Fumonisin B_1 Carcinogenicity in a Two-Year Feeding Study Using F344 Rats and B6C3F_1 Mice

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Fumonisin B_1 (FB_1) is a mycotoxin isolated from *Fusarium* fungi that contaminate crops worldwide. A previous study demonstrated that FB_1 promoted preneoplastic foci in initiated rats and induced hepatocellular carcinomas in BD IX rats at 50 parts per million (ppm), but fundamental dose–response data were not available to assist in setting regulatory guidelines for this mycotoxin. To provide this information, female and male F344/N/Nctr BR rats and B6C3F_1/Nctr BR mice were fed for two years a powdered NIH-31 diet containing the following concentrations of FB_1: female rats, 0, 5, 15, 50, and 100 ppm; male rats, 0, 5, 15, 50, and 150 ppm; female mice, 0, 5, 15, 50, and 80 ppm; male mice, 0, 5, 15, 80, and 150 ppm. FB_1 was not tumorigenic in female F344 rats with doses as high as 100 ppm. Including FB_1 in the diets of male rats induced renal tubule adenomas and carcinomas in 0/48, 0/40, 9/48, and 15/48 rats at 0, 5, 15, 50, and 150 ppm, respectively. Including up to 150 ppm FB_1 in the diet of male mice did not affect tumor incidence. Hepatocellular adenomas and carcinomas were induced by FB_1 in the female mice, occurring in 5/47, 3/48, 1/48, 19/47, and 39/45 female mice that consumed diets containing 0, 5, 15, 50, and 80 ppm FB_1, respectively. This study demonstrates that FB_1 is a rodent carcinogen that induces renal tubule tumors in male F344 rats and hepatic tumors in female B6C3F_1 mice. *Key words:* fumonisin B_1, hepatocarcinogenicity, renal carcinoma, rodent bioassay. — *Environ Health Perspect* 109(suppl 2):277–282 (2001).
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Fumonisins are a group of hydrophilic mycotoxins produced by fungi of the *Fusarium* species. Fumonisin B_1 (FB_1) was the first fumonisin identified, and this led to the discovery of several different homologues (*1–4*). The *Fusarium* fungi have been shown to contaminate crops (primarily corn or maize) worldwide (*5*), and the severity of the infection depends on environmental conditions such as drought and heat. *Fusarium moniliforme* Sheldon (= *F. verticillioides*) is considered the dominant species of *Fusarium* on crops that produces FB_1, although other species have been shown to produce FB_1 and the other fumonisins in culture (*6–13*). The predominant fumonisin homologues (B_1, B_2, B_3) differ on the basis of hydroxyls at positions C5 and C10 (*1–3*).

The discovery of FB_1 was the result of long-term investigations into the reasons for regiospecificity in the occurrence of esophageal cancer in South Africa. Areas in the Transkei that are only 150 km apart have esophageal cancer incidences that differ 2- to 3-fold (*14,15*). Cytologic analysis of esophageal scrapings from patients in these areas showed that cytologically abnormal esophageal mucosal cells were present in patients from the high-risk area (*16*). Furthermore, investigators demonstrated a higher incidence of *F. moniliforme* in maize from households in the high-risk area than in the low-risk area of the Transkei (*16,17*). These field investigations led to the isolation from household corn of several isolates of *F. moniliforme* (*18–20*). Including isolate *F. moniliforme* MRC 826 in the diet of BD IX rats for 22–27 months led to formation of esophageal hyperplasia, forestomach papillomas and carcinomas, hepatocellular carcinomas, and cholangiocarcinomas (*21*).

FB_1 was isolated as the compound present in cultures of *F. moniliforme* MRC 826 that promoted the formation of preneoplastic altered enzyme foci in the livers of dimethylnitrosamine-initiated rats (*22*). In a subsequent study, including FB_1 at 50 mg/kg in the diet of BD IX rats led to development of liver tumors (*23*). Hepatic regenerative nodules, cholangiofibrosis, and cirrhosis developed in all 10 rats maintained on the FB_1 for 20–26 months, whereas only 7 of 10 rats developed hepatocellular carcinomas (*23*). Although this study was limited to a single dose of FB_1 in the diet and included only a small number of rats, when combined with the tumor promotion studies (*22*) it strongly suggested that FB_1 was a rodent carcinogen.

FB_1 was nominated for tumorigenesis testing under the auspices of the National Toxicology Program because of the carcinogenesis of FB_1 and *F. verticillioides* MRC 826 in rats, the presence of FB_1 in maize in areas of the world with high incidences of esophageal cancer (*5*) attributed to the presence of FB_1 in food destined for human consumption (maize), and because of the lack of fundamental dose–response data available to assist with setting regulatory levels.

Materials and Methods

Study Material and Feed

FB_1 was produced by aqueous cultures of *F. proliferatum* on corn. The FB_1 was extracted from the autoclaved material using methanol and purified as the ammonium salt using high-performance liquid chromatography (HPLC). The purity of the FB_1 was established as > 96% using ^1H and ^{13}C nuclear magnetic resonance spectroscopy, mass spectrometry, and HPLC with evaporative light scattering detection (*24*).

Autoclaved-powdered NIH-31 rodent feed (Purina Corp., St. Louis, MO) was the test diet in the study, and FB_1 was added as a water-based component using a Patterson-Kelley V-blender (Patterson-Kelley Co., East Stroudsburg, PA). The FB_1 content of the control diet was below 0.06 parts per million (ppm).

Animals and Housing

Female and male Fischer 344/N/Nctr BR rats and B6C3F_1/Nctr BR mice were obtained from the National Center for Toxicological Research (NCTR) breeding colony at 4 weeks postpartum. The rats and mice were allocated to the study dose groups in a random manner that controlled for weight bias and minimized occurrence of littermates in the same dose

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group. The rats were housed two per cage and the mice four per cage in polycarbonate cages with autoclaved hardwood chip bedding. Cagemates were identified using an ear-clip identification system. Water was available *ad libitum*. The powdered feed was available *ad libitum* in custom feeders designed for powdered feed. The cages and water were changed twice weekly for the rats and weekly for the mice.

Study Design

The doses of FB_1 for the rats were based on toxicities detected in 28-day and 90-day subchronic studies (*24–26*). The doses for the bioassay were 0, 5, 15, 50, and 100 ppm FB_1 for female F344 rats and 0, 5, 15, 50, and 150 ppm FB_1 for the male F344 rats.

The doses of FB_1 for the $B6C3F_1$ mice were based on the response of the mice in 28- and 90-day subchronic studies (*24,25*). The FB_1 doses used with the female mice were 0, 5, 15, 50, and 80 ppm FB_1, whereas the doses used in the diets for the male mice were 0, 5, 15, 80, and 150 ppm FB_1.

The study was conducted in accordance with the guidelines of the National Toxicology Program (*27*) and the U.S. Food and Drug Administration (*28*). Animals were allocated 48 for each sex per dose group except for the groups receiving diets containing 5 ppm FB_1, in which there were 40 rats of each sex. The mice and rats were acclimated to the cage and control powdered feed until 6 weeks postpartum, when the dosed feed was added.

Animals were necropsied after 104 weeks of consumption of the dosed feed or upon removal as moribund or dead. The livers and kidneys from all the animals were examined microscopically, whereas other tissues were examined only for animals receiving control or high-dose diets (*24*). Pathologic examinations were conducted as described for the National Toxicology Program (*27*).

Statistical Analysis

Tests of pairwise comparisons of the neoplastic and non-neoplastic lesions for each exposed group with the controls was conducted using the Poly-k test (*24,29,30*). A k-value of 3 was used in these analyses (*30*). The analysis includes a risk–weight adjustment on animals that died before completion of the study and reports an adjusted rate of lesion incidence.

Results

The female F344 rats that consumed diets containing 100 ppm FB_1 had decreased weights compared to the body weights of the female F344 rats on the control diets (Figure 1A). No body weight differences were detected in the female F344 rats that consumed diets containing 5, 15, and 50 ppm FB_1 compared to the female F344 rats on the control diet (Figure 1A). The daily mean consumption of diet by the male and female rats during the course of the study was indistinguishable from the dietary consumption of other F344/N/Nctr BR rats at this facility (data not shown) The mean consumption rates of compound in the female rats between weeks 51 and 104 on the dosed feed were 0, 0.27, 0.78, 2.57, and 5.24 mg FB_1 per kg body weight per day (mg/kg bw/day) for 0, 5, 15, 50, and 100 ppm diets, respectively. There were no FB_1-dependent changes in the body weights of the male F344 rats that consumed up to 150 ppm FB_1 (Figure 1B). The mean consumption rates of FB_1 between weeks 51 and 104 of the study were 0, 0.22, 0.67, 2.24, and 6.60 mg/kg bw/day for male rats consuming diets containing 0, 5, 15, 50, and 150 ppm FB_1.

There were no dose-related differences in the survival of the female F344 rats at 104 weeks (Figure 2A). The survival for female F344 rats on the control diet was 52%, whereas the survival of female F344 rats consuming the 100 ppm FB_1 diet was 60%. Consumption of the FB_1 dose used in the study did not induce toxicity that could be detected by changes in body weight throughout the 2-year study.

There were no dose-related differences in the survival rates of the male F344 rats, with 35% of the male F344 rats on the control diet and 52% of the male F344 rats consuming diets with 150 ppm FB_1 surviving for 2 years (Figure 2B).

Necropsy and microscopic evaluation of the tissues of the male F344 rat revealed an increase in renal tubule adenomas and carcinomas (Table 1). No tumors were present in the kidneys of the male F344 rats that consumed diets containing 0, 5, or 15 ppm FB_1. Renal tubule adenomas were present in 2 of the 48 male F344 rats consuming 50 ppm FB_1, and renal tubule carcinomas were present in 7 of the 48 rats on this dose. This produced an adjusted incidence of 25.7% for renal adenomas and carcinomas in the male rats at the 50 ppm FB_1 dose. The development of renal tubule adenomas and carcinomas was more pronounced in male F344 rats that consumed 150 ppm FB_1 (Table 1), with 5 of the 48 rats developing adenomas and 10 of the 48 rats developing carcinomas, for an adjusted incidence of 38.1% of the rats at the 150 ppm FB_1 dose with adenomas or carcinomas. These increases in adenomas at 150 ppm, carcinoma at 50 and 150 ppm, and adenoma or carcinoma at 50 and 150 ppm were significant for the control group (Table 1).

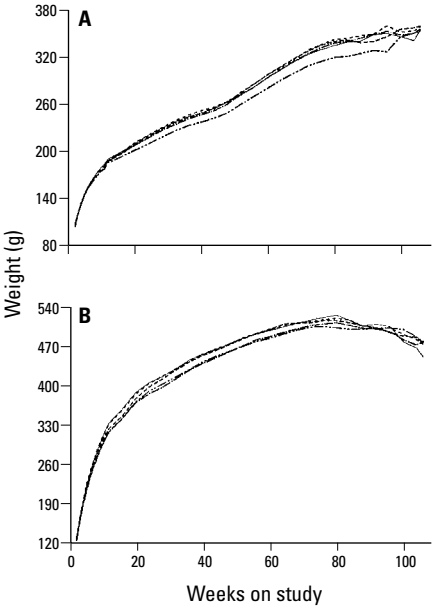

Figure 1. Body weights of female (*A*) and male (*B*) F344/N/Nctr BR rats in the 2-year tumor study. The body weights were measured weekly, and are shown at 4-week intervals starting at 20 weeks. The following FB_1 doses were given to the female F344 rats: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 100 ppm (– • • –). The following FB_1 doses were given to the male F344 rats: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 150 ppm (– • • –).

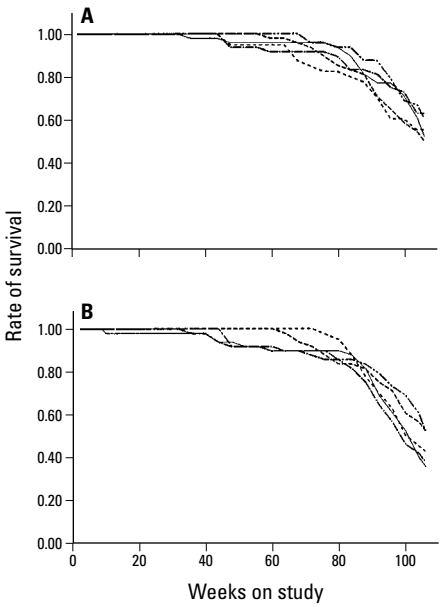

Figure 2. Survival of female (*A*) and male (*B*) F344/N/Nctr BR rats in the 2-year tumor study. Survival rates are expressed as the fraction of rats initially loaded into the study that survived to the end of the indicated week. The following FB_1 doses were given to the female F344 rats: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 100 ppm (– • • –). The following FB_1 doses were given to the male F344 rats: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 150 ppm (– • • –).

Among the female F344 rats, there were no FB_1-dependent changes in the incidence of tumors. One renal adenoma was detected in a female F344 rat consuming 50 ppm FB_1, and one renal tubule carcinoma was detected in a female F344 rat that consumed 100 ppm FB_1; however, from the Poly-k analysis, the low frequency of these tumors did not indicate a dose-related trend.

The renal tubule adenomas were characterized by a defined focus of expansive tubule cells. The nuclear and cell volumes were increased in adenoma cells compared to normal adjacent cells. The cytoplasm of the adenoma cells stained clear to basophilic. A representative adenoma is shown in Figure 3.

The renal tubule carcinomas were characterized as growths of abnormal and atypical cells that compressed and invaded neighboring normal tissue (Figure 4). The cells within the growing boundary of the carcinoma contained basophilic cytoplasm with typically increased volume and hyperchromatic nuclei. Necrosis was evident within the interior of the larger carcinomas. In many of the carcinomas, small renal tubule-like structures were evident. These renal tubule carcinomas metastasized to the lung and lymphatic tissues.

We detected no differences in the body weights of the female $B6C3F_1$ mice (Figure 5A) or in the consumption of the diets containing FB_1 (data not shown) when compared to those of mice receiving control diets. The mean daily consumption of FB_1 between weeks 51 and 104 of the study were 0, 0.65, 1.91, 6.62, and 12.76 mg/kg bw/day for the groups receiving 0, 5, 15, 50 and 80 ppm FB_1, respectively. Similarly, the body weights of the male $B6C3F_1$ mice consuming diets containing FB_1 did not differ from the body weights of the male $B6C3F_1$ mice on control diets (Figure 5B). The mean daily consumption of FB_1 in the male mice receiving 0, 5, 15, 50, and 150 ppm FB_1 was 0, 0.53, 1.55, 9.04, and 15.41 mg/kg bw/day, respectively, between weeks 51 and 104 of the study. The body weights of the male mice were approximately 15% less than the body weights of $B6C3F_1$/Nctr BR mice in other studies conducted at NCTR, whereas the body weights of the female mice were 30% less than expected. Analysis of the feed consumption rates indicated that the mice were consuming approximately 30% less feed than mice in other studies at NCTR. The lower consumption of feed apparently was not caused by palatability, because feed consumption was reduced in the control groups. Availability of the feed through the screen feeders was reduced, although particle size analysis of the feed did not indicate that the powdered feed was altered by the addition of FB_1.

Survival of the female $B6C3F_1$ mice consuming 80 ppm FB_1 decreased compared to survival of mice consuming control diets or diets containing 0–50 ppm FB_1 (Figure 6A). This decrease in survival started at approximately 1 year of age and continued until the end of the study. Female $B6C3F_1$ mice consuming the other FB_1 diets had survival rates indistinguishable from those of female mice consuming control diets (Figure 6A). Exposure to FB_1 had no effect on survival of male $B6C3F_1$ mice at any of the doses (Figure 6B).

Hepatocellular adenomas were present in 11.7% of the female $B6C3F_1$ mice given control diet for 2 years (Table 2). Hepatocellular adenomas were present at adjusted rates of 6.5 and 2.1% at 5 and 15 ppm FB_1; these values were not statistically significantly different from the incidence in the control group. In females consuming 50 and 80 ppm FB_1, the adjusted rates for the incidence of adenoma increased to 36.3 and 73.7%, respectively. Hepatocellular carcinomas were not present in female $B6C3F_1$ mice given 0, 5, or 15 ppm FB_1. Hepatocellular carcinomas were present at adjusted rates of 22.5 and 23% among female $B6C3F_1$ mice that consumed 50 and 80 ppm FB_1, respectively (Table 2). Consumption of FB_1 increased the adjusted rate of incidence of hepatocellular adenomas and carcinomas from 11.7% of the

Table 1. Incidences of neoplastic and non-neoplastic lesions in male rat kidneys.

	Number of rats examined				
Type of lesion	48	40	48	48	48
Renal tubule epithelial hyperplasia	2	1	4	14[a]	8[a]
Renal tubule adenoma	0	0	0	2	5
Adjusted rate (percent)[b]	0	0	0	5.7	12.9
Significance[b]	–	–	–	$p = 0.2293$	$p = 0.0314$
Renal tubule carcinoma	0	0	0	7	10
Adjusted rate (percent)	0	0	0	20.0	25.4
Significance	–	–	–	$p = 0.0059$	$p = 0.0008$
Renal tubule adenoma or carcinoma	0	0	0	9	15
Adjusted rate (percent)	0	0	0	25.7	38.1
Significance	–	–	–	$p = 0.0011$	$p = 0.0001$

[a]Significantly different from control group ($p < 0.05$) using the Poly-k test described in "Methods." [b]The adjusted incidence rate and significance were determined using the Poly-k test.

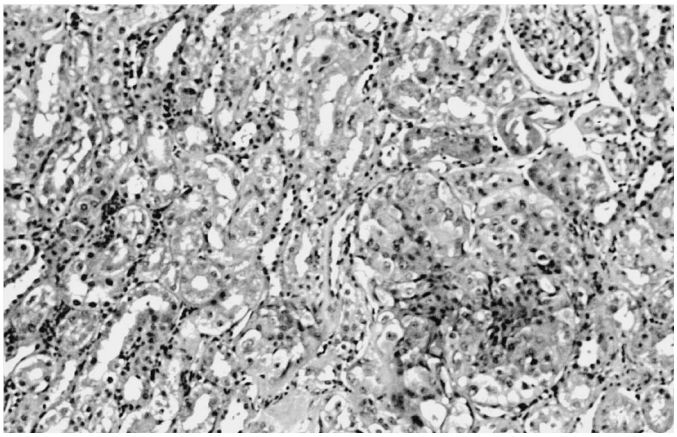

Figure 3. Photomicrograph of renal tubule adenoma from a male F344/N/Nctr BR rat that consumed a diet containing 150 ppm FB_1. The adenoma is present in the lower left side of the photomicrograph as a focus of cells that disrupted normal tubule organization.

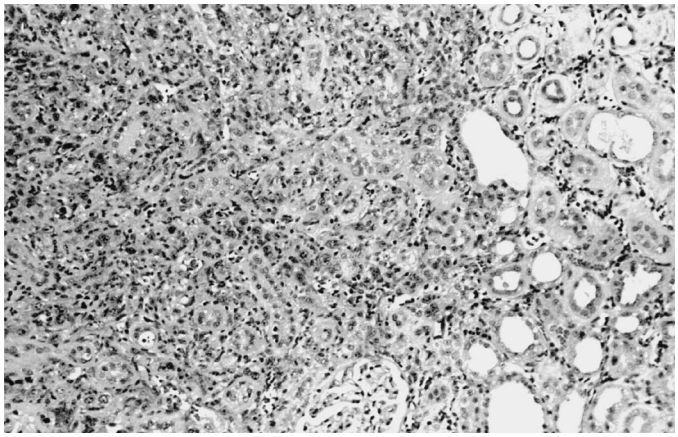

Figure 4. Photomicrograph of a renal tubule carcinoma from a male F344/N/Nctr BR rat that consumed a diet containing 150 ppm FB_1. The normal kidney is present on the right side of the photomicrograph where renal tubules are present. The carcinoma (left side of photomicrograph) contains cells of atypical morphology, although some tubule-like organization is found within the tumor.

female B6C3F$_1$ mice on control diets to 6.5, 2.1, 42.7, and 88.3% of mice consuming diets that contained 5, 15, 50, and 80 ppm FB$_1$, respectively (Table 2). The increased rates of adenomas or carcinomas at 50 and 80 ppm FB$_1$ were statistically significantly different from those of the control group.

The hepatocellular adenomas were characterized by distinct foci of cells that were either eosinophilic or basophilic and that routinely compressed the adjacent normal parenchymal cells (Figure 7). The carcinomas were characterized by poorly differentiated and anaplastic cells within the liver (Figure 8).

Among male B6C3F$_1$ mice, exposure to FB$_1$ did not affect the incidence of neoplasia of any type, including in the liver, where approximately 25% of the mice had hepatocellular adenomas or carcinomas (Table 2).

The increase in numbers of hepatocellular adenomas or carcinomas in female mice was accompanied by increases in hepatocellular hypertrophy at 50 and 80 ppm FB$_1$ (Table 2). Although hypertrophy correlated with tumor incidence in female mice, it was present in the livers of male mice at 80 and 150 ppm (Table 2), but there was no increase in tumor incidence.

Discussion

These studies show that FB$_1$ is a renal carcinogen when included in the diets of male F344 rats. Renal tubule adenomas or carcinomas were absent in the male F344 rats consuming control diets and diets containing 5 or 15 ppm FB$_1$ for 2 years (Table 1). Induction of renal tubule carcinomas was evident at doses of 50 and 150 ppm FB$_1$ (Table 1), with adjusted rates of 22.5 and 23.0%, respectively. This suggests that a no-observed-effect-level (NOEL) for the induction of renal tumors in male rats lies between 15 and 50 ppm FB$_1$. The highest dose of FB$_1$ included in the diets of the female F344 rats was 100 ppm; this did not increase tumor incidence.

In the present study, including FB$_1$ in doses as high as 150 ppm in the diets of male F344 rats and as high as 100 ppm in the diet of female F344 rats over the 2-year feeding period did not increase mortality compared to that of rats consuming control diets (Figure 2). Similarly, the body weights of the male and female rats consuming diets containing FB$_1$ did not decrease compared to the body weights of rats on the control diets (Figure 1). Therefore, we can conclude that the dietary levels of FB$_1$ that induced tumors in male F344 rats (15 ppm < NOEL ≤ 50 ppm) were not close to the maximum tolerated dose (MTD), as evidenced by an absence of effect on the growth and survival of the dosed rats.

In a feeding study using male BD IX rats, Gelderblom et al. (*23*) included 50 ppm FB$_1$ in the diet for up to 26 months. The diet contained 75% sifted white corn, and the purity of the FB$_1$ was reported as > 90% (*23*). The FB$_1$-fed rats developed hepatic regenerative nodules and cholangiofibrosis (synonymous with adenofibrosis), whereas rats on the control diets did not. The liver dysplasia progressed to hepatic cirrhosis and hepatocellular carcinomas in 10 of the 15 rats sacrificed between 18 and 26 months. In an additional study, FB$_1$ was fed to male BD IX rats at 0, 1, 10, and 25 ppm for 24 months (*31*). These levels of FB$_1$ failed to induce the hepatocellular carcinomas that were induced in the previous study with 50 ppm FB$_1$. As a result, the studies with male BD IX rats suggest that a NOEL exists between 25 and 50 ppm FB$_1$ for the formation of hepatocellular tumors in male BD IX rats. Renal tumors in male rats

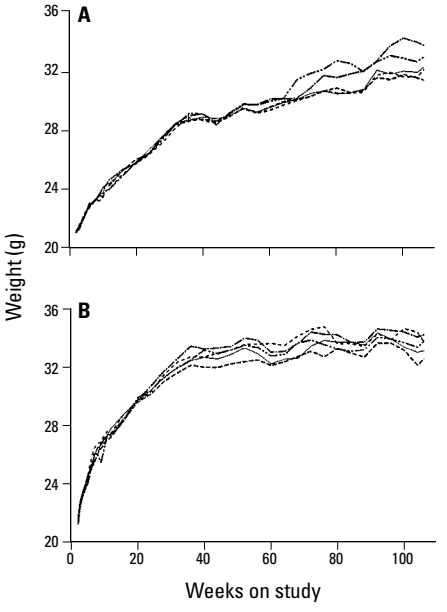

Figure 5. Body weights of female (*A*) and male (*B*) B6C3F$_1$/Nctr BR mice in the 2-year tumor study. The body weights were measured weekly and are shown at 4-week intervals starting at 20 weeks. The following FB$_1$ doses were given to the female mice: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 80 ppm (– • • –). The following FB$_1$ doses were given to the male mice: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 80 ppm (– • –), and 150 ppm (– • • –).

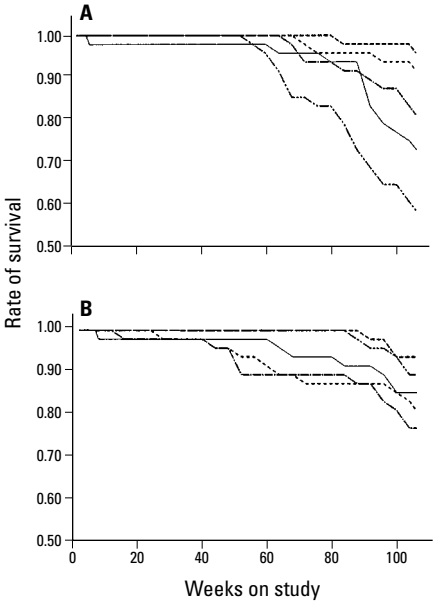

Figure 6. Survival of the female (*A*) and male (*B*) B6C3F$_1$/Nctr BR mice in the 2-year tumor study. Survival rates are expressed as the fraction of mice initially loaded into the study that survived to the end of the indicated week. The following FB$_1$ doses were given to the female mice: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 50 ppm (– • –), and 80 ppm (– • • –). The following FB$_1$ doses were given to the male mice: control diets (——), 5 ppm (– – –), 15 ppm (— — —), 80 ppm (– • –), and 150 ppm (– • • –).

Table 2. Incidences of neoplastic and non-neoplastic lesions in mouse liver.

	Number of female mice examined					Number of male mice examined				
Type of lesion	47	48	48	47	45	47	47	48	48	48
Diffuse hepatocellular hypertrophy	0	0	0	27[a]	31[a]	10	9	24[a]	25[a]	30[a]
Hepatocellular adenoma	5	3	1	16	31	9	7	7	6	8
Adjusted rate (percent)[b]	11.7	6.5	2.1	36.3	73.7					
Significance[b]	–	$p = 0.3314$	$p = 0.0862$	$p = 0.0047$	$p = 0.0001$					
Hepatocellular carcinoma	0	0	0	10	9	4	3	4	3	2
Adjusted rate (percent)	0	0	0	22.5	23.0					
Significance	–	–	–	$p = 0.0007$	$p = 0.0007$					
Hepatocellular adenoma or carcinoma	5	3	1	19	39	12	9	9	9	10
Adjusted rate (percent)	11.7	6.5	2.1	42.7	88.3					
Significance[b]	–	$p = 0.3314$	$p = 0.0862$	$p = 0.0005$	$p = 0.0001$					

[a]Significantly different from control group ($p < 0.01$) using the Poly-k test described in "Methods." [b]The adjusted incidence rate and significance were determined using the Poly-k test.

can be induced by compounds that bind to $\alpha_{2\mu}$-globulin (*32,33*). The morphology of the tumors was not consistent with this type of mechanism. The reasons for the different organospecificities in tumor formation (i.e., livers in the male BD IX rats and kidneys in the male F344 rats) remain to be elucidated and warrant additional studies.

The current study is the first to examine the carcinogenicity of FB_1 in mice. Our results show that FB_1 was hepatocarcinogenic in the female $B6C3F_1$ mice at doses of 50 and 80 ppm, with carcinoma formation in 22.5 and 23% of the mice, respectively (Table 2). There was an increased incidence of hepatocellular adenomas in the mice fed 50 and 80 ppm FB_1 (Table 2). These results suggest that the NOEL for adenoma or carcinoma in the female $B6C3F_1$ mouse is between 15 and 50 ppm. Failure to increase the incidence of hepatocellular adenomas and carcinomas in male $B6C3F_1$ mice and the lack of an increase in tumors in any other sites in response to the consumption of FB_1-containing diets suggests a NOEL for tumor formation in male $B6C3F_1$ mice > 150 ppm.

Little information is available on the MTD of FB_1 in mice to allow comparison of the FB_1 doses required for tumor induction versus MTD. In a 90-day feeding study with $B6C3F_1$ mice, Voss et al. (*25*) demonstrated that 81 ppm FB_1 in the diet was not toxic to male or female $B6C3F_1$ mice. In male $B6C3F_1$ mice fed FB_1 for 28 days, decreased body weights were detected in the group that consumed 484 ppm FB_1 but not in the group fed 234 ppm FB_1 (*24*). The body weights of female $B6C3F_1$ mice that consumed up to 484 ppm FB_1 for 28 days were not affected (*24*). Additionally, there were no deaths among the male or female $B6C3F_1$ mice consuming FB_1 for 28 days. The body weights and survival rates of the male $B6C3F_1$ mice in our 2-year study were not affected by the FB_1 (Figures 5 and 6), suggesting that the MTD for FB_1 in a chronic study is > 150 ppm. Although the body weights of the female mice were unaffected by doses of FB_1 as high as 80 ppm, their survival rates decreased beginning after approximately 1 year of consuming diets containg FB_1 (Figure 6). The cause of death in the mice consuming 80 ppm FB_1 was primary liver cancer (data not shown). Therefore, the MTD for FB_1 in female $B6C3F_1$ mice seems to be between 50 and 80 ppm FB_1 because of the appearance of hepatocarcinogenicity at 80 ppm.

The body weights of the male and female $B6C3F_1$ mice in the 2-year study were low when compared to the historical body weights of $B6C3F_1$ mice at NCTR. This apparently was caused by an inadvertent restriction of feed in the feeders used in this study. With a commercial blender, the FB_1 was added as an aqueous solution to predried powdered NIH-31 rodent feed. Particle analysis of the feed did not demonstrate any difference in the particle size before or after application of the FB_1 (data not shown), but the free flow of the powdered feed in the mouse feeders apparently was restricted, which meant that the mice consumed only about 70% of the amount of feed expected for an *ad libitum* study. This explains a reduction in body weight of approximately 15% for the male $B6C3F_1$ mice and approximately 20% for the female mice at 52 weeks in this study, compared to *ad libitum* studies with other $B6C3F_1$/Nctr BR mice at NCTR. The effects of reduced body weight through feed restriction were increased longevity and reduced incidence of spontaneous tumor formation (*34*). Given the body weight of the male mice at 52 weeks, a liver tumor incidence of 20% would have been expected (*35*). The liver tumor rate in the control group of male $B6C3F_1$ mice in this study was 26%; therefore, it appears that the inadvertent feed restriction resulted in the predicted liver tumor rate among the male mice. In another study (*36*), dietary restriction of female $B6C3F_1$ mice to 60% of *ad libitum* reduced liver tumors from 55 to 12% of the mice. The adjusted liver tumor rate in our study was 11.7% for the female $B6C3F_1$ mice consuming control diets. Therefore, it appears that the feed restriction in our study resulted in a decrease in liver tumor rates in the female $B6C3F_1$ mice similar to those previously reported (*36*).

F. moniliforme MRC 826 culture material was isolated from an area of South Africa that has a high incidence of esophageal cancer (*18–20*). Including this fungal isolate at 0.5% (wt/wt) in the diet of BD IX rats led to the development of esophageal hyperplasia and the development of forestomach and liver tumors (*21*). Further studies with the MRC 826 fungal isolate led to the discovery of FB_1 as the compound responsible for the induction of preneoplastic foci in initiated rats (*22*) and eventually to hepatic tumor formation in BD IX rats (*23*). The purification of FB_1 from *F. moniliforme* MRC 826 has led researchers to conclude that FB_1 is a potential human esophageal carcinogen. In this study we were unable to detect any hyperplasia or tumors in the esophageal tissue of rats or mice treated for 2 years with FB_1. Although transient increases in esophageal epithelium labeling index have been reported following gavage administration of FB_1 to rats (*37*), other reports have indicated a lack of effect of FB_1 consumption on rat esophageal epithelial tissue (*22–26*).

Esophageal tumors have been induced by many compounds in rat feeding studies. Most of these compounds are *N*-nitrosamines (*38*), which are structurally dissimilar from the

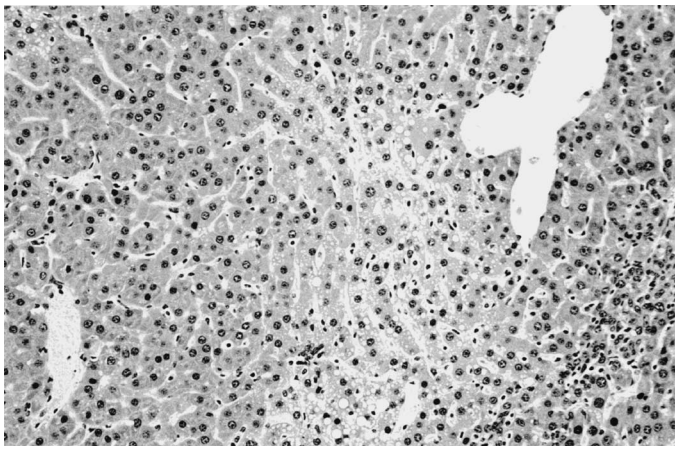

Figure 7. Photomicrograph of a hepatocellular adenoma in a $B6C3F_1$/Nctr BR mouse that consumed a diet containing 80 ppm FB_1. The adenoma is present in the upper center of the photomicrograph as a focus of basophilic cells of altered morphology.

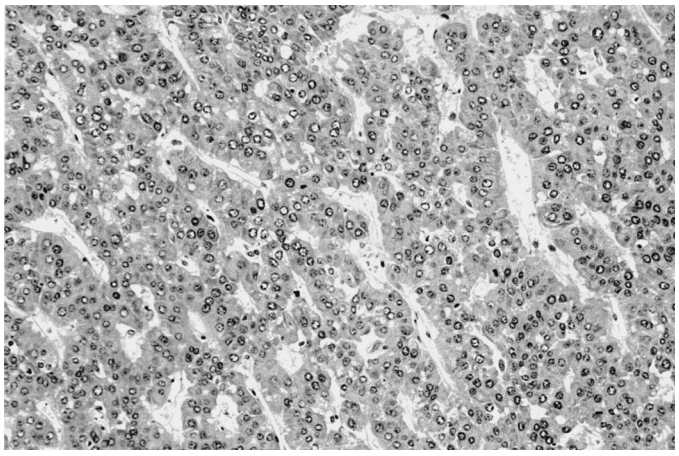

Figure 8. Photomicrograph of a hepatocellular carcinoma in a female $B6C3F_1$/Nctr BR mouse that consumed a diet containing 80 ppm FB_1. Some normal hepatic tissue is shown at the right side of the photomicrograph; the carcinoma is present in the left center of the photomicrograph.

fumonisins. Whereas *N*-nitrosamines are DNA alkylators, FB_1 is a nongenotoxic compound. We have reported that the incubation of methanolic extracts of *Fusarium* cultures with DNA in the presence of rat liver *S*9 proteins results in the formation of DNA adducts (*39*). The chromatographic characteristics of these unidentified DNA adducts suggest they are hydrophobic (*39*). Therefore, the possibility exists that compounds present in *Fusarium* fungi might alkylate DNA and participate in the induction of *Fusarium*-induced rodent esophageal dysplasia and forestomach tumors. Further research is required to establish whether FB_1 has a role in the development of esophageal cancer in humans.

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