Urinary Naphthol Metabolites and Chromosomal Aberrations in 5-Year-Old Children

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Abstract

Background: Exposure to naphthalene, an International Agency for Research on Cancer (IARC)-classified possible carcinogen and polycyclic aromatic hydrocarbon (PAH), is widespread, though resulting health effects are poorly understood. Metabolites of naphthalene, 1- and 2-naphthol, are measurable in urine and are biomarkers of personal exposure. Chromosomal aberrations, including translocations, are established markers of cancer risk and a biodosimeter of clastogenic exposures. Although prenatal (maternal) PAH exposure predicts chromosomal aberrations in cord blood, few studies have examined chromosomal aberrations in school-age children and none has examined their association with metabolites of specific PAHs.

Methods: Using Whole Chromosome Paint Fluorescent *in situ* Hybridization, we documented chromosomal aberrations including translocations, in 113 five-year-old urban minority children and examined their association with concurrent concentrations of PAH metabolites measured in urine.

Results: We report that in lymphocytes, the occurrence and frequency of chromosomal aberrations including translocations are associated with levels of urinary 1- and 2-naphthol. When doubling the levels of urinary naphthols, gender-adjusted OR for chromosomal aberrations are 1.63 [95% confidence interval (CI), 1.21–2.19] and 1.44 (95% CI, 1.02–2.04) for 1- and 2-naphthol, respectively; and for translocations OR = 1.55 (95% CI, 1.11–2.17) and 1.92 (95% CI, 1.20–3.08) for 1- and 2-naphthol, respectively.

Conclusion: Our results show that markers of exposure to naphthalene in children are associated with translocations in a dose-related manner, and that naphthalene may be a clastogen.

Impact: Indoor exposure to elevated levels of naphthalene is prevalent in large regions of the world. This study is the first to present an association between a marker of naphthalene exposure and a precarcinogenic effect in humans. *Cancer Epidemiol Biomarkers Prev; 21(7); 1191–202. ©2012 AACR.*

Introduction

Naphthalene, the smallest polycyclic aromatic hydrocarbon (PAH) and an International Agency for Research on Cancer (IARC) possible carcinogen, is ubiquitous in ambient air with high volumes of vehicular traffic (1) and is elevated in indoor air when mothballs or stoves burning biomass fuels are used (2–4). Naphthalene, a 2-ring PAH, is derived from petroleum and biofuel products and, like other small PAHs, is found entirely in gas phase rather than as a particulate. Human exposure to naphthalene is primarily through inhalation, though ingestion and dermal absorption can be major contributors to exposure in both occupational and nonoccupational settings (5–7). Because of the volatile nature of naphthalene, a box of naphthalene-containing mothballs can elevate indoor naphthalene levels to levels compatible with mid to upper level occupational exposure, with higher ambient concentrations in smaller apartments or enclosed areas (2). Some Caribbean immigrant families in New York City (NYC) report using naphthalene-containing mothballs as air fresheners (with resulting increased rates of hemolytic anemia; ref. 8). Indoor exposure to naphthalene affects more than half of the world's population and represents a potentially important environmental contributor to the global burden of disease (3, 4, 7). Understanding potential downstream effects of naphthalene exposure has become increasingly relevant.

Once inhaled or ingested, naphthalene is metabolized by cytochrome P450 enzymes to form naphthalene oxide, which are rearranged to 1- and 2-naphthol. These metabolites are then conjugated with glucuronic acid and

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sulfate to be excreted in the urine and have been used extensively as biomarkers for exposure (9, 10). Measurements of urinary 1- and 2-naphthol have shown consistently detectable levels in almost all samples analyzed including 100% of National Health and Nutrition Survey (NHANES) samples tested (11). Urinary levels of 1- and 2-naphthol are markers of occupational (12), vehicular traffic, household (5), and infant mothball exposure (13) and correlate significantly with naphthalene vapor levels in personal air monitors (5, 14).

Urinary metabolites of naphthalene can be useful markers for measuring carcinogenically relevant exposures to naphthalene, as elevated levels of urinary naphthols predict levels of sperm DNA damage (15). Downstream metabolites of 1-naphthol include 1,4-dihydroxynaphthalene and 1,4-naphthoquinone; whereas 2-naphthol is metabolized to dihydrodiol and 1,2-naphthoquinone (16) which can also be generated through a 1,2-epoxide intermediate (17). These quinone metabolites have been associated with DNA adducts and marrow stem cell toxicity (8, 13).

Little is known about the direct carcinogenicity of naphthalene; though, there have been reports of laryngeal carcinoma with occupational exposure and colorectal carcinoma in young adults after naphthalene ingestion (18, 19). In occupationally exposed agricultural workers, levels of 2-naphthol in urine predict levels of DNA damage in sperm (15). Increasing levels of DNA strand breakage in lymphocytes correlate with increased air levels of naphthalene (20). Naphthyl-keratin adducts (derived from naphthalene oxide) have been documented in skin of exposed jet fuel workers (21).

Ex vivo exposure of cord blood mononuclear cells to naphthalene and its metabolites has resulted in impaired formation of granulocyte-monocyte colony-forming units (22), suggesting potential stem cell susceptibility and oxidative damage with elevated exposure, whereas exposure to mothballs causes hemolytic anemia in individuals with G6PD deficiency (8, 13). In rodent models using inhalational exposure to naphthalene, dose-dependent cytotoxic effects of naphthalene, mediated by an oxidative mechanism, have been noted in bronchiolar epithelial Clara cells (23), with concentration-dependent increases in bronchiolar-alveolar adenomas in mice (24), and olfactory epithelial neuroblastomas in rats (25). *In vitro* and *in vivo* work has showed both stable and depurinating glutathione adducts derived from a topical exposure to 1,2-naphthoquinone in mice (23, 26). However, there has not been evidence associating naphthalene with clastogenic damage in humans.

Chromosomal aberrations are an established marker of cancer risk and are a biodosimeter of genotoxic exposures in adults (27). Translocations, the most persistent subtype of chromosomal aberrations, with half-lives of 2 to 4 years (28, 29) documented after either ionizing radiation or mixed chemical occupational exposures, are considered the most meaningful cytogenetic endpoint for assessing cancer risk (30, 31). In newborns from the Columbia

Center for Children's Environmental Health (CCCEH), an urban birth cohort of underprivileged Dominican and African-American children in NYC, we have documented that chromosomal aberrations and translocations in cord blood are associated with prenatal maternal exposure to air PAH (32).

Levels of urinary PAH metabolites in spot urines from a subset of 221 school age CCCEH participants were previously compared with data from NHANES (01-02) and were reported to range 1.6- to 2.5-fold higher for metabolites of naphthalene and 1-hydroxypyrene (1-OHP) whereas levels for metabolites for other fuel derived semivolatile 3-ring PAHs, phenanthrene and fluorene, were consistent with national data (11, 33).

The objectives of this study were to evaluate (i) whether urinary PAH metabolite measurements predicted occurrence and frequency of chromosomal aberrations and translocations in young school-age children, (ii) whether the association between chromosomal aberrations and PAH might differ depending on the family of small-ringed PAHs examined, and (iii) whether risk of translocations varied with levels of naphthalene metabolites.

Materials and Methods

Study population

The Harlem, Bronx, and Washington Heights—CCCEH—longitudinal birth cohort consists of 697 mother and child pairs followed since pregnancy to examine prenatal effects of air pollutants on health outcomes. Many CCCEH mothers lack a high school diploma (25%), and 45% reported annual household incomes below $10,000 during pregnancy (33). The cohort has 83% retention at age 3 years (34). Children who reached their fifth birthday between February 2005 and December 2007 were entered into an additional study (N = 222) that examined predictors of asthma and allergy at age 5 (33). Blood and spot urine samples and PAH-exposure questionnaires querying about the 48 hours before the urine collection were collected as previously described (33). We processed an aliquot of fresh blood from those children whose blood and urine samples were collected concurrently after January 2006 (N = 113) using Whole Chromosome Paint Fluorescent *in situ* hybridization (WCP-FISH). All available fresh blood samples that had corresponding spot urine sample measurements were included. All participating mothers signed an approved consent in accordance with the Institutional Review Board of the Columbia University Medical Center (New York, NY). The Centers for Disease Control and Prevention (CDC) laboratory's role was determined to not constitute engagement in human subjects research.

PAH metabolites in urine

In the CCCEH laboratory, spot urine samples were aliquotted and frozen ($-80°C$) before shipping to the CDC NCEH Laboratories to be analyzed for PAH metabolites including 1- and 2-naphthol as previously described (11, 33). Enzymatic deconjugation, followed by automated

liquid–liquid extraction and quantified by gas chromatography/isotope dilution high-resolution mass spectrometry (GC-IDHRMS) was used for analytical determination of urinary PAH concentrations. To control for differences in urine dilution, specific gravity was measured using a handheld refractometer as previously described (33).

Chromosome aberration scoring by WCP-FISH

Aberrations are scored in T lymphocytes that have a half-life of up to a year *in vivo*. Therefore, detected aberrations should reflect exposures occurring in recent months. At the time of routine follow up visits for 5-year-old CCCEH participants, 0.8 mL of blood was collected in a heparinized tube and kept at room temperature until processed for WCP-FISH. Fresh samples were cultured and hybridized using the procedures described previously (32). In brief, samples were cultured for 72 hours in PB-Max Complete Media (Invitrogen) at 37°C using standard techniques which preferentially expand T lymphocytes and including replicate cultures for each sample when possible. We used individual WCP (Cytocell) for chromosomes 1, 2, and 4 in red and chromosomes 3, 5, and 6 in green. The colors were chosen to facilitate distinguishing individual chromosomes by a combination contrasting colors and morphology. We focused on chromosomes 1 to 6, which together comprise 39% of the human genome, to be comparable with prior work from our group and others (30, 35–38). For each case, chromosomes 1 to 6 were hybridized on slides using 4′,6-diamidino-2-phenylindole (DAPI) counter stain (Cytocell). A 6q subtelomere-specific probe (RP11-307K1 and RP11-292F10) was incorporated to assist in differentiating chromosomes 5 and 6.

The 6q subtelomere-specific probe (RP11-307K1 and RP11-292F10) was generated as previously described (32) using Spectrum Red dUTP with a nick translation labeling kit (Vysis). One microliter of the resulting red 6qtel probe was applied to each slide at the time of applying the WCP mixture for chromosomes 1 to 6 with hybridization and washing as described previously (32). FISH signals were visualized using a fluorescence microscope (Olympus Bx-UCB; Olympus) equipped with appropriate filters (FITC, TRITC, and DAPI) and CytoVision software (Genetix).

Scoring was carried out by trained clinical cytogenetic technicians who were blinded to identification or exposures of the samples. Inclusion criteria for metaphases to be scored included good spread, absence of broken metaphases, readable color signal intensity, and complete visualization of 12 chromosomes and visualization of each centromere. Metaphases containing aneuploidy were not scored for aberrations. All abnormalities found were recorded with their given coordinates and photographed using CytoVision. Questionable aberrations were confirmed using the inverse DAPI feature of CytoVision. All potential chromosomal aberrations were reviewed independently by the principal study cytogeneticist (D. Warburton) who also was blinded to exposure and subject identity. Only those approved and classified by D. Warburton were entered into the study database.

Chromosomal aberrations were classified morphologically and breaks were tabulated according to the PAINT system (30, 39). Only chromosomal aberrations containing clearly visible centromeres were considered "stable" (able to persist in subsequent cell divisions) and were counted in subsequent analyses. Translocations (unbalanced and balanced) were counted as chromosomal aberrations but also were analyzed separately given their recognized advantage for documenting carcinogenically relevant changes (30, 31). For each sample, 750 metaphases (>425 cell equivalents) were scored and all potential chromosomal aberrations were reviewed. Aberration frequency was calculated per 100 cell equivalents to be consistent with other cohorts in which WCP-FISH is measured (30) and based on the proportion of the genome painted (40). Cells with aneuploidy were not included in those scored for stable aberrations and thus did not go into the calculation of aberration frequency or genome equivalence.

Unidentified red chromosomes were presumed to represent either chromosomes 1, 2, or 4, and unidentified green chromosomes were presumed to be composed of either 3, 5, or 6p. Fragments without centromeres and aneuploidies were not considered stable and were excluded from subsequent analyses.

WCP-FISH scoring results were noted by study staff and entered along with chromosome location, slide quadrant, and aberration type. The aberration and translocation frequencies were calculated from the number of chromosomal aberrations per number of cells (metaphases) analyzed, adjusted for 100 cell equivalents using the correction factors calculated from the proportion of the genome painted simultaneously in a given slide using the formula for 2 color paints by Lucas (40). Ascribed genetic content was based on the Human Genome Project bp delineation as previously described (32).

Statistical analysis

Summary statistics were calculated to describe sample characteristics. To compare 2 groups such as those with and without WCP-FISH, or the 2 ethnic groups, Dominicans and African-Americans, we used the *t* test or Wilcoxon test for continuous variables and the χ^2 or Fisher exact tests for categorical variables. Variables for naphthalene exposure were also compared between the children with and without chromosomal aberrations and with or without translocations. Spearman correlation coefficient was calculated for describing bivariate associations between quantitative variables.

Chromosomal aberration frequency was defined as number of chromosomal aberrations per 100 cell equivalents and translocation frequency as the number of translocations per 100 cell equivalents (32, 37). Urinary PAH levels had a skewed distribution and were logarithmically transformed to meet assumptions for *t* tests and to reduce the impact of extreme values when used as the main predictor in the models for presence or frequencies of

Table 1. Study population with urine PAH measures at age 60 months, comparison of those with and without WCP-FISH

Variables	Blood sample analyzed with WCP-FISH (n = 113) % (*n*)	Blood sample not analyzed with WCP-FISH (n = 108) % (*n*)
Ethnic groups		
Dominican	58 (66)	60 (65)
African-American	42 (47)	40 (43)
Sex		
Female	56 (63)	54 (58)
Male	44 (50)	46 (50)
Child around smokers during 48 hbefore urine collection	25 (27)[a]	11 (12)
Mother received medicaid during pregnancy[b]	89 (100)	91 (98)
Mother with less than HS diploma[b]	38 (43)	36 (38)
	Geometric mean (min–max)	Geometric mean (min–max)
SG adjusted 1-naphthol (ng/L)	3,400 (78.0–161,000)	2,489 (78–154,000)
SG adjusted 2-naphthol (ng/L)	4,170 (147–132,000)	3,930 (703–27,600)
SG adjusted 1-OH pyrene (ng/L)	171 (15–2,580)	151 (20.0–2,420)

Abbreviation: SG, specific gravity.

[a]Proportion of children with smokers in the house between children analyzed by WCP-FISH and those without WCP-FISH differs by χ^2, $P = 0.02$.

[b]Data obtained during pregnancy.

chromosomal aberration or translocation. Overall exposure variables were created by summing the metabolites derived from each parent PAH as done for analyses with urinary PAH data from NHANES (11). NAPH, the overall exposure variable for naphthalenes, is the sum of 1- and 2-naphthol; for fluorenes, FLUOR is the sum of 2-, 3-, 9-OH fluorene. For pyrene, PYR is 1-OH pyrene as it is the only pyrene metabolite measured. For phenanthrene, PHEN is the sum of 1-, 2-, 3-, and 4-OH phenanthrene.

To examine the effect of naphthalene exposure variables, logistic regression models were used for binary outcomes for chromosomal aberrations and translocations (presence vs. absence) and negative binomial models were used for the outcome variables of frequencies of chromosomal aberration and translocations. To aid in the interpretation of the associations with 1- and 2-naphthol, covariate adjusted ORs, along with their confidence intervals (CI), were derived from the parameters in logistic models. Similarly, covariate adjusted mean ratios of frequencies of chromosomal aberrations and translocations, along with their 95% CI, were derived from the parameters obtained in negative binomial models. Because the naphthalene metabolite levels varied between the 2 ethnic groups in our sample and patterns of exposure to naphthalene might vary by gender, we controlled for ethnicity and child's sex in the analysis with the whole sample (N = 113) and controlled for child's sex in ethnic group-specific analyses. We used the Wald test to detect ethnic group differences in the model parameters documenting the effect of exposure. Statistical significance level of the tests was set at 0.05 and statistical analyses were conducted with SAS 9.3.1 and SPSS18.

Results

Of our 221 children with spot urine samples, 113 children had fresh blood samples that met criteria for cytogenetic analysis. These 113 children did not differ in sex, ethnicity, levels of maternal education, or use of medicaid, compared with the 108 children that did not have blood samples processed for WCP-FISH. Children whose blood samples were processed for WCP-FISH were more likely to have been in the presence of a smoker in the 48 hours before urine collection. Levels of urinary 1- and 2-naphthol as well as urinary 1-OHP did not differ between the 2 groups (Table 1).

The 5-year olds have mean frequencies of 0.154 (SD, 0.272) for chromosomal aberrations and 0.079 (SD, 0.197) for translocations, which is in the reported range for children ages 5 to 9 (mean, 0.15; range, 0.07–0.32; ref. 30). However, aberration data on children (after birth) have only been reported for 38 children ages 5 to 9 and only 7 with >300 cell equivalents scored (30). Table 2 includes the characteristics of children with chromosomal aberrations and translocations. In keeping with the PAINT convention (30, 39), translocations included balanced and unbalanced translocations. One translocation had a dicentric chromosome. There did not appear to be clonal rearrangements, nor were there recurring chromosomal rearrangements.

Mothers of African-American (N = 47) and Dominican (N = 66) participants did not differ in demographic characteristics such as education or eligibility for medicaid during pregnancy (Table 2). At age 5 years, African-American children were more likely to live in a home with a smoker, but the 2 groups did not differ in the proportion

Table 2. Demographic characteristics and biomarker levels by ethnic group

Demographic variables	Dominican (n = 66) % (n)	African-American (n = 47) % (n)
Sex (n = 66, 47)		
Female	57.6 (38)	53.2 (25)
Male	42.4 (28)	46.8 (22)
Smoker in home (n = 66, 46)[a]		
Yes	16.7 (11)	37.0 (17)
No	83.3 (55)	63.0 (29)
Child around smoker during 48 hours before urine collection (n = 63, 46)		
Yes	23.8 (15)	26.1 (12)
No	76.2 (48)	73.9 (34)
Mother without a high school diploma[b] (n = 65, 47)		
Yes	38.5 (25)	38.3 (18)
No	61.5 (40)	61.7 (29)
Mother received medicaid during pregnancy[b] (n = 66, 46)		
Yes	90.9 (60)	87.0 (40)
No	9.1 (6)	13.0 (6)
Specific gravity adjusted urinary naphthol levels	Geometric mean (min–max); 95% CI	Geometric mean (min–max); 95% CI
1-Naphthol	3,340 (329–138,000); (2,330–4,770)	3,490 (78.0–161,000); (2,250–5,430)
2-Naphthol[c]	5,260 (200–132,000); (3,960–6,970)	3,010 (147–17,000); (2,340–3,860)
NAPH[d]	10,800; 95% CI (8,210–14,200)	7,660; 95% CI (5,440–10,800)
Unadjusted urinary naphthol levels	Geometric mean (Min–Max)	Geometric mean (Min–Max)
1-Naphthol	2,970 (113–138,000)	2,890 (82.0–67,700)
2-Naphthol	4,680 (246–52,900)	2,470 (150–10,300)
Chromosomal aberrations	% (n)	% (n)
Translocation		
Yes	16.7 (11)	19.1 (9)
No	83.3 (55)	80.9 (38)
Stable aberration		
Yes	30.3 (20)	31.9 (15)
No	69.7 (46)	68.1 (32)
Mean aberration frequency	Mean (SD)	Mean (SD)
Translocation	0.072 (0.179)	0.092 (0.233)
Stable aberration	0.148 (0.252)	0.155 (0.299)

[a]Proportion of children with smokers in the house between African-American and Dominican children differs by χ^2, $P = 0.02$.
[b]Data obtained during pregnancy.
[c]Difference in 2-naphthol levels (ln-transformed) between 2 ethnic groups is significant, $P = 0.007$; no other variables differed between the 2 ethnic groups.
[d]For NAPH, only 95% CI values are given.

of children who were in the presence of a smoker during the 48 hours before the urine collection. Levels of 1-naphthol did not differ by ethnicity, but 2-naphthol levels were significantly higher in Dominicans. Neither chromosomal aberration or translocation frequency, nor the proportion of children with chromosomal aberrations or translocations differed between the 2 ethnic groups.

Comparison of CCCEH versus NHANES urinary PAH metabolites

Adjustments for dilution were conducted using specific gravity to avoid misclassification reported previously with adjustments using spot creatinine in children with lower muscle mass, pulmonary disease, or using corticosteroids or β agonist inhalers (33, 41). Specific gravity levels ranged from 1.003 to 1.035 and did not vary with presence or frequency of chromosomal aberrations or translocations.

We examined the ranges of unadjusted urinary metabolite levels from our subgroup of 113 of these 5-year olds compared with fresh weight (unadjusted) levels from the youngest members of the 01-02 NHANES cohort (ages 6–11) measured in the same laboratory.

Table 3. Comparison of geometric mean specific gravity adjusted summed urinary metabolite levels (ng/L) in urine samples between children with and without chromosomal aberrations (stable) or translocations

	Stable aberration [geometric mean (min–max)]		Translocation [geometric mean (min–max)]	
	Present (n = 35)	Absent (n = 78)	Present (n = 93)	Absent (n = 20)
NAPH	12,900[a] (972–191,000)	8,100 (224–168,000)	17,300[b] (1,840–191,000)	8,200 (224–168,000)
PYR	205.14 (25.0–2,580)	157 (15.0–1,580)	195 (25.0–2,580)	166 (15.0–1,900)
FLUO	815 (96.7–9,610)	669 (71.0–5,020)	816 (128–9,610)	691 (71.0–5,010)
PHEN	476 (63.4–3,850)	411 (29.0–3,390)	468 (70.2–3,850)	423 (29.0–3,390)

[a]Difference between ln-transformed NAPH levels for presence and absence of stable aberration is significant ($P < 0.05$).
[b]Difference between ln-transformed NAPH levels for presence and absence of translocation is significant ($P = 0.009$).

Geometric means of 1- and 2-naphthol were 2,871 and 2,472 ng/L, respectively, among African-American children, and 2,967 and 4,675, respectively, in Dominican children.

Among the NHANES cohort geometric means of 1-naphthol was 1,430 ng/L (95% CI, 1,170–1,730) and 2-naphthol was 1,690 ng/L (95% CI, 1,560–1,840; ref. 11). Levels of naphthalene metabolites, in particular, 2-naphthol, in these 113 CCCEH children range higher than those of children in NHANES, though when compared with the equivalent ethnic groups in NHANES, our median levels appear in the range of the 75th percentile of NHANES for both non-Hispanic blacks and Hispanics (11).

Urinary naphthalene metabolites (specific gravity adjusted) and chromosomal aberrations

Among those factors examined in our study, the only difference noted between children with blood samples collected for analysis by WCP-FISH and those whose blood was not processed for FISH was that those collected for FISH were from children that were more likely to be in the presence of a smoker during the 48 hours before their urine collection (Table 1). However, a child's presence in the company of a smoker during the 48 hours before the urine collection did not predict a child's 1- and 2-naphthol levels, chromosomal aberration, translocation, or the relationship between naphthol levels and chromosomal aberration (data not shown). As a result, whether or not the child was in the company of a smoker was not used as a control variable. The proportion of smoking at home in 47 African-Americans (36.96%) was higher than in 66 Dominicans (16.67%; χ^2 test, $P = 0.025$), however presence of chromosomal aberrations and presence of translocations did not differ between genders or ethnic groups. Consistent with findings in newborns, both presence and frequency of chromosomal aberrations did not differ with exposure to passive smoking (8, 21).

In the 113 children with measurements for both urinary PAH metabolites and chromosomal aberrations, we examined whether levels of urinary PAH metabolites predicted whether a child would have at least one chromosomal aberration (of any type) or a translocation (Table 3). Levels of NAPH and 1- and 2-naphthol differed significantly between children with and without translocations. Levels for NAPH and 1-naphthol differed significantly between children with and without chromosomal aberrations. Urinary metabolite levels for PYR, FLUO, and PHEN did not differ with occurrence of either chromosomal aberrations or translocations (Table 3).

Associations between frequencies of chromosomal aberrations and urinary PAH metabolites for our population of 113 5-year olds are shown in Table 4. Bivariate correlations between frequencies of chromosomal aberrations and 1- and 2-naphthol as well as the NAPH summed term, stratified by ethnicity suggest an ethnic disparity in

Table 4. Bivariate association between naphthalene and frequencies of chromosomal aberrations and translocations

	Spearman correlation (r)	
All 5-year olds (n = 113)	Chromosomal aberration frequency	Translocation frequency
1-Naphthol	0.204[a]	0.165
2-Naphthol	0.053	0.173
NAPH	0.192[a]	0.235[a]
African-American (n = 47)		
1-Naphthol	−0.090	0.006
2-Naphthol	−0.163	0.086
NAPH	−0.080	0.147
Dominican (n = 66)		
1-Naphthol	0.387[b]	0.276[a]
2-Naphthol	0.180	0.270[a]
NAPH	0.380[b]	0.334[b]

Abbreviation: r = Spearman coefficient.
[a]Correlation is significant at the 0.05 level.
[b]Correlation significant at the 0.01 level.

Table 5. OR for presence of chromosomal aberrations and translocation with doubling of naphthalene metabolite levels and mean ratio of frequency of chromosomal aberrations and translocation with doubling of naphthalene metabolite levels

Occurrence of	Total (n = 113)		Dominican (n = 66)	African-American (n = 47)	Ethnic group difference[c]
	OR (95% CI)	AOR[a] (95% CI)	AOR[b] (95% CI)	AOR[b] (95% CI)	P
Aberration					
1-Naphthol	1.23 (1.02–1.48)[e]	1.23 (1.01–1.48)[e]	1.63 (1.21,2.19)[f]	0.90 (0.68–1.20)	0.005
2-Naphthol	1.17 (0.90–1.51)	1.19 (0.90–1.56)	1.44 (1.02–2.04)[e]	0.77 (0.46–1.27)	0.04
NAPH	1.27 (.997–1.63)[d]	1.29 (1.00–1.65)[e]	1.95 (1.29–2.95)[f]	0.84 (0.58–1.23)	0.003
Translocation					
1-Naphthol	1.25 (1.00–1.56)[d]	1.27 (1.01–1.59)[e]	1.55 (1.11–2.17)[e]	1.03 (0.74–1.43)	0.09
2-Naphthol	1.50 (1.08–2.09)[e]	1.69 (1.16–2.46)[f]	1.92 (1.20–3.08)[f]	1.24 (0.65–2.39)	0.29
NAPH	1.47 (1.09–1.99)[e]	1.55 (1.13–2.12)[f]	2.15 (1.30–3.57)[f]	1.12 (0.73–1.73)	0.06
Frequency of	Total (n = 113)		Dominican (n = 66)	African-American (n = 47)	Ethnic group difference[c]
	MR (95% CI)	MR[g] (95% CI)	MR[h] (95% CI)	MR[h] (95% CI)	P
Aberration					
1-Naphthol	1.16 (0.97–1.39)	1.18 (0.98–1.42)[i]	1.38 (1.10–1.72)[f]	0.90 (0.65–1.25)	0.049
2-Naphthol	1.09 (0.87–1.37)	1.11 (0.87–1.41)	1.18 (0.89–1.57)	0.98 (0.62–1.55)	0.54
NAPH	1.19 (0.95–1.50)	1.23 (0.96–1.57)[i]	1.43 (1.07–1.92)[e]	0.95 (0.63–1.44)	0.14
Translocation					
1-Naphthol	1.13 (0.87–1.47)	1.16 (0.89–1.51)	1.33 (0.95–1.85)[i]	0.93 (0.60–1.45)	0.24
2-Naphthol	1.28 (0.93–1.78)	1.43 (1.02–1.99)[e]	1.38 (0.96–1.98)[i]	1.79 (0.78–4.08)	0.61
NAPH	1.30 (0.92–1.84)	1.42 (1.00–2.02)[e]	1.53 (1.04–2.26)[e]	1.24 (0.64–2.42)	0.62

Abbreviation: MR, mean ratio.
[a]AOR: gender and ethnicity adjusted OR.
[b]AOR: gender adjusted OR.
[c]Wald test was used for difference between ethnic groups.
[d]$P < 0.06$.
[e]$P < 0.05$.
[f]$P < 0.01$.
[g]MR: gender and ethnicity adjusted.
[h]MR: gender adjusted.
[i]$0.05 < P < 0.10$.

the association between chromosomal aberrations and translocations with urinary naphthols. Higher 1-naphthol was associated with higher frequency of chromosomal aberrations though this association was only observed in the Dominican 5-year olds ($r = 0.387$, $P < 0.01$). Translocations were higher with higher levels of NAPH, particularly in Dominican children ($r = 0.334$, $P < 0.01$). There were no recurrent breakpoints among the translocations observed.

Predictors of chromosomal aberrations or translocations

Among the study subjects, 35 children had chromosomal aberrations. Of these, 20 children had translocations. Levels of 1- and 2-naphthol did not vary with presence of a smoker in the child's home, nor did they vary with a child's reported consumption of smoked meat or charbroiled hamburgers (data not shown). Levels of 2-naphthol (but not 1-naphthol) seemed higher in girls ($P = 0.08$) and in Dominicans ($P = 0.01$).

To describe the effects of exposures on frequency of chromosomal aberration and translocations, mean ratios and ORs were derived for a doubling of naphthol levels (Table 5). After adjusting for sex and ethnicity, presence of both chromosomal aberrations and translocations were associated with higher levels of either 1- or 2-naphthol. For all children (combining both genders and ethnicities), a doubling of 1-naphthol was associated with increased odds for having chromosomal aberrations (OR, 1.23; 95% CI, 1.01–1.48). Similarly, for 2-naphthol, doubling the 2-naphthol levels appeared to associate with nonsignificantly increased odds for chromosomal aberrations

(OR, 1.19; 95% CI, 0.90–1.56). However, when we examined Dominican children separately, the effect was stronger for both 1-naphthol (OR, 1.63; 95% CI, 1.21–2.19) and 2-naphthol (OR, 1.44; 95% CI, 1.02–2.04).

In contrast, for translocations, the odds of having a translocation were significantly elevated when either 1- or 2-naphthol were higher. The odds for translocations were also significantly associated with higher levels of the sum variable (NAPH) when all children were examined together. However, when examined separately by ethnicity, the odds of having translocations were significantly elevated only among Dominicans. In Dominican children, for a doubling of 2-naphthol levels, the odds of having translocations were significantly increased (OR, 1.92; 95% CI, 1.20–3.08).

When we examined the effect of naphthol on the mean frequencies of chromosomal aberrations, the mean frequency of chromosomal aberrations increased by doubling 1-naphthol (mean ratio, 1.38; 95% CI, 1.10–1.72) in Dominicans but not in African-Americans. For this doubling, the group difference between the 2 ethnic groups was significant ($P < 0.05$). In contrast, the association between naphthols and frequency of translocations did not differ by ethnicity.

To examine possible dose–response, levels of 2-naphthol were trichotomized by tertiles using the lowest tertile as the referent group. After adjusting for gender and ethnicity, children with 2-naphthol in the highest group (above 5,800 ng/L) were significantly more likely to have translocations (OR, 4.29; 95% CI, 1.11–16.55) when compared with those in the lowest group (below 2,540 ng/L). Similarly, children in the highest group of 2-naphthol exposure also appeared to have a higher frequency of translocations (mean ratio, 3.23; 95% CI, 0.85–12.50; Table 6), though the results were not significant. The effect of increasing naphthol on chromosomal aberrations or translocations was not dominated by any one chromosome, in contrast to our findings in newborns, which were dominated by chromosome 6 (32).

Although 2-naphthol is derived exclusively from naphthalene, 1-naphthol can result from metabolism of carbaryl as well as from metabolism of naphthalene. Levels of

carbaryl in prenatal air samples from mothers in our population were detectable in only one mother and consequently are not measured in children our cohort (42). To determine the source of 1-naphthol in our samples, we examined the ratio of 1- to 2-naphthol in the urine samples of the 113 participants as described by Meeker and colleagues (15). Carbaryl was an unlikely contributor in 75% of the children, whose ratio are below the threshold level of 2 (15). This proportion is similar to the proportion described among Hispanic pregnant women (15, 43). The proportion with ratios greater than 2 did not differ between those with and without aberrations or between those with and without translocations. The mean ratio of 1- to 2-naphthol also does not differ between those with and without aberrations or between those with and without translocations.

Discussion

Our results show that markers of exposure to naphthalene in young children are associated with translocations and stable chromosomal aberrations in lymphocytes in a dose-related manner. Childhood is a period of heightened susceptibility when exposure to environmental toxins can result in molecular changes that act as determinants for later disease. Exposures to low levels of common environmental toxins such as naphthalene during key periods of development may increase long-term risk of disease. Chromosomal aberrations in lymphocytes are used as a biodosimeter of protracted personal exposure to low-dose radiation (37) and of occupational exposure to genotoxins (31). Air levels of PAHs predict chromosomal aberrations in occupationally exposed adults (30). In studies on older children (8–19 years), frequencies of chromosomal aberrations correlate with levels of ambient pollutants (40). Translocations, the most persistent aberrations (half-life, 2–4 years), are a biodosimeter of low-dose clastogenic exposures and can persist 10 to 13 years after exposure (29). WCP-FISH has been used to facilitate documentation of translocations. Previous studies have shown that frequency of translocations increases with age and smoking exposure (30) and that they can persist over years in serially measured occupational cohorts (27–31).

Table 6. Dose response pattern for presence and frequency of translocations by levels of 2-naphthol

Translocation	Tertiles of 2-naphthol levels (ng/L)			
	147–<2,540 (n = 38)	2,540–<5,800 (n = 38)	5,800–132,000 (n = 37)	P trend
% presence (N)	10.5% (4)	15.8% (6)	27.0% (10)	0.16
OR (95% CI)	1	1.85 (0.46–7.36)	4.29[a] (1.11–16.6)	0.09
Mean frequency (SD)	0.048 (0.15)	0.080 (0.221)	0.114 (0.227)	0.20
Mean ratio (95% CI)	1	1.65 (0.44–6.19)	3.23[b] (0.850–12.5)	0.22

NOTE: OR and MR derived from sex and ethnicity adjusted regression models.
[a]$P < 0.05$.
[b]$P = 0.08$.

Few studies have used WCP-FISH in peripheral blood to examine effects of environmental exposures in children postnatally. In prior work, we have shown that occurrence of stable aberrations is nonrandom and is not proportional to the genomic content of any given chromosome (32). In addition, the frequency of stable chromosomal aberrations detectable by WCP-FISH in chromosomes 1 to 6 and 11, 12, 14, and 19 in cord blood was associated positively with higher levels of PAH measured in maternal prenatal air samples (32). The ambient prenatal PAH measured include 8 potentially carcinogenic PAHs (32), but naphthalene could not be measured in the prenatal samples because of its volatility. These present findings suggest that exposure to some PAHs may lead to clastogenesis. In particular, exposure to naphthalene appears associated with clastogenesis, though at a low rate. Metabolites of other measured PAHs, including fluorene, phenanthrene, and pyrene, do not appear to have this association. Our findings are consistent with recent findings of elevated chromosomal aberrations documented in airline workers exposed to jet fuels (44, 45), which are also a source of naphthalene exposure.

Naphthalene, a PAH and IARC-classified possible carcinogen, is the primary ingredient in some mothballs (2). It is a byproduct of wood and gasoline combustion and is contained in gasoline exhaust, jet fuel, and cigarette smoke and is ubiquitous in ambient air with high volumes of vehicular traffic (1). U.S. indoor levels of naphthalene can exceed outdoor ambient levels by 10-fold (6), particularly when naphthalene-containing household products are used (2, 5). Naphthalene like other small PAHs, is found entirely in gas phase rather than as a particulate. One study in U.S. preschoolers found that indoor air levels of naphthalene in daycare and home settings were 5- to 10-fold higher than outdoor air levels (6). Naphthalene levels vary depending on the source of exposure: mothball exposure is equivalent to low level occupational exposure and is likely an important exposure source for children (2, 46). A box of "old fashioned" mothballs contains 396 g of naphthalene, which is sufficient to raise the average residential indoor air concentration to approximately 200 μg per cubic meter over a period of 1 year, though in small homes or apartments this would be expected to be much higher (2, 46). Exposures of this magnitude are commensurate with industrial exposures to coal tar, coke, or jet fuels (2). Families in the CCCEH may be culturally predisposed to use naphthalene in their homes (47). Mothball use as air-fresheners has been reported by 27% of NYC families using one inner city emergency room NYC (8). Some Latino households also use mothballs as air-fresheners, pesticides, and in traditional remedies (47). Household exposures may present greater risk for inner city school-aged children because they spend larger proportions of their day indoors, largely due to the relative insecurity of their neighborhoods (48).

Despite the widespread exposure to naphthalene, human effects resulting from naphthalene exposure are poorly understood (49). These results are the first to correlate a clastogenic and precarcinogenic effect with a specific PAH in human populations. By using a marker of excretion, we are able to correlate our results with individual levels of exposure and metabolism (49). Naphthalene undergoes metabolism by the cytochrome P450 monooxygenases to reactive metabolites, which may be critical to its toxicity. Naphthalene metabolites could contribute to development of chromosomal aberrations through adduct formation. The 1,2-naphthoquinone metabolite of naphthalene has been associated with depurinating adduct formation in rodent models (26). Other metabolites (naphthalene-1, 2-epoxide, 1,2-dihydrodiol-3,4-epoxide, and 1,4-napthoquinone) may be potential sources of oxidative damage as exposure to these metabolites may result in DNA strand breakage and/or DNA base damage through epoxide formation. DNA damage can also result from bulk DNA-adduct formation. Although in a recent small occupational cohort 2-naphthol levels did not correlate with DNA adducts in peripheral blood mononuclear cells or in urothelial cells, the adducts measured were not naphthalene metabolite–specific (50). Mammalian models have documented naphthalene metabolite–specific adducts in skin cells (21, 26) as well as cytotoxicity and tumor development in cells lining nasal passages and bronchial epithelial cells (23, 51, 52). Our preliminary data suggest that translocations are associated with naphthalene exposure. Together with *in vitro* data showing toxicity in myeloid precursors after exposure to naphthalene metabolites (22), our results in lymphocytes suggest that naphthalene might also contribute to damage in hematogenous cells.

Our data show associations between 2 urinary metabolites of naphthalene and both chromosomal aberrations and translocations. Although 1-naphthol can be derived from both naphthalene and carbaryl, 2-naphthol is derived only from naphthalene. The dose–response association seen with translocations in our data is specifically with 2-naphthol. Although the correlation between chromosomal aberrations frequency and 2-naphthol is not significant (Table 4), the associations between increasing levels of 2-naphthol and presence of chromosomal aberrations and translocations are stronger than those seen with 1-naphthol (Table 5). Our data suggest that the clastogenicity we document may result from exposure to naphthalene itself. For overall chromosomal aberrations, we do see a significant association between both 1- and 2-naphthol, however as noted above, translocations are considered more predictive of carcinogenic potential. Given the extremely low prevalence of carbaryl in the prenatal air samples in our cohort it is unlikely that children in our cohort are exposed to airborne carbaryl, though they may have some exposure through ingestion of contaminated produce.

The ethnic differences in the levels 1- and 2-naphthol, as well as the differences in associations between naphthol levels and incidence of chromosomal aberrations and especially translocations suggest differences in both exposures to sources of naphthalene, as well as in response to

this exposure. However, the relatively small number of participants in the 2 ethnic groups limits our ability to examine this further. Our results are similar to those noted in the much larger NHANES cohort in which levels of urinary naphthalene metabolites are highest among Mexican-Americans (11) suggesting possible cultural variations in patterns of exposure. Differences in response to exposure to naphthalene might reflect differences in incidence of functional polymorphisms of PAH-metabolizing enzymes. Polymorphisms in CYP2E1 and GSTM1 have been associated with elevated 2-naphthol levels in coke oven workers (53). Similarly, polymorphisms in CYP1A1, GSTP1, EPHX1, p53 MspI, and MTHFR have been associated with translocations in urban smokers occupationally exposed to ambient PAH (54).

Although our study was limited by a relatively small sample size, our findings are consistent with other those of other studies examining adult populations (15,20). The urine samples, which we analyzed, were collected as single spot measurements; hence they may not be representative. However, because they were collected during home visits, they may reflect exposures present chronically in the homes. Future efforts to build on our initial findings will need to examine larger numbers of children with urinary samples reflecting longer periods of exposure.

In summary, our data suggest that children in the CCCEH cohort have higher levels of urinary naphthalene metabolites and that these are associated with chromosomal aberrations (including translocations), which are precancerous changes in adults. The widespread exposure to naphthalene in the United States and worldwide supports the need for further study. Levels of nonoccupational exposure are high in the United States, particularly in indoor air (7). DNA strand breakage has been associated with occupational exposure to naphthalene (20). We have found chromosomal breaks measured in peripheral blood of school age children associated with increased levels of naphthalene metabolites. Occupational cohort studies have showed that such breaks are predictive of a more than 2-fold increased risk of later cancer (27). While the age difference between the NHANES population and the children in our cohort did not allow for a direct comparison, our data and that of NHANES, nevertheless, document that exposure to naphthalene is widespread in the United States (11). Exposure to this potential carcinogen appears particularly elevated in poor U.S. households (7).

In less industrialized societies, cooking with biomass fuel stoves is recognized as a source of indoor air PAH exposure, including naphthalene (3, 4). Recent measurements of Peruvian women using biomass fuels for indoor cooking showed median levels of 1-naphthol and 2-naphthol exceeding the 75th percentile of NHANES. The frequent use of biomass for cooking and heating in less industrialized communities illustrates the need for future studies to confirm the findings presented here and to better assess the risk of occupational and residential exposure to naphthalene worldwide.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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