

Application of a Geographic Information System to Explore Associations Between Air Pollution and Micronucleus Frequencies in African American Children and Adults

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Exposure to air pollution has been associated with adverse respiratory and cardiovascular health outcomes in both children and adults. In this study, we used geographic information systems (GISs) to explore possible associations between chromosomal damage in 65 African American children and their mothers from Oakland, California, and both proximity to traffic and regional ozone levels. Study participants were interviewed at the Healthy Child Clinic of Children's Hospital, Oakland, and their blood and buccal cells were collected for assessment of chromosomal damage by the micronucleus (MN) assay. Regional ozone levels, which decreased from April to November with a secondary peak in late summer, were highly correlated with season by month ($r = -0.84$, $P = 0.02$) and strongly associated with MN frequency (frequency ratio (FR): 3.37, 95% confidence interval (CI): 1.30–8.72) in both cell types of children and

adults. Additionally, MN frequencies were modestly associated with individual measures of traffic density in children (FR = 2.45, 95% CI = 0.86–7.10), but not in adults; this suggests a greater vulnerability to traffic-related air pollution in children. Smoking in the household also increased MN frequency in the lymphocytes of children (FR: 1.13, 95%CI: 1.01–1.24) and adults (FR: 1.06, 95%CI: 0.99–1.13), whereas vitamin use in adults decreased MN frequency in both lymphocytes and buccal cells (FR: 0.17, 95%CI: 0.02–1.31; FR: 0.18, 95%CI: 0.03–1.18, respectively). Our data indicate that GIS-generated measures of traffic density for individual households augment regional ozone monitoring data used to assess effects of air pollution. This approach helped to demonstrate elevated cytogenetic damage in exposed minority children. *Environ. Mol. Mutagen.* 47:236–246, 2006. © 2006 Wiley-Liss, Inc.

Key words: micronucleus frequency; traffic; vitamins; smoking; ETS; ozone

INTRODUCTION

Epidemiological studies have demonstrated associations between air pollution and health effects like asthma exacerbations and cardiovascular disease [Brunekreef and Holgate, 2002]. In California, motor vehicle exhaust accounts for a large portion of ambient air pollution [CARB, 2005]. Exhaust emissions are a complex mixture of gases and fine particles, including respiratory irritants and carcinogens. In animal studies, inhalation of diesel exhaust particles, which contain polycyclic aromatic hydrocarbons (PAHs), is associated with lung and other types of cancer [IARC, 1989]. Therefore, exposure to traffic pollutants is an important contributor to the adverse health effects of outdoor air pollution.

Since traffic pollutant concentrations tend to be higher near and downwind of major roads [Zhu et al., 2002], studies that explore the effect of traffic pollution on children's health often employ a surrogate measure of proxim-

ity to traffic. Utilizing a measure of traffic counts within 50 m of a residence, a study of German school children found strong associations between traffic counts and increased respiratory symptoms such as asthma, wheezing, and coughing [Nicolai et al., 2003]. Another study reported a higher risk for childhood leukemia in children whose homes were heavily exposed to road traffic emis-

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sions as compared to children from unexposed homes [Crosignani et al., 2004].

In addition to health outcomes, studies have also looked for evidence of genetic damage as a result of traffic pollution exposure. The frequency of micronuclei, formations resulting from chromosome breakage or loss during mitosis, serves as a useful measure of genetic damage [Fenech et al., 1999]. The micronucleus (MN) assay has been used to study effects of environmental, occupational, genetic, and lifestyle factors on genomic stability [Norppa et al., 1992, 1993; Bonassi et al., 2003; Neri et al., 2005]. For instance, occupational studies have examined MN frequency in adults exposed to traffic pollution. Zhao et al. [1998] reported an increased MN frequency in Chinese traffic policemen compared with office workers, while other studies did not detect a difference in Italian traffic workers as compared with office workers [Bolognesi et al., 1997; Leopardi et al., 2003].

In children, studies examining the effects of genotoxic agents like ionizing radiation and chemicals have reported an increase in MN frequency in exposed populations [Neri et al., 2003]. However, few studies have examined genetic damage in children exposed to air pollution. A study in Mexico found evidence of greater DNA damage by Comet assay in the nasal epithelium of children living in areas of high air pollution vs. children living in relatively low pollution areas [Calderon-Garciduenas et al., 1997]. In Russia, Pelevina et al. [2001] reported a higher MN frequency in the lymphocytes of children exposed to greater levels of environmental pollution compared to referents (lower levels of environmental pollution). These studies imply that air pollution may affect levels of genomic stability and potentially cancer in children.

Some studies of genetic damage also suggest that children may be more susceptible to the effects of air pollution than adults [Neri et al., 2005]. In a cohort of mothers and newborns from New York City, Perera et al. [2004] reported that despite an estimated 10-fold lower dose of PAHs, the mean level of DNA damage (benzo[*a*]pyrene DNA adducts) in cord blood surpassed the levels in mothers. This indicates that children may be a more vulnerable segment of the population. Furthermore, children's increased respiratory rates expose them to proportionately greater volumes of air pollution than adults and thus they breathe in more pollutants per unit body weight [Plunkett et al. 1992]. Behaviorally, children tend to spend more time outdoors and closer to the ground where some pollutants settle and concentrate [Wiley et al., 1991; Lipsett, 1995].

Children of the inner city may be at even greater risk of exposure to air pollution and traffic. In southern California, low income areas experience greater concentrations of ozone than higher income areas [Korc, 1996]. Gunier et al. [2003] reported that in the state of California, children of color were three times more likely to live

in areas of high traffic density as compared to Caucasian children. In addition, the percentage of children living in high traffic density areas was negatively associated with median income [Gunier et al., 2003]. These data suggest that inner city children live in more high risk environments; their increased exposures to air pollution may leave them more vulnerable to its hazardous effects.

In this study, we used geographic information systems (GIS) methods and data from regional ozone monitoring to explore how exposure to outdoor air pollution affects cytogenetic damage in African American children and adults from Oakland, California. We also explored whether additional factors, including season, environmental tobacco smoke (ETS), and the use of gas appliances, contribute to cytogenetic damage in our study population. Preliminary analyses of this data established background levels and explored variability in the cytogenetic markers of these children and mothers [Gunn, 1999; Holland, 2003].

METHODS

Study Description

Healthy children and their mothers were enrolled to this study at the Pediatric Clinical Research Center at Children's Hospital, Oakland. Informed consent, approved by the IRB of Children's Hospital, Oakland, and the University of California Human Subjects Committee, was obtained for each study participant. The study population was restricted to African Americans (self-identified) to avoid potential confounding by race. In total, 65 children from 39 different households (36 females and 29 males) and 39 mothers provided a small blood sample and buccal swabs. Twenty-two of the 39 households had 2 children enrolled in the study and one of the households had 5 children in the study. At the time of sample collection, a brief questionnaire was administered. Collected information included the age and gender of the children, residential and school locations, diet, smoking in the household, proximity to gas stations, and use of gas appliances. Median income levels were estimated by using census level data based on the census tract of the subject's residence location.

The study area, which encompasses Oakland, California, is located among several major freeways and arterial roads (Fig. 1). In addition, the urban setting can be characterized by several areas of densely-traveled local roads. Residence and school locations of subjects were along the traffic corridors with varying traffic density surrounding them.

Cell Collection and MN Assay

Exfoliated Buccal Cells

Methods of cell collection, cell processing, and subsequent scoring criteria were performed as described previously [Titenko-Holland et al., 1998]. At least 1,000 cells were scored for MN frequency for 64 children and 38 mothers.

Lymphocytes

Lymphocytes were isolated from blood specimens using a Ficoll density gradient and washed twice in PBS and once in RPMI 1640 media. They were cultured at an initial density of 1×10^6 cells in 2.0 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 1.5% phy-

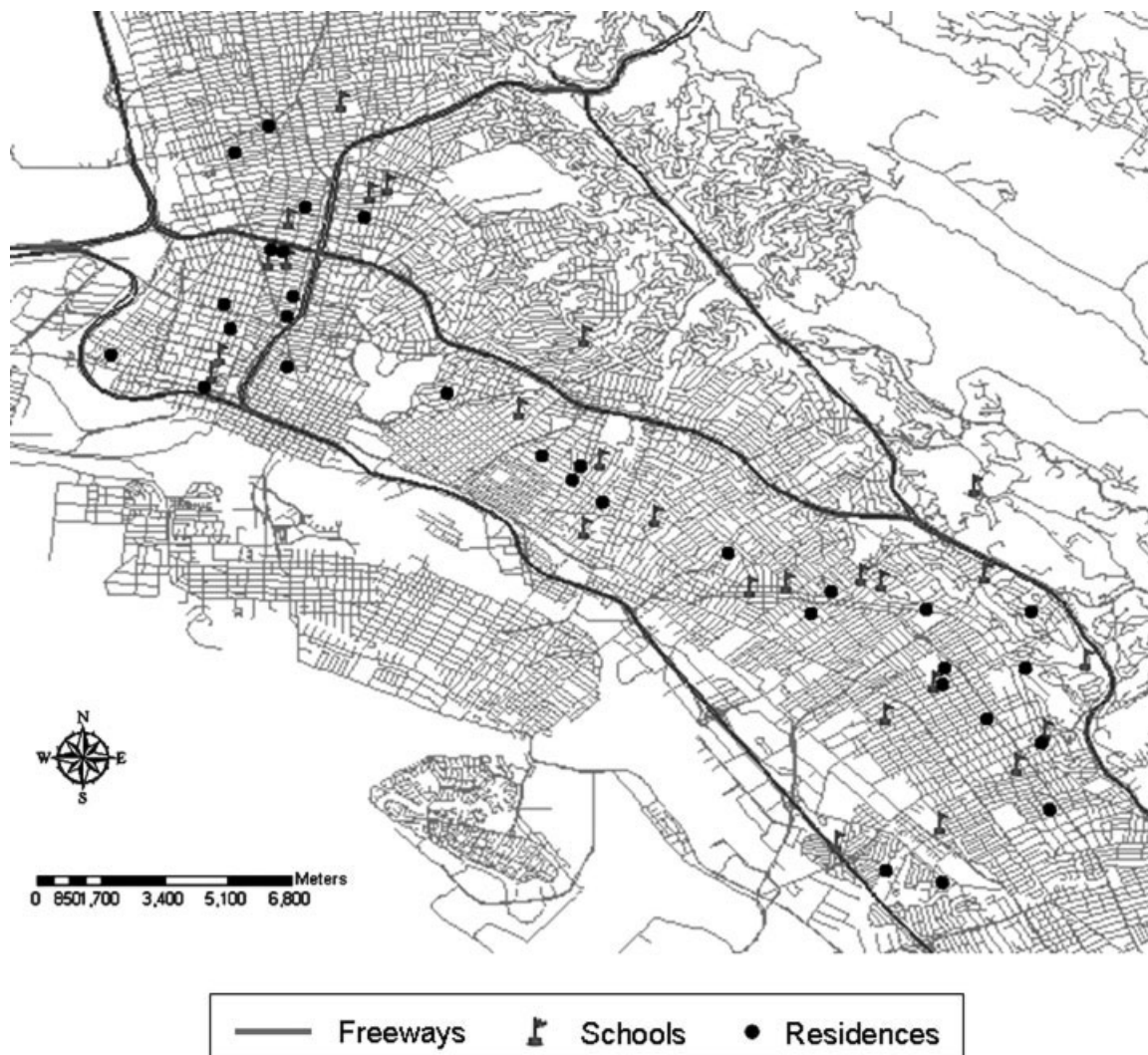


Fig. 1. Map of study area. Locations of residences and schools in Oakland, California.

tohemagglutinin. Cultures were grown in a humidified incubator with 5% CO₂ at 37°C. At 72 hr, cells were spun directly onto slides in a cytocentrifuge. The slides were allowed to air dry and then fixed with methanol. Cells were stained with 4'-diamidino-2-phenylindole (DAPI). Before scoring, the slides were randomized and coded to eliminate the potential for scoring bias. At least 1,000 cells per subject were scored for MN frequency in mononucleated lymphocytes in 40 children and 31 mothers, according to criteria described in Fenech et al. [2003].

Traffic Exposure Assessment

Residential addresses and school/daycare addresses from questionnaire data were geocoded using ArcView GIS software (ESRI, Redlands, CA) and a commercial road data layer obtained from GDT (Geographic Data Technology, Lebanon, NH). Four addresses were not included because they were outside of Alameda County and their traffic information was not available. School addresses were not available for 13 children and 10 children did not yet attend school or daycare. Annual average daily traffic (AADT) count information for roads in Alameda County during 2001 were obtained from the Highway Performance Monitoring System (HPMS) data maintained by the California Department of Transportation.

These data estimate AADT counts for freeways, state highways, and most major roads. The HPMS data were conflated onto a GDT street map layer of Alameda County. Using the geocoded addresses and the traffic data, traffic metrics for each address were generated in ArcView GIS.

To produce the traffic exposure variable, distance-weighted traffic density (DWTD), we first constructed a 300 m radius buffer around each residential and school address. For each road captured in the buffer, distance from the residence to the road and AADT counts (vehicles per day on weekdays) were recorded. The model developed by Pearson et al. [2000] was used to estimate how motor vehicle exhaust disperses from a source. This method assumes that exhaust pollutants follow a Gaussian probability distribution in which 96% of the pollutants disperse at 152.4 m (500 feet) from the road. This is modeled by the following equation:

$$Y = \left(\frac{1}{0.4\sqrt{2\pi}} \right) \times \exp \left[- \left(\frac{(0.5)(\frac{D}{500})^2}{(0.4)^2} \right) \right]$$

Y is used to weight the AADT count for each road within the buffer. D represents the shortest distance in feet from the road to the address. Thus, roads with a shorter distance D will yield a larger weight Y .

TABLE I. Demographic and Exposure Characteristics of Study Population

| | Children (<i>n</i> = 65) | | | | | Mothers (<i>n</i> = 39) | | | | |
|---|---------------------------|------|-------|-----|-------|--------------------------|------|-------|-----|-------|
| | <i>N</i> | % | Mean | Min | Max | <i>N</i> | % | Mean | Min | Max |
| <i>Continuous variables</i> | | | | | | | | | | |
| Age (years) | 63 | | 7.3 | 4 | 12 | | | | | |
| Boys | 29 | | 7.1 | 4 | 12 | | | | | |
| Girls | 36 | | 7.5 | 4 | 12 | | | | | |
| Daily fruit servings | 59 | | 2.2 | 0.0 | 6.0 | 36 | | 1.9 | 0.0 | 6.0 |
| Daily vegetable servings | 59 | | 1.7 | 0.0 | 4.5 | 32 | | 1.8 | 0.0 | 5.0 |
| Cigarettes smoked in household ^a | 65 | | 3.9 | 0.0 | 40 | 39 | | 4.0 | 0.0 | 40.0 |
| <i>Dichotomous variables</i> | | | | | | | | | | |
| Vitamin use | | | | | | | | | | |
| No | 50 | 84.7 | | | | 28 | 77.8 | | | |
| Yes | 9 | 15.3 | | | | 8 | 22.2 | | | |
| Proximity to gas station ^b | | | | | | | | | | |
| No | 21 | 32.3 | | | | 13 | 33.3 | | | |
| Yes | 44 | 67.7 | | | | 26 | 66.7 | | | |
| Use of gas appliances | | | | | | | | | | |
| No | 29 | 44.6 | | | | 18 | 46.2 | | | |
| Yes | 36 | 55.4 | | | | 21 | 53.8 | | | |
| <i>Traffic exposure variables</i> | | | | | | | | | | |
| DWTD at residence | 62 | | 11813 | 0 | 55143 | 37 | | 11115 | 0 | 55143 |
| DWTD at school | 51 | | 7209 | 0 | 64230 | | | | | |
| Weighted DWTD ^c | 48 | | 11082 | 1 | 55143 | | | | | |

DWTD: Distance weighted traffic density.

^aCigarettes smoked in the household was measured using the number of cigarettes smoked in the entire household per day.

^bSubjects living within 2 blocks of a gas station as reported by questionnaire were considered in close proximity of a gas station.

^cDWTD was weighted by time at both the residence and the school.

DWTD is the sum of the weighted AADT ($Y \cdot AADT$) counts for each road captured in the buffer and has the units average cars per day on weekdays. A weighted DWTD taking into account exposures at both schools and residences weighted by time at each location was also calculated. Briefly, the fraction of time (hour per day divided by 24 hr) in the day spent at school was multiplied by the school DWTD and the fraction of time spent at home ($[24 \text{ hr} - \text{time at school}] / 24 \text{ hr}$) was multiplied by the residential DWTD. The final weighted measure was the sum of these two calculations and represented a measure of exposure for the children at both locations.

Ozone Data

Data on maximum 8-hr ozone measurements in this region were obtained from the California Air Resources Board website [CARB, 1998], which provided daily air monitoring concentrations from the Alice Street station in Oakland. Ozone levels were measured using the ultraviolet photometry method. Daily measurements were summarized by a monthly average in subsequent statistical analysis.

Statistical Methods

SAS 9.1 (SAS, Cary, NC) and STATA 8.0 (STATA, College Station, TX) were used for statistical analysis. Negative binomial regression that treats the outcome, MN frequency (MN/1,000 cells scored), as count data was used because it takes into account the effect of over dispersion. The PROC GENMOD function in SAS was used to fit the model. For children, a random-effects negative binomial regression model to account for the clustering of data for children living at the same address was also performed using the xtnbreg function in STATA. However, the likelihood-ratio test revealed no evidence that the pooled analysis by household was different than the unpooled analysis ($P = 0.43$). Therefore, only the unpooled analysis is reported.

A backward selection method was used to determine which variables should be included in the negative binomial regression model for lymphocytes and buccal cells. Analyses in children and mothers were carried out separately. For the initial full model, factors previously linked to genetic damage in the literature were included: consumption of fruits (servings/day), consumption of vegetables (servings/day), vitamin use (yes/no), number of cigarettes smoked per day in the household, use of gas appliances (yes/no), age (children only), gender (children only), presence of gas stations within 2 blocks (yes/no), and median income by census tract [US Census Bureau, 2001]. A season variable by month of collection was also included because of the general trend of decreasing MN frequency from April to November. Since season was highly correlated to ozone levels, we also used a model that adjusted for regional ozone during the month of collection instead of season. Age and gender were not included in the models for mothers because fathers were not included in the collection and mothers' ages were not requested in the questionnaire. The traffic exposure variables used in the models included DWTD at residences, DWTD at schools, and a weighted DWTD at schools and residences. A two-sided P -value less than 0.05 was considered statistically significant, while a P -value less than 0.10 was considered marginally significant. After final models were established, frequency ratios (FRs) were calculated by exponentiation of the β coefficients. The FR represents the proportional increase of MN frequency in the study group vs. the referent group for categorical variables. For continuous variables, the FR represents the proportional increase of MN frequency due to a one unit increase in the variable. Since the DWTD variable units are difficult to define, FRs were expressed as a proportional MN frequency per increase in interquartile range (25th percentile to 75th percentile) of DWTD.

Likelihood-ratio tests were also used to test for interactions between several variables: traffic exposure \times cigarettes per day, traffic exposure \times season (month), and season \times cigarettes per day. However, none of these interactions were significant ($P \gg 0.05$, two-sided test).

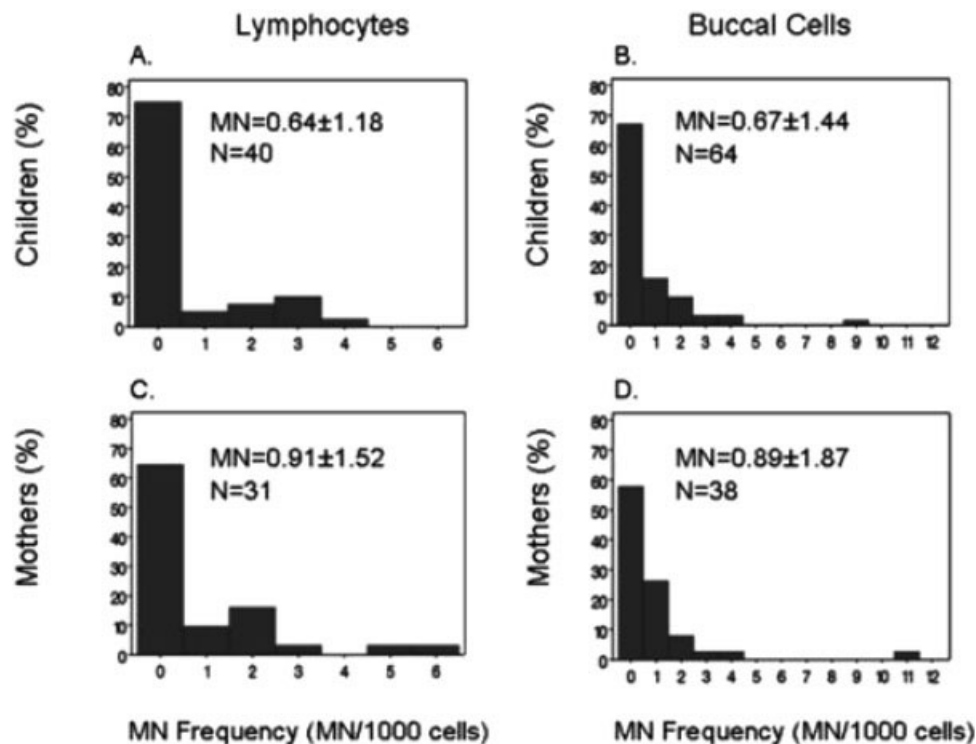


Fig. 2. Distribution of MN frequency (MN/1,000 cells) in lymphocytes and buccal cells of mothers and children. (A) MN frequency in lymphocytes of children, (B) MN frequency in buccal cells of children, (C) MN frequency in lymphocytes of mothers, and (D) MN frequency in buccal cells of mothers. The distribution of MN frequency did not differ significantly between children and mothers ($P = 0.4$).

RESULTS

Description of Study Subjects, Exposure Characteristics, and MN Frequency

Demographic and exposure characteristics of children and mothers are summarized in Table I. The average age of children was 7.3 years. Twenty-six children (40%) came from households with cigarette smoking. Nine out of 65 children (14%) and 8 out of 39 mothers (21%) took vitamins. Approximately half of the families used gas appliances and two-thirds lived in close proximity to a gas station. The median income of subjects' census tracts ranged from \$20,000–\$50,000. The average median income for the participants' census tracts (\$33,000) was about 40% lower than the overall median income for Alameda county (\$56,000)[US Census Bureau, 2001].

MN frequency was almost identical in the buccal epithelia and blood, although the ranges were wider in buccal cells (Fig. 2). For instance, MN frequency in children ranged from 0–9 MN/1,000 cells in buccal cells and 0–4 MN/1,000 cells in mononucleated lymphocytes. MN frequency in mothers was ~30% higher than in children in both cells types (0.9 vs. 0.65 MN/1,000 cells respectively). However, because of the broad interindividual variability, this difference was not statistically significant ($P = 0.4$) for either cell type.

Measures of traffic proximity also varied widely. For instance, some homes did not have attributable traffic within 300 m (DWTD = 0), while a family living close to I-880 had a DWTD of 55,143 (cars/day). DWTD at schools was significantly lower than DWTD at residences for this study population (mean DWTD: 7,209 vs. 11,813 cars/day, respectively; $P = 0.04$, paired t-test).

GIS-Modeled Traffic Exposure and Other Factors in Children

In children, several traffic exposure variables were moderately associated with mean MN frequency in lymphocytes (Table II). We found marginally significant associations between mean MN frequency and DWTD at schools ($P = 0.10$) and the weighted DWTD ($P = 0.08$). Traffic density near schools was negatively associated (FR: 0.19, 95% confidence interval (CI): 0.03–1.40), while the weighted measure was positively associated (FR: 2.45, 95% CI: 0.86–7.10). However, in buccal cells (Table III), DWTD near residences and the weighted DWTD yielded negative associations with MN frequency (FR: 0.47, 95%CI: 0.24–0.94; FR: 0.35, 95%CI: 0.11–1.10, respectively).

We also detected associations between MN frequency in lymphocytes and buccal cells of children and season

TABLE II. Associations Between Exposures and Cytogenetic Damage in Lymphocytes: Negative Binomial Regression Results

| | <i>N</i> | β (SE) | FR ^a | 95% CI | <i>P</i> | Scale deviance | Values per d.f. |
|------------------------------|----------|---------------|-----------------|--------------|----------|----------------|-----------------|
| Children | | | | | | | |
| Intercept | | 1.69 (1.46) | | | 0.25 | 26.63 | 0.76 |
| Season | | -0.52 (0.29) | 0.59 | (0.34,1.05) | 0.07 | | |
| No of cigarettes per day | | 0.06 (0.03) | 1.06 | (1.00,1.14) | 0.06 | | |
| DWTD residences ^b | 39 | 0.03 (0.02) | 1.44 | (0.77,2.74) | 0.25 | | |
| Intercept | | 3.60 (2.23) | | | 0.11 | 20.31 | 0.78 |
| Season | | -0.65 (0.36) | 0.52 | (0.26,1.05) | 0.07 | | |
| No of cigarettes per day | | 0.07 (0.03) | 1.07 | (1.00,1.14) | 0.05 | | |
| DWTD schools ^c | 30 | -0.19 (0.12) | 0.19 | (0.03,1.40) | 0.10 | | |
| Intercept | | 3.71 (2.34) | | | 0.11 | 17.99 | 0.75 |
| Season | | -1.06 (0.54) | 0.35 | (0.11,1.06) | 0.05 | | |
| No of cigarettes per day | | 0.12 (0.05) | 1.13 | (1.01,1.24) | 0.02 | | |
| DWTD weighted ^d | 28 | 0.05 (0.03) | 2.45 | (0.86,7.10) | 0.08 | | |
| Intercept | | -10.13 (4.51) | | | 0.03 | 16.4 | 0.68 |
| Ozone | | 2.60 (1.23) | 13.50 | (1.21,150.9) | 0.04 | | |
| No of cigarettes per day | | 0.08 (0.04) | 1.09 | (1.01,1.17) | 0.03 | | |
| DWTD weighted ^d | 28 | 1.20 (0.62) | 3.33 | (0.99,11.2) | 0.05 | | |
| Mothers | | | | | | | |
| Intercept | | 2.48 (1.22) | | | 0.04 | 24.74 | 1.24 |
| Season | | -0.30 (0.17) | 0.74 | (0.53,1.05) | 0.09 | | |
| No of cigarettes per day | | 0.06 (0.03) | 1.06 | (0.99,1.13) | 0.08 | | |
| Vitamin use | | | | | | | |
| Yes | | -1.78 (1.04) | 0.17 | (0.02,1.31) | 0.09 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| Gas appliances | | | | | | | |
| Yes | | -1.08 (0.63) | 0.34 | (0.10,1.16) | 0.08 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| DWTD residences ^b | 26 | -0.01 (0.02) | 0.92 | (0.53,1.59) | 0.76 | | |
| Intercept | | -3.10 (1.57) | | | 0.05 | 24.38 | 1.22 |
| Ozone | | 1.21 (0.19) | 3.37 | (1.30,8.72) | 0.01 | | |
| No of cigarettes per day | | 0.05 (0.03) | 1.05 | (0.99,1.11) | 0.08 | | |
| Vitamin use | | | | | | | |
| Yes | | -1.69 (1.01) | 0.18 | (0.03,1.35) | 0.10 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| Gas appliances | | | | | | | |
| Yes | | -0.91 (0.58) | 0.40 | (0.13,1.25) | 0.12 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| DWTD residences ^b | 26 | 0.13 (0.28) | 1.14 | (0.66,1.97) | 0.63 | | |

SE, standard error.

^aFrequency ratio is the exponentiated value of the beta coefficients. It represents the proportional change in MN frequency per unit increase for continuous variables. For categorical variables, it represents the change in MN frequency as compared to the referent group. FR for DWTD was expressed as the proportional increase for an increase of interquartile range (IQR: 25th percentile to 75th percentile).

^bInterquartile range of DWTD at residences: 1797–16032.

^cInterquartile range of DWTD at schools: 111–8535.

^dInterquartile range of DWTD weighted by time at residences and schools: 2736–19404.

(FR: 0.35, 95%CI: 0.11–1.06; FR: 0.70, 95%CI: 0.53–0.94, respectively) as MN frequency decreased from April to November (Tables II and III). In addition, we found a strong correlation (Pearson) between season by month and average monthly ozone levels (maximum 8-hr average) measured at the Alice Street air monitoring station in Oakland ($r = -0.84$, $P = 0.016$) [CARB, 1998]. Since the trends of regional ozone levels by month and MN frequency by month (lymphocytes and buccal cells) were very similar (Fig. 3), we also examined the association of ozone levels by month of collection with MN frequency (adjusted for weighted DWTD and smoking in the household in a negative binomial regression model) in both cell

types of children. This analysis indicated that higher ozone levels increased MN frequencies significantly (FR: 13.50, 95%CI: 1.21–150.9 for lymphocytes; FR: 2.56, 95%CI: 1.12–5.86 for buccal cells; FR expressed as proportional increase of micronuclei per increase of interquartile range of ozone: 0.008 ppm).

Second-hand smoke in the household also was associated with MN frequency in lymphocytes in children (FR: 1.13, 95%CI: 1.01–1.24). The following factors were not significantly associated with MN frequency in children: age and gender of children, proximity to gas stations, gas appliance use, vitamin use, fruit and vegetable intake, and median income by census tract.

TABLE III. Associations Between Exposures and Cytogenetic Damage in Buccal Cells: Negative Binomial Regression Results

| | <i>N</i> | β (SE) | FR ^a | 95% CI | <i>P</i> | Scale deviance | Value per d.f. |
|------------------------------|----------|--------------|-----------------|-------------|----------|----------------|----------------|
| Children | | | | | | | |
| Intercept | | 2.07 (0.81) | | | 0.01 | 46.15 | 0.84 |
| Season | | -0.32 (0.13) | 0.73 | (0.57,0.93) | 0.01 | | |
| DWTD residences ^b | 58 | -0.05 (0.02) | 0.47 | (0.24,0.94) | 0.03 | | |
| Intercept | | 1.54 (0.96) | | | 0.11 | 37.32 | 0.85 |
| Season | | -0.30 (0.15) | 0.74 | (0.55,0.98) | 0.04 | | |
| DWTD schools ^c | 47 | 0.00 (0.02) | 1.00 | (0.45,2.21) | 0.99 | | |
| Intercept | | 2.28 (0.94) | | | 0.02 | 33.64 | 0.82 |
| Season | | -0.35 (0.15) | 0.70 | (0.53,0.94) | 0.02 | | |
| DWTD weighted ^d | 44 | -0.06 (0.03) | 0.35 | (0.11,1.10) | 0.07 | | |
| Intercept | | 2.67 (1.32) | | | 0.04 | 33.04 | 0.85 |
| Ozone | | 0.95 (0.42) | 2.56 | (1.12,5.86) | 0.03 | | |
| DWTD weighted ^d | 44 | -0.87 (0.57) | 0.42 | (0.14,1.29) | 0.13 | | |
| Mothers | | | | | | | |
| Intercept | | 1.22 (1.17) | | | 0.30 | 26.62 | 0.95 |
| Season | | -0.33 (0.16) | 0.72 | (0.53,0.98) | 0.03 | | |
| Vitamin use | | | | | | | |
| Yes | | -1.71 (0.96) | 0.18 | (0.03,1.18) | 0.07 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| Gas appliances | | | | | | | |
| Yes | | 1.16 (0.64) | 3.19 | (0.92,11.1) | 0.07 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| DWTD residences | 33 | 0.01 (0.02) | 1.10 | (0.59,2.04) | 0.77 | | |
| Intercept | | -4.55 (1.91) | | | 0.02 | 23.36 | 0.90 |
| Ozone | | 1.10 (0.53) | 3.01 | (1.06,8.57) | 0.04 | | |
| Vitamin use | | | | | | | |
| Yes | | -1.30 (1.04) | 0.27 | (0.36,2.08) | 0.21 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| Gas appliances | | | | | | | |
| Yes | | 1.52 (0.72) | 4.57 | (1.11,18.9) | 0.04 | | |
| No | | 0.00 (0.00) | 1.00 | (1.00,1.00) | 0.00 | | |
| DWTD Residences | 33 | 0.35 (0.39) | 1.42 | 0.67,3.04) | 0.36 | | |

SE, standard error.

^aFrequency ratio is the exponentiated value of the beta coefficients. It represents the proportional change in MN frequency per unit increase for continuous variables. For categorical variables, it represents the change in MN frequency as compared to the referent group. FR for DWTD was expressed as the proportional increase for an increase of interquartile range (IQR: 25th percentile to 75th percentile).

^bInterquartile range of DWTD at residences: 1797–16032.

^cInterquartile range of DWTD at schools: 111–8535.

^dInterquartile range of DWTD weighted by time at residences and schools: 2736–19404.

Variables Affecting MN Frequency in Mothers

Traffic density was not associated with MN frequency in mononucleated lymphocytes or buccal cells of the mothers (Tables II and III). However, similar to children, both season and regional ozone levels (FR: 3.37, 95%CI: 1.30–8.72 for lymphocytes; FR: 2.99, 95%CI: 1.05–8.54 for buccal cells) were highly associated with MN frequency (adjusted for vitamin use, smoking in the household, and DWTD in a negative binomial regression model). Additionally, we found a positive association between smoking in the household and MN frequency in lymphocytes (FR: 1.06, 95%CI: 0.99–1.13). Vitamin use appeared to have a protective effect on MN frequency in adults. Compared to non-vitamin users, the mean MN frequency in vitamin users was more than 80% lower in both lymphocytes and buccal cells (FR: 0.17, 95% CI: 0.02–1.31; FR: 0.18 95% CI: 0.03–1.18, respectively). Gas appliance

use was marginally significant for MN frequency in lymphocytes; gas appliance users had lower MN frequencies compared to those who did not use gas appliances (FR: 0.34, 95%CI: 0.10–1.16). In contrast, gas appliance users had higher MN frequencies in buccal cells as compared to nongas appliance users (FR: 3.19, 95%CI: 0.92–11.1). We did not detect significant associations between MN frequency in adults and proximity to gas stations, fruit and vegetable consumption, and median income by census tract.

DISCUSSION

In this study of African American children and adults in the inner city of Oakland, California, we demonstrated strong associations between regional ozone levels and MN frequency, a common biomarker of chromosome loss or breakage, in two cell types (lymphocytes and oral epithelia) while adjusting for traffic exposure. Additionally,

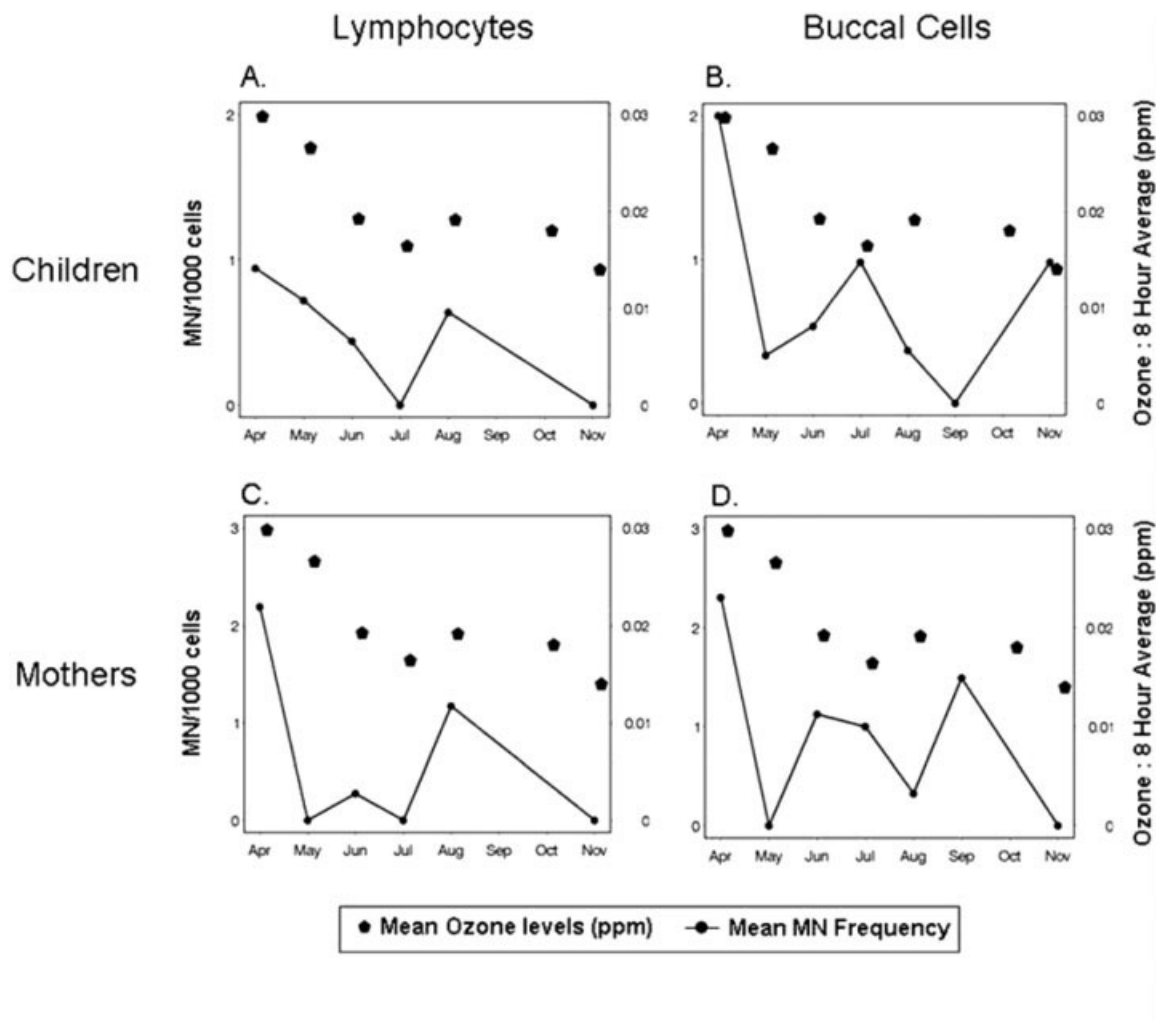


Fig. 3. Seasonal influence on cytogenetic damage. Ozone levels were measured at the Alice Street monitoring station in Oakland, California. Mean ozone levels (ppm) gradually decreased from April to November with a peak in late summer and were highly correlated with season (month), $r = -0.84$, $P = 0.016$. This seasonal trend by month of sample collection was negatively associated with cytogenetic damage in both

cell types of children and mothers: (A) lymphocytes in children, FR: 0.35, 95%CI: 0.10–1.16; (B) buccal cells in children, FR: 0.70, 95%CI: 0.53–0.94; (C) lymphocytes in mothers, FR: 0.74, 95%CI: 0.53–1.05; and (D) buccal cells in mothers, FR: 0.72, 95%CI: 0.53–0.98. MN frequencies plotted at each time point represented the average MN frequencies for all subjects recruited during that month.

we applied GIS methods to create individual measures of traffic-related exposures at residential and school/daycare addresses for each child and identified modest associations with MN frequency.

Overall, we observed similar effects of ozone in blood, most commonly used for cytogenetic monitoring in humans, and epithelial cells of the same individuals. Exfoliated buccal and urothelial epithelial cells are becoming increasingly popular for population monitoring, because the methods of cell collection are noninvasive and MN frequencies are reflective of damage to the target tissues (i.e., buccal cells in case of air pollution). In adults, MN levels in mononucleated lymphocytes are comparable to epithelial cells: 0.5–5 MN/1,000 cells, but MN frequencies in commonly studied binucleated cells are generally higher

and range from 5–25 MN/1,000 cells [Fenech et al., 1999; Kirsch-Volders and Fenech, 2001; Holland, 2003]. The similarity in response to ozone in different cells types is in agreement with studies that reported similar effects in both epithelial and blood cells in subjects exposed to metals and pesticides [Burgaz et al., 2002; Pastor et al., 2002]. However, other studies have reported dissimilar effects in cells from different tissues. For instance, studies that examined the effect of formaldehyde exposure on MN frequency observed changes in exfoliated buccal and nasal cells, but not in lymphocytes; this was later confirmed by fluorescent in situ hybridization (FISH) staining with a pancentromeric probe for two types of exfoliated cells [Suruda et al., 1993; Titenko-Holland et al., 1996].

Although cytogenetic effects of ozone were present in both adults and children, moderate associations between traffic proximity and MN frequency were detected only in children, suggesting that children may be more vulnerable to traffic pollution. In lymphocytes, we found a suggestive (FR: 1.44) positive association between chromosomal damage and DWTD at homes, but the opposite effect was seen with DWTD at schools (FR: 0.19). Further, the weighted DWTD (FR: 2.45) accounting for traffic at schools and residences was also positively associated with MN and had a stronger point estimate than DWTD at residences. While exposures to traffic exhaust at schools were lower than exposures at residences, the instability of the point estimates between the weighted DWTD and the DWTD at homes may be attributable to the relatively modest size of our cohort.

We also found a consistent effect of season among children and mothers in both cell types. Mean MN frequencies were higher in samples collected in April and steadily decreased through November with a smaller peak in July/August. Similar patterns were observed for regional ozone levels, which were typical for the Bay Area, California. Including season in the models changed regression estimates by greater than 10%; thus, season was a confounder of the relationship between traffic proximity variables and MN frequency in our models. Few studies have reported on the influence of seasonality on MN frequency. Barale et al. [1998] studied MN frequency in a large Italian cohort and reported collection time by month accounted for seasonal and experimental factors; it explained 1–4% of MN variability in their models. Pendzich et al. [1997] also saw a seasonal influence on measures of sister chromatid exchange in lymphocytes in a study of ambient air pollution in occupationally exposed men in Poland. Samples collected in February had higher mean values of cytogenetic damage than those collected in September. Neither of these studies explored possible causes of seasonal effects on air pollution.

Seasonal variation involving factors like temperature, sunlight, and wind may affect regional ozone levels [Alexis and Cox, 2005]. Our correlation analysis demonstrated a clear relationship between variation of ozone levels and the season in Oakland, California. Additionally, DWTD may also be related to ozone exposure as traffic exhaust contains ozone precursors. Thus, adjusting for seasonal ozone variation in addition to traffic density yields a more accurate exposure measure. Utilization of such a model revealed a strong association between ozone levels during the month of sample collection and genetic damage measured by MN frequency. This agrees with a study that reported cytogenetic damage in lymphocytes and buccal cells of healthy young adults as a result of acute ozone exposure [Chen et al., 2004].

In addition to the effects of air pollution via traffic-related exposure, our analysis also identified several addi-

tional variables that influenced MN frequency in children and adults. The number of cigarettes smoked per day in the household increased MN frequencies in the lymphocytes of both children and adults. Unfortunately, the questionnaire administered to subjects did not ask mothers to specify who was smoking in the household. Therefore, we could not distinguish whether the smoking variable accounted for direct or passive exposure for the mothers; in the future, we can use banked samples to measure cotinine levels in these mothers as a method to accurately determine their exposures. For children, this variable represented exposure to ETS. The results agree with Baier's study that detected differences in MN frequency between ETS exposed and unexposed children [Baier et al., 2002]. In adults, our study corroborates with Bonassi et al. [2003], who reported higher MN frequencies in the lymphocytes of heavy smokers.

Two factors were only identified as significant with mothers' MN frequency: vitamin use and gas appliance use. The effect of vitamin use, which was protective in both cell types, corroborated with prior studies [Titenko-Holland et al., 1998; Fenech, 2002]. Gas appliance use was marginally significant in both cell types. However, we saw a positive association in buccal cells and a negative association in lymphocytes. Since indoor cooking on gas stoves exposes mothers to toxic products of combustion, we would expect greater exposure to buccal cells through inhalation as compared to lymphocytes.

This study has some limitations. First, although DWTD has been used in previous studies exploring the health effects of residential proximity to traffic [English et al., 1999; Wilhelm and Ritz, 2003], this variable is a relatively simple surrogate for exposure to traffic-related pollutants. For instance, it does not account for individual components of traffic pollution, such as NO_x and particulate matter or the effect of wind and climate. Another limitation is that the traffic counts used to calculate DWTD do not distinguish between the type, fuel use, or age of the vehicles. This may result in some degree of exposure misclassification. However, since our calculations of exposure are made without knowledge of outcome (MN frequency), any misclassification is nonsystematic; this would bias our results towards the null. Additionally, our study population was limited to African American participants; further studies should explore whether similar associations can be identified in other ethnic groups. Finally, only mononucleated lymphocytes were available for analysis in this study. Several studies have used mononucleated lymphocytes to study genotoxic effects (MN assay) in human cells and reported similar trends in binucleated lymphocytes [Titenko-Holland et al., 1998; Kirsch-Volders and Fenech, 2001]. Nevertheless, it would be interesting to expand this type of analysis to binucleated lymphocytes, additional endpoints of the MN assay [Fenech et al., 2003], and other cytogenetic markers.

We used a novel approach to evaluate the effects of air pollution exposure on genetic damage. Most cytogenetic studies have been based on occupational exposures or compared groups of residents from high pollution areas vs. low pollution areas. The application of GIS methods allows us to better differentiate between individual exposures to traffic pollution via proxy measures of proximity to major roads. Further, we employed regional ozone monitoring data, which proved to be highly predictive of cytogenetic damage in children and mothers. We also adjusted for additional sources of indoor pollution like household smoking and gas appliance use. This enabled us to assess exposures to air pollution in children and their mothers more accurately. The San Francisco/Oakland Bay Area is a geographic region of relatively good air quality despite high traffic volumes. Yet, we were still able to discern some associations between air pollution exposure and chromosomal damage in children. Similar methods may yield even stronger effects in other areas of the world where traffic pollution and ozone levels are much higher. Thus, the fusion of GIS and cytogenetic methods in combination with regional ozone monitoring data appears to be a promising tool for detecting genetic damage in environmentally-exposed populations.

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REFERENCES

- Alexis A, Cox P. 2005. The California Almanac of Emissions and Air Quality. Sacramento, CA: CARB.
- Baier G, Stopper H, Kopp C, Winkler U, Zwirner-Baier I. 2002. Respiratory diseases and genotoxicity in tobacco smoke exposed children. *Laryngorhinootologie* 81:217–225.
- Barale R, Marrazzini A, Bacci E, Di Sibio A, Tessa A, Cocchi L, Scarcelli V, Lubrano V, Vassalle C, Landi S. 1998. Sister chromatid exchange and micronucleus frequency in human lymphocytes of 1,650 subjects in an Italian population: I. Contribution of methodological factors. *Environ Mol Mutagen* 31:218–227.
- Bolognesi C, Merlo F, Rabboni R, Valerio F, Abbondandolo A. 1997. Cytogenetic biomonitoring in traffic police workers: micronucleus test in peripheral blood lymphocytes. *Environ Mol Mutagen* 30:396–402.
- Bonassi S, Neri M, Lando C, Ceppi M, Lin YP, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Fenech M. 2003. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. *Mutat Res* 543: 155–166.
- Brunekreef B, Holgate ST. 2002. Air pollution and health. *Lancet* 360:1233–1242.
- Burgaz S, Demircigil GC, Yilmazer M, Ertas N, Kemaloglu Y, Burgaz Y. 2002. Assessment of cytogenetic damage in lymphocytes and in exfoliated nasal cells of dental laboratory technicians exposed to chromium, cobalt, and nickel. *Mutat Res* 521:47–56.
- Cairns J. 1975. Mutational selection and the natural history of cancer. *Nature* 255:197–200.
- Calderon-Garciduenas L, Osnaya N, Rodriguez-Alcaraz A, Villarreal-Calderon A. 1997. DNA damage in nasal respiratory epithelium from children exposed to urban pollution. *Environ Mol Mutagen* 30:11–20.
- CARB. 1998. Air Quality Data Statistics. Sacramento, CA: California Air Resources Board.
- CARB. 2005. California Almanac of Emissions and Air Quality. Sacramento, CA: California Air Resources Board. (Datafile).
- Chen CL, Arjomandi M, Balmes J, Tager I, Shigenaga M, Kadze M, Holland NT. 2004. Cytogenetic and oxidative damage from acute ozone exposure. In: Thirty-Fifth Annual Meeting of EMS, Pittsburgh, PA, October 2–6.
- Crosignani P, Tittarelli A, Borgini A, Codazzi T, Rovelli A, Porro E, Contiero P, Bianchi N, Tagliabue G, Fissi R, Rossitto F, Berrino F. 2004. Childhood leukemia and road traffic: A population-based case-control study. *Int J Cancer* 108:596–599.
- English P, Neutra R, Scalf R, Sullivan M, Waller L, Zhu, L. 1999. Examining associations between childhood asthma and traffic flow using a geographic information system. *Environ Health Perspect* 107:761–767.
- Fenech M. 2002. Micronutrients and genomic stability: a new paradigm for recommended dietary allowances (RDAs). *Food Chem Toxicol* 40:1113–1117.
- Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. 1999. The Human MicroNucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat Res* 428:271–83.
- Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat Res* 534:65–75.
- Gunier RB, Hertz A, Von Behren J, Reynolds P. 2003. Traffic density in California: socioeconomic and ethnic differences among potentially exposed children. *J Expo Anal Environ Epidemiol* 13:240–246.
- Gunn L. 1999. Biomarkers of Genetic Damage in Children of Inner City. Berkeley: University of California, Berkeley. p 48.
- Holland N. 2003. Human biomonitoring using micronucleus assay in blood and exfoliated cells. In: Cebulka-Wasilewska A, editor. *Human Monitoring for Genetic Effects*. Amsterdam: IOS Press. pp 92–102.
- IARC. 1989. IARC monographs on the evaluation of carcinogenic risk to humans. Diesel and gasoline engine exhausts and some nitroarenes. Lyon: IARC.
- Kirsch-Volders M, Fenech M. 2001. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. *Mutagen* 16:51–58.
- Korc ME. 1996. A socioeconomic assessment of human exposure to ozone in the South Coast Air Basin of California. *J Air Waste Manag Assoc* 46:547–557.
- Leopardi P, Zijno A, Marcon F, Conti L, Carere A, Verdina A, Galati R, Tomei F, Baccolo TP, Crebelli R. 2003. Analysis of micronuclei in peripheral blood lymphocytes of traffic wardens: effects of exposure, metabolic genotypes, and inhibition of excision repair in vitro by ARA-C. *Environ Mol Mutagen* 41:126–130.
- Lipsett M. 1995. The hazards of air pollution to children. In: Brooks S, editor. *Environmental Medicine*. St. Louis: Mosby.

- Neri M, Fucic A, Knudsen LE, Lando C, Merlo F, Bonassi S. 2003. Micronuclei frequency in children exposed to environmental mutagens: a review. *Mutat Res* 544:243–254.
- Neri M, Ugolini D, Bonassi S, Fucic A, Holland N, Knudsen L, Šrám R, Ceppi M, Bocchini V, Merlo D. 2005. Children's exposure to environmental pollutants and biomarkers of genetic damage. I. Overview and critical issues. *Mutat Res*. (Epub) PMID: 16027031.
- Nicolai T, Carr D, Weiland SK, Duhme H, von Ehrenstein O, Wagner C, von Mutius E. 2003. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. *Eur Respir J* 21:956–963.
- Norppa H, Heikänen H, Pfaffli P, Virtanen H. 1992. Increased micronuclei in buccal mucosa cells but not in blood lymphocytes of formaldehyde exposed workers. *Pharmacol Toxicol* 70:22
- Norppa H, Luomahaara S, Heikänen H, Roth S, Sorsa M, Renzi L, Lindholm C. 1993. Micronucleus assay in lymphocytes as a tool to biomonitor human exposure to aneuploidogens and clastogens. *Environ Health Perspect* 101(Suppl 3):139–143.
- Pastor S, Creus A, Xamena N, Siffel C, Marcos R. 2002. Occupational exposure to pesticides and cytogenetic damage: results of a Hungarian population study using the micronucleus assay in lymphocytes and buccal cells. *Environ Mol Mutagen* 40:101–109.
- Pearson RL, Wachtel H, Ebi KL. 2000. Distance-weighted traffic density in proximity to a home is a risk factor for leukemia and other childhood cancers. *J Air Waste Manag Assoc* 50:175–180.
- Pelevina II, Aleshchenko AV, Antoshchina MM, Kudriashova OV, Kurneshova LE, Gotlib V, Noskin LA, Noskin VA, Semanova LP, Serebrianyi AM. 2001. [Level of spontaneous and radiation-induced cytogenetic damage in blood lymphocytes of children depending on age and life style]. *Radiats Biol Radioecol* 41:573–579.
- Pendzich J, Motykiewicz G, Michalska J, Wang LY, Kostowska A, Chorazy M. 1997. Sister chromatid exchanges and high-frequency cells in men environmentally and occupationally exposed to ambient air pollutants: an intergroup comparison with respect to seasonal changes and smoking habit. *Mutat Res* 381:163–170.
- Perera FP, Tang D, Tu YH, Cruz LA, Borjas M, Bernert T, Whyatt RM. 2004. Biomarkers in maternal and newborn blood indicate heightened fetal susceptibility to procarcinogenic DNA damage. *Environ Health Perspect* 112:1133–1136.
- Plunkett L, Turnbull D, Rodricks J. 1992. Differences between adults and children affecting exposure assessment. In: Guzelian P, Henry C, Olin S, editors. *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*. Washington, DC: ILSI Press. pp 79–96.
- Suruda A, Schulte P, Boeniger M, Hayes RB, Livingston GK, Steenland K, Stewart P, Herrick R, Douthit D, Fingerhut MA. 1993. Cytogenetic effects of formaldehyde exposure in students of mortuary science. *Cancer Epidemiol Biomarkers Prev* 2:453–460.
- Titenko-Holland N, Levine AJ, Smith MT, Quintana PJ, Boeniger M, Hayes R, Suruda A, Schulte P. 1996. Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat Res* 371:237–248.
- Titenko-Holland N, Jacob RA, Shang N, Balaraman A, Smith MT. 1998. Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. *Mutat Res* 417:101–114.
- US Census Bureau. 2001. 2000 Census. Washington, DC: US Census Bureau.
- Wiley J, Robinson J, Piazza T. 1991. Activity patterns of California residents: final report. Report nr Publication No. A6-177–33. California Air Resources Board, Sacramento, CA.
- Wilhelm M, Ritz B. 2003. Residential proximity to traffic and adverse birth outcomes in Los Angeles county, California, 1994–1996. *Environ Health Perspect* 111:207–216.
- Zhao X, Niu J, Wang Y, Yan C, Wang X, Wang J. 1998. Genotoxicity and chronic health effects of automobile exhaust: a study on the traffic policemen in the city of Lanzhou. *Mutat Res* 415:185–190.
- Zhu Y, Hinds WC, Kim S, Sioutas C. 2002. Concentration and size distribution of ultrafine particles near a major highway. *J Air Waste Manag Assoc* 52:1032–1042.

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