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Exposure to Fumonisins and the Occurrence of Neural Tube Defects along the Texas–Mexico Border

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Along the Texas–Mexico border, the prevalence of neural tube defects (NTDs) among Mexican-American women doubled during 1990–1991. The human outbreak began during the same crop year as epizootics attributed to exposure to fumonisins, a mycotoxin that often contaminates corn. Because Mexican Americans in Texas consume large quantities of corn, primarily in the form of tortillas, they may be exposed to high levels of fumonisins. We examined whether or not maternal exposure to fumonisins increases the risk of NTDs in offspring using a population-based case–control study. We estimated fumonisin exposure from a postpartum sphinganine:sphingosine (sa:so) ratio, a biomarker for fumonisin exposure measured in maternal serum, and from maternal recall of periconceptional corn tortilla intake. After adjusting for confounders, moderate (301–400) compared with low (≤ 100) consumption of tortillas during the first trimester was associated with increased odds ratios (ORs) of having an NTD-affected pregnancy (OR = 2.4; 95% confidence interval, 1.1–5.3). No increased risks were observed at intakes higher than 400 tortillas (OR = 0.8 for 401–800, OR = 1.0 for > 800). Based on the postpartum sa:so ratio, increasing levels of fumonisin exposure were associated with increasing ORs for NTD occurrences, except for the highest exposure category (sa:so > 0.35). Our findings suggest that fumonisin exposure increases the risk of NTD, proportionate to dose, up to a threshold level, at which point fetal death may be more likely to occur. These results also call for population studies that can more directly measure individual fumonisin intakes and assess effects on the developing embryo. Key words: case–control study, corn, fumonisins, Mexican Americans, mycotoxins, neural tube defects. Environ Health Perspect 114:237–241 (2006). doi:10.1289/ehp.8221 available via http://dx.doi.org/[Online 29 September 2005]
matched to case women by facility and year. Facilities included hospitals and midwife-attended birthing centers.

**Data collection.** The Texas Department of Health Institutional Review Board approved the study design. English–Spanish consent forms, English–Spanish interview instruments, specimen collection, and study procedures. In cooperation with hospital staff, field teams contacted women at the time of delivery or termination of pregnancy to inform them of the study and obtain consent. All women in the study gave written informed consent, in their preferred language (English or Spanish), before participation in the study.

Women were interviewed in person about 5–6 weeks postpartum with an interview instrument modeled after the 1993 Centers for Disease Control and Prevention (CDC) mother questionnaire for birth defects risk factor surveillance. The questionnaire assessed maternal health history, demographics, use of medications and nutritional supplements, and environmental and occupational exposures during the periconceptional period—the 3 months before conception and the 3 months after conception (first trimester of pregnancy). Before each interview, the staff obstetrician/gynecologist and interviewer estimated the date of conception for the index pregnancy using all gestational age estimates from the medical records. Women were specifically asked about corn tortilla consumption during periconception, including the type (brand name, homemade), the month consumed, the frequency (number of days per month), and quantity (number of tortillas per day). We calculated body mass index (BMI; kilograms ÷ meter2) from self-reported prepregnancy height and weight. Women were paid $20 for the 2-hr interview. Blood and urine samples were also collected, for which women were paid an additional $20.

**Laboratory procedures.** Maternal blood specimens were collected in 13 mL tubes without anticoagulant. After coagulation and centrifugation, 3 mL aliquots of serum were apportioned into cryovials. These aliquots were frozen at 20°C and shipped overnight on dry ice to Emory University Laboratory (Atlanta, GA).

Analysis of the sa:so ratio in serum was conducted by high-performance liquid chromatography with fluorescent detection of the sa:so ratio. To estimate fumonisin levels in tortillas, we collected samples of tortillas from participating homes and local grocery stores throughout the study period. A total of 146 households contributed tortilla samples, and another 114 samples were obtained from grocery stores. Tortillas were placed in plastic recyclable sandwich bags and labeled with the date of collection, brand of tortilla, and place of tortilla purchase. The samples were frozen at 0°C until shipped, and then shipped in cold packs overnight to the Division of Natural Products laboratory at the U.S. Food and Drug Administration (FDA). Of the multiple structural isomers of fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), we report only FB1 levels; FB2 and FB3 levels were essentially nondetectable. FB1 levels were determined using high-pressure liquid chromatography (Stack 1998).

**Statistical analysis.** Of the 225 Mexican-American women with NTD-affected pregnancies and 378 Mexican-American women with healthy live births identified for study, 184 case women and 225 control women participated in the interview. Twenty-six case women (12%) and 101 control women (27%) refused to be interviewed, and 15 case women (7%) and 52 control women (14%) had moved out of the study area without being interviewed. Of those interviewed, 163 case women (89%) and 189 control women (84%) provided blood specimens for the sa:so assay.

We evaluated two direct exposure metrics: fumonisin exposure as measured by the sa:so ratio assayed from the maternal blood sample, and total number of corn tortillas eaten during the first trimester of pregnancy as reported on the mothers’ questionnaires. We chose categori- cal cut-points for presentation of the fumonisins and continuous corn exposure variables by calculating the effect of finely categorized variables and then coalescing adjacent categories based on the observed effect estimates (Greenland and Rothman 1998).

### Table 1. Distribution of selected maternal factors by NTD status.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 184)</th>
<th>Controls (n = 225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age [years; median (5th, 95th)]</td>
<td>23 (16, 35)</td>
<td>23 (15, 34)</td>
</tr>
<tr>
<td>Annual household income [n (%)]</td>
<td>11 (5, 15)</td>
<td>12 (6, 16)</td>
</tr>
<tr>
<td>Serum folate (ng/mL)</td>
<td>429 (222, 935)</td>
<td>497 (280, 1,038)</td>
</tr>
<tr>
<td>Serum folate (ng/mL)</td>
<td>27 (14.7)</td>
<td>39 (16.9)</td>
</tr>
<tr>
<td>Maternal BMI [kg/m2; median (5th, 95th)]</td>
<td>25.6 (18.8, 39.7)</td>
<td>24.8 (18.3, 34.5)</td>
</tr>
<tr>
<td>Maternal education [years of school; median (5th, 95th)]</td>
<td>11 (6.0)</td>
<td>10 (4.4)</td>
</tr>
<tr>
<td>Maternal education [years of school; median (5th, 95th)]</td>
<td>26 (14.1)</td>
<td>37 (16.4)</td>
</tr>
</tbody>
</table>

**Percentiles.**

### Table 2. Distribution of corn-related exposures in women by NTD status.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 184)</th>
<th>Controls (n = 225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tortillas eaten during the 3 months before conception</td>
<td>261 (17, 900)</td>
<td>215 (9.2, 10.9)</td>
</tr>
<tr>
<td>No. of tortillas eaten during the first trimester</td>
<td>252 (15, 900)</td>
<td>200 (9.2, 10.9)</td>
</tr>
<tr>
<td>Source of corn tortillas [n (%)]</td>
<td>144 (78.3)</td>
<td>192 (85.3)</td>
</tr>
<tr>
<td>Purchased</td>
<td>25 (13.6)</td>
<td>23 (10.2)</td>
</tr>
<tr>
<td>Homemade</td>
<td>4 (2.2)</td>
<td>4 (1.8)</td>
</tr>
<tr>
<td>Both</td>
<td>23 (12.5)</td>
<td>14 (6.2)</td>
</tr>
<tr>
<td>Serum sa:so ratio</td>
<td>0.16 (0.08, 0.33)</td>
<td>0.14 (0.07, 0.35)</td>
</tr>
<tr>
<td>Serum sa:so ratio</td>
<td>21 (11.4)</td>
<td>30 (16.0)</td>
</tr>
</tbody>
</table>

**Percentiles.**
In addition, we calculated a third metric based partially on ecologic data: nanograms of fumonisins ingested per day during the periconceptional period as estimated from grouped 6-month averages for the tortilla samples. To control for possible seasonal variability, we categorized dates of conception into 6-month blocks, beginning with February–July 1994 and ending with August 1999–January 2000. We chose these categories based upon the timing of the entry of new corn crops into the U.S. market for human purchase and consumption (Miller D, personal communication). We linked each woman’s date of conception to the average fumonisin levels found in tortillas collected during that 6-month block. Daily dose of fumonisin exposure was calculated by multiplying the average fumonisin level (nanograms per gram) by 24 g per tortilla (the average weight of collected samples) and then by the number of tortillas the woman reported eating per day during the first trimester of pregnancy. That number was then divided by the woman’s weight in kilograms.

Using the SAS statistical software package (Version 8, SAS Institute 1991), we fit a logistic regression to calculate deconfounded odds ratios (ORs) and 95% confidence intervals (CIs) to estimate prevalence ratios of NTDs. We considered other possible risk factors for NTDs as potential confounders if addition of that variable to the model changed the OR by 10% or greater. Confounding checks were performed in both univariate and final multivariate models. If a factor was identified as a confounder of any estimated main effect, it was kept in all models. Based on these criteria, we controlled for BMI (kilograms per square meter, continuous), serum B_{12} (picograms per milliliter, continuous), and dates of conception (6-month blocks). In addition, the sa:so values were found to differ systematically by batch (batch 1: n = 172, ratio median = 0.19, 5th percentile = 0.08, 95th percentile = 0.36; batch 2: n = 180, ratio median = 0.13, 5th percentile = 0.08, 95th percentile = 0.24), and therefore we adjusted for batch in all sa:so analyses. Known NTD risk factors that were not confounders within this population included maternal age, maternal birthplace, annual household income, folate intake (dietary plus multivitamin), and prior pregnancy loss. Finally, because it is unclear whether serum folate levels are a measure of an intermediate variable between the main corn/fumonisin effects and NTDs or whether serum folate is a potential confounder, we fit models with and without adjustment for serum folate.

### Results

The demographic profile of control women illustrates the low socioeconomic conditions prevalent on the border. More than a third reported a household income of < $10,000 per year, and only about half had completed high school (Table 1). Control women were leaner and more educated than were case women but comparable in age. Few women reported ever taking preconceptional folic acid supplements.

Nearly all women consumed corn tortillas (96% of case women and 93% of controls). As seen in Table 2, control women reported an average consumption of two tortillas per day during their first trimester of pregnancy (180 total during this 3-month period). That number was nearly identical to the intake reported during the 3 months before conception. Most women purchased rather than made corn tortillas.

After adjustment for BMI, serum B_{12}, and date of conception, moderate (301–400) compared with low (≤ 100) consumption of tortillas during the first trimester was associated with an increased OR of having an NTD-affected pregnancy (OR = 2.4, 95% CI, 1.1–5.3) (Table 3). Higher intakes (401–800 and > 800) were associated with either a slight decrease in occurrence (OR = 0.8 for 401–800) or no effect (OR = 1.0 for > 800). The type of tortilla usually consumed appeared to affect risk. NTD risk increased with exposure to homemade tortillas (OR = 2.9; 95% CI, 1.4–5.9) (Table 3). Among those who purchased tortillas, the effect estimates differed slightly by brand, but the CIs for individual brands were wide (data not shown). As shown in Table 3, increased exposure to fumonisins, based on postpartum sa:so ratio, was associated with an increased NTD occurrence except for the highest exposure category. The highest exposure category (sa:so > 0.35) was related to less frequent occurrence (OR = 0.7, 95% CI, 0.2–2.9) but was based on the fewest number of subjects and therefore had a wide CI. Results differed negligibly when serum folate was added to the model.

The mean fumonisin level measured in the 240 tortilla samples was 234 ng/g (range = 0–1,690 ng/g; SD = 256). When fumonisin levels by season were linked to dates of conception, the median daily fumonisin exposure (nanograms per day per kilogram of weight) was 172.6 for case women and 156.1 for control women. Using the imputed exposure levels for individual women, we also observed an inverted U-shape relation to risk, reflecting the tortilla consumption pattern (Table 4).

### Discussion

Our findings from the postpartum serum sa:so measure suggest that fumonisin exposure increases the risk of NTD, proportionate to dose, up to a threshold level. Data on the corn tortilla consumption and fumonisin ingestion appear to support an inverted U-shaped pattern of occurrence. This pattern may reflect the formation of NTDs up to a threshold of damage, at which point fetal death is more likely to
occur. Because true biologic incidence is impossible to determine, the prevalence of birth defects is a function of embryologic incidence as well as intrauterine survival (Weinberg and Wilcox 1998). Additionally, experiments demonstrate that fetal resorption can occur in folate-deficient pregnant mice (Burgoon et al. 2002) as well as in hamsters and mice exposed to high doses of FB1 (Floss et al. 1994; Gelineau-van Waes et al. 2005; Reddy et al. 1996).

We also observed a difference in risk effect between manufactured tortillas and homemade tortillas. The variations in small-scale tortilla preparation, especially the corn-to-lime ratio, results in wide variations in residual fumonisins (De La Campa et al. 2004). If tortillas made at home have a consistently lower concentration of time or poorer quality corn is used, this could potentially explain some of the increased effect seen in homemade tortillas (De La Campa et al. 2004).

Alternative explanations for the effects that we observed include concern about the potential for recall bias in the estimate of tortillas consumed and lack of a true biomarker of fumonisins at the time of neural tube closure. Case and control women were asked to recall corn consumption as much as a year earlier. However, dietary recall over a much longer period has been shown to be generally reliable (Byers et al. 1987). Although it might seem intuitively that case women were more motivated to remember some events as they sought explanations for having a child with a birth defect, this has not proved to be a consistent bias in studies comparing prospective and retrospective measurement of exposures (Khoury et al. 1994; Mackenzie and Lippman 1989). It seems unlikely that an event as routine as eating corn tortillas would have been differentially recalled. Furthermore, recall bias would not easily account for the consistent observation of an inverted U-shaped relation between questionnaires and laboratory-measured exposures.

Temporality of the blood sample collection is also an important concern. The sa:so ratio measures acute exposure to fumonisins, with levels returning to normal when the exposure is removed. In our sample, the sa:so ratio was measured 5–6 weeks postpartum and would reflect periconceptional levels at neural tube closure only if study participants were exposed to fumonisins at a constant level. We note, however, that in rats and mice, a subtoxic fumonisin dose will maintain elevated sphingolipids (De La Campa et al. 2004). We also observed that the increased risk seen in homemade tortillas (De La Campa et al. 2004)

References


