Fumonisin Toxicosis in Swine: An Overview of Porcine Pulmonary Edema and Current Perspectives

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Fumonisin toxicosis in swine was named porcine pulmonary edema (PPE) after outbreaks of a fatal disease in pigs fed *Fusarium verticillioides* (*F. moniliforme*)-contaminated corn screenings from the 1989 corn crop in Iowa, Illinois, and Georgia. Pigs that died had severe pulmonary edema, which has not been identified in other species after exposure to fumonisins. The disease has been reproduced experimentally by feeding of naturally contaminated corn, *F. verticillioides* culture material, and by intravenous administration of fumonisin B_1 (FB_1). Hepatic lesions consisting of apoptosis, necrosis, and hepatocyte proliferation also are observed. As in other species, alterations in clinical pathology reflect hepatic injury as well as elevated serum cholesterol concentration. In chronic studies, esophageal plaques, hyperplastic hepatic nodules, and right ventricular hypertrophy were found. In pigs, as in other species, fumonisin alters sphingolipid biosynthesis, with the greatest alterations in sphingosine and sphinganine concentrations in kidney, liver, lung, and heart. Our recent studies on fumonisin toxicosis in pigs have focused on immune effects and the pathogenesis of pulmonary edema. The specific immune system was not affected; however, FB_1 inhibited phagocytosis and sphingolipid biosynthesis in pulmonary macrophages. Fumonisin induced an accumulation of membranous material in pulmonary capillary endothelial cells; this change appears specific to this cell type and to swine. In short-term cardiovascular studies, fumonisin decreased left ventricular dP/dt_{max} (an index of cardiac contractility), mean systemic arterial pressure, heart rate, and cardiac output, and increased mean pulmonary artery pressure and pulmonary artery wedge pressure. These changes are compatible with the inhibition of L-type calcium channels by increased sphingosine and/or sphinganine concentration. Therefore, fumonisin-induced pulmonary edema in swine appears to result from acute left-sided heart failure mediated by altered sphingolipid biosynthesis. *Key words*: cardiovascular, fumonisin, immune system, liver, pulmonary edema, sphinganine, sphingosine, swine. — *Environ Health Perspect* 109(suppl 2):251–257 (2001).
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Fumonisins—mycotoxins produced by several Fusarium species, especially *Fusarium verticillioides* (*F. moniliforme*) and *proliferatum*—have been reported in animal foods and corn-based human foods from around the world (*1,2*). Fumonisins alter sphingolipid biosynthesis, induce hepatotoxicity, and elevate serum cholesterol concentration in all species studied. Other effects are species specific: pulmonary edema and cardiovascular changes in pigs, leukoencephalomalacia in horses, and renal injury in sheep, rabbits, and rats (*3–10*). Mycotoxin-induced pulmonary edema was first documented in swine in 1981 after they were exposed experimentally to corn contaminated by *F. verticillioides* (*11*). In the midwestern and southeastern parts of the United States, thousands of pigs died from pulmonary edema after ingesting corn contaminated by fumonisins from the 1989 corn crop. Thus, the disease was designated porcine pulmonary edema (PPE). Abortions also were reported in some affected herds (*12*). This outbreak was caused by high concentrations of fumonisins present in corn following a hot dry summer. Corn screenings obtained from Georgia and Iowa farms with outbreaks of fatal pulmonary edema contained fumonisin (F)B_1 at 155 and 92 parts per million (ppm), respectively, and, when experimentally fed to pigs, produced severe pulmonary edema and hydrothorax within 7 days, as well as liver damage (*4,12,13*). A recent report suggests that this disease has been recognized since the 1950s in Hungary, where it is known as "fattening or unique pulmonary edema of pigs" (*14*). Pulmonary edema has been reproduced following experimental exposure of swine to fumonisin-containing culture material and purified FB_1 given orally or intravenously (*4,15,16*).

Abnormal climatic conditions in the U.S. Midwest were responsible for the very high levels of fumonisins in the 1989 corn crop. Since that time, the overall levels of fumonisins in corn have been lower. In a U.S. Department of Agriculture (USDA) study of the 1995 Midwest corn crop (preharvest samples), 7% of the crop contained > 5 ppm FB_1, 40% 0.5–5 ppm, and 54% < 0.5 ppm (*17*). The highest concentration of FB_1 was 24 ppm and the average 1.5 ppm. In a survey carried out from 1996 to 1999 in south Georgia, up to 91% of the samples contained fumonisins; 30 % of samples had concentrations > 1 ppm, and the highest concentration was 33 ppm (*18*). A World Health Organization working group found that globally 59% of corn and corn product samples were contaminated by FB_1 (*19*).

Whereas initial studies focused on reproduction of the spontaneous disease and its characterization, subsequent research has addressed the pathogenesis of pulmonary edema and effects of longer-term ingestion of low doses of fumonisin. Physiologically, the two main causes of pulmonary edema are left ventricular failure, which increases pulmonary capillary hydrostatic pressure, and increased vascular permeability after injury to the alveolar capillary endothelium or alveolar epithelium. Structural changes may be absent in either case. The pathogenesis of fumonisin-induced pulmonary edema in swine must account for the species specificity of the lesion because pulmonary edema has been documented only in swine. In addition, the role of altered sphingolipid biosynthesis must be considered. Understanding the pathogenesis of pulmonary edema is important in both preventing and treating PPE as well as in understanding the mechanism of fumonisins' species-specific effects. Perhaps more important than PPE, which occurs only at high-level exposure, are the effects of long-term exposure to low concentrations of fumonisins in the diet, because this occurs more frequently and involves larger numbers of animals. Mycotoxins commonly are immunosuppressive, and an association between

fumonisin contamination and infectious disease in swine has been reported (*20*).

Because swine are excellent models for human cardiovascular and other diseases, the mechanism for fumonisin toxicosis in swine must be characterized to permit assessment of its potential toxicity in human populations. This assessment includes characterizing the hepatic, cardiovascular, and hypercholesterolemic effects of fumonisin ingestion in swine as well as interaction with other agents, both infectious and toxic. In humans, epidemiologic association of high-level fumonisin exposure with esophageal cancer (*21*) is well known and has recently been described for neural-tube defects linked to fumonisin-induced folate deficiency (*22*).

Pharmacokinetics of FB_1 and Residue Localization

Because availability of purified FB_1 is limited, the most extensive studies have been performed in rodents. In rats, fumonisins are absorbed poorly and excreted rapidly (apparently as parent compound) by the liver and to a much lesser degree through the kidney (*23–26*). The pharmacokinetics of $[^{14}C]FB_1$ in swine are as follows:

- Half-life: α, 2 min; β, 10 min; γ, 182 min
- Volume of distribution: 2.4 L/kg (widely distributed)
- Plasma clearance: 9.1 mL/min/kg (moderate rate)
- Absorption: 3–6% (poor bioavailability)
- Excretion (recovery at 72 hr)—mainly fecal (60%), urine (20%), total (80%)—biliary excretion with enterohepatic recycling
- Distribution (specific activity at 72 hr): liver >> kidney > large intestine > brain > lung, heart, adrenal gland, spleen (*27,28*).

In swine, plasma radioactivity declined in a triexponential manner following intravenous administration of $[^{14}C]FB_1$ with a large volume of distribution (2.4 L/kg) and moderately rapid plasma clearance (9.1 mL/min/kg) (*27*). Only trace levels of radioactivity remained in plasma after 3 days. As in rats, FB_1 was eliminated primarily in the feces of swine (58% recovery) and to a much lesser extent in the urine (21%). Fumonisin tissue residues 72 hr after administration were greatest in liver >> kidney > large intestine > brain > lung, heart, and adrenal gland. In bile-cannulated pigs, bile recovery was 71% and the γ phase was absent, indicating enterohepatic recirculation of the toxin. When FB_1 was given intragastrically, bioavailability was 3–6%. After 3 days, the total remaining tissue residues were 10- to 20-fold lower than after the intravenous dose (*27*). Fumonisin residues accumulated in liver and kidney when pigs were fed $[^{14}C]FB_1$ at dietary concentrations of 2–3 ppm for 24 days (*28*). When this was

followed by clean food, radioactivity decreased rapidly to 35% of peak values after 3 days, with only trace levels after 9 days. Interestingly, fumonisin excretion was < 35% of intake during the first 3 days of feeding, but then increased to 70–85% of the daily intake for the remainder of the trial. Therefore, FB_1 has very poor oral bioavailability in swine, although the fraction that is absorbed undergoes wide distribution and has specific affinity for the liver and to a lesser extent the kidney. Furthermore, the half-life of absorbed fumonisins is relatively long because of enterohepatic recirculation and to a lesser extent the uptake and slow release of the toxin by the liver and kidney. Therefore, diets containing FB_1 that are consumed over extended periods may result in the accumulation of potentially toxic residues in liver and kidney. Pigs exposed to dietary FB_1 at 2–3 ppm would require a withdrawal period of at least 2 weeks to ensure that only minimal residue levels remained (*28*).

Overview of Fumonisin Toxicosis

Fumonisin toxicosis in swine is characterized by injury to the pulmonary, hepatic, cardiovascular, and immune systems as well as early and widespread alteration of sphingolipid metabolism and effects on growth rate and carcass composition. Pigs develop lethal pulmonary edema within 4–7 days when fed FB_1 containing feed or culture material at concentrations of $\geq$ 92 ppm or $\geq$ 16 mg/kg body weight/day (*4,15,16,29,30*). Similarly, lethal pulmonary edema occurs within 7 days after daily intravenous administration of FB_1 (*4*). In many studies using feed or culture material, only the concentration of FB_1 is given. However, generally FB_1 and FB_2 are produced together, with FB_2 representing 15–35% of the amount of FB_1 (*31*). In rats, similar effects were produced by FB_1, FB_2, and FB_3 in culture material, with severity of response in the order $FB_1 > FB_2 > FB_3$ (*32*). We recommend that equal consideration be given to FB_1, FB_2, and FB_3 for risk assessment and management purposes until data to the contrary are obtained (*32*). Therefore, the presence of FB_1, FB_2, and FB_3 should be taken into account in any toxicity calculations.

In contrast to most studies in which pulmonary edema is observed in pigs only during the first week of high-dose FB_1 ingestion, pulmonary edema of increasing severity was identified by computer tomographic and pathologic examination in weanling pigs fed lower doses of FB_1 as culture material (10–40 ppm) for 4 weeks (*33*). However, pigs did not show any clinical signs of toxicity. Pulmonary edema also was observed in piglets from two sows fed 300 ppm FB_1 as culture material for 14 days,

from day 107 of gestation to 7 days after parturition (*34*).

Clinically, inactivity, increased respiratory rate, and decreased heart rate occur approximately 12 hr before the development of pulmonary edema and death (*35*). Respiratory distress, manifested as increased respiratory rate and effort with abdominal and open-mouth breathing, occurs within hours of death; vomiting and diarrhea also have been observed (*15,16*). At necropsy, pulmonary edema is present and can be quantitated as increased lung wet/dry weight ratio. Although cardiovascular abnormalities were not detected using electrocardiographic and pathologic evaluation (*15,29*), we recently identified hemodynamic changes indicating cardiovascular injury that precede the onset of pulmonary edema and presumably are causative (*9,10,35–37*).

Pigs ingesting fumonisin develop hepatic injury that is dose- and time-dependent and can be detected as elevated hepatic serum enzyme activity and morphologic alteration (*15,16,29,30*). Serum biochemical changes indicate hepatic necrosis, cholestasis, and decreased hepatic function. Clinically, pigs with severe liver disease become anorexic and icteric, may lose weight, show signs of encephalopathy, and become moribund (*12,15*). The earliest and most specific biochemical change is an increase in serum and tissue sphingoid bases (sphingosine and sphinganine) that is time- and dose-dependent (*16,35,38*). This is caused by the inhibition of ceramide synthase by fumonisin that inhibits sphingolipid biosynthesis with resultant increases in sphingoid base concentrations in tissues and serum (*39*).

Pigs fed 100–193 ppm FB_1 in culture material for at least 93 days developed hepatic nodular hyperplasia (2/3 studies), hyperplastic esophageal plaques (1/3 studies), gastric ulceration, hypertrophy of the heart and medial hypertrophy of the pulmonary arteries (1/3 studies) (*6,40,41*). The cardiovascular changes occurred in four pigs receiving 150–170 ppm FB_1 from culture material in the diet for up to 217 days (*6*). Histopathologic alterations were not observed in the hearts. The cardiovascular changes suggest pulmonary hypertension, although pulmonary hypertension has been documented only in short-term, high-dose studies (*9,10,35,37*).

Variable effects of fumonisins on food consumption and feed efficiency have been reported. Although decreased food consumption often is seen with naturally contaminated corn, presumably due to palatability problems (*29,30*), observations with FB_1, either as pure compound or in culture material, have been variable; no effect as well as increased and decreased feed consumption and weight gain have been reported (*28,42–44*). Decreased

feed efficiency may be caused by increased oxygen consumption (*10,35*). Increased variability of carcass quality was reported with ingestion of FB_1 at 1 ppm (*44*).

Clinical Pathology

Alterations in hepatic serum biochemical parameters, including serum bile acids, bilirubin concentration, and aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase, and arginase activities reflect toxicity to the liver that is time- and dose-dependent (*9,10,15, 16,29,30,40*). Serum hepatic biochemical alterations were observed with FB_1 and FB_2 as low as 12 ppm in the diet (*30*). Increased serum cholesterol concentration is a feature of fumonisin toxicity and has been reported at ≥ 1 ppm FB_1 (*15,16,29,30,40,43,44*). Slight increases were reported after 11 weeks in nonfasted (but not in fasted) pigs at a dietary concentration of 1 ppm FB_1 (*44*); however, the same research group fed pigs a ration containing 10 ppm, and after 8 weeks the mean cholesterol values in both sexes were less than those of controls (*43*). Hypercholesterolemia appears to be the most sensitive serum biochemical parameter to fumonisin exposure. Hematologic profiles remain within the normal range in fumonisin-treated pigs (*30,43*).

Sphingolipid Alterations

Fumonisins and fumonisin analogs inhibit sphinganine (sphingosine) *N*-acyltransferase (ceramide synthase), an enzyme of critical importance in sphingolipid biosynthesis, increasing the concentration of sphingoid bases (sphinganine and sphingosine) and depleting complex sphingolipids (*39,45*). Because the alteration in cellular concentrations of these molecules is proposed as the mechanism of fumonisin toxicosis (*46,47*), it has been important to document the organ specificity, dose response, and temporal changes in these compounds and to relate these changes to the effects of fumonisins in cells and tissues.

Fumonisin exposure in swine causes a dose- and time-dependent elevation in the concentration of free sphinganine, and to a lesser extent sphingosine, with a corresponding increase in the sphinganine-to-sphingosine ratio, in serum and tissues (*16,35,38*). Increased sphinganine-to-sphingosine ratios were present in pig tissues (lung, liver, and kidney) at ≥ 23 (but not 5) ppm and in serum at ≥ 5 ppm total fumonisins from naturally contaminated corn after a 14-day exposure (*38*). Similar increases in sphinganine and sphingosine concentrations were induced with purified toxin in tissues and serum of pigs fed FB_1 at 1.5 mg/kg/day for 5 days (*42*) and in tissues at 10 (but not ≤ 1) ppm for 8 weeks (*43*). In a temporal study, the concentration of sphingoid bases increased in pig tissues after only one day of feeding culture material containing FB_1 at 20 mg/kg body weight and before appearance of histologic changes in liver and lung, which were detectable at day 2 [Figure 1 (*16*)]. Sphinganine levels plateaued in virtually all organs, as well as serum, by day 2 or 3 while sphingosine continued to increase progressively. Depletion of sphingosine containing complex sphingolipids with an increase in sphinganine containing complex sphingolipids in the liver has been reported (*38*). Similar alterations in sphingosine and sphinganine concentrations have been observed in other species (*8,48,49*) but not with any other toxicant class.

Based on pharmacokinetic studies, there is some correlation between the magnitude of sphingolipid alterations in tissues and the distribution of FB_1, which is greatest in liver and kidney (*27*). Changes in the sphinganine-to-sphingosine ratio in short-term swine studies generally have been in the following order: kidney, liver > heart > lung >> other tissues such as lymph node and skeletal muscle (*16,35,38,42*); however, in a low-dose, longer-term study the magnitude was lung >> kidney, liver (*43*). In general, the kidney appears to be the most sensitive organ to fumonisin-induced sphingolipid alterations in all species examined, regardless of its sensitivity to morphologic and functional damage in that species (*38,48,49*). Unlike in rabbits, sheep, and rats, where kidney is the primary target organ (*5,7,8*), in swine, renal injury has been reported inconsistently (*14,15*). This may relate to cell-specific functions of sphingolipids in the same organ in different species. Sphingoid bases increased in serum earlier (within 24 hr) (*16*) and at lower doses of fumonisin (≥ 5 ppm) (*38*) than serum liver enzymes and before morphologic tissue alterations, suggesting that serum concentrations of sphingoid bases could be used as a sensitive biomarker of fumonisin exposure. The role of altered sphingolipid biosynthesis in the pathogenesis of fumonisin toxicity currently is under investigation.

Pulmonary Pathology and *in Vitro* Studies

Pigs that die in respiratory distress have pulmonary edema characterized grossly by heavy, wet lungs with widened interlobular septa as well as fluid in the airways and the thoracic cavity; this fluid is a modified transudate (*4,16,29,30*). Histologically, the major alteration is the presence of clear fluid and markedly dilated lymphatics in connective tissue around vessels and bronchi, subpleurally, and in interlobular septa (Figure 2). In more severe cases, protein may be present in the edema fluid and this fluid may be observed in alveoli and even in airways. Inflammation is not a feature of the disease.

Ultrastructurally, alterations that indicate permeability changes have not been observed in the pulmonary vasculature. However, accumulation of membranous material (multilamellar bodies) was found within pulmonary intravascular macrophages in pigs with pulmonary edema (*15,29*). In a more detailed study where lung was fixed intravascularly, the primary site of these membranous accumulations was identified as the pulmonary capillary endothelial cell; this alteration was observed as early as 2 days after initiation of exposure (*16*). Occasionally, affected endothelial cells were electron dense and shrunken, i.e., undergoing apoptosis. Phagocytosis of these dying endothelial cells by pulmonary intravascular macrophages resulted in the membranous material being localized within phagosomes of these cells. This membranous material may be the result of sphingoid base accumulation with damage to the cytocavitary network. This membranous material was observed in pigs given
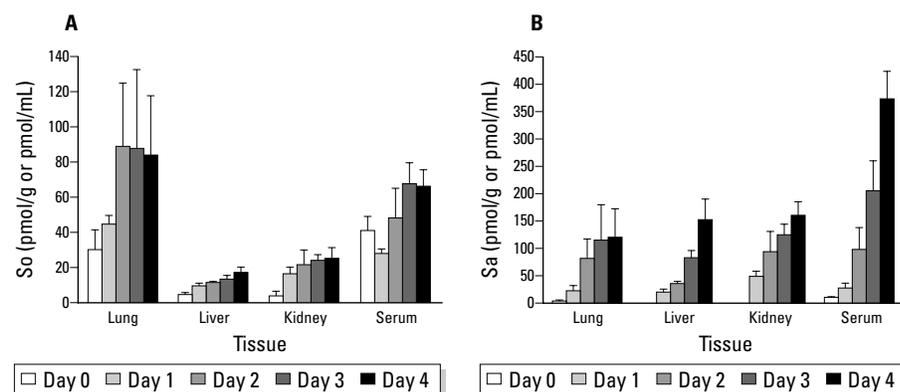
Figure 1. Sphingosine (*A*) and sphinganine (*B*) concentrations in tissues and serum of pigs fed fumonisin-containing culture material at a dose of 20 mg FB_1/kg/day (*16*). Sphinganine increases over time reaching a plateau or declining on the last day, whereas the increase in sphingosine is progressive and continual. Bars represent the mean value (error bars = SD); $n = 4$–6 pigs.
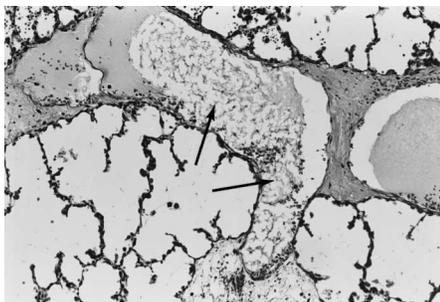
Figure 2. Lung from a pig that died from porcine pulmonary edema after ingesting corn naturally contaminated with fumonisins. Edema is characterized by massive dilation of lymphatics within the interlobular septa (arrows). In control lung, lymphatics are barely discernible. Hematoxylin and eosin × 100.

purified FB_1 intravenously (*50*) and in pigs fed fumonisin containing culture material at doses that did not induce pulmonary edema (*15,50*), indicating that these alterations were induced by FB_1 but did not necessarily cause pulmonary edema. Similar capillary endothelial changes were not observed in any other species (*50*) nor have they been reported for any other toxin; therefore this alteration appears specific for fumonisin exposure in swine. Following chronic exposure to fumonisins, medial pulmonary arterial hypertrophy indicative of hypertension has been observed (*6*).

The effects of fumonisins on pulmonary endothelial cells have been examined in *in vitro* studies. Increased albumin permeability occurred in cultured porcine pulmonary artery endothelial cells within 12 hr of exposure to 30 or 50 mM FB_1 (*51*), doses much higher than expected in plasma of pigs dying from pulmonary edema. Cell viability appeared to decrease proportionately to the increase in albumin permeability, suggesting that cell death was responsible for the increased permeability. The cell cultures incubated with FB_1 at 50 mM had elevated sphinganine concentrations by 3 hr and an elevated sphinganine-to-sphingosine ratio by 24 hr (*51*). Neither toxicity nor ultrastructural changes were observed when we incubated porcine pulmonary endothelial cells with FB_1 at doses within the range anticipated for *in vivo* exposure, 5–20 µg/mL for 10 or 7 days, respectively (*52*).

Cardiovascular Toxicity

In early acute studies of fumonisin exposure, the cardiovascular system was examined using morphology and electrocardiography because pulmonary edema frequently is secondary to cardiovascular damage (*15,29*). Because alterations were not observed in these studies, the focus of subsequent studies examining the pathogenesis of pulmonary edema shifted to the lung. However, right ventricular

hypertrophy and medial hypertrophy of small pulmonary arteries, suggesting pulmonary hypertension, were observed in a chronic study where pigs were fed 100–190 ppm FB_1 for up to 210 days (*6*). Cardiovascular changes were not observed in a similar study lasting 93 days (*40*).

The chronic cardiovascular findings stimulated more detailed examination of the potential effects of fumonisins on cardiovascular function, using pigs instrumented to facilitate hemodynamic measurements (*9,10,35–37,53*). Pigs fed culture material containing fumonisins at ≤ 20 mg/kg body weight for 3–7 days had increased mean pulmonary arterial pressure and decreased heart rate, cardiac output, and mixed venous oxygen tension (*9,10,35,37*). In addition, treated pigs had decreased cardiac contractility as assessed by left ventricular dP/dt_{max} (*10*) and end-systolic elastance (*36,37*), the gold standard *in vivo* measure of cardiac contractility. Plasma sphinganine and sphingosine concentrations were increased by 24 hr and continued to increase over time; sphinganine and sphingosine also markedly increased in cardiac muscle (*35*). Intravenous administration of purified FB_1 to swine similarly decreased cardiovascular function; however, the permeability of the alveolar-capillary membrane, as measured by Evans blue dye clearance, was not altered (*35*). Fumonisin ingestion also directly relaxed systemic arteries, leading to systemic arterial hypotension (*53*). Therefore, FB_1 is a negative inotropic and chronotropic agent that also relaxes vascular smooth muscle, leading to decreased cardiovascular reserve in pigs. Decreased cardiac contractility would appear responsible for increasing pulmonary artery wedge pressure (an index of pulmonary capillary pressure) and subsequent pulmonary edema.

Hepatic Pathology

Fumonisins cause hepatic injury in all species regardless of the route of administration. In swine, morphologic alterations were observed following ingestion (≥ 23 ppm) (*30*) or intravenous administration (*4,12,29*). Acute hepatic changes include disorganization of hepatic cords, hepatocellular vacuolation, megalocytosis, apoptosis, necrosis, and cell proliferation [Figure 3 (*4,12,15,16,29,30*)]. These changes appear to be most marked in centrilobular and midzonal regions (*16*). Ultrastructurally, accumulations of membranous material (multilamellar bodies) similar to those found in the lung have been reported within hepatocytes and Kupffer cells (*29*).

In pigs with clinical manifestation of liver failure, similar but more severe liver changes were observed (*15*). Liver damage was reversible following removal of the toxin in a small group of pigs (*15*). In chronic

feeding studies—100–193 ppm FB_1 in culture material for a minimum of 93 days—hepatic necrosis (without bile duct proliferation), fibrosis, and nodular hyperplasia were observed (*6,40,41*).

Other Organ-Specific Alterations

Miscellaneous and inconsistent findings reported include pancreatic acinar cell apoptosis and necrosis (*4,12,29,30*) with membranous intracytoplasmic material (multilamellar bodies) similar to that found in the pulmonary capillary endothelial cells (*29*). Histologic evidence of renal tubular injury (*14,15*) has been inconsistently reported and could be secondary to severe hepatic injury (bile nephrosis). More extensive evaluation of renal function, including clearance studies, indicates that direct fumonisin-induced nephrotoxicity does not occur (*35*). Encephalomalacia in one of two pigs that developed fatal pulmonary edema was reported in one study using culture material (*14*). Hyperplasia of the basal cell layer of the distal esophageal mucosa was reported in 6/6 pigs and hyperplastic plaques in 1/6 pigs fed 100–193 ppm FB_1 in culture material for 93 days (*40*). In the same study, gastric ulceration was present in 6/6 pigs. Esophageal changes are of interest because of the epidemiologic association between ingestion of FB_1 and esophageal cancer in humans (*18*).

Immunotoxicity

Although the immunosuppressive effects of mycotoxins such as T-2 toxin and aflatoxin are well known (*54,55*), only limited information is available regarding the effects of fumonisins on the immune system, especially in swine. In mice, fumonisins can be either immuno-stimulatory or immuno-suppressive depending on the dose of FB_1 and period of exposure (*56*). In swine, short-term ingestion of fumonisin (up to 3 weeks) did not affect specific immunity (*12,57*) but longer-term ingestion (4–8 weeks) produced an adverse effect (*40,58,59*). We fed pigs 2 mg FB_1/kg/day as culture material for 5 weeks and then vaccinated them with a killed pseudorabies virus vaccine (PRV/Marker Gold-KV; SyntroVet, Inc., Lenexa, KS, USA). There were no significant changes in the PrV stimulation index, response to phytohemagglutinin, or PrV antibody titers, in total lymphocyte numbers, nor in numbers of $CD3^+$, $CD4^+$, $CD8^+$, or $CD4^+/8^+$ subpopulations (*60*). Therefore, fumonisin did not affect either the cell-mediated or humoral immune responses in our study.

We also examined the effect of fumonisins on nonspecific immunity. The accumulation of membranous material in pulmonary intravascular macrophages and Kupffer cells,
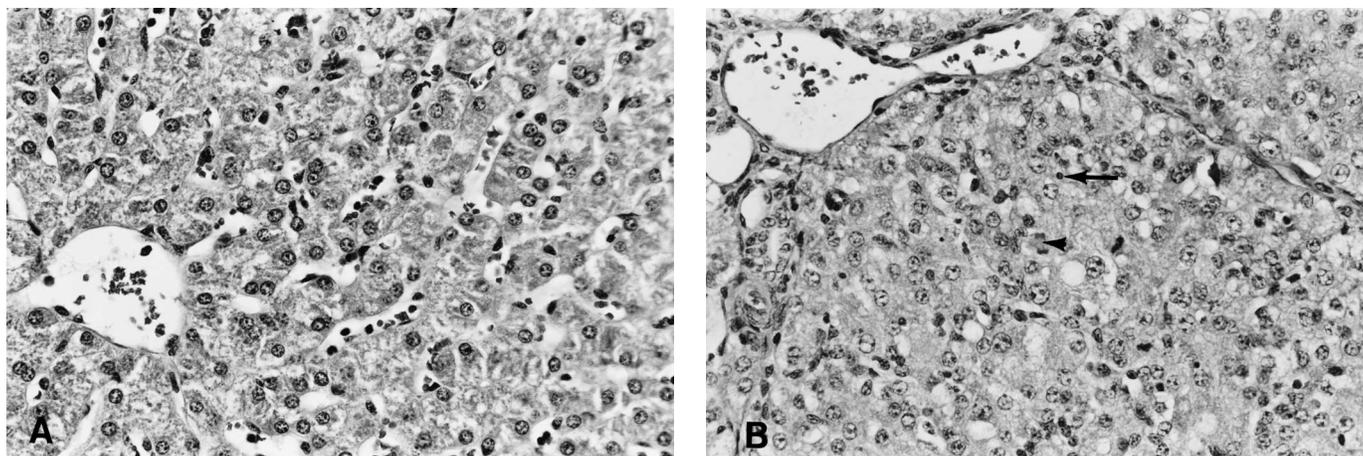
Figure 3. Liver from a control (*A*) pig and a pig that died from porcine pulmonary edema (*B*) after ingesting corn naturally contaminated with fumonisins. In control liver, the central vein (lower left) is surrounded by hepatocytes arranged in cords separated by sinusoids. In the treated pig, the hepatic cords are disorganized, and hepatocytes contain vacuoles and apoptotic bodies (arrow). A hepatocyte is undergoing mitosis (arrowhead). Hematoxylin and eosin × 300.

and to a lesser extent in alveolar macrophages (*29*), suggested an effect of fumonisin on macrophage function. Pulmonary intravascular macrophages are phagocytic cells that adhere to the pulmonary capillary endothelium. In pigs as well as in ruminants, pulmonary intravascular macrophages are numerous and are the primary cells responsible for clearing particulates from the circulation, as opposed to the hepatic Kupffer cells, which are responsible for clearance in most species such as dogs, humans, and rodents (*61*). Pigs fed a sublethal dose of fumonisin from culture material for 7 days had decreased clearance of bacteria (*Pseudomonas aeruginosa*) and particulate material (copper phthalocyanine as Monastral blue) from the pulmonary circulation (*62*). Therefore, we investigated phagocytosis by alveolar macrophages using flow cytometry of fluorescein isothiocyanate (FITC)-labeled opsonized *Salmonella typhimurium*. In an *in vitro* study, we demonstrated dose-dependent fumonisin inhibition of *S. typhimurium* phagocytosis by a murine monocyte/macrophage (RAW264) cell line exposed to FB_1 at $\geq$ 25 mM for 24 hr (*63*). Similar findings were obtained in RAW cells using luminal-dependent chemiluminescence (*64*). In addition, phagocytosis of *S. typhimurium* was decreased in alveolar macrophages from pigs fed fumonisin from culture material and isolated before pulmonary edema developed (*63*). Therefore, fumonisin exposure can suppress an important surveillance system (nonspecific immunity) in pigs. Decreased ability of pulmonary intravascular macrophages to remove bacteria from the circulation and of alveolar macrophages to remove bacteria inhaled into the lung could predispose pigs to infectious diseases, both pulmonary and systemic. In fact, an association between increased risk of infectious disease and fumonisin-contaminated swine food has been reported (*17*). Additional studies are

needed to determine the clinical relevance of these findings.

Pathogenesis of Porcine Pulmonary Edema

The pathogenesis of fumonisin-induced pulmonary edema must consider the pulmonary and cardiovascular findings presented above. *In vivo* pulmonary changes relevant to the pathogenesis of fumonisin-induced pulmonary edema include histologic evidence of pulmonary interstitial edema, pulmonary capillary endothelial alterations (*16*), and the lack of effect on the permeability of pulmonary endothelium *in vivo* (*35*). The ultrastructural findings raised questions regarding the role of the endothelial changes in the development of PPE, the involvement of altered sphingolipids in the development of the endothelial lesion, and functional perturbations in the affected endothelium. The *in vitro* studies in which increased permeability was shown are considered not physiologically relevant (*51*). The rapid and dose-dependent increases in sphingoid bases observed in lung, *in vivo* and *in vitro*, and in endothelial cells *in vitro* (*16,38,42,51*) must be considered.

The ultrastructural accumulation of membranous material in porcine pulmonary capillary endothelial cells is believed to be specifically induced by FB_1 and caused by altered sphingolipid metabolism, resulting in disruption of the endoplasmic reticulum and Golgi (*16*). However, the role of these endothelial changes in the development of PPE is unclear since only a few affected cells underwent degeneration and cell death in pigs with pulmonary edema. Only a significant loss of capillary endothelial cells would lead to increased permeability and alveolar edema. Increased permeability was not observed *in vivo*, and interstitial rather than alveolar edema is the most prominent morphologic change. However, this capillary

endothelial change would cause thickening of the alveolar-capillary membrane and thus contribute to the oxygen diffusion abnormalities induced by the edema that lead to systemic arterial hypoxemia. Other functional perturbations in the affected endothelium cannot be ruled out.

The cardiovascular hemodynamic changes induced by FB_1 in swine and documented by our laboratory indicate that FB_1 is a negative inotropic agent that decreases the cardiovascular reserve of pigs (*9,10,35,37*). The decreased cardiac contractility appears responsible for increasing pulmonary artery wedge pressure (an index of pulmonary capillary pressure) and the subsequent pulmonary edema caused by left-sided heart failure. We propose that the fumonisin-induced increase in heart and/or serum sphingosine concentration inhibits the L-type calcium channels of cardiac myocytes, thereby decreasing Ca^{2+}-induced Ca^{2+} release and thus decreasing cardiac contractility.

We also observed a terminal increase in oxygen consumption (*10,35*). The cellular mechanism for the increased oxygen consumption in fumonisin-treated pigs may be due to sphingosine-mediated inhibition of L-type Ca^{2+} channels or sphingosine-1-phosphate activated Ca^{2+} release from the endoplasmic reticulum, increasing the occurrence of futile cycles. Alternatively, inhibition of protein kinase C may result in the use of energetically less efficient pathways for cellular metabolism.

Current Issues

During the USDA's National Animal Health Monitoring System (NAHMS) Swine '95 study, mycotoxins (aflatoxins, zearalenone, deoxynivalenol, and fumonisins) were detected in nearly 77% of the corn-based rations and 45% of noncorn-based rations such as milo and barley. Fumonisins are of

special concern because of their widespread occurrence, potential carcinogenicity, and species-specific effects. Although much information is available regarding the effects of fumonisins from *in vivo* and *in vitro* studies, these significant gaps in our knowledge remain to be addressed:

• Pathogenesis of pulmonary edema
• Mechanism of cardiovascular and immune effects
• Dose–response studies for cardiovascular, hepatic, and immune effects
• *In vivo* challenge studies for immunosuppression
• Reproductive effects
• Interaction with other mycotoxins/diseases
• Hypercholesterolemia: pathogenesis and potential role in atherosclerosis.

When considering the effects of fumonisins in swine, the following areas need to be addressed: swine safety and productivity, the safety of swine-derived products consumed by humans, and the potential role of fumonisins in human disease. It is well known that fumonisins at high doses can cause morbidity and mortality in pigs and horses. In the United States, the 1989 corn crop was heavily contaminated with fumonisins, and large numbers of pigs died of PPE; abortions also were reported. In 1998, the southern U.S. corn crop also was heavily contaminated with fumonisins and aflatoxins; however, outbreaks of PPE were not reported, perhaps because of a change in feeding practices. In 1993, the Mycotoxin Committee of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) recommended that the total swine diet contain less than 10 ppm FB_1 [quoted in Miller et al. (*65*)]. In addition, corn screenings, usually heavily contaminated with fumonisins, are no longer commonly used as animal feed. However, outbreaks of PPE continue to be reported sporadically from Europe. Equine leukoencephalomalacia continues to occur sporadically within the United States, presumably because horses are much more sensitive to the toxic effects of fumonisins than are swine and cattle. Of greater economic concern in swine are the low-dose effects of fumonisins, which include subclinical hepatic disease; altered carcass quality; adverse immune effects with potential predisposition to infectious diseases; and potential interactions with other toxins, including mycotoxins and other diseases. Potential reproductive effects also need further study in swine.

Mycotoxins present in animal feed can enter the human food supply as residues present in animal tissues and other animal-derived products. The primary current public health concern with fumonisins is cancer because of the induction of tumors in laboratory animals (*66*) and the epidemiologic association of fumonisins with esophageal cancer in humans (*18*). Although FB_1 has poor bioavailability in swine, the little that is absorbed undergoes extensive distribution and can remain in the body for an extended time. FB_1 and its metabolites accumulate primarily in the liver and to a lesser extent in the kidney, and accumulation appears to increase with exposure time (*27,28*). This suggests that market-weight pigs could contain residual levels even if only exposed to low dietary concentrations. However, because fumonisin residues in generally edible products are low and organ meats typically constitute a very small percentage of the diet, it is believed that pork products do not represent a public health concern when swine are exposed to fumonisins at or below the guidelines (10 ppm) set by the AAVLD (*65*). In addition to fumonisin residues in swine products, the effect of fumonisins on the swine immune system also must be considered in evaluation of fumonisins for their food safety. The potential increase in susceptibility of swine to infectious agents, such as *Salmonella* sp., which has become one of the top foodborne health concerns in the United States, is of importance. FB_1 has been shown to increase susceptibility to *Escherichia coli* infection in pigs (*67*).

Swine are physiologically similar to humans and are used widely as models for human diseases, particularly cardiovascular disease (*68*). Understanding the pathophysiology of fumonisin toxicosis in swine is important not only in the context of prevention and therapy of outbreaks of PPE, but also in understanding whether human exposure to fumonisins may have adverse chronic effects.

Low-dose, long-term studies are needed to investigate further the cardiovascular and pulmonary alterations induced by fumonisins in swine. In addition, further investigation of hypercholesterolemia and its potential role in the development of atherosclerosis is needed. FB_1 increases serum cholesterol concentration in all species examined. This is one of the earliest detectable serum biochemical change and has been reported at dietary concentrations as low as 1 ppm in swine (*44*). Moreover, long-term feeding of FB_1 as culture material to nonhuman primates (Vervet monkeys) caused hypercholesterolemia and an atherogenic plasma lipid profile (*69*). The potential contribution of dietary fumonisins to cardiovascular disease in humans must be examined given that the pig is the best model for cardiovascular disease and that hypercholesterolemia is an independent risk factor for heart disease in humans.

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