Depletion and Bioavailability of [14C]Furazolidone Residues in Swine Tissues

David W. Gottschall* and Richard Wang

Department of Developmental Pharmacokinetics and Drug Metabolism,
SmithKline Beecham Animal Health, 1600 Paoli Pike, West Chester, Pennsylvania 19380-1169

Six swine (three male, three female) were dosed orally with [14C]furazolidone for 14 days at a dose level of 16.5 mg kg^{-1} day^{-1}. At 0-day (10-h) withdrawal, liver contained the highest residues (41.1 ppm) followed by kidney (34.4), muscle (13.2), and fat (6.2). After a 45-day withdrawal, all tissues had total residues of approximately 2.0 ppm. The amount of aminooxazolidinone (AOZ) released from the bound tissue residues, on average, represented 7.5% of the total residues. Additional tissue samples were lyophilized, pelleted, and fed to rats for determination of bound residue bioavailability using the Gallo-Torres model. Bioavailability of liver residues decreased from 40% at 0-day withdrawal to 19% after 45 days, while muscle residues showed no change in relative bioavailability with corresponding values of 37% and 41%. Pretreatment of tissue samples with organic solvents to generate a nonextractable pellet removed 10−20% of the initial radioactivity but had no effect on the overall bioavailability of the remaining residues.

Keywords: *Furazolidone; tissue residues; bioavailability; Gallo-Torres*

INTRODUCTION

Furazolidone [*N*-5-(nitro-2-furfurylidene)-3-amino-2-oxazolidinone] belongs to a class of chemicals broadly known as the nitrofurans. The compound has been widely used as an antibacterial and antiprotozoal feed additive for poultry and swine and has provided veterinary practitioners with a low-cost solution to the control of *Salmonella* and *Escherichia coli* infections. Marketing approval for furazolidone was recently withdrawn by the FDA (Kessler, 1991) due to the mutagenicity/carcinogenicity concerns of the parent drug, lack of sufficient information on the metabolites, and absence of methodology required for appropriate monitoring of tissue residues.

The concern over the nitrofurans was not limited to the United States. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the status of nitrofurazone and furazolidone in June 1992 but failed to allocate acceptable daily intake (ADI) or maximum residue levels (MRL) for these compounds. The JECFA concerns centered on (1) the carcinogenicity of the parent drug, (2) the lack of data on the quantity and nature of the metabolites and tissue residues, and (3) the absence of an identified marker residue and regulatory method (JECFA, 1992).

The research studies described in this paper were performed between 1988 and 1992 as part of an overall effort to provide new information on furazolidone tissue residues for worldwide regulatory agencies. Although total residue and bioavailability studies using [14C]-furazolidone-dosed swine were conducted previously (Vroomen *et al.*, 1986b, 1988a, 1990), these investigators used tissues from animals held for relatively short withdrawal times (0−14 days). The present strategy involved extended withdrawal periods of up to 45 days to allow additional time for the depletion of tissue residues. This study examined whether the longer withdrawal period, which was still acceptable from a marketing perspective, would result in an overall reduction of residue bioavailability.

MATERIALS AND METHODS

[14C]Furazolidone was synthesized by the Radiochemistry Department of SmithKline Beecham Pharmaceuticals (Shu *et al.*, 1991). Because of the expected hydrolysis of the imine group linking the nitrofuran and aminooxazolidinone rings *in vivo*, radiolabels were incorporated into both portions of the molecule. Two independent synthetic procedures were followed to label each ring of the furazolidone molecule. [14C]-Furazolidone I was prepared using [1,2-14C]2-chloroethanol to label the oxazolidinone ring. [14C]Furazolidone II used $Ba^{14}CO_3$ to label the formyl carbon attached at position 2 of the nitrofuran ring via the intermediate 2-furan[14C]carboxylic acid. [14C]Furazolidone I and II were each diluted to identical specific activities using a commercially available lot of nonradiolabeled furazolidone. The final material, [14C]furazolidone III, was subsequently prepared by combining both radiolabeled compounds in equal amounts. Two batches of [14C]furazolidone III were prepared according to this technique to yield specific activities of 1.0 and 3.0 mCi/g. The HPLC radiochemical purity (≥98%) was determined using a silica gel, 5 μm, 4.6 mm × 25 cm column (J. T. Baker). The chromatography was performed at ambient temperature with 1,2-dichloroethane/acetonitrile (90:10 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Detection was by UV at 240 nm coupled with on-line radioactivity detection (Ramona Tru-Count scintillator, 5.0 mL/min flow rate, 0.75 mL flow cell). The [14C]furazolidone was injected at a concentration of 0.2 mg/mL and eluted with a retention time of 8.9 min. The HPLC chemical purity (≥98%) was performed with a Partisil 5 ODS-3, 5 μm, 4.6 mm × 25 cm column (Whatman) using a mobile phase of 0.25 N triethylammonium phosphate, pH 2.5/water/acetonitrile (150:150:100 v/v/v) at a flow rate of 1.0 mL/min. Detection was by UV at 220 nm with elution occurring at 9.9 min. The labeling pattern of the final dosing material is shown in Figure 1.

Swine Residue Study. *Animals and Dose Administration.* Ten crossbred swine (5 barrows, 5 gilts; 40−45 kg on day of dosing) were acclimated to individual swine metabolism cages in a temperature-controlled room (average minimum 66 °F, maximum 74 °F, humidity 59%). The standard diet given *ad libitum* via individual feeders consisted of Purina 2349 Complete Sow Chow III W/O containing not less than 13% crude protein, not less than 2.5% crude fat, and not more than 5.0% crude fiber. Water was available *ad libitum* via automatic water nipples. During the 2-week acclimation period, the swine were trained to accept food treats (peanut butter,
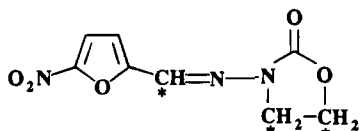
Bioavailability of [14C]Furazolidone Residues in Swine Tissues

J. Agric. Food Chem., Vol. 43, No. 9, 1995 **2521**

Figure 1. [14C]Furazolidone **III** labeling pattern.

doughnut pieces, apple pieces, etc.) containing empty gelatin capsules (Lilly brand 000). Following acclimation, the swine were assigned to four groups based on body weight distribution and temperament. Groups I–III each contained one male and one female per group and were subsequently dosed with [14C]furazolidone twice per day for 14 days at a dose rate of 16.5 mg kg^{-1} day^{-1} (equivalent to consumption of medicated feed at 300 g/ton). The four remaining swine (group IV) comprised the controls and were sham dosed with empty gelatin capsules. Half of the daily dose was administered at 8:00 a.m. with the remainder being given at 4:00 p.m. The gelatin capsules containing the [14C]furazolidone were embedded in the food treats to facilitate oral delivery of the drug. Feed was withdrawn 2 h prior to dosing to increase acceptance of the food treats. Groups I and II received the [14C]furazolidone having the lower specific activity of 1.0 mCi/g. Group III, however, received the higher specific activity (3.0 mCi/g) material since these animals were designated for the extended withdrawal time. To minimize stress and handling, body weights for groups I–III were obtained every 3 days post-arrival to calculate the required daily dosages. Estimates of individual body weights were obtained by linear regression using the three most recent values when actual weight data were not available.

Following administration of the final dose, groups I, II, and III were subjected to withdrawal times of 0 (10 h), 21, and 45 days, respectively. The animals were terminated by stunning via captive bolt pistol followed by immediate exsanguination. The entire liver, both kidneys, muscle (composite from leg and loin), and fat (subcutaneous from back and neck region) were removed during necropsy and stored frozen.

Combustion Analysis. Liver, muscle, and kidney tissues were thawed, processed through a meat grinder, composited, reground two more times, and divided into subsamples. Each subsample was combined with a known amount of water, thoroughly homogenized (Tekmar Tissumizer), shell frozen, and lyophilized (Labconco Model 3 freeze-dryer). The dried tissue samples (5 × 200 mg) were analyzed for total radioactive residues by combustion analysis (Packard oxidizer Model 306) and scintillation counting (Packard Model 2250CA). All remaining bulk lyophilized tissues were stored frozen. Fat samples were thawed and ground similarly but were analyzed directly (5 × 100 mg) without lyophilization. The combustion results are reported in parts per million based on [14C]furazolidone equivalents.

Analysis of Aminooxazolidinone (AOZ) Released from Bound Residues. The release of AOZ was determined from lyophilized samples (250 mg) of liver, kidney, and muscle tissues (Hoogenboom *et al.*, 1991b, 1992b). Briefly, samples were exhaustively extracted with organic solvents. The resuspended pellets were mixed with 4 mL of 0.1 N HCl containing 0.5 mM 2-nitrobenzaldehyde and subsequently incubated in a water bath at 37 °C for 16 h. The released and derivatized AOZ (NPAOZ) was extracted with ethyl acetate, taken to dryness under nitrogen, redissolved in acetonitrile/water (1:1), and analyzed by HPLC using a C$_{18}$ Spher, 4.6 mm × 25 cm column (Chrompack, The Netherlands). The chromatography was performed at ambient temperature with acetonitrile/water (30:70 v/v) as the mobile phase at a flow rate of 1.0 mL/min. NPAOZ was detected by UV at 275 nm (Spectroflow 783, Applied Biosystems), UV–vis diode array (HP1041A, Hewlett-Packard), or on-line radioactivity (LB 506C, Berthold). Alternatively, fractions containing NPAOZ, as detected by UV at 275 nm, were collected, mixed with scintillation cocktail, and counted for total radioactivity. This figure was then corrected for sample size, dry weight, and the specific activity of AOZ (2.45 or 7.35 dpm/ng for groups I and II and for group III,

respectively) to obtain the micrograms of AOZ released per gram of wet tissue.

Rat Bioavailability Study. *Animals and Dose Administration.* The bioavailability of the radioactive residues present in the swine tissues was examined using adult male CD rats (Gallo-Torres, 1977). A total of 44 rats was used in this study with 4 rats assigned to each of 11 groups (A–K). Prior to surgery, the animals (8–9 weeks of age, *ca.* 220 g; Charles River, Ltd., Margate, Kent, U.K.) were placed in stainless steel cages with suspended mesh floors. Food and water were supplied continually before surgery. Room temperature was kept at 22 ± 2 °C and relative humidity at 40–60%, along with a 12 h light/dark cycle. Surgery was performed to cannulate the common bile duct and to allow for recirculation of sodium taurocholate (25 mg 0.9 mL^{-1} h^{-1}) via continuous infusion into the stomach. The rats were placed in restraining cages designed for the separate collection of urine and feces and allowed to recover from surgery for 24 h while being fed a standard laboratory diet or the appropriate pelleted control tissues. Subsequently, between 24 and 48 h post-surgery, the rats were permitted to spontaneously consume (1) the pelleted radiolabeled swine tissue residues, (2) the control swine tissues fortified with [14C]furazolidone, or (3) the [14C]furazolidone medicated laboratory diet (300 ppm). The four rats in group A received a single 16.5 mg/kg dose of [14C]furazolidone (6.6 mg/mL) in polyethylene glycol 200 via a stomach tube. Consumption of the medicated tissues and diets continued for 24 h followed by a 48-h withdrawal period, after which time the animals were sacrificed (cervical dislocation under halothane/oxygen anesthesia). Bioavailability was assessed by the amount of radioactivity recovered in the bile, urine, tissues, carcass, and intestinal wall, while nonbioavailable residues included counts associated with the luminal contents and feces (Gallo-Torres, 1977). Bioavailable residues were determined for both swine liver and muscle tissues obtained following the 0- and 45-day withdrawal periods (groups I and III from the residue depletion study). In addition, 0- and 45-day swine liver samples as well as 0-day swine muscle samples were subjected to exhaustive extraction with organic solvents followed by the determination of the bioavailability of the remaining nonextractable residues.

Preparation and Assay of Medicated Diets. Medicated doses were prepared by mixing Labsure Laboratory Diet II, control muscle (lyophilized), or control liver (lyophilized) with [14C]furazolidone at a rate of 0.3 mg/g. Medicated rat diet was prepared by weighing [14C]furazolidone into a small glass container (*ca.* 20 mL). An appropriate quantity of diet was weighed and 10 g was added to the [14C]furazolidone. The mixture was shaken and transferred to a mortar along with an additional amount of diet, and all contents were ground with a pestle. This mixture was added to a polythene bag containing the remaining quantity of diet necessary to prepare the complete dose. The bag was shaken by inversion (*ca.* 200 times) for at least 2 min, and the concentration and homogeneity were confirmed by combustion. The medicated diet was pelleted (0.5-g pellets) using a hydraulic press. To achieve a suitable consistency, the diet was mixed with water (20% w/w) prior to pelleting and the pellets were freeze-dried. Final concentration and homogeneity were assessed once again by combustion analysis. The measured specific activity was used in conjunction with feed intake to calculate the administered dose.

The lyophilized liver and muscle tissues (control, dosed, and nonextractable) were used directly without the addition of the Labsure Laboratory Diet II and thus comprised 100% of the material consumed by the rats. Fortification of the control tissues with [14C]furazolidone was performed according to a procedure similar to that used for the medicated rat diet except that water addition and freeze-drying were unnecessary to achieve a suitable pellet.

Nonextractable tissues were prepared by repeated treatment with organic solvents. Liver (0-day withdrawal) was extracted with methanol/water (1:1 v/v, three times), methanol (three times), ether (one time), and ethyl acetate (one time). The residual solvent was removed from the solid residue under vacuum. The solid residue was lyophilized, blended, analyzed

for homogeneity by combustion, pelleted, and reanalyzed by combustion for homogeneity and radioactivity content. Other tissues were prepared similarly, except that slight modifications in the general extraction procedures were employed as follows: 45-day withdrawal liver (six times methanol, one time acetonitrile, one time ethyl acetate); 45-day withdrawal muscle (six times methanol, one time acetonitrile, two times ethyl acetate); 0- and 21-day withdrawal muscle (seven times methanol, one time acetonitrile, two times ethyl acetate).

The pelleted, medicated feeds were ground and weighed (*ca.* 5 g) into Soxhlet thimbles and extracted with dimethylformamide/acetone (2:8 v/v) for 5 h. The mixture was transferred to a 100 mL volumetric flask and made to volume with DMF washings of the extraction thimble. The radiochemical purity of the [^{14}C]furazolidone in the extract was determined by TLC using chloroform/methanol/water/formic acid (75:25:3:3 v/v) on silica gel 60 F$_{254}$ plates, 0.25 mm layer (E. Merck AG, Darmstadt, Germany). Radioactivity was analyzed using a Berthold Automatic-TLC-Linear Analyzer linked to an IBM Chroma data system. The concentration of furazolidone in the extract was determined by HPLC using a 12.5 cm Apex ODS 5 μm column (Jones Chromatography, Ltd., Llanbradach, U.K.). The chromatography was performed at ambient temperature with a water/acetic acid/acetonitrile (392:8:100) mobile phase at a flow rate of 1.0 mL/min. Furazolidone standards of 0.25, 2, 4, 7, and 10 μg/mL were prepared in dimethylformamide.

Sample Collection and Analysis. During the 72-h experimental period, bile was collected during 0–6, 6–24, 24–30, 30–48, 48–54, and 54–72 h, while urine and feces were collected separately during 0–24, 24–48, and 48–72 h. Urine and bile were stored at 4 °C, analyzed within 24 h, and stored frozen. The liver and the gastrointestinal tract were removed from the carcass, and the contents of the GI tract were separated by washing with 0.15 M saline (50 mL). The stomach was slit open and washed with saline from a 50-mL syringe. Saline was introduced into the anterior end of the small intestine and forced through by gentle pressure, washing out the contents (repeat five or six times). The caecum was treated as the stomach. The large intestine was voided of residual fecal material and flushed with saline (5–8 mL). All washings were combined and kept separate from the gastrointestinal walls. The liver, GI tract and its contents, and the remaining carcass were stored at −20 °C prior to analysis for radioactivity. Cages were washed with wet paper tissues and water at the end of each study, and any fine residual dose material remaining under the restraining cage was collected with a damp paper tissue as well (dose wash).

Bile (0.5 mL), urine (1.0 mL), and cage washings (1.0 mL) were counted directly in MI 31 Special scintillation cocktail (Canberra Packard). Feces were mixed with distilled water, homogenized, shell-frozen, lyophilized, blended, and analyzed by sample oxidation (0.05–0.1 g). Liver and GI tract were weighed and then homogenized with 2 volumes of distilled water/g of wet weight of tissue. The GI tract contents were homogenized in 50 mL of saline. All homogenates were shell-frozen, lyophilized, blended, and analyzed by sample oxidation. Carcasses were digested at 55 °C in a water/Triton X405/ methanol/sodium hydroxide solution (600;100;300:80 v/v/v/w). Carcass digestion samples (1 mL) were mixed with water (1 mL) and MI 31 (14 mL) and counted. All cage and dose washing tissues were dried and combusted *in toto.* The radioactivity found in the dose washing tissues was subtracted from the calculated dose to give the true ingested dose.

RESULTS AND DISCUSSION

Total Tissue Residues in Swine. The total radioactive residues in tissues from the six swine treated with [^{14}C]furazolidone (groups I–III) for 14 days at a dose level 16.5 mg kg^{-1} day^{-1} are shown in Table 1. At 0-day withdrawal, the highest residues were found in the liver followed by kidney, muscle, and fat. Residues depleted rapidly (50–90%) between the 0- and 21-day time points but more slowly between 21 and 45 days.

Table 1. Total Radioactive Residues (Parts per Million) Based on [^{14}C]Furazolidone Equivalents in Tissues from Swine Medicated for 14 Days at a Dose Level of 16.5 mg kg^{-1} day^{-1}

	group ID (withdrawal time)		
tissue	group I (0 days)	group II (21 days)	group III (45 days)
liver (M)	42.1	3.7	1.8
liver (F)	40.0	5.0	2.3
kidney (M)	34.7	2.8	1.6
kidney (F)	34.0	3.9	2.3
muscle (M)	12.2	2.7	2.0
muscle (F)	14.1	3.8	2.8
fat (M)	6.1	2.9	2.1
fat (F)	6.2	3.1	1.7

This biphasic residue depletion is indicative of an early and rapid removal of free drug and metabolites followed by turnover and elimination of residues bound to tissue proteins and other macromolecules. After 45 days, the longest withdrawal time used in this study, all tissue residues were approximately equal at 2 ppm. No sex differences were observed in the total residues at any time point. The tissues from the four swine comprising group IV were used to correct for background radioactivity in the combustion experiments and as a source of control tissue (lyophilized) for the rat bioavailability study (groups C–K).

In an earlier study (Vroomen *et al.,* 1986), piglets were administered [^{14}C]furazolidone (labeled only in the oxazolidinone ring) orally for 10 days at a dose level of 12 mg kg^{-1} day^{-1} followed by sacrifice after 0 (2 h) and 14 days. The residue data from the present study (Table 1) are, in general, higher than those reported previously. This difference may be due to the higher dose level (16 *vs* 12 mpk) but may also reflect the use of the dual radiolabeled [^{14}C]furazolidone, which provides a more accurate assessment of the total residue profile.

Although tissue assays for furazolidone were not conducted in this study, previous data (Vroomen *et al.,* 1986a) reported that the parent drug could not be detected (<2 ppb) in muscle, kidney, fat, and liver even after 0 day (2 h) withdrawal.

Extractable Tissue Residues. A nonextractable pellet was generated to allow comparisons between the relative bioavailability of residues present in the total tissue and that of residues present only in a nonextractable form. Percent extractability was determined by treating the lyophilized tissues containing the [^{14}C]-furazolidone residues with organic solvents. The radioactivity remaining in the pellet was considered to be nonextractable. The residues in the liver of group I (0-day withdrawal) were found to be 44.0% extractable, although this value may be slightly elevated in comparison to the other data points. The procedure utilized for these samples involved initial extraction with water/ methanol (50:50), possibly causing the solubilization of residues bound to small proteins, and thus confounding the interpretation of the bioavailability results. For all subsequent samples, the aqueous component was excluded and the extraction initiated with 100% methanol. When this modified procedure was used for the group III liver samples (45-day withdrawal), extractability decreased to 8.3%. For muscle, the majority of the radioactivity remained bound at all time points as only 21.8%, 18.6%, and 13.7% of the total residues were extractable after 0, 21, and 45 days of withdrawal (groups I–III), respectively. The extractable residues

Bioavailability of [14C]Furazolidone Residues in Swine Tissues

J. Agric. Food Chem., Vol. 43, No. 9, 1995 **2523**

Table 2. Aminooxazolidinone Residues Released from Swine Tissues Containing Bound Residues Derived from [14C]Furazolidone

withdrawal time	liver ppm[a]	liver av % total residue[b]	kidney ppm[a]	kidney av % total residue[b]	muscle ppm[a]	muscle av % total residue[b]
0 days (M)	3.154 3.056	16.3	1.681 1.743	10.9	1.149 1.207	21.3
0 days (F)	3.858 3.548	20.4	2.215 1.984	13.6	1.432 1.377	22.0
21 days (M)	0.241 0.212	13.5	0.115 0.114	9.0	0.086 0.084	6.9
21 days(F)	0.247 0.289	11.8	0.078 0.089	4.4	0.135 0.134	7.8
45 days (M)	0.085 0.085	10.4	0.033 0.037	4.8	0.046 0.053	5.5
45 days (F)	0.072 0.077	7.1	0.025 0.030	2.6	0.075 0.073	5.8

[a] Aminooxazolidinone (μg) released per gram of wet tissue based on recovery of radioactivity in the NPAOZ fraction from the HPLC. [b] Fraction of AOZ released from protein-bound metabolites, as related to total residues on a molecular basis, assuming that all residues contain an intact side chain.

decreased with increasing withdrawal time since any free residues (e.g. metabolites) would be eliminated more rapidly than bound residues.

Analysis of Aminooxazolidinone (AOZ) Released from Bound Residues. The results of the bound residue analyses are shown in Table 2 and indicate that AOZ was released from all tissues tested. The fractions of AOZ were calculated on a molecular basis, assuming that all residues still contain an intact and releasable side chain, which in practice is very unlikely (Hoogenboom *et al.*, 1991b, 1992b). The fraction of AOZ released tends to decrease as withdrawal time increases, with this difference being more pronounced between the 0- and 21-day periods. When expressed as a percentage of the maximum amount of releasable AOZ, the values are relatively uniform when compared across tissues and withdrawal times, especially at the latter time points. The 21- and 45-day data, obtained following depletion of all free metabolites, are remarkably consistent and averaged 10.7%, 5.2%, and 6.5%, respectively, for liver, kidney, and muscle (overall average $\approx$ 7.5%). These results parallel those reported previously (Hoogenboom *et al.*, 1991b) for tissues from piglets sacrificed at 0- and 14 days of withdrawal, when the release of AOZ averaged 13.6% and 10.6% in liver, respectively. In that study, a similar decrease in releasable AOZ was observed with increasing withdrawal time.

Prior to the development of this technique (Hoogenboom *et al.*, 1991b, 1992b), the only methods available for the analysis of furazolidone were based on the detection of the parent drug (Winterlin *et al.*, 1981; Vroomen *et al.*, 1986a). These methods were sensitive (<2 ppb) but were of little value in monitoring total residues since furazolidone depletes rapidly in tissues with half-lives on the order of 7–63 min (Nouws and Laurensen, 1990). The data presented in Table 2, however, suggest that the method for release and determination of AOZ from bound residues may be well suited for this application with the released AOZ serving as the marker residue. An important criterium for any regulatory method is that a consistent ratio of marker residue (AOZ) to total residue be maintained. The small variation in this ratio observed for the tissues analyzed

Table 3. Study Design: Rat Bioavailability Study

group[a]	dose	food source 0–24 h	food source 24–48 h
A	[14C]furazolidone in PEG	lab diet	lab diet
B	[14C]furazolidone in lab diet	lab diet	dose
C	[14C]furazolidone in control liver	control liver	dose
D	[14C]furazolidone in control muscle	control muscle	dose
E	0-day pelleted swine liver	control liver	dose
F	45-day pelleted swine liver	control liver	dose
G	0-day pelleted swine muscle	control muscle	dose
H	45-day pelleted swine muscle	control muscle	dose
I	nonextractable 0-day swine liver	control liver	dose
J	nonextractable 45-day swine liver	control liver	dose
K	nonextractable 45-day swine muscle	control muscle	dose

[a] Four male rats per group.

Table 4. Bioavailability of [14C]Furazolidone in Male Rats When Administered Orally in Solution via Gastric Catheter or through Consumption of Medicated Diet, Fortified Lyophilized Control Swine Liver, or Fortified Lyophilized Control Swine Muscle

fraction	experiment[a,b] catheter A	diet B	liver C	muscle D
feces	6.85	11.93	33.92	10.76
cage washings	0.44	0.67	1.14	0.71
GIT contents	0.35	0.26	0.93	0.47
total nonabsorbed	7.64	12.86	35.99	11.94
bile	36.51	34.60	17.91	35.27
urine	46.56	51.47	50.83	55.45
liver	0.75	0.71	0.82	0.92
GIT	0.19	0.14	0.24	0.29
carcass	2.79	3.43	2.87	3.68
total absorbed	86.79	90.35	72.66	95.60
total recovery	94.43	103.20	108.65	107.54

[a] All data expressed as percent recovery of radioactivity based on the total administered dose. [b] Data represent the average of four male rats.

in the present study supports the utility of this procedure in providing estimates of total furazolidone residues (e.g. [total furazolidone derived residues] $\approx$ [AOZ released]/0.075).

Bioavailability of [14C]Furazolidone Residues. The overall design of the rat bioavailability study using the Gallo-Torres model is summarized in Table 3. This technique has been recognized by the FDA as an important tool for assessing the bioavailability of bound residues in a second species (FDA Guidelines, 1990) and has been used previously for other animal health drugs (MacDonald *et al.*, 1990) as a means of obtaining information on the nature of the residues.

The current study was divided into three parts. Part I (Table 4) essentially represents a set of control experiments and illustrates the effect of various matrices on the bioavailability of the parent drug. When administered orally in a solution of PEG 200 (A), furazolidone and/or its metabolites were 87% bioavailable. When the drug was fortified into control laboratory rat chow (B) or control lyophilized swine muscle

Table 5. Bioavailability of Radioactive Residues in Male Rats Consuming Lyophilized Liver or Muscle from Swine Treated with [^{14}C]Furazolidone for 14 Days at 16.5 mg kg^{-1} day^{-1} and Subjected to 0- and 45-Day Withdrawal Periods

	experiment[a,b]			
fraction	liver (0 days) E	liver (45 days) F	muscle (0 days) G	muscle (45 days) H
feces	47.93	66.88	54.77	50.89
cage washings	1.37	3.20	0.70	1.22
GIT contents	1.19	2.18	1.03	1.38
total nonabsorbed	50.49	72.26	56.50	53.48
bile	2.71	1.58	2.35	2.71
urine	27.49	10.45	26.89	22.44
liver	1.59	1.58	1.50	2.78
git	0.51	0.55	0.60	1.12
carcass	7.27	5.31	5.63	12.18
total absorbed	39.56	19.46	36.97	41.24
total recovery	90.05	91.72	93.47	94.72

[a] All data expressed as percent recovery of radioactivity based on the total administered dose. [b] Data represent the average of four male rats.

Table 6. Bioavailability of Nonextractable Radioactive Residues in Male Rats Consuming Lyophilized Liver or Muscle from Swine Treated with [^{14}C]Furazolidone for 14 Days at 16.5 mg kg^{-1} day^{-1} and Subjected to 0- and 45-Day Withdrawal Periods

	experiment[a,b]		
fraction	NE liver (0 days) I	NE liver (45 days) J	NE muscle (45 days) K
feces	52.19	70.13	60.23
cage washings	0.58	0.26	1.29
GIT contents	1.04	3.05	3.12
total nonabsorbed	53.81	73.43	64.64
bile	2.10	1.15	2.97
urine	20.61	10.28	18.67
liver	1.94	1.89	3.61
git	0.60	0.66	1.38
carcass	6.06	2.22	10.45
total absorbed	31.29	16.19	37.08
total recovery	85.11	89.62	101.72

[a] All data expressed as percent recovery of radioactivity based on the total administered dose. [b] Data represent the average of four male rats.

(D), the extents of absorption were similar at 90% and 96%, respectively. However, bioavailability decreased to 73% when furazolidone was fortified into lyophilized control swine liver (C), indicating a matrix interference to absorption, the nature of which is unknown. In all cases, the major excretion pathway was via the urine, which is consistent with previous results (White, 1989). The bile also accounted for a significant portion of the radioactivity. For the fortified control liver (C), fecal elimination was increased at the expense of biliary excretion.

Part II (Table 5) assesses the bioavailability of the swine liver and muscle tissue residues obtained from the residue depletion study. While bioavailability was reduced compared to that of the parent drug, significant absorption of the radioactive residues was still evident. The 0-day liver (E) and muscle (G) tissues had relatively high bioavailabilities of 40% and 37%, respectively. These results were expected since the tissues had high total residue levels (Table 1) and still contained a substantial amount of free metabolites. However, the finding that significant residue absorption occurred after an extended withdrawal time was unexpected. The bioavailable liver residues after 45 days of withdrawal (F) only decreased to approximately half (20%) of the 0-day levels, while the corresponding muscle residues (H) actually showed a slight increase (41%). Compared to furazolidone itself, biliary excretion was minimal for these residues, while elimination via the urine remained at a relatively high level. Fecal excretion accounted for the majority of the nonabsorbed radioactivity.

The bioavailability of the *nonextractable* tissue residues (part III, Table 6) was similar to that observed for the whole tissues. Although pre-extraction with organic solvents removed between 8% and 44% of the total radioactivity, no effect on the extent of absorption of the remaining nonextractable residues was apparent. Once again, urine elimination remained the predominant pathway for excretion of the absorbed radioactivity. Bioavailability values of the nonextractable 0-day liver (I), 45-day liver (J), and 45-day muscle (K) residues were 31%, 16%, and 37%, respectively, whereas the corre-

sponding values for the intact tissues (E, F, and H) were 40%, 19%, and 41%.

Nature of the Residues. The results (Tables 4–6) clearly show that furazolidone tissue residues are bioavailable in the rat, regardless of the withdrawal time. This conclusion obviously leads to the question of the nature of the absorbed residues and their potential impact to the consumer from a human food safety viewpoint. Although the exact nature of the bioavailable residues is unknown, several inferences can be made on the basis of previous knowledge.

Since furazolidone is rapidly eliminated and rapidly metabolized and is known to degrade in tissues with a short half-life (Nouws and Laurensen, 1990; White, 1989), it is unlikely that the parent drug is available for absorption, especially after a 45-day withdrawal period. Although unproven in this study, it is also unlikely that any nitro-containing metabolites of furazolidone remain among the tissue residues. The 5-nitro group of furazolidone is a primary site of metabolism in the molecule, and many of the metabolites previously identified *in vivo* and *in vitro* (Swaminathan and Lower, 1978; Tatsumi *et al.*, 1978, 1981, 1984; Tatsumi and Takahashi, 1982; Vroomen *et al.*, 1987) result from nitro group reduction.

Many compounds are known to interact extensively with thiol groups, including glutathione, mercaptoethanol, and cysteine. While, in general, a reaction with glutathione is classified as a detoxification mechanism, reactive thiol conjugates have been reported. A concern with human food safety may result if reactive thiol conjugates are released following ingestion of tissue residues. The FDA has expressed concern for this possibility and now requires that an assessment of the gastro intestinal binding potential of released bound residues be part of an overall residue assessment (SOM Final Rule, 1987; FDA Guidelines, 1990). The generation of reactive conjugates of furazolidone metabolites has been reported in microsomal incubations (Vroomen *et al.*, 1988b); however, this could not be confirmed using hepatocytes (Hoogenboom *et al.*, 1992a), possibly due to their instability. The glutathione and mercaptoethanol adducts of furazolidone, isolated from microsomal

Bioavailability of [¹⁴C]Furazolidone Residues in Swine Tissues

J. Agric. Food Chem., Vol. 43, No. 9, 1995 **2525**

incubations, were also tested and shown to be negative in the *Salmonella* mutagenicity (Ames) test.

The present study confirms that the AOZ moiety can be released from the bound residues and thus would become available for absorption. While AOZ itself has been shown to be nonmutagenic (SmithKline Beecham Animal Health, NOTOX Research and Consulting Co., Project 049073, unpublished data, 1991), it is postulated to be a precursor of the metabolite β-hydroxyethylhydrazine (HEH), which is known to inhibit the enzyme monoamine oxidase (MAO) (Hoogenboom *et al.*, 1991a; Stern *et al.*, 1967). However, the relevance of this process with regard to human food safety is unclear. While MAO inhibition *in vivo* resulting from the release of bound furazolidone-derived residues has not been demonstrated, the release of AOZ followed by its conversion to HEH and subsequent binding to macromolecules remains a possibility.

The 40th JECFA (held in June 1992) was unable to assign an MRL for furazolidone due to concerns over the lack of information on the metabolites and the absence of an identified marker residue. While the development of the method for the release and determination of AOZ from bound residues potentially resolves the latter point, a new comprehensive metabolism study for furazolidone is clearly necessary since many metabolites remain unidentified. A followup to the bioavailability study, designed to determine the identity of the absorbed residues, is also appropriate. The release of the FDA guidelines (SOM Final Rule, 1987; FDA Guidelines, 1990) for the evaluation of bound residues derived from a carcinogenic drug represents a very positive step and now allows a mechanism for the design of experiments to evaluate the relative human food safety concerns of residues from drugs such as furazolidone in the future.

ACKNOWLEDGMENT

LITERATURE CITED

FDA Guidelines. Part VIII. Guideline for the Human Food Safety Evaluation of Bound Residues Derived from Carcinogenic New Animal Drugs. In *General Principles for Evaluating the Safety of Compounds Used in Food Producing Animals*; U.S. GPO: Washington, DC, 1986; revised Sept 21, 1990.

Gallo-Torres, H. E. *J. Toxicol. Environ. Health* **1977**, *2*, 827.

Hoogenboom, L. A. P.; Tomassini, O.; Oorsprong, M. B. M.; Kuiper, H. A. *Food Chem. Toxicol.* **1991a**, *29* (3), 185.

Hoogenboom, L. A. P.; Kammen, M. van; Berghmans, M. C. J.; Koeman, J. H.; Kuiper, H. A. *Food Chem. Toxicol.* **1991b**, *29* (5), 321.

Hoogenboom, L. A. P.; Kammen, M. van; Oorsprong, M. B. M.; Kuiper, H. A. *Toxicol. in Vitro* **1992a**, *6* (3), 227.

Hoogenboom, L. A. P.; Berghmans, M. C. J.; Polman, T. H. G.; Parker, R.; Shaw, I. C. *Food Addit. Contam.* **1992b**, *9* (6), 623.

JECFA. Joint FAO/WHO Expert Committee on Food Additives, 40th Report; FAO: Rome, 1992.

Kessler, D. A. *Fed. Regist.* **1991**, *56* (164), 41902.

MacDonald, A.; Laurencot, H.; Elsom, L. The Use of Bioavailability Studies in the Evaluation of Tissue Residues. Presented at the EuroResidue Conference on Residues of Veterinary Drugs in Food, Noordwijkerhout, The Netherlands, 1990.

Nouws, J. F. M.; Laurensen, J. *Vet. Q.* **1990**, *12* (1), 56.

Shu, A. Y. L.; Villani, A. J.; Levinson, S. H.; Heys, J. R. Synthesis of Carbon-14 Labeled Furazolidone. In *Synthesis and Applications of Isotopically Labelled Compounds*; Buncel, E., Kabalka, G. W., Eds.; Elsevier Science Publishers: Amsterdam, 1991; p 214.

SOM Final Rule. Sponsored Compounds in Food Producing Animals; Criteria and Procedures for Evaluating the Safety of Carcinogenic Residues, Animal Drug Safety Policy, Final Rule, Availability of Guidelines for Human Food Safety Evaluation; Notices. *Fed. Regist.* **1987**, *251*, 49572.

Stern, I. V.; Hollifield, R. D.; Wilk, S.; Buzard, J. A. *J. Pharmacol. Exp. Ther.* **1967**, *156*, 492.

Swaminathan, S.; Lower, G. M. Biotransformations and excretions of nitrofurans. In *Carcinogenesis, Vol. 4, Nitrofurans*; Bryan, G. T., Ed.; Raven Press: New York, 1978; p 59.

Tatsumi, K.; Takahashi, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3435.

Tatsumi, K.; Ou., T.; Yamada, H.; Yoshimura, H.; Koga, H.; Horiuchi, T. *J. Pharmacobio-Dyn.* **1978**, *1*, 256.

Tatsumi, K.; Yamada, H.; Yoshimura, H.; Kawazoe, Y. *Arch. Biochem. Biophys.* **1981**, *208*, 167.

Tatsumi, K.; Nakabeppu, N.; Takahashi, Y.; Kitamura, S. *Arch. Biochem. Biophys.* **1984**, *234*, 112.

Vroomen, L. H. M.; Berghmans, M. C. J.; Stuijs, T. D. B. van der. *J. Chromatogr.* **1986a**, *362*, 141.

Vroomen, L. H. M.; Berghmans, M. C. J.; Leeuwen, P. van; Struijs, T. D. B. van der; Vries, P. H. U. de; Kuiper, H. A. *Food Addit. Contam.* **1986b**, *3* (4), 331.

Vroomen, L. H. M.; Berghmans, M. C. J.; Hekman, P.; Hoogenboom, L. A. P.; Kuiper, H. A. *Xenobiotica* **1987**, *17* (12), 1427.

Vroomen, L. H. M.; Berghmans, M. C. J.; Bladeren, P. J. van; Groten, J. P.; Wissink, H. C. G.; Kuiper, H. A. Veterinary Pharmacology, Toxicology, and Therapy in Food Producing Animals. In *Proceedings from the 4th Congress of the European Association for Veterinary Pharmacology and Toxicology*; Simon, Lees, Semjen, Eds.; University of Veterinary Science: Budapest, 1988a; p 259.

Vroomen, L. H. M.; Berghmans, M. C. J.; Groten, J. P.; Koeman, J. H.; Bladeren, P. J. van. *Toxicol. Appl. Pharmacol.* **1988b**, *95*, 53.

Vroomen, L. H. M.; Berghmans, M. C. J.; Bladeren, P. J. van; Groten, J. P.; Wissink, H. C. G.; Kuiper, H. A. *Drug Metab. Rev.* **1990**, *22*, 79.

White, A. H. *Scand. J. Gastroenterol.* **1989**, *24* (Suppl. 169), 4.

Winterlin, W.; Hall, G.; Mourer, C. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1055.