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Research Article

Conception Residences near Interstate Highways and the Neural Tube Defects Births

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Abstract

Objectives: In response to an anencephaly cluster on the Texas-Mexico border, we conducted an incident matched case-control study of Neural Tube Defects (NTD). Data are presented regarding the conception residences near interstate highways and biomarkers of chemical exposures to metals, organophosphorus and organochlorine pesticides.

Methods: Maternal biological samples (blood, sera, urine, and hair) were obtained at the time of NTD diagnosis for cases and a comparable time for controls with unaffected pregnancies. Conception addresses of NTD cases and matched controls were geocoded during residential visits for environmental sampling. An objective GIS subroutine was used to ascertain the distance at conception from major highways. Conditional logistic regression was used to evaluate odds ratios for distance and biological markers of chemical exposure to six dialkyl phosphate metabolites, eleven organochlorine metabolites, and three metals.

Results: Infants with NTD were conceived significantly more often within ¼ mile from the highways (OR=3.0, 95% CI 1.05, 8.83; p=0.041). A proximity to birthing clinic pattern was unlikely. Significant odds ratios for urinary dialkyl phosphate metabolites of organophosphorus pesticides were associated with NTD (4th quartile ORs for creatinine-adjusted Diethyl phosphate (DEP), Diethyl thiophosphate (DETP), and Diethyl dithiophosphate (DEDTP) were 3.5, 2.8, and 2.9, respectively). Serum organochlorine pesticides were also significantly associated with NTD (4th quartile ORs for lindane, dieldrin, and trans-nonachlor were 11.1, 3.8, and 9.4, respectively). There was no parallel gradient indicated for metals with the distance from the major highways.

Conclusion: We invite attention to potential risks for NTD involving large-scale applications of pesticides for cropland, mosquito, and brush control on the shoulders of highways.

Keywords: Neural tube defects; Matched case-control study; Conception residence; Distance from interstate highways; Chemical risk factors

Introduction

Evidence in the US and Europe pointed to increased risks of pulmonary symptoms associated with living near busy roads [1-4]. Researchers in California reported associations between maternal exposure to traffic air pollution, preterm births, and low birth weight [5,6].

Our present communication addresses the distance at conception from the interstate highways obtained as part of a larger investigation into the etiology of NTD births on the Texas-Mexico border. To our knowledge, no other study has been reported evaluating whether an increased risk of NTD is associated with the conception residence near the interstate highways.

Methods

Controls were individually matched to cases on geographic region, maternal age, race/ethnicity, gestational age, and type of health insurance (including none). The matched ratio ranged from 1 to 4, depending on the availability of controls.

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The controls were enrolled from the same prenatal care clinics or delivery hospitals as the cases. Since controls were selected concurrently with the case, there was implicit matching on date of conception. Maternal biological specimens (blood, sera, urine, and hair samples) were obtained at the time of NTD diagnosis for cases and at matching gestational ages for controls. Cord blood was collected at delivery or termination of the pregnancy. A strict specimen collection and processing protocol was developed to minimize contamination. Blood samples were drawn by venipuncture, centrifuged, and the sera frozen at -20˚C within one hour of the phlebotomy. The frozen specimens were sent to the laboratory, where they were kept at -70˚C for prompt analysis. Biological specimens were analyzed for selected metals and pesticides, lipids, serum folate, and vitamin B12. Analyses were carried out at the National Center for Environmental Health laboratories per established protocols. The U.S. Environmental Protection Agency laboratory in Houston, Texas, assisted with the hair analyses for metals.

Face-to-face maternal interviews were conducted in English and Spanish languages. Controls were interviewed before they know the status of their pregnancy, to avoid influencing responses.

Because the NTD is formed in the first 28 days after ovulation, the residential address around the time of conception was a variable of interest. Environmental sampling was conducted at conception residences of case and controls. Tap water, soil, soil gas, and air samples were tested for the EPA priority pollutants [7-9]. Latitude and longitude of conception residences were geocoded during this home visit or later, using Street Info database by MapInfo Co [10]. For addresses on the Mexico side, coordinates were obtained during home visits and cross-referenced using the MapInfo databases "Mexico

Streets, Boundary, and Demographic Bundle" (for Cd. Juarez) and "Mexico Municipalities" elsewhere in Mexico. On a few occasions (all outside the State of Texas), locations at conception were geocoded as the county centroids.

Addresses at conception were mapped and interstate highways superimposed (Figures 1, 2 and 3). The shortest distance from major highways was calculated using a subroutine in MapInfo. Case *vs.* control odds ratios were calculated for distances of conception residences from highways. Due to the better precision for in-state locations, only Texas addresses were used to calculate odds ratios. Concentrations of metals and pesticides in biological specimens and environmental samples were evaluated as quartiles of the control distributions for their association with NTD. Confounding and interaction with the distance from highway (less than ¼ mile) dichotomy was evaluated for categories of chemical biomarkers using a likelihood ratio test.

Results

A total of 539 women were contacted for enrollment into this study, 166 cases (30.8%) and 373 controls (69.2%). Of the women contacted, 446 (82.7%) were eligible for inclusion and of that, 382 border area residents (107 women with NTD affected pregnancies and 275 individually matched controls) provided biological specimens (85.6% participation). Of 107 cases, 77 (72%) were anencephaly and 30 (28%) were open spina bifida. Thirty-two cases (30%) were matched at a 1:4 ratio, 28 at 1:3, 16 at 1:2, and 31 at 1:1. Upon medical records verification, none of the controls was excluded.

The median age for case mothers (and matched referent mothers) was 23 years (Table 1), and most of the women were of Hispanic decent (94.4%). More than 60% of the cohort was born outside the U.S., but the majority, 80 to 85%, resided in this country at the time of conception of index babies. More case mothers (32.7%) than controls (23.6%) changed residence between conception and enrollment (Table 2). Forty-seven percent of case mothers were enrolled from the Lower Rio Grande Valley; 38% from El Paso-Cd. Juarez area, and 15% from Laredo-Nuevo Laredo. The majority (61.7%) of case mothers had Medicaid insurance, 24.3% had private insurance and 14.0% had none. There was no difference in educational attainments of cases and control families (not shown).

Table 1: Matching Characteristics, Cases and Controls, Texas-Mexico Border, 1993-2000.

Matching Criteria	Cases (n=107)		Controls (n=275)	
	Number	%	Number	%
Mother's age (years)*				
<19	22	20.8	62	22.5
>19- to ≤23	20	10.3	48	17.5
>23 to ≤26	26	17.8	55	20
>26 to ≤30	21	15	61	22.2
>30	18	16.8	47	17.1
Gestational age (weeks)*				
≤14	4	3.7	8	2.9
15-29	54	50.5	136	49.4
30-36	18	16.8	66	24
37+	31	29	65	23.7
Mother ethnicity				
Hispanic	101	94.4	266	96.7
Non-Hispanic	6	5.6	9	3.3
Mother's insurance type				
Self-pay/none	15	14	40	14.5
Medicaid	66	61.7	180	65.5
Private	26	24.3	55	20

*At enrollment

Area where a case was identified: A=El Paso, USA, - Cd. Juarez, Mexico; B=Laredo, USA, - Nuevo Laredo, Mexico; C=Lower Rio Grande Valley near Harlingen-Brownsville, USA, and Reynosa-Matamoros, Mexico.

The odds ratio for country of conception Mexico *vs.* U.S. was greater than one (OR=1.4), but this was not statistically significant. Residence at conception for the majority of participants (66.4% cases and 81.1% controls) was in urban areas (towns and cities of more than 10,000 inhabitants). However, a larger proportion of NTD cases than controls conceived in rural areas, 11.2% *vs.* 1.8% (OR=6.4, 95% CI 2.00-20.9, p=0.002) and significantly more lived near agricultural fields (OR=4.2, 95% CI 2.0-8.8, p<0.001). Fewer case families (50.5% *vs.* 59.3%) lived in the single-family dwellings, with 10.3% of the cases *vs.* 5.4% controls lived in trailer homes. Roughly 22% of all participants were apartment dwellers.

Distance from highways

More cases (11.1%) compared to controls (6.6%) resided directly near interstate highways, such as within the first quarter mile. Overall, the conception residences of NTD cases were closer to the major highways, with a median distance of 1.1 miles to the nearest interstate thorough fares for the cases and 1.3 miles for controls (Table 3). By the study regions, the median distance from the highways was also less for case residences than controls, i.e., 0.9 *vs.* 1.3 miles in El Paso; 1.1 *vs.* 1.2 miles in Laredo; and 1.2 *vs.* 1.3 miles in the Lower Rio Grande Valley.

This proximity to the highways for the cases was particularly noticeable along U.S. Highway #83 in the Lower and Middle Rio Grande Valley (Figure 2 and 3), and a highway connecting Texas and Mexico in the El Paso area (Figure 1). However, at the extreme, one case lived as far as 104 miles from an interstate highway, more than twice than any control.

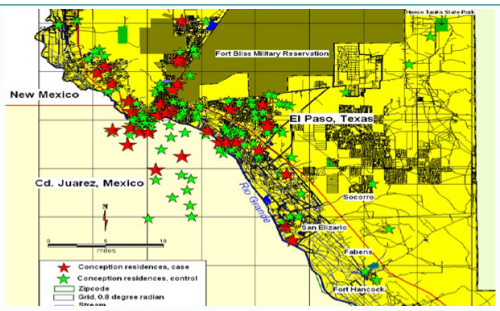

Figure 1: Residences at Conception, Neural Tube Defect Cases and Controls, El Paso-Cd. Juarez.

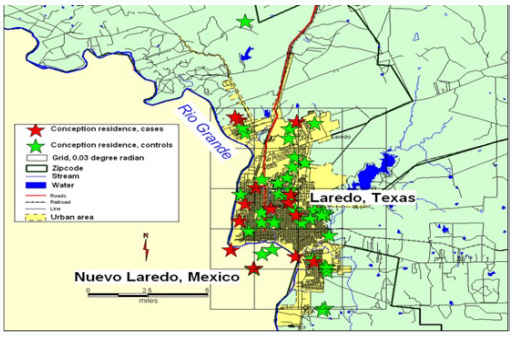

Figure 2: Residences at Conception, Neural Tube Defect Cases and Controls, Laredo-Nuevo Laredo.

Table 2: Index Baby, Place, and Season of Conception.

Variables	Cases (n=107)	Controls (n=275)	OR[1] (95% CI)	P
Residence at conception of index baby				
United States	88 (82.2%)	236 (85.8%)	Reference	
Mexico	17 (15.9%)	31 (11.3%)	1.4 (0.72, 2.84)	0.309
Place of residence at the time of conception				
Same	69 (64.5%)	205 (74.5%)	Reference	
Changed	35 (32.7%)	65 (23.6%)	1.6 (0.96, 2.64)	0.07
Area of residence around conception				
Large town and city (Pop. >10,000)	71 (66.4%)	223 (81.1%)	Reference	
Small town (Pop. ≤10,000)	15 (14%)	21 (7.6%)	1.7 (0.78, 3.84)	0.174
Colonia	5 (4.7%)	15 (5.5%)	1.1 (0.38, 3.04)	0.895
Ranch	1 (0.9%)	1 (0.4%)	~	~
Rural	12 (11.2%)	5(1.8%)	6.4 (2.00, 20.9)	0.002**
Type of dwelling				
Single family house	54 (50.5%)	163 (59.3%)	Reference	
Apartment	24 (22.4%)	61 (22.2%)	1.6 (0.85, 2,84)	0.154
Trailer/mobile home	11 (10.3%)	15 (5.4%)	2.0 (0.81, 4.89)	0.131
Multiple family house	10 (9.3%)	20 (7.3%)	2.2 (0.90, 5.42)	0.086
Season at conception of index baby				
Cold (October-March)	59 (55.1%)	148 (53.8%)	Reference	
Warm (April-September)	48 (44.9%)	127(46.2%)	0.9 (0.54, 1.38)	0.533
Lived near agricultural field				
Yes	25	21	4.2 (2.0, 8.8)	<0.001***
No	61	201	Reference	

[1]OR: Odds Ratio; CI: Confidence Interval; Significance **$P<0.01$ and ***$P<0.005$.
Note: Total percentage not add up to 100% because of missing data, † ~ Too few exposed subjects to calculate a meaningful odds ratio.

Odds ratios were calculated comparing residences within the first ¼ mile from the major highways *vs.* further (Table 4). The odds ratio was 3.0 (95% CI 1.05-8.83, p=0.041). Each of the three regions indicated similar tendencies. This supports the significance of the proximity to highways as a possible risk indicator for NTD.

Chemical exposures

A parallel focus of our study was on chemical exposures. Results for maternal levels of metals in blood, urine, and hair are given in Table 5. Blood lead levels were associated with mild increase risk of NTD (OR, 4th quartile, 2.4; 95% CI 1.0-5.9), but hair lead level was not (OR, 4th quartile, 1.4; 95% CI 0.5-3.8). Increase of mercury levels in blood, urine, and hair were not associated with an increased risk of NTDs, neither was an association evident for arsenic in urine. A non-significant elevated risk was observed for hair arsenic (OR, 3rd tertile, 1.7; 95% CI 0.8-4.0).

Six dialkyl phosphate metabolites that would indicate an exposure to organophosphorus (OP) insecticides were assessed. The 50th percentile concentrations of most of the metabolites (diethyl phosphate, dimethyl phosphate, diethyl thiophosphate, and dimethyl thiophosphate) in our cohort were considerably greater than the 50th percentile for a sample of the U.S. population and a subset sample of Mexican-Americans [11]. When adjusted for creatinine (Table 6), all six OP metabolites were positively associated with risk of NTD. This associations was statistically significant for diethyl phosphate (OR, 4th quartile, 3.5; 95% CI 1.4-8.9), diethyl dithio phosphate (OR, 4th quartile, 2.9; 95% CI 1.3-6.6), and diethyl thiophosphate (OR, 4th quartile, 2.8; 95% CI 1.0-7.5).

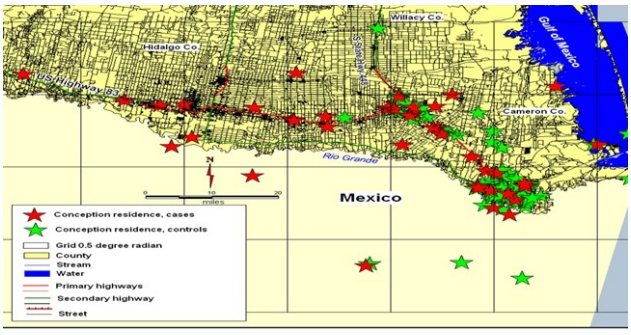

Figure 3: Residences at Conception, Neural Tube Defect Cases and Controls, Lower Rio Grande.

Table 3: Distance from Major Highways (miles) to Residences at Conception, by Geographic Regions.conception residence.

Distance	Obs	Median	Mean	St. Dev	Min	Max
Cases						
All sites	81	1.07	5.92	17.3	0.04	103.8
Region A	29	0.88	4.89	19.1	0.04	103.8
Region B	14	1.08	12.1	28	0.24	78.9
Region C	38	1.21	4.44	8.7	0.06	46.2
Controls						
All sites	237	1.29	2.34	5	0.04	49.6
Region A	85	1.28	1.64	2	0.04	13.6
Region B	46	1.2	4.2	10.2	0.13	49.6
Region C	105	1.31	2.12	2.84	0.09	20

Region A is El Paso; Region B is Laredo; Region C is the Lower Rio Grande.
Note: One more control conceived outside the three border regions. The distance was estimate from the appropriate thoroughfare at the location of conception residence.

Table 4: Odds Ratios Associated with the Distance of Residence at Conception from the Major Highways, Miles, Texas Only.

Distance, all sites	Cases	Controls	OR	95% CI	P
≤ 0.25 miles	9 (11.1%)	15 (6.6%)	3	1.05 - 8.83	0.041*
>0.25 miles	72 (88.9%)	214 (93.4%)	1	Reference	

*Statistically significant compared to >0.25 miles

Table 5: Odds ratios associated with metals in maternal biological specimens.

Exposure	Cases	Controls	Odds Ratio[1] (95% CI)	P-value
Blood lead (mg/dl)				
<1.2 (reference)	17	60	1	
>1.2 -<1.7	12	53	1.3 (0.5, 3.5)	0.55
>1.7 -<2.9	18	58	2.0 (0.8, 5.0)	0.11
>2.9	18	52	2.4 (1.0, 5.9)	0.06
Hair lead (mg/g)				
<0.4 (reference)	14	54	1	
>0.4 -<0.7	12	50	1.3 (0.5, 3.6)	0.58
>0.7 -<1.4	10	51	1.0 (0.4, 2.9)	0.93
>1.4	14	50	1.4 (0.5, 3.8)	0.54
Blood mercury (ug/L)				
<LOD[2] (reference)	16	66	1	
> LOD - <0.7	10	43	1.4 (0.5, 3.8)	0.87
>0.7 -<1.8	18	52	1.2 (0.5, 2.8)	0.69
>1.8	11	53	0.5 (0.2, 1.3)	0.15
Hair mercury (mg/g)				
<0.1 (reference)	24	53	1	
>0.1 -<0.2	11	51	0.4 (0.2, 1.1)	0.08
>0.2 - <0.4	8	51	0.4 (0.1, 1.0)	0.06
>0.4	7	50	0.3 (0.1, 0.8)	0.02
Urine mercury (ug/g creatinine)				
<LOD2 (reference)	12	53	1	
>LOD -<1.0	15	39	3.1 (1.1, 8.8)	0.03
>1.0 -<1.9	6	47	0.7 (0.2, 2.2)	0.55
>1.9	17	45	2.2 (0.7, 6.5)	0.17
Urine arsenic (mg/g creatinine)				
<LOD2 (reference)	38	93	1	
>LOD - <9.2	4	32	0.3 (0.1, 1.3)	0.11
>9.2	9	62	0.3 (0.1, 0.9)	0.03
Hair arsenic (mg/g)				
<0.01 (reference)	21	79	1	
>0.01 - <0.06	11	61	0.9 (0.4, 2.2)	0.81
>0.06	18	65	1.7 (0.8, 4.0)	0.2

Odds ratio obtained by conditional logistic regression Limit of detection

Table 7 presents the results for analyses of ten serum organochlorine pesticides. Monotonically and significantly increased risks with increasing serum levels were observed for y-HCCH (lindane) (4th quartile OR, 11.1; 95% CI 2.2-55.4), dieldrin (4th quartile OR, 3.8; 95% CI 1.4-10.5), trans-nonachlor (4th quartile OR, 9.4; 95% CI 2.6-33.8), op-DDT (4th quartile OR, 2.2; 95% CI 0.8-6.0) and pp-DDT (4th quartile OR, 2.5; 95% CI 0.9-7.0). Elevated (non-significant) 4th quartile ORs were observed for several other organochlorine pesticides, such as oxychlordane, heptachlor epoxide, and mirex. Median concentrations of pp-DDE and beta-hexachlorocyclohexane (β-HCCH) considerably exceeded the median concentrations (and their 95% upper limit) for a sample of the U.S. population and a subset sample of Mexican-Americans [11]. However, elevated risks for NTD with increasing concentrations were not observed for these latter chemicals.

Confounding was evaluated between the indicated biological specimen dichotomies and the ¼ mile distance from highway dichotomy (Table 8). Significant confounding was indicated between highway proximity, the organophosphorus metabolites (DEP, DEDTP and DETP), and the organochlorine compounds (lindane and mirex), urine arsenic, but not for metals.

Table 6: Odds ratios associated with concentrations of organophosphate metabolites in maternal urine (ug/gcreatinine).

Exposure	Cases	Controls	Odds Ratio[1] (95% CI)	P-value
Diethyl Phosphate (DEP)				
<0.5 (reference)	8	63	1	-
>0.5 -<1.7	9	57	1.0 (0.3, 3.1)	0.98
>1.7 -<4.4	11	54	1.6 (0.5, 4.7)	0.43
>4.4	28	58	3.5 (1.4, 8.9)	<0.010**
Diethyl Dithiophosphate (DEDTP)				
<LOD[2] (reference)	22	109	1	-
>LOD -<0.1	8	45	1.1 (0.4, 3.1)	0.89
>0.1 -<0.2	3	31	0.7 (0.2, 2.9)	0.65
>0.2	23	47	2.9 (1.3, 6.6)	<0.010**
Diethyl Thiophosphate (DETP)				
<0.7 (reference)	8	70	1	-
>0.7 -<1.1	9	49	1.3 (0.4, 3.7)	0.68
>1.1 -<2.3	23	57	3.8 (1.5, 9.7)	<0.010**
>2.3	16	56	2.8 (1.0, 7.5)	0.040*
Dimethyl Phosphate (DMP)				
<1.35 (reference)	10	58	1	-
>1.4 -<4.2	13	59	1.2 (0.5, 3.0)	0.74
>4.2 - <8.6	13	57	1.3 (0.5, 3.6)	0.56
>8.6	20	58	1.8 (0.7, 4.8)	0.21
Dimethyl Dithiophosphate (DMDTP)				
<LOD[2] (reference)	27	125	1	-
>LOD -<1.8	5	32	0.7 (0.2, 2.3)	0.55
>1.8	24	75	1.9 (0.9, 4.1)	0.1
Dimethyl Thiophosphate (DMTP)				
<0.4 (reference)	8	59	1	-
>0.4 -<2.9	14	57	1.7 (0.6, 5.1)	0.33
>2.9 -<14.7	18	58	2.6 (0.9, 7.3)	0.06
>14.7	16	57	1.8 (0.7, 4.8)	0.24

Odds ratio obtained by conditional logistic regression Limit of detection.

Discussion

To our knowledge, this is the first study reporting residential proximity to highways as a potentially associated with NTD. The strength of this study was that analyses focused on objectively derived addresses at conception rather than at birth, and that GIS subroutines were used to calculate distances, rather than recall estimates. With notable exceptions [12,13], most previous NTD studies used residence at birth, rather than at conception. Since the critical time in the NTD pregnancy is the first month after ovulation, our protocol stressed reconstructing the residential history back to the periconceptual period, to avoid misclassification of exposures. Although cases and controls were matched on region at enrollment, the residence at conception was the variable of research interest, and all environmental and geo-spatial analyses were done with respect to it. For the majority of participants, the residential address at conception was validated during a house visit.

A string of NTD conceptions was detected that extended SE-to-NW between Harlingen-Brownsville and Laredo. Upon overlaying digitized coordinates of major interstate highways, it became evident that this chain followed the interstate highway #83. Importantly, this pattern was not observed for control residences. The distances from highways was investigated by sub-regions and overall, using a

Table 7: Odds ratios associated with lipid-adjusted concentrations of organochlorine in maternal sera (ng/g).

Pesticide	# Cases	# Controls	2nd Quartile OR[1] (95% CI)	3rd Quartile OR[1] (95% CI)	4th Quartile OR[1] (95% CI)
Lindane	69	217	4.9 (1.0, 25.1)	10.8 (2.2, 53.9)	11.1 (2.2, 55.4)
Dieldrin	64	211	1.6 (0.5, 5.0)	1.9 (0.7, 5.4)	3.8 (1.4, 10.5)
T- Nonachlor	70	223	4.1 (1.3, 13.2)	4.2 (1.1, 15.4)	9.4 (2.6, 33.8)
op-DDT	60	191	0.8 (0.3, 2.5)	1.2 (0.4, 3.7)	2.2 (0.8, 6.0)
pp-DDT	70	221	1.3 (0.5, 3.5)	2.4 (1.0, 6.3)	2.5 (0.9, 7.0)
Oxychlordane	69	220	2.0 (0.7, 5.7)	1.6 (0.6, 4.6)	3.2 (1.0, 9.6)
Heptachlor Epoxide	68	218	1.0(0.85,5.01)	2.1(0.85,5.00)	1.8(0.69,4.41)
Mirex	67	207	1.4 (0.5, 3.8)	3.1 (1.2, 8.1)	2.3 (0.8, 6.1)
pp-DDE	69	222	0.9 (0.4, 2.1)	1.2 (0.5, 3.0)	0.7 (0.2, 2.1)
p-HCCH	70	219	2.3 (1.0, 5.4)	1.7 (0.7, 4.3)	1.1 (0.4, 2.9)
HCB	70	223	3.2 (1.1, 8.9)	3.3 (1.1, 9.9)	2.0 (0.7, 6.3)

Odds ratio obtained by conditional logistic regression.

Table 8: Confounding between Selected Maternal Characteristics and the Distance of Conception Residences Dichotomy (<¼ mile *vs.* >¼ mile) from Major Highways, Texas-Mexico Border, 1993-2000.

	Covariate		N	Distance		Test for Confounding	
Name	Threshold	Unit	Collapsed Model	OR	P	OR	P
Blood Lead	1.7	µg/dL	158	2.7	0.132	1.7	0.182
Blood Lead	2.9	µg/dL	158	2.7	0.133	1.3	0.5
Blood Hg	1.7	µg/L	146	2.2	0.262	0.5	0.089
Urine As	10	µg/L	128	4.2	0.081	0.3	0.036*
Urine As	9.2	µg/g creatinine	124	4.8	0.061	0.3	0.041*
DEP	4.45	µg/g creatinine	146	3	0.155	3.4	0.006*
DEDTP	0.2	µg/g creatinine	146	2.4	0.254	2.7	0.024*
DETP	1.1	µg/g creatinine	146	3.6	0.103	4.2	0.002*
DETP	2.3	µg/g creatinine	146	2.5	0.202	1.3	0.538
DMP	8.65	µg/g creatinine	146	2.5	0.213	1	0.939
DMDTP	1.85	µg/g creatinine	146	2.5	0.229	2	0.095
DMTP	2.9	µg/g creatinine	145	2.7	0.181	1.6	0.218
HCB	38	ng/g lipid	153	4.7	0.031*	1.5	0.484
HCB	64.9	ng/g lipid	153	4.9	0.026*	1	0.992
β-HCCH	7.85	ng/g lipid	151	5	0.028*	1.8	0.261
γ-HCCH	0.92	ng/g lipid	147	5.7	0.023*	6.1	0.045*
γ-HCCH	1.27	ng/g lipid	147	6.4	0.012*	4.4	0.006*
γ-HCCH	1.74	ng/g lipid	147	5.1	0.025*	1.9	0.116
Oxychlordane	11.4	ng/g lipid	152	5	0.027*	1.3	0.613
T- Nonachlor	12	ng/g lipid	153	5.7	0.018*	1.8	0.227
T- Nonachlor	19	ng/g lipid	153	5.2	0.023*	1.9	0.126
Dieldrin	7.8	ng/g lipid	142	4.7	0.031*	1.9	0.08
op-DDT	1.99	ng/g lipid		3.2	0.129	1.5	0.423
pp-DDT	7.5	ng/g lipid	152	4.9	0.029*	1.8	0.215
Mirex	0.8	ng/g lipid	138	5.3	0.024*	2.4	0.040*
Serum Folate	9.5	ng/mL	141	11.2	0.035*	0.3	0.006*
Serum Folate	15.3	ng/mL	141	11.9	0.031*	0.4	0.044*
Vitamin B12	248	pg/mL	139	13.1	0.027*	0.3	0.030*
Total Cholesterol	193	mg/dL	165	4.8	0.037*	0.3	0.003*
Total Cholesterol	220	mg/dL	165	6.8	0.013*	0.2	0.003*
Total Cholesterol	254	mg/dL	165	7.2	0.011*	0.3	0.087

conditional logistic regression analysis. Comparing residences <¼ mile *vs.* a reference distance >¼ mile, a statistically significant odds ratio of 3.0 was indicated. It was unlikely that such a distribution was merely a reflection of access to the birthing hospitals and clinics, as controls enrolled from the same prenatal care facilities did not exhibit this pattern. Additionally, the median distance for controls was greater, overall and by sub-regions.

It is difficult to extrapolate findings in older US cities to the Texas-Mexico border communities - the latter having a much more recent history and a different urban growth pattern. Importantly also, a significantly greater proportion of NTD case families in our study compared to controls lived in rural communities stretched along the highways. This type of housing might have created a greater need and likelihood of applications of pest and weed control measures. This explanation agrees with findings by Shalat and co-workers [14] who reported an association between OP pesticide in the house dust, hands, and urine of infants residing in one of the colonies in Texas Southwest. In another study [15] it was shown that lawn-applied herbicides are transferred from turf to home and the turf residues were correlated with floor dust.

The combustion of gasoline containing tetraethyl lead and tires wear containing lead oxide might have resulted in lead contamination of soil along the roads. However, we found no statistically significant evidence that concentrations of total lead in the blood, hair, soil, and water of the study participants increased in proportion with the decreasing distance from interstate highways (Tables 5 and 8). The same was true for two other metals.

The drift of insecticides from fields situated along the highways and road shoulder' applications of herbicides for brush and weed control would have been more likely factors associated with road proximity. Herbicides are widely applied along highways to control roadside vegetation [16], and surface water is frequently affected nearby application sites. Studies in California showed that the percentage of the applied herbicide that was detected in surface runoff over a season ranged from 0.05% to 43.5%, and the most critical factor in controlling the variation were the solubility of the herbicide and the runoff volume [16].

Agricultural fields in the Southwest Texas are typically stretched along the interstate highway, with the associated uses of pesticides, and significantly more ($p<0.001$) cases in our study lived near agricultural fields. Dispersal of certain types of mosquito along the highway system has also been reported in Texas [17], necessitating the use of insecticides.

We accounted for pesticide exposures in biological samples that were obtained at the time of NTD diagnosis for cases and controls. Urinary dialkyl phosphate metabolites of OP pesticides were significantly associated with NTD (4^{th} quartile ORs for creatinine adjusted DEP, DETP, and DEDTP were 3.5, 2.8, and 2.9, respectively). Several serum organochlorine pesticides (OC) were also significantly associated with NTD (4^{th} quartile ORs for lindane, dieldrin, and trans-nonachlor were high, 11.1, 3.8, and 9.4, respectively). OC chemicals were banned in US, but apparently are still used and/or persist in the soil. Most home uses of OP insecticides also have been phased out in the US, but some OP insecticides (e.g. Malathion and naled) are still used for cropland and mosquito control.

Herbicides are used for control of grass weeds in a wide variety of crops and in non-cropland applications, such as control of brush and woody plants along highways, bridges, railroads, electric power lines right-of-ways, and other large green spaces. Active ingredients are usually applied in solution, which is either sprayed in strips/bands or through truck-mounted foggers, depending on the desired outcomes [18-22] The use of herbicides and insecticides for trash grass, weeds, and mosquito control would be particularly commonplace considering the hot climate of the Texas Gulf Coast and a year-around rapid grass and brush growing season. Since major interstate highways have larger shoulders, the insecticide and herbicide treatment of these grassy spaces might have been far more substantial than it would have been for minor roads. Per our personal communication with the Texas Department of Transportation in 2006, the following herbicides were used in Texas along the highways, with commercial names: of Roundup Pro (54% Glyposate); Excort XP; Outrider; Landmark XP; Translite; Pathfinder II; and Habitat.

We have noticed that in the City of El Paso, three cases of NTD occurred within a very short distance from each other, about 2,000 feet (!) (Figure 1). It would have been unlikely for this to be a chance occurrence. A feature of this small neighborhood was that it bordered an expanse of a cemetery, and we speculated that mosquito, weeds, and brush control on this grassy acreage might have been a factor of relevance. Several other cases in El Paso (Figure 1) were conceived in houses proximal to a large green expanse of a military base.

The finding of an association between NTD and the residential proximity to highways warrants further biologically-bases study. Currently, there is limited knowledge regarding the mechanism of action for most herbicides, and still less about herbicide metabolite. It is known though, that the commonly used pesticides and herbicides have neurotoxic and endocrine-disrupting properties. For instance, a study reported significant neurologic and neurobehavioral developmental defects in children born to applicators of herbicide glyphosate (of organophosphorus family), with an OR of 3.6 (CI 1.3-9.6), and particularly those children conceived in the spring [19]. Studies with animals indicated that herbicides have teratogenic effects solely or by interaction with other chemicals [20,21], as well as affecting male reproductive organs [22]. In our study, we looked for urine metabolites that would have been indicative of organophosphorus and organochlorine exposures, which are components of both insecticides and herbicides.

The concept of proximity to highways as a marker for the development of the neuro-skeletal defects is supported by an interesting fact that the incidence of orofacial cleft (also a midline birth defect) has been shown to increase in families who resided within a close convergence of four major highways in another part of Texas [23,24]. We suggested that perhaps the application of chemicals on the shoulders of these interstate highways, solely or in tandem with other pollution could be responsible for this pattern.

We have previously reported the significantly lower concentrations of serum folate and vitamin D in NTD case mothers compared with unaffected pregnancies [8]. The Table 8 showed that there was a confounding / interaction of odds for conception residence being near interstate highways and serum folate/vitamin D concentrations. While several factors may explain the choice of conception residence, it is unlikely that mothers with lower folate and lower vitamin D would have chosen locations near highways. It is reasonable to question whether folate and vitamin D deficiencies are not products of herbicide exposure along the major highways.

Despite of obvious limitations related to more than one possible risk factors and their interaction, the clinical and public health implications of the association that we observed between decreased distance from the interstate highways and NTD is important in its own right. This variable is objectively measureable and could be very useful to alert neonatologists and primary care physicians.

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