Fumonisin B<sub>1</sub> Carcinogenicity in a Two-Year Feeding Study Using F344 Rats and B6C3F<sub>1</sub> Mice

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Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is a mycotoxin isolated from Fusarium fungi that contaminate crops worldwide. A previous study demonstrated that FB<sub>1</sub> 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/Nctr BR rats and B6C3F<sub>1</sub>/Nctr BR mice were fed for two years a powdered NIH-31 diet containing the following concentrations of FB<sub>1</sub>: 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<sub>1</sub> was not tumorigenic in female F344 rats with doses as high as 100 ppm. Including FB<sub>1</sub> in the diets of male rats induced renal tubule adenomas and carcinomas in 0/48, 0/40, 9/48, and 19/48 rats at 0, 5, 15, 50, and 150 ppm, respectively. Including up to 150 ppm FB<sub>1</sub> in the diet of male mice did not affect tumor incidence. Hepatocellular adenomas and carcinomas were induced by FB<sub>1</sub> in the female mice, occurring in 5/48, 3/48, 1/48, 19/47, and 39/45 female mice that consumed diets containing 0, 5, 15, 50, and 80 ppm FB<sub>1</sub>, respectively. This study demonstrates that FB<sub>1</sub> is a rodent carcinogen that induces renal tubule tumors in male F344 rats and hepatic tumors in female B6C3F<sub>1</sub> mice.

Key words: fumonisin B<sub>1</sub>, hepatocarcinogenicity, renal carcinoma, rodent bioassay. — Environ Health Perspect 109(suppl 2):277–282 (2001).

http://ehpnet1.niehs.nih.gov/docs/2001/suppl-2/277-282/howard/abstract.html

Fumonisins are a group of hydrophilic mycotoxins produced by fungi of the Fusarium species. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) 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<sub>1</sub>, although other species have been shown to produce FB<sub>1</sub> and the other fumonisins in culture (6–13). The predominant fumonisin homologues (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>) differ on the basis of hydroxyls at positions C5 and C10 (1–3).

The discovery of FB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> in the diet and included only a small number of rats, when combined with the tumor promotion studies (22) it strongly suggested that FB<sub>1</sub> was a rodent carcinogen.

FB<sub>1</sub> was nominated for tumorigenesis testing under the auspices of the National Toxicology Program because of the carcinogenicity of FB<sub>1</sub> and *F. verticillioides* MRC 826 in rats, the presence of FB<sub>1</sub> in maize in areas of the world with high incidences of esophageal cancer (5) attributed to the presence of FB<sub>1</sub> 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<sub>1</sub> was produced by aqueous cultures of *F. proliferatum* on corn. The FB<sub>1</sub> 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<sub>1</sub> was established as > 96% using ¹H and ¹³C 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<sub>1</sub> was added as a water-based component using a Patterson-Kelley V-blender (Patterson-Kelley Co., East Stroudsburg, PA). The FB<sub>1</sub> content of the control diet was below 0.06 parts per million (ppm).

Animals and Housing

Female and male Fischer 344/Nctr BR rats and B6C3F<sub>1</sub>/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 littersmates 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-chip identification system. Water 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\textsubscript{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\textsubscript{1} for female F344 rats and 0, 5, 15, 50, and 150 ppm FB\textsubscript{1} for the male F344 rats.

The doses of FB\textsubscript{1} for the B6C3F\textsubscript{1} mice were based on the response of the mice in 28- and 90-day subchronic studies (24,25). The FB\textsubscript{1} doses used with the female mice were 0, 5, 15, 50, and 80 ppm FB\textsubscript{1}, whereas the doses used in the diets for the male mice were 0, 5, 15, 80, and 150 ppm FB\textsubscript{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\textsubscript{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 on 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\textsubscript{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\textsubscript{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 0.27, 0.78, 2.57, and 5.24 mg FB\textsubscript{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\textsubscript{1}-dependent changes in the body weights of the male F344 rats that consumed up to 150 ppm FB\textsubscript{1} (Figure 1B). The mean consumption rates of FB\textsubscript{1} between weeks 51 and 104 of the study were 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\textsubscript{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\textsubscript{1} diet was 60%. Consumption of the FB\textsubscript{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\textsubscript{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\textsubscript{1}. Renal tubule adenomas were present in 2 of the 48 male F344 rats consuming 50 ppm FB\textsubscript{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\textsubscript{1} dose. The development of renal tubule adenomas and carcinomas was more pronounced in male F344 rats that consumed 150 ppm FB\textsubscript{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\textsubscript{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).
Among the female F344 rats, there were no FB1-dependent changes in the incidence of tumors. One renal adenoma was detected in a female F344 rat consuming 50 ppm FB1, and one renal tubule carcinoma was detected in a female F344 rat that consumed 100 ppm FB1; 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. In many of the carcinomas, necrosis was evident within the interior of the carcinoma (Figure 4). The cells within the growing boundary of the carcinoma contained basophilic cytoplasm with typically increased volume and hyperchromatic nuclei. The nuclear and cell volumes were characterized by a defined focus of expansive tubule organization. These renal tubule carcinomas metastasized to the lung and lymphatic tissues.

We detected no differences in the body weights of the female B6C3F1 mice (Figure 5A) or in the consumption of the diets containing FB1 (data not shown) when compared to those of mice receiving control diets. The mean daily consumption of FB1 between weeks 51 and 104 of the study were 0.65, 1.91, 6.62, and 12.76 mg/kg bw/day for the groups receiving 0, 5, 15, 50 and 80 ppm FB1, respectively. Similarly, the body weights of the male B6C3F1 mice consuming diets containing FB1 did not differ from the body weights of the male B6C3F1 mice on control diets (Figure 5B). The mean daily consumption of FB1 in the male mice receiving 0, 5, 15, 50, and 150 ppm FB1 was 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 B6C3F1/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 FB1.

Survival of the female B6C3F1 mice consuming 80 ppm FB1 decreased compared to survival of mice consuming control diets or diets containing 0–50 ppm FB1 (Figure 6A). This decrease in survival started at approximately 1 year of age and continued until the end of the study. Female B6C3F1 mice consuming the other FB1 diets had survival rates indistinguishable from those of female mice consuming control diets (Figure 6A). Exposure to FB1 had no effect on survival of male B6C3F1 mice at any of the doses (Figure 6B).

Hepatocellular adenomas were present in 11.7% of the female B6C3F1 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 FB1; these values were not statistically significantly different from the incidence in the control group. In females consuming 50 and 80 ppm FB1, the adjusted rates for the incidence of adenoma increased to 36.3 and 73.7%, respectively. Hepatocellular carcinomas were not present in female B6C3F1 mice given 0, 5, or 15 ppm FB1. Hepatocellular carcinomas were present at adjusted rates of 22.5 and 23% among female B6C3F1 mice that consumed 50 and 80 ppm FB1, respectively (Table 2). Consumption of FB1 increased the adjusted rate of incidence of hepatocellular adenomas and carcinomas from 11.7% of the

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

| Type of lesion                              | Number of rats examined | Significance |
|---------------------------------------------|-------------------------|--------------|
| Renal tubule epithelial hyperplasia         |                         |              |
| Renal tubule adenoma                        |                         |              |
| Renal tubule carcinoma                      |                         |              |
| Renal tubule adenoma or carcinoma           |                         |              |

*Significantly different from control group (*p* < 0.05) using the Poly-k test described in “Methods.” *The adjusted incidence rate and significance were determined using the Poly-k test.*
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female B6C3F1 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 FB1, respectively (Table 2). The increased rates of adenomas or carcinomas at 50 and 80 ppm FB1 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 (Figure 8). The carcinomas were absent in the male F344 rats consuming control diets, but were 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 FB1 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 diets that contained 5, 15, 50, and 80 ppm (Table 2). The hepatocellular adenomas were characterized by poorly differentiated and anaplastic cells (Figure 7). The carcinomas were characterized by distinctly foci of cells that were either eosinophilic or basophilic and that routinely compressed the adjacent normal parenchymal cells (Figure 8).

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

| Type of lesion                        | Number of female mice examined | Number of male mice examined |
|--------------------------------------|--------------------------------|----------------------------|
|                                      | 47                             | 48                          | 48                           | 47 | 48 | 48 | 48 |
| Diffuse hepatocellular hypertrophy   | 0                              | 0                           | 0                            | 27 | 31 |
| Hepatocellular adenoma               | 5                              | 3                           | 1                            | 16 | 31 |
| Adjusted rate (percent)              | 11.7                           | 3.314                       | 2.1                          | 36.3| 73.7|
| Significance                        | –                              | p = 0.0033                   | p = 0.0086                   | p = 0.0007 | p = 0.00001|
| Hepatocellular carcinoma             | 0                              | 0                           | 0                            | 10 | 9  |
| Adjusted rate (percent)              | 11.7                           | 6.514                       | 2.1                          | 42.7| 88.3|
| Significance                        | –                              | p = 0.0033                   | p = 0.0086                   | p = 0.0005 | p = 0.0001|

*Significantly different from control group (p < 0.01) using the Poly-k test described in “Methods.” *The adjusted incidence rate and significance were determined using the Poly-k test.

In the present study, including FB1 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 FB1 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 FB1 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 FB1 in the diet for up to 26 months. The diet contained 75% sifted white corn, and the purity of the FB1 was reported as > 90% (23). The FB1-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, FB1 was fed to male BD IX rats at 0, 1, 10, and 25 ppm for 24 months (31). These levels of FB1 failed to induce the hepatocellular carcinomas that were induced in the previous study with 50 ppm FB1. As a result, the studies with male BD IX rats suggest that a NOEL exists between 25 and 50 ppm FB1 for the formation of hepatocellular tumors in male BD IX rats. Renal tumors in male rats...
can be induced by compounds that bind to α2M-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 FB1 in mice. Our results show that FB1 was hepatocarcinogenic in the female B6C3F1 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 FB1, (Table 2). These results suggest that the NOEL for adenoma or carcinoma in the male B6C3F1 mouse is between 15 and 50 ppm. Failure to increase the incidence of hepatocellular adenomas and carcinomas in male B6C3F1 mice and the lack of an increase in tumors in any other sites in response to the consumption of FB1-containing diets suggests a NOEL for tumor formation in male B6C3F1 mice > 150 ppm.

Little information is available on the MTD of FB1 in mice to allow comparison of the FB1 doses required for tumor induction versus MTD. In a 90-day feeding study with B6C3F1 mice, Voss et al. (25) demonstrated that 81 ppm FB1, in the diet was not toxic to male or female B6C3F1 mice. In male B6C3F1 mice fed FB1 for 28 days, decreased body weights were detected in the group that consumed 484 ppm FB1 but not in the group fed 234 ppm FB1 (24). The body weights of female B6C3F1 mice that consumed up to 484 ppm FB1 for 28 days were not affected (24). Additionally, there were no deaths among the male or female B6C3F1 mice consuming FB1 for 28 days. The body weights and survival rates of the male B6C3F1 mice in our 2-year study were not affected by the FB1 (Figures 5 and 6), suggesting that the MTD for FB1 in a chronic study is > 150 ppm. Although the body weights of the female mice were unaffected by doses of FB1 as high as 80 ppm, their survival rates decreased beginning after approximately 1 year of consuming diets containing FB1 (Figure 6). The cause of death in the mice consuming 80 ppm FB1 was primary liver cancer (data not shown). Therefore, the MTD for FB1 in female B6C3F1 mice seems to be between 50 and 80 ppm FB1, because of the appearance of hepatocarcinogenicity at 80 ppm.

The body weights of the male and female B6C3F1 mice in the 2-year study were low when compared to the historical body weights of B6C3F1 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 FB1 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 FB1 (data not shown), but the free flow of the powdered feed in the mouse feeders apparently was restricted, which means 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 B6C3F1 mice and approximately 20% for the female mice at 52 weeks in this study, compared to ad libitum studies with other B6C3F1/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 B6C3F1 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 B6C3F1 mice to 60% of ad libitum reduced liver tumors from 55 to 12% of the year of consuming diets containing FB1 (Figure 6). The cause of death in the mice consuming 80 ppm FB1 was primary liver cancer (data not shown). Therefore, the MTD for FB1 in female B6C3F1 mice seems to be between 50 and 80 ppm FB1, because of the appearance of hepatocarcinogenicity at 80 ppm.

Further studies with the MRC 826 fungal isolate led to the discovery of 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 FB1. Although transient increases in esophageal epithelium labeling index have been reported following gavage administration of FB1 to rats (37), other reports have indicated a lack of effect of FB1 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
fumonisins. Whereas  \textit{N}-nitrosamines are DNA alkylators, FB\textsubscript{1} is a nongenotoxic compound. We have reported that the incubation of methanolic extracts of \textit{Fusarium} cultures with DNA in the presence of rat liver S9 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 \textit{Fusarium} fungi might alkylate DNA and participate in the induction of \textit{Fusarium}-induced rodent esophageal dysplasia and forestomach tumors. Further research is required to establish whether FB\textsubscript{1} has a role in the development of esophageal cancer in humans.

**REFERENCES AND NOTES**

1. Bezuidenhout SC, Gelderblom WCA, Gorst-Allman CP, Horak RM, Marasas WFO, Spittle G, Vleggaar R. Structure elucidation of the fumonisins, mycotoxins from \textit{Fusarium moniliforme}. J Chem Soc Chem Commun 1988;743–745 (1988).

2. Plattner RD, van der Greef J, Schreiber G, Janssen HE, Hoefsloot LH, van der Weerd J, Bobbert A, Meuwly M. A new fumonisin from liquid cultures of \textit{Fusarium moniliforme}. Mycopathologia 117:23–29 (1992).

3. Scott PM. Fumonisins. Int J Food Microbiol 18:257–270 (1993).

4. Branham BE, Plattner RD. Isolation and characterization of a new fumonisin from solid cultures of \textit{Fusarium moniliforme}. J Nat Prod 56:1630–1633 (1993).

5. Dutton MF. Fumonisins, mycotoxins of increasing importance: their nature and their effects. Pharmacol Ther 70:137–161 (1997).

6. Nelson PE. Fumonisins—Mycotoxins produced by \textit{Fusarium moniliforme}. Appl Environ Microbiol 54:1806–1811 (1988).

7. Thiel PG, Marasas WFO, Sydenham EW, Shepherd GS, Gelderblom WCA, Nieuwenhuis JJ. Survey of fumonisin production by \textit{Fusarium species}. Appl Environ Microbiol 57:1090–1093 (1991).

8. Nelson PE. Taxonomy and biology of \textit{Fusarium moniliforme}. Mycopathologia 117:29–36 (1992).

9. Norred WP. Fumonisins—Acytostosins produced by \textit{Fusarium moniliforme}. J Toxicol Environ Health 28:309–328 (1989).

10. Bullerman LB, Tsai WT. J. Incidence and level of \textit{Fusarium moniliforme}, Fusarium proliferatum and fumonisins in corn. J Food Prot 57:541–547 (1994).

11. Meinekes MCA, Cornea B, Freshman D, Gambaie W, Paulus CR, Chacon-Reche NG, Pozzi CR. Mycotoxins of the toxic feeds associated with equine leucoencephalomalacia (ELEM) outbreaks in Brazil. Mycopathology 107:165–188 (1994).

12. Abbas HK, Scamb CM, Xie WP, Mirsho CM, Shen WT. First report of fumonisin B\textsubscript{1} and B\textsubscript{2} produced by \textit{Fusarium oxysporum var. redolens}. Plant Dis 79:968 (1995).

13. Abbas HK, Scamb CM. First report of production of fumonisin B\textsubscript{1} by \textit{Fusarium polyphialidium} collected from seeds of \textit{Punica granatum}. Plant Dis 79:962 (1995).

14. Rose EF, McGlashan ND. The spatial distribution of esophageal carcinoma in the Transkei, South Africa. Br J Cancer 31:197–206 (1975).

15. Rose EF. Esophageal cancer in Transkei—the pattern and associated risk factors. In: Cancer of the Esophagus (Pfiffer CJ, ed). Boca Raton, FL:CRC Press, 1982;19–28.

16. Marasas WFO, Jadwitzek K, Venter FS, van Schalkwyk DJ. \textit{Fusarium moniliforme} contamination of maize in esophageal cancer areas in Transkei. S. African Med. J 74:110–114 (1998).

17. Rhodep JF, Marasas WFO, Thiel PG, Sydenham EW, Shepherd GS, van Schalkwyk DJ. \textit{Fusarium moniliforme} and fumonisins in corn in relation to esophageal cancer in Transkei. Phytopathology 82:353–357 (1992).

18. Kriek NPJ, Jaskiewicz K, Marasas WFO. A comparative study of the toxicity of \textit{Fusarium} verticilloides (\textit{F. moniliforme}) to horses, primates, sheep and rats. Understootsept: J Vet Res 48:129–133 (1981).

19. Kriek NPJ, Marasas WFO, Thiel PG. Hepato- and cardiotoxicity of \textit{Fusarium verticilloides} (\textit{F. moniliforme}) isolates from southern African maize. Food Cosmet Toxicol 18:447–456 (1981).

20. Jaskiewicz K, van Rensburg SJ, Marasas WFO, Gelderblom WC. Carcinogenicity of \textit{Fusarium moniliforme} culture material in rats. J Nut Cancer Test 78:321–325 (1987).

21. Marasas WFO, Kriek NPJ, Fincham JE, van Rensburg SJ. Primary liver cancer and esophageal basal cell hyperplasia in rats caused by \textit{Fusarium moniliforme}. Int J Cancer 34:383–387 (1984).

22. Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, Kriek NPJ. Fumonisins—Novel mycotoxins with cancer-promoting activity produced by \textit{Fusarium moniliforme}. Appl Environ Microbiol 54:1806–1811 (1988).

23. Gelderblom WCA, Kriek NPJ, Marasas WFO, Thiel PG. Toxicity and carcinogenicity of the \textit{Fusarium moniliforme} metabolite, fumonisin B\textsubscript{1}, in rats. Carcinogenesis 12:247–251 (1991).

24. NTP. Toxicology and Carcinogenesis Studies on Fumonisin B\textsubscript{1} in B6C3F\textsubscript{1} mice and Fischer 344 rats. Fund Appl Toxicol 24:102–110 (1995).

25. Voss KA, Chamberlain WJ, Bacon CW, Norred WP. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B\textsubscript{1}. Natural Toxins 1:222–228 (1993).

26. Voss KA, Chamberlain WJ, Bacon CW, Norred WP. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin B\textsubscript{1}. Natural Toxins 1:222–228 (1993).

27. NTP. Specification for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological, and Physical Agents in Laboratory Animals for the National Toxicology Program. Research Triangle Park, NC:National Toxicology Program, 1994.

28. U.S. FDA. Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II. Washington, DC:U.S. Food and Drug Administration, 1992.

29. Porter CJ, Hedges JC, Hoel DG. Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program’s carcinogenicity experiments. Cancer Res 46:4372–4378 (1986).

30. Baker AJ, Porter CJ. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 44:417–431 (1988).

31. Gelderblom WC, Smuts CM, Abel S, Snyman SD, van der Westhuizen L, Huber WW, Swanevelder S. Effect of fumonisin B\textsubscript{1} on the levels and fatty acid decomposition of selected lipids in rat liver in vivo. Food Chem Toxicol 35:647–656 (1997).

32. Short BG, Burnett VL, Swenberg JA. Elevated proliferation of proximal tubule cells and localization of accumulated \textit{oxytetracycline} in \textit{F. moniliforme} rats during chronic exposure to unlabeled gasoline and 2,2,4-trimethylpentane. Toxicol Appl Pharmacol 101:414–431 (1989).

33. Dietrich DR, Swenberg JA. The presence of \textit{oxytetracycline} is necessary for \textit{d-limonene} promotion of male rat kidney tumors. Cancer Res 51:3521–3525 (1991).

34. Sheldon WG, Buz T, Hart RW, Turman T. Age-related neoplasia in a lifetime study of ad libitum-fed and food-restricted B6C3F\textsubscript{1}, mice. Toxicol Pathol 23:438–476 (1995).

35. Leakey IA, Seng JE, Ramos CR, Baker VM, Hart RW. A mechanistic basis for the beneficial effects of caloric restriction on longevity and disease: consequences for the interpretation of rodent toxicity studies. Int J Toxicol 17:5–56 (1998).

36. Haseman JK. National Toxicology Program experience with dietary restriction: does the manner in which reduced body weight is achieved affect tumor incidence? Int J Toxicol 17:123–134 (1998).

37. Lim CW, Parker HM, Vosendorf RF, Hascheck WM. Intravenous fumonisin \textit{B}\textsubscript{1} induces cell proliferation and apoptosis in the rat. Nat Toxins 4:34–41 (1996).

38. Summary of the Carcinogenic Potency Database by Target Organ. Available: [http://patcent.db.de/pathtest/pathology.table.html](http://patcent.db.de/pathtest/pathology.table.html) [cited 1 August 2000].

39. Bever RJ Jr, Couch LH, Sutherland JT, Williams AJ, Beger RD, Churchill MI, Doerge DR, Howard PC. DNA adduct formation by \textit{Fusarium} culture extracts: lack of role of fumacin C. Chem-Biol Interact 128:141–157 (2000).