been applied to incurred chicken tissues (see above).

APPRAISAL

There is not sufficient information to establish an MRL. The major difficulty is the complete lack of information on the nature or quantity of residues in tissues of target animals. There is no radiometric study in target animals. The metabolism is probably somewhat similar to that for furazolidone in that there is extensive metabolism to numerous metabolites many of which form bound residues. There is some evidence that the 5-nitro group is reduced to the amine by the xanthine-oxidoreductase system. Whereas the parent drug rapidly disappears as a residue, the bound residues and maybe other metabolites could have long half lives. There is a need for information on the quantity, nature and toxicity of the total residues.

Following the in-feed administration of a commercial dose of drug, residues of the parent drug were measured in tissues of chickens at zero withdrawal time. The levels were highest in liver (113 μg per kg) and lowest in muscle tissue (0.7 to 9 μg per kg). At two days withdrawal time no residues (< 1 μg per kg) of parent drug were detectable. In an older study residues were found at the 2 μg per kg level in the kidneys of one chicken at 3 days withdrawal time and another bird at 5 days withdrawal time. No residues at the 0.1 mg per kg level were detected in pigs at zero withdrawal time after feeding at the 0.011% inclusion level. There are several well validated methods for measuring residues of the parent drug at the 1 to 2 μg per kg level.

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