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Abstract

14C-Fumonisin B1 (FB1) was produced by *Fusarium proliferatum* M-5991 in modified Myro liquid medium and purified to >95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 μ mol of 14C-FB1, 14C-hydrolyzed FB1, or 14C-FB1-fructose/kg body weight. Urinary excretion of 14C-FB1 and 14C-FB1-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of 14C-hydrolyzed HFB1 was significantly greater ($P > 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the 14C-FB1, 14C-hydrolyzed FB1, and 14C-FB1-fructose was similar, increased urinary excretion of the 14C-hydrolyzed FB1 as compared to 14C-FB1 and 14C-FB1-fructose indicated a greater absorption of the hydrolyzed form.

Keywords

Laboratory Animal Resources, 14C-Fumonisin B1; hydrolyzed fumonisin B1; fumonisin B1-fructose; excretion

Disciplines

Comparative and Laboratory Animal Medicine | Food Science | Human and Clinical Nutrition | Other Veterinary Medicine

Comments

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Excretion of ^{14}C -Fumonisin B₁, ^{14}C -Hydrolyzed Fumonisin B₁, and ^{14}C -Fumonisin B₁-Fructose in Rats

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^{14}C -Fumonisin B₁ (FB₁) was produced by *Fusarium proliferatum* M-5991 in modified Myro liquid medium and purified to >95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 μmol of ^{14}C -FB₁, ^{14}C -hydrolyzed FB₁, or ^{14}C -FB₁-fructose/kg body weight. Urinary excretion of ^{14}C -FB₁ and ^{14}C -FB₁-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of ^{14}C -hydrolyzed HFB₁ was significantly greater ($P > 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the ^{14}C -FB₁, ^{14}C -hydrolyzed FB₁, and ^{14}C -FB₁-fructose was similar, increased urinary excretion of the ^{14}C -hydrolyzed FB₁ as compared to ^{14}C -FB₁ and ^{14}C -FB₁-fructose indicated a greater absorption of the hydrolyzed form.

Keywords: ^{14}C -Fumonisin B₁; hydrolyzed fumonisin B₁; fumonisin B₁-fructose; excretion

INTRODUCTION

The fumonisins (FBs) are a family of mycotoxins including FB₁, FB₂, FB₃, and FB₄ (Gelderblom et al., 1988; Cawood et al., 1991) with FB₁ predominant. The FBs are produced by the maize pathogens *Fusarium proliferatum* and *Fusarium moniliforme*. Fumonisin consumption causes equine leucoencephalomalacia (Kellerman et al., 1990) and porcine pulmonary edema (Osweiler et al., 1992; Colvin and Harrison, 1992). Fumonisin also cause embryopathogenicity in chickens (Javed et al., 1993), developmental toxicity in hamsters (Floss et al., 1994), and kidney toxicity and liver cancer in rats (Gelderblom et al., 1991; Voss et al., 1993). Sharma et al. (1997) demonstrated in vivo apoptosis in mouse liver and kidney after doses of 0.35–8.7 μmol of FB₁/kg body weight (bw) were given subcutaneously. The effects of FBs on humans are not known. But epidemiological studies show significant correlations between high levels of FBs in corn consumed by humans and esophageal cancer (Sydenham et al., 1990; Rheeder et al., 1992; Chu and Li, 1994). Fumonisin B₁ is listed as a Class 2B carcinogen, a probable human carcinogen (IARC, 1993).

The study of fumonisin metabolism has been facilitated by the production of ^{14}C -FB₁, which permits rapid analysis of the biological disposition of this compound. Fumonisin B₁ has been radiolabeled using ^{14}C -acetate or -methionine in cultures of *F. moniliforme* or *F. proliferatum* in liquid medium (Norred et al., 1993;

Blackwell et al., 1994; Lebepe-Mazur, 1993). In fasted rats, Norred et al. (1993) detected 80% and 2.3% of FB₁ administered by gavage (1.4 μmol of ^{14}C -FB₁/kg bw) in feces and urine, respectively. Liver, kidney, and blood retained a total of 0.6% of the dose 96 h after treatment. In fed rats at 24 h after a dose administered by gavage (10.4 μmol of ^{14}C -FB₁/kg bw), Shephard et al. (1992) detected 100% and trace levels of FB₁ in feces and urine, respectively. Trace levels of ^{14}C -FB₁ were detected in liver, kidney, and blood of these rats. From these two studies, FB₁ absorption may be greater in fasted rats than in fed rats. Biliary excretion of FB has been suggested by observations of fecal recovery of ^{14}C -FB given by intravenous or intraperitoneal routes (Norred et al., 1993; Shephard et al., 1992). Shephard et al. (1994) recovered 67% of an intraperitoneal dose (10.4 μmol of ^{14}C -FB₁/kg bw) after 24 h in bile whereas 0.2% of a 0.4- μmol dose administered by gavage was detected in bile of fed rats. Other forms of fumonisins such as hydrolyzed FB₁ have not been evaluated for biliary excretion. Hopmans et al. (1997) analyzed by HPLC the excretion of unlabeled FB₁ at three doses. Administration of 0.69, 6.93, and 69.3 μmol of FB₁/kg bw to fed rats resulted in 7.4, 1.2, and 0.5% of the dose excreted in urine, respectively, confirming previous findings with radiolabeled FB₁ but also suggesting dose differences in absorption.

FB₁ may undergo reactions in food systems that alter its chemical structure and its toxicity. Alkaline hydrolysis of FB-containing corn produced hydrolyzed FB₁ (HFB), which was found to promote diethylnitrosamine (DEN)-initiated hepatocarcinogenesis nearly as well as FB₁ when fed to rats (Hendrich et al., 1993). Urinary excretion of HFB₁ was 2-fold greater than FB₁ in rats (Hopmans et al., 1997), suggesting a significant role for increased bioavailability in HFB₁ toxicity as compared

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with FB₁. A Maillard-like reaction between FB₁ and a reducing sugar such as fructose can occur during heating (Murphy et al., 1996). Feeding FB₁-fructose reaction products caused no development of altered hepatic foci in DEN-initiated rats, whereas an equimolar amount of FB₁ promoted hepatocarcinogenesis readily (Lu et al., 1997). The seeming lack of toxicity of FB₁-fructose product(s) could not be explained by lesser absorption in Hopmans et al. (1997) because such products were absorbed to a greater extent than FB₁ in rats, based on relative urinary excretion. A FB₁-glucose reaction product, *N*-(carboxymethyl)-FB₁ has recently been isolated (Howard et al., 1998), but its toxicity is unknown. The mechanism of formation and the nature of the first products in the Maillard reaction are not well-characterized even after over 50 years of study (Ge and Lee, 1997). But fumonisin-sugar products might form during food processing and could diminish or alter fumonisin toxicity.

This study was designed to determine, with ¹⁴C-FB₁, the extent of urinary excretion of the low doses of 0.69 μmol of FB₁, HFB₁, and FB₁-fructose/kg bw used by Hopmans et al. (1997). We also analyzed biliary circulation of these three forms of fumonisin most likely to be found in foods.

MATERIALS AND METHODS

Reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise. Milli-Q water (Millipore-Waters, Bedford, MA) was used throughout. All animal procedures and protocols were approved by the Iowa State University Animal Care and Use Committee. The FB₁ produced from *Fusarium* cultures is a class 2B carcinogen and was handled accordingly. We treated HFB₁ similarly. We followed Iowa State University Environmental Health and Safety guidelines for the use of ¹⁴C.

The 500-mL liquid cultures of *F. proliferatum* M5991 were prepared as in Dantzer et al. (1996a). These cultures were inoculated with Myro liquid medium (LM), containing MgSO₄ at only 0.5 g/L and 1.00% corn hull extract (modified Myro LM). The inoculum culture was incubated for 4 days on a rotary shaker at 220 rpm at 25 °C. Aliquots of 0.5 mL of inoculum were transferred to three replicate rubber-stoppered 125-mL Erlenmeyer flasks containing 50 mL of modified Myro LM. Compressed air was cleaned by passage through five 2-L plastic bottles containing air, 2 N KOH, 2 N KOH, distilled H₂O, and 2 N H₂SO₄, followed by a moisture trap and a 0.2-μm in-line filter. The purified air was bubbled through the *F. proliferatum*-inoculated culture flasks at 0.5 mL/min while the flasks were shaken on a rotary shaker at 220 rpm and 23 °C for 30 days. Aliquots of 1 mL were removed from the cultures every 7 days and frozen at -20 °C until analyzed for FB₁.

Glassware was cleaned with Nochromix laboratory glass cleaner (GODAX Laboratories, Inc. Takoma Park, MD) for > 15 min. An 0.5-mL aliquot of a 4-day-old *F. proliferatum* M 5991 LM culture was transferred to a rubber-stoppered 125-mL Erlenmeyer flask. Air exiting the culture was passed through two CO₂ traps containing 2 N KOH. The flask was incubated with shaking at 220 rpm and 23 °C for 10 days. A 100-μL aliquot of culture was removed to obtain baseline FB₁ concentration. The ethanol containing 56 mCi/mmol universally labeled (U)-¹⁴C-acetate (American Radiolabeled Chemicals, Inc., St. Louis, MO; Lot No. 960214) was evaporated, and the residue was dissolved in Milli-Q water. Preliminary results indicated that ethanol decreased production of FB₁ and cell density of *F. proliferatum* culture. Four equal aliquots of 250 μCi of U-¹⁴C-acetate were transferred into the *F. proliferatum* culture after 12, 15, 18, and 21 days for a total of 1.00 mCi of U-¹⁴C-acetate. The culture was agitated on a shaker at 220 rpm and harvested at 25 days. The KOH traps were replaced every 24 h and counted in ScintiVerse BD scintillation fluid for 4 min using a Packard liquid scintillation analyzer, model 1900TR (Packard Instruments Co., Downers Grove, IL).

¹⁴C-Fumonisin B₁ purification was performed according to Dantzer et al. (1996b) with modifications. Briefly, the 50 mL of ¹⁴C-FB₁-containing LM was filtered through a Whatman No. 1 filter, followed by 0.8- and 0.45-μm MSI filters (4.5 cm, nylon, Micron Separations, Inc., Westboro, MA). The ¹⁴C-FB₁ was fractionated on XAD-16, C₈, DEAE-Sepharose, and C₁₈ columns. The XAD-16 column was 30 × 2 cm with 50 g of Amberlite XAD-16 (Sigma Chemical Co., St. Louis, MO). The XAD column was washed with 150 mL of Milli-Q water followed by 150 mL of 50% acetonitrile in water. All other steps were as reported previously. After elution of the FB fraction from the C₁₈ column, the sample was concentrated by rotary evaporation at 45 °C, freeze-dried, weighed, rehydrated in 50% acetonitrile/water, and quantified for FB₁. Three aliquots, each containing 1.39 μmol of ¹⁴C-FB₁ were taken to dryness by rotary evaporation at 45 °C and used to prepare ¹⁴C-FB₁, ¹⁴C-HFB₁, and ¹⁴C-FB₁-fructose doses. The ¹⁴C-FB₁ dose was rehydrated in 10 mL of Milli-Q water. The ¹⁴C-HFB₁ was prepared by rehydration of 1.39 μmol of FB₁ in 5 mL of Milli-Q water and hydrolysis in a marble-capped test tube with 1 mL of 2 N KOH for 2 h in a boiling water bath. The pH of the HFB₁ solution was adjusted to <3 with 12 N HCl, loaded onto a PrepSep P479-C₁₈ extraction column, and washed with 20 mL of Milli-Q water. The HFB₁ was eluted with 10 mL of methanol (Hopmans et al., 1997). The methanol was removed by rotary evaporation at 45 °C. The ¹⁴C-HFB₁ was redissolved in 10 mL of Milli-Q water. The ¹⁴C-FB₁-fructose was prepared by rehydration of 1.39 μmol of ¹⁴C-FB₁ in 10 mL of 50 mM K₂HPO₄, pH 7.0, containing 100 mM D-fructose and heating at 80 °C for 48 h (Hopmans et al., 1997). Ten percent of unreacted FB₁ remained in the FB₁-fructose mixture. The volume of the FB₁-fructose was adjusted to 10 mL with Milli-Q water. All three ¹⁴C-FB forms were stored at -20 °C until use.

The concentrations of ¹⁴C-FB₁ and -HFB₁ were estimated by HPLC analysis of *o*-phthalaldehyde derivative using a Turner fluorometer (Corning 7-60 primary filter and Wratten 2a secondary filter, 15 μL flow cell) (Dantzer et al., 1996b). The purity of ¹⁴C-FB₁ was determined by comparing the amounts as measured by HPLC with the freeze-dried mass of the purified ¹⁴C-FB₁. The ¹⁴C-HFB₁ was quantified in a similar manner by comparison with an HFB₁ standard curve. The HFB₁ standard was prepared according to Hopmans et al. (1997). The HFB₁ standard was shown to be >95% pure by FAB-mass spectrometry (Hopmans et al., 1997; Mirocha, personal communication). Residual FB₁ in the ¹⁴C-FB₁-fructose dose was analyzed by HPLC (Dantzer et al., 1996b).

Eighteen Fisher 344/NHsd rats, nine male and nine female, at 7–8 and 9–10 weeks of age (bw 135–160 g), respectively, were randomly assigned to one of the treatment groups for the excretion study. All rats were housed individually and given AIN-93M diet (Reeves et al., 1993) and water ad libitum for 1 wk under a 12-h light/dark cycle. At the end of a 12-h light cycle, groups of three males and three females were administered 0.69 μmol of ¹⁴C-FB₁, ¹⁴C-HFB₁, or ¹⁴C-FB₁-fructose/kg bw by gavage. The rats were housed individually in metabolic cages, and fecal and urine samples were collected at 12-h intervals. At 84 h, the rats were sacrificed by CO₂ asphyxiation; blood was drawn by heart puncture; and the hearts, livers, lungs, kidneys, and brains were removed for ¹⁴C analysis.

For the biliary excretion study, nine female 15-week-old Sprague–Dawley rats (bw 240–270 g) were used. All rats were housed individually and given AIN-93M diet and tap water ad libitum for 1 week under 12-h light cycle. Rats were anesthetized with 5 mL/kg bw of a 25% urethane (Sigma) solution intraperitoneally, and their bile ducts were surgically cannulated with silicone rubber tubing (0.3 mm i.d. × 0.6 mm o.d.; BrainTree Scientific, Braintree, MA). After cannulation, the abdomen was surgically closed, and 3 rats/treatment were immediately gavaged with 0.69 μmol of either ¹⁴C-FB₁, -HFB₁, or -FB₁-fructose/kg bw. All rats were placed on their left side on a hot water heating pad set at medium heat. Bile from each rat was collected into 1.5-mL graduated microcentrifuge tubes every 30 min until rats died or were sacrificed 9.5 h after dosing. Livers, kidneys, stomach washes, stomachs, and the

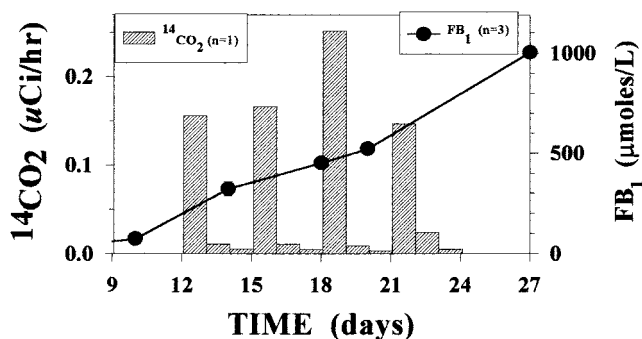


Figure 1. Production of FB₁ (μmol/L) and ¹⁴CO₂ (μCi/h) by *Fusarium proliferatum* M-5991. Bars represent ¹⁴CO₂ production in 50 mL of modified Myro media spiked with 250 μCi of U-¹⁴C-acetate at days 12, 15, 18, and 21; *n* = 1. Line represents FB₁ production (μmol/L); *n* = 3.

first, second, and third sections (I, II, and III) of small intestines were collected after the rats died or at sacrifice, 9.5 h after dosing. All samples were stored at -20 °C until analysis.

Fecal samples, oven dried at 60 °C overnight, and intestinal tissues, frozen in liquid N₂, were ground in a porcelain mortar and pestle. Ground feces, intestinal tissues, stomach, stomach wash, kidney, liver, lung, brain, and blood were separately blended in a tissue homogenizer (model TR-10, Tekmar Co., Cincinnati, OH) at 60% power for 0.5–1 min in 5 mL of Milli-Q water, brought to a known volume of 10–35 mL with Milli-Q water, and quantified for ¹⁴C by scintillation analyzer. Quenching of tissues was measured with 0.5–1 mL of tissue extract with and without 18 400 dpm ¹⁴C-FB₁. Data were corrected using percent quenching in the respective sample.

A completely randomized design was used for statistical evaluation of urine and fecal excretion data in the 18 Fisher rats and for the biliary excretion data in the 9 Sprague Dawley rats. Differences among treatments were assessed by a Student's *t* statistic (*P* ≤ 0.05) using SAS (version 6.03, 1995, Cary, NC).

RESULTS AND DISCUSSION

F. proliferatum yielded 1200 μmol of FB₁/L between days 10 and 31 of culture (Figure 1). There was an average production of 0.18 μCi of ¹⁴CO₂/h within the first 24-h period after each addition of 250 μCi of ¹⁴C-acetate. The production of ¹⁴CO₂ decreased to undetectable levels 3 days after each addition of the ¹⁴C-acetate at an apparent logarithmic rate, suggesting that the production of acetate by the *F. proliferatum* culture was significantly greater than the addition of the labeled acetate. The culture was harvested at 24 days. The FB₁ was purified to >95% purity with a yield of 24 μmol and a specific activity of 1.7 mCi/mmol. ¹⁴C-Fumonisin B₁ measurement in biological samples allowed for improved monitoring of FB₁ as compared to HPLC fluorescence detection. But scintillation counting of ¹⁴C-FB₁ could not reveal any information on metabolic modification of FB₁ or its related forms.

The doses for the urine and fecal excretion study were 0.14 μmol of ¹⁴C-FB₁, ¹⁴C-HFB₁, or ¹⁴C-FB₁-fructose/mL with specific activities of 1.7, 1.2, and 1.8 mCi/mmol, respectively. The lower specific activity of the HFB₁ suggested that part of the U-¹⁴C-acetate was incorporated into the tricarboxylic side chains of FB₁ that were removed during hydrolysis to produce HFB₁.

Quenching in the blood and liver fractions was determined to be 86 and 28%, respectively, for added ¹⁴C-FB₁. All other tissues had negligible quenching (data not shown). All data were corrected for quenching.

Recently, Howard et al. (1998) identified *N*-(carboxymethyl)-FB₁ as a reaction product of FB₁ and glucose. Yaylayan and Huyghues-Despointes (1994) reported that nonenzymatic browning reaction products from fructose are much more complicated than those from glucose due to the nature of the products formed from a ketose. In addition, our reaction time between FB₁ and fructose was 4-fold longer than that reported by Howard et al. (1998), which probably means that our FB₁-sugar adducts were a much more complex mixture, as is usually seen with the formation of Amadori products (Labuza, 1994). For the sake of simplicity in this paper, we named our fructose-FB₁ reaction product mixture, FB₁-fructose. Our limited attempts to identify the products produced in the FB₁-fructose (and -glucose) model systems by mass spectrometry have been unsuccessful to date. A number of *o*-phthalaldehyde (OPA) fluorescent peaks were detected in the FB-fructose reaction mixture, but we would not expect anything reacting with the FB amine group to be detected by amine derivatization.

Excretion of ¹⁴C in urine over time did not differ significantly between genders, except for HFB₁ during the first excretion interval [female > male (Table 1)], so data were combined (*n* = 6) (Figure 2). Fifteen percent, 4.4%, and 0.6% of the total ¹⁴C dose was excreted in urine for HFB₁, FB₁-fructose, and FB₁ respectively (Table 1). The average half-life for excretion of FB compounds was 10 h. There were only trace amounts of ¹⁴C-FB₁ excreted in urine with most of the urinary excretion occurring in the first 12 h (Figure 2). The urinary excretion of HFB₁ persisted for up to 60 h after dosing with maximum excretion occurring between 12 and 24 h. The urinary excretion of ¹⁴C-FB₁-fructose persisted for 24 h with the maximum excretion occurring in the first 12 h. The pattern of excretion of HFB₁ and of FB₁-fructose resembled one-compartment models of elimination. Amounts of 2–5-fold greater ¹⁴C-HFB₁ than FB₁ were excreted in urine, suggesting that HFB₁ was better absorbed than FB₁ in these rats. Dietary HFB₁ was nearly as toxic as FB₁ during the promotion phase of a two-stage model of rat hepatocarcinogenesis (Hendrich et al., 1993). Because the similarity between HFB₁ and FB₁ in toxicity may be accounted for by the greater bioavailability of HFB₁, FB₁ would seem to be more toxic at the cellular level than HFB₁. But in primary rat hepatocytes, HFBs were more cytotoxic than FBs as measured by lactate dehydrogenase leakage (Gelderblom et al., 1993). The toxic dose of FBs was 1 mM or more in the hepatocyte cultures. Such high concentrations would be highly unlikely to be achievable through dietary exposures, given the very limited apparent absorption of FB₁. The hepatocyte culture studies are probably not helpful in explaining FB₁ toxicity in vivo.

Total excretion of ¹⁴C-FB₁-fructose was 8-fold greater than FB₁, suggesting that FB₁-fructose may have greater bioavailability than FB₁ in these rats (Table 1). Fumonisin B₁-fructose product(s) seem to be detoxified forms of FB₁ during the promotion phase of a two-stage rat hepatocarcinogenesis model (Lu et al., 1997). The mechanism cannot be due to reduced absorption of FB-fructose. Blocking the FB amine group prevented toxicity in primary rat hepatocyte cultures as well as in vivo (Gelderblom et al., 1993). Perhaps the blocked amine group sterically prevents the interaction of FBs with their molecular sites of action.

Table 1. Percent Recovery of ^{14}C from 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, and $^{14}\text{C}\text{-FB}_1\text{-Fructose/kg}$ Body Weight in Rats^a

total dose	percent dose							
	urine		feces		tissues		recovery	
	male	female	male	female	male	female	male	female
FB_1	0.4 ^a	0.7 ^a	85	95	0.28	2.72	86	99
HFB ₁	12.8 ^c	17.3 ^d	87	91	0.14	1.31	100	110
$\text{FB}_1\text{-fructose}$	4.2 ^b	4.6 ^b	86	97	0.19	0.42	91	102

^a Different superscripts indicate that the means were significantly different at $\alpha = 0.05$; $n = 3$ per gender and treatment.

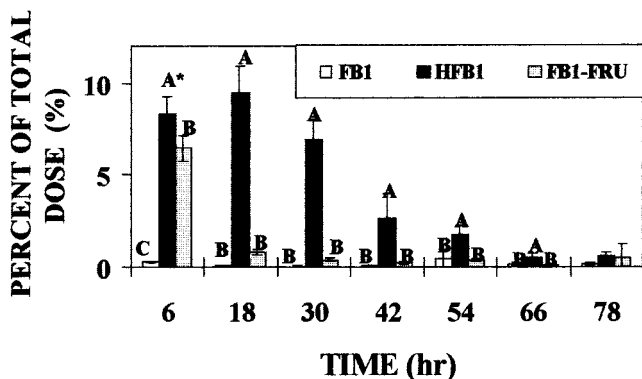


Figure 2. Urine excretion of ^{14}C from 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, and $^{14}\text{C}\text{-FB}_1\text{-fructose/kg}$ body weight by rats per 12-h interval. Error bars represent ± 1 standard deviation; $n = 6$. Bars within a time interval with different superscripts were different at $\alpha = 0.05$. $\text{FB}_1\text{-FRU} = \text{FB}_1\text{-fructose}$.

The total urinary excretion of 0.69 μmol of HFB₁ and $\text{FB}_1\text{-fructose}$ as compared to FB_1 over 84 h was comparable to the previous study over 96 h at three dose levels (Hopmans et al., 1997). Both studies reported the same total percent urinary excretion of HFB₁ and relative absorptions of HFB₁ and $\text{FB}_1\text{-fructose}$ as compared to FB_1 . In the study by Hopmans et al. (1997), rats dosed with 0.69 μmol of $\text{FB}_1\text{-fructose}$ or FB_1/kg bw excreted 4.2% of the total dose of $\text{FB}_1\text{-fructose}$ and 7.4% of the total dose of FB_1 in the urine as compared with 4.4% and 0.6%, respectively, in our current study. Hopmans et al. (1997) correlated FB_1 and $\text{FB}_1\text{-fructose}$ to the amount of OPA-HFB₁ in the hydrolyzed rat urine. Hydrolysis of urine produced a free amine group on the FB_1 molecule for OPA derivatization. In the current study, FB_1 and $\text{FB}_1\text{-fructose}$ were measured as the amount of ^{14}C detected in the rat urine. The differences in these two studies may reflect a difficulty in accurate estimation of very small quantities of FBs by sample extraction and HPLC analysis. Norred et al. (1993) found that 2–3% of a gavaged dose of 1.4 μmol of $^{14}\text{C}\text{-FB}_1/\text{kg}$ bw was excreted in the urine of fasted Sprague–Dawley rats after 96 h, suggesting that their fasted rats had a greater absorption of FB_1 than our rats. Our dosing of rats occurred at the end of the light cycle. Rats do not eat much during the light cycle, so our rats should have been fairly comparable to rats fasted for 12 h. The apparent higher absorption by Norred's rats as compared to our rats could be attributed to differences in strain of rat or, more likely, to differences in absorption of FBs with the extent of food deprivation. In fed rats, food may make FBs less available for GI tract absorption.

The data for fecal excretion of ^{14}C in male and female rats, dosed with the same compound, were combined because they were not significantly different according to gender (Figure 3). The pattern of fecal excretion of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, and $^{14}\text{C}\text{-FB}_1\text{-fructose}$ followed a

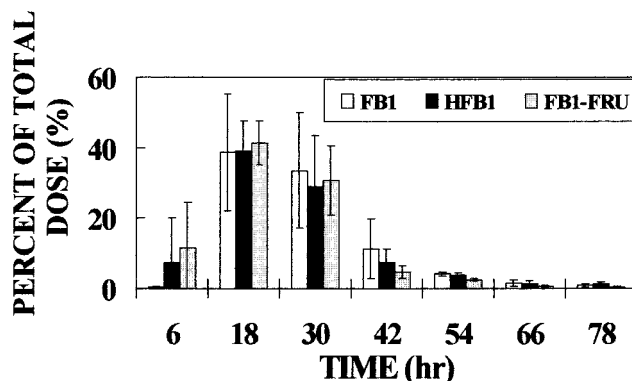


Figure 3. Fecal excretion of ^{14}C from 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, and $^{14}\text{C}\text{-FB}_1\text{-fructose/kg}$ body weight by rats per 12-h interval. Error bars represent ± 1 standard deviation; $n = 6$.

Table 2. Biliary Excretion of ^{14}C from 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, and $^{14}\text{C}\text{-FB}_1\text{-Fructose/kg}$ Body Weight in Rats over 4 h^a

dose	percent excretion
FB_1	1.55 \pm 2.51
HFB ₁	1.71 \pm 1.99
$\text{FB}_1\text{-fructose}$	0.80 \pm 0.82

^a $n = 3$.

normal excretion of a compound through the fecal route (Casarett and Doull, 1991) with maximum excretion of ^{14}C from the three FB forms between 12 and 24 h. After 60 h, only trace amounts of ^{14}C were recovered in the feces from these rats. Total fecal excretion was not significantly different between FB compounds or gender and averaged 90% recovery of total dose after 84 h (Table 1).

The total ^{14}C recovered from the hearts, brains, livers, blood, kidneys, or lungs of rats dosed with 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, or $^{14}\text{C}\text{-FB}_1\text{-fructose/kg}$ bw was not significantly different from zero for all rats, indicating that accumulation of these compounds did not occur after 84 h (data not shown).

In the biliary excretion study, Sprague–Dawley rats were used because the Fisher rats were not large enough for successful cannulation. The same FB treatments were used for the biliary excretion study as in the urinary excretion study. However, to obtain proper volumes, the 0.14 μmol of $^{14}\text{C}\text{-HFB}_1$ and $^{14}\text{C}\text{-FB}_1\text{-fructose/mL}$ treatments were diluted with unlabeled 0.14 μmol of HFB₁ or $\text{FB}_1\text{-fructose/mL}$, which resulted in specific activities of 0.7 and 1.0 mCi/mmol, respectively. All nine of the cannulated female rats survived for 4 h after dosing. Biliary excretion of 0.69 μmol of $^{14}\text{C}\text{-FB}_1$, $^{14}\text{C}\text{-HFB}_1$, or $^{14}\text{C}\text{-FB}_1\text{-fructose/kg}$ bw by female rats were not significantly different with an average of 1.35% and a range of 0.80 ($\text{FB}_1\text{-fructose}$) to 1.17 (HFB₁) of the total ^{14}C dose excreted 4 h after dosing (Table 2). Biliary excretion of the three FB compounds increased

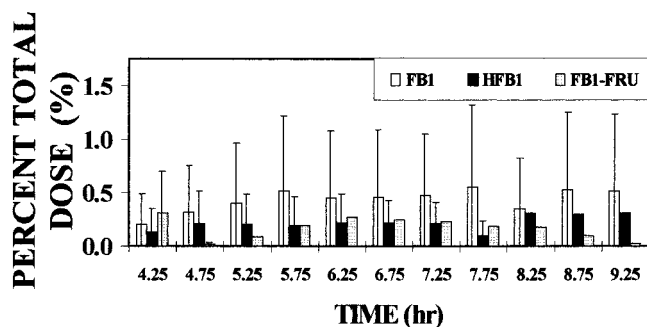


Figure 4. Biliary excretion of ¹⁴C from 0.69 μmol of ¹⁴C-FB₁, ¹⁴C-HFB₁, and ¹⁴C-FB₁-fructose/kg body weight by female rats per 30-min interval. Error bars represent ± range; n = 2. Bars without error bars represent n = 1.

from 0 to 0.5% of the total dose per 0.5-h interval within 2 h after dosing. The three forms of FB continued to be excreted in the bile by the rats up to 9.5 h after dosing (Figure 4). There seemed to be no cyclical nature to ¹⁴C excretion in the bile after administration of the dose by gavage. The large range seen in the biliary excretion data suggest the presence of fluorescent compounds that have been previously observed in rat bile (Hicks et al., 1984). Only two rats from the ¹⁴C-FB₁ dose and one rat from the ¹⁴C-HFB₁ and ¹⁴C-FB₁-fructose doses survived for 9.5 h. Shephard et al. (1994) recovered almost 7-fold less, 0.2% of their total ¹⁴C dose in bile duct cannulated rats gavaged with ¹⁴C-FB₁ as compared to our rats. The rats used by Shephard et al. (1994) were not under anesthesia during gavage or bile collection. Half of the ¹⁴C dose of the three forms of FB₁ were recovered in the stomach tissue and contents of our rats (50 ± 23% of the total dose), indicating that only about half of the dosed-FB compounds could have reached the small intestine of these rats (data not shown). Because these rats were under anesthesia throughout the duration of the experiment, there may have been a slower rate of absorption as compared with Shephard et al. (1994). The pooled intestines and bile samples from the rats contained 3 ± 6 and 3 ± 4% of the total dose, respectively. Kidneys and livers contained less than 1% of the ¹⁴C dose. Total recovery of the ¹⁴C doses from these rats of the bile excretion study averaged 63 ± 17%.

The 25-fold greater absorption of ¹⁴C-HFB₁ than that of ¹⁴C-FB₁ in male and female Fisher rats suggested that, once in circulation, HFB₁ was less toxic than FB₁, on a molar basis, because both have been shown to be equally toxic on a dietary basis to rats (Hendrich et al., 1993). Detoxification of FB₁ by the formation of FB₁-fructose was not the result of decreased absorption since ¹⁴C-FB₁-fructose was absorbed 8-fold more than ¹⁴C-FB₁ by these rats. These data complement and extend the findings of Hopmans et al. (1997), suggesting that HFB₁ or FB₁-fructose were absorbed more than FB₁. In addition, there were no differences in biliary excretion of ¹⁴C-FB₁, ¹⁴C-HFB₁, or ¹⁴C-FB₁-fructose in female Sprague-Dawley rats, lending additional support to the likelihood that the observed decrease in urinary excretion of FB₁ as compared with HFB₁ or FB₁-fructose was due to decreased absorption of FB₁ relative to HFB₁ or FB₁-fructose in rats.

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