

## MICROBIOLOGICAL TOXINS

### THE INDUCTION OF NEURAL TUBE DEFECTS WITH FUMONISINS IN LM/Bc AND CD1 MICE BY THE ORAL AND INTRAPERITONEAL EXPOSURE ROUTES

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The human health effects of *Fusarium verticillioides* and fumonisins are uncertain. There is emerging evidence suggesting that fumonisins disrupt folate utilization and increase the risk of neural tube defects (NTDs = birth defects resulting from failure of the neural tube to close) in populations depending heavily on fumonisin-contaminated corn as a food source. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) was not teratogenic when given orally (gavage) to pregnant CD1 mice on gestation days (GD) 7-15 whereas intraperitoneal injection of  $\geq 5$  mg FB<sub>1</sub>/kg BWt on GD7 and GD8, the critical time for neural tube closure, to pregnant LM/Bc mice caused a dose-dependent increase in NTDs. Therefore, the incidences of NTDs in the litters of LM/Bc and CD1 dams given FB<sub>1</sub> by two different dosing regimens were compared: (a) dietary exposure provided by adding *F. verticillioides* culture material (CM=corn molded under controlled conditions with a single fungal isolate) to the diet beginning 5 weeks before mating and (b) intraperitoneal injection of FB<sub>1</sub> on GD7 and GD8. Results of the feeding studies were inconclusive. Diets containing 50 ppm FB<sub>1</sub> did not cause NTDs. At the maternally hepatotoxic dose of 150 ppm FB<sub>1</sub>, one of five LM/Bc litters was NTD positive. No NTDs were found at this dose in the CD1 litters, however, fetal death rates were higher when compared to the LM/Bc strain. Increased sphinganine to sphingosine ratios were found in the livers of LM/Bc, but not CD1, fetuses suggesting that *in utero* exposure to fumonisins was greater in the LM/Bc than in the CD1 strain. In a second study, however, no NTDs were found in the fetuses of LM/Bc dams fed diets containing 150 or 300 ppm FB<sub>1</sub>.

A dose-related increase in NTDs was found in the litters of CD1 dams given FB<sub>1</sub> by intraperitoneal injection: 0, 11, 0, and 40% of the litters were NTD positive at doses of 0, 15, 30 and 45 mg FB<sub>1</sub>/kg BWt, respectively. Susceptibility of CD1 fetuses to fumonisin-induced NTD was corroborated in a second experiment. Specifically, the defect was induced in 0, 8.3, 16.6, 36.4, and 54.5% of the litters of CD1 dams injected with 0, 10, 23, 45 or 100 mg FB<sub>1</sub>/kg BWt. In the affected litters of CD1 dams given  $\leq 45$  mg FB<sub>1</sub>/kg BWt, 33% or less of the fetuses were positive for NTD. The number of affected fetuses per NTD-positive litter tended to be higher when the CD1 dams were given 100 mg FB<sub>1</sub>/kg BWt: 15 to 100% exhibited NTD (average mean for the group = 42%). In contrast, 100% of the litters and  $\geq 50\%$  of the fetuses from LM/Bc dams given  $\geq 15$  mg FB<sub>1</sub>/kg BWt using this dosing protocol were NTD positive. These results indicate that (a) both mouse strain and dosing regimen affect NTD induction; (b) induction of NTDs by intraperitoneal FB<sub>1</sub> exposure during the critical time for neural tube closure is not unique to the inbred LM/Bc mouse strain; (c) LM/Bc mice are more sensitive to NTD induction by fumonisins; and (d) unequivocal induction of NTDs by dietary exposure to fumonisins remains to be shown. Comparative studies in fumonisin-exposed LM/Bc and CD1 mice will be a useful research tool for elucidating the physiological and biochemical events involved in NTD formation *in vivo*.

### FUMONISIN CONCENTRATION AND CERAMIDE SYNTHASE INHIBITORY ACTIVITY OF CORN, MASA, AND TORTILLA CHIPS

(K. A. Voss, W. P. Norred, F. I. Meredith, R. T. Riley, and D. S. Saunders)

Fumonisin is a mycotoxin that is found worldwide in corn and corn-based foods. Nixtamalization is the process by which corn is converted to masa for tortillas and has been shown to reduce fumonisins in cooked corn and in masa and tortilla products made from that corn. Fumonisin concentrations and potential toxicity might be underestimated in processed foods such as tortillas however if unknown, but biologically active fumonisins are present therein. Therefore, the relative amount of fumonisins in the

extracts of fumonisin-contaminated corn and its masa and tortilla chip nixtamalization products was compared using an *in vitro* ceramide synthase inhibition bioassay with increased sphinganine (Sa) and sphinganine to sphingosine ratio (Sa/So) as endpoints. African Green Monkey kidney cells (Vero cells ATCC CCL-81) were grown in 1 ml wells and exposed to 4  $\mu$ l of the concentrated extracts for 48 hr. The corn extract inhibited ceramide synthase as both Sa (mean=132 pmole/well) and Sa/So (mean=2.24) were high compared to vehicle controls (Sa=9 pmole/well; Sa/So=0.10). Inhibitory activity (mean Sa=14-24 pmole/well; mean Sa/So=0.17-0.28) of the masa and tortilla chip extracts was significantly reduced,  $\geq 80\%$ , compared to the corn extract. Results were corroborated in a second experiment in which Sa and Sa/So of the wells treated with masa or tortilla chip extracts were reduced  $\geq 89\%$  compared to those treated with the corn extract. Masa and tortilla chip FB<sub>1</sub> concentrations were also markedly reduced (ca. 80-90%) when compared to those of the corn by HPLC analysis for fumonisins B<sub>1</sub> (FB<sub>1</sub>). Nixtamalization therefore reduced both the measured amount of FB<sub>1</sub> and the ceramide synthase inhibitory activity of masa and tortilla chips extracts to a similar extent, indicating that the masa and tortilla chip extracts contained no significant amounts of unknown fumonisins having ceramide synthase inhibitory activity.

### **THE ROLE OF TUMOR NECROSIS FACTOR *ALPHA* AND THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR *ALPHA* AS MODULATORS OF FUMONISIN HEPATOTOXICITY IN MICE**

(K. A. Voss, R. T. Riley, C. Dunn, and J. C. Corton)

Fumonisin mycotoxins are produced by *Fusarium verticillioides*. They are found in maize worldwide and in maize-based foods. Fumonisin is suspected risk factors for human esophageal cancer in some populations heavily dependent on maize-based foods containing high amounts of fumonisins as a dietary staple. Exposure to fumonisin B<sub>1</sub> (FB<sub>1</sub>), the most common fumonisin, causes hepatotoxicity and alters the balance between cell proliferation and apoptosis in the liver of rodents. The cytokine tumor necrosis factor *alpha* (TNF) and the nuclear receptor peroxisome proliferator-activated receptor *alpha* (PPAR) are known modulators of hepatocyte proliferation and apoptosis and there is evidence suggesting that they play a role in fumonisin toxicity. Experiments were therefore done to further examine the effects of dietary exposure to a fumonisin-containing culture material (CM=corn molded under laboratory conditions using a single fungal isolate) of the fungus *F. verticillioides* in the livers of genetically altered (null) mice lacking either TNF or PPAR. Compared to wild-type mice, TNF-null mice exhibited more severe hepatotoxicity including increased hepatocyte proliferation and apoptosis when fed the CM for eight days. In contrast, the PPAR-null and wild-type mice showed similar patterns of hepatocyte apoptosis and proliferation when fed the CM for eight days or five weeks. These findings provide further evidence that TNF-dependent signaling pathways have a modulating role in liver toxicity in mice consuming fumonisin-contaminated diets. In contrast, the PPAR experiment corroborates the results of other feeding studies which likewise indicated that hepatotoxicity induced in mice by *F. verticillioides* CM and fumonisin B<sub>1</sub> is similar, but not dependent on PPAR.