Journal of Exposure Science and Environmental Epidemiology (2007) 17, 314–320

www.nature.com/jes

Utility of urinary 1-naphthol and 2-naphthol levels to assess environmental carbaryl and naphthalene exposure in an epidemiology study

JOHN. D MEEKER[a], DANA B. BARR[b], BERRIN SERDAR[c], STEPHEN M. RAPPAPORT[d] AND RUSS HAUSER[e]

[a]*Department of Environmental Health Sciences, University of Michigan, Ann Arbor, Michigan, USA*
[b]*Centers for Disease Control and Prevention, Atlanta, Georgia, USA*
[c]*Department of Environmental and Occupational Health, Florida International University, Miami, Florida, USA*
[d]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina, USA*
[e]*Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA*

We recently reported associations between urinary 1-naphthol (1N) levels and several intermediate measures of male reproductive health, namely sperm motility, serum testosterone levels, and sperm DNA damage. However, because 1N is a major urinary metabolite of both naphthalene and the insecticide carbaryl, exposure misclassification stemming from differences in exposure source was probable and interpretation of the results was limited. As naphthalene, but not carbaryl, is also metabolized to 2-naphthol (2N), the relationship of urinary 1N to 2N within an individual may give information about source of 1N. Utilizing data from two previous studies that measured both 1N and 2N in urine of men exposed to either carbaryl or naphthalene, the present study employed several methods to differentiate urinary 1N arising from exposures to carbaryl and naphthalene among men in the reproductive health study. When re-evaluating the reproductive health data, techniques for identifying 1N source involved exploring interaction terms, stratifying the data set based on 1N/2N ratios, and performing an exposure calibration using a linear 1N to 2N relationship from a study of workers exposed to naphthalene in jet fuel. Despite some inconsistencies between the methods used to distinguish 1N source, we found that 1N from carbaryl exposure is likely responsible for the previously observed association between 1N and sperm motility, whereas 1N from naphthalene exposure is likely accountable for the association between 1N and sperm DNA damage. We demonstrate that studies of health effects associated with carbaryl should utilize a 1N/2N ratio to identify subgroups in which carbaryl is the primary source of 1N. Conversely, studies of naphthalene-related outcomes may utilize 2N levels to estimate exposure.
Journal of Exposure Science and Environmental Epidemiology (2007) **17,** 314–320; doi:10.1038/sj.jes.7500502; published online 24 May 2006

Keywords: *biomarker, carbaryl, exposure measurement error, naphthalene, naphthol, reproductive health.*

Introduction

Exposure biomarkers are useful in environmental epidemiology as indicators of internal dose that account for all routes of exposure. Urine is often the preferred matrix for exposure biomarkers because it is easy to collect and can provide large sample volumes (Barr et al., 1999). As 1-naphthol (1N) is a major urinary metabolite of both naphthalene and the insecticide, carbaryl (1-naphthyl-*N*-methylcarbamate, widely known as Sevin®), levels of urinary 1N can arise from exposure to one or both of these compounds. Urinary 1N is observed in a large proportion of the general population. For example, the Second National Report on Human Exposure to Environmental Chemicals (CDC 2003) found that over 75% of the United States male population had detectable levels of 1N in urine. However, as the sources and health effects of carbaryl and naphthalene differ, it would be useful to differentiate the particular sources of 1N in human urine.

Carbaryl is a broad-spectrum carbamate insecticide commonly applied to residential lawns and gardens, including fruits and vegetables. An estimated 2–4 million pounds (0.9–1.8 million kg) of carbaryl were applied in the home and garden market sector in 1999 (EPA, 2002). Carbaryl has been measured in the dust of homes (Rudel et al., 2003; Colt et al., 2004) and in food residues (USDA, 2002; FDA, 2003) throughout the US. Thus, residential exposure to carbaryl would commonly involve multiple media and routes of exposure, including inhalation, dermal contact, and ingestion, making a biomarker of internal exposure desirable in epidemiology studies. Urinary 1N is the primary biomarker used to estimate carbaryl exposure in human studies as it accounts for more than 85% of all carbaryl metabolites in urine (Maroni et al., 2000).

Naphthalene, a polycyclic aromatic hydrocarbon (PAH), is an ubiquitous environmental contaminant that is discharged into the environment by incomplete combustion of

1. Address all correspondence to: Dr. J. Meeker, Department of Environmental Health Sciences, University of Michigan School of Public Health, Room M6226 SHP II, 109 S. Observatory St Ann Arbor, MI 48109, USA. Tel.: +1-734-764-7184; Fax: +1-734-763-8095.
E-mail: meekerj@umich.edu
Received 15 February 2006; accepted 21 April 2006; published online 24 May 2006

hydrocarbons from industrial, domestic, and natural sources and from direct vaporization from diesel and jet fuels (Preuss et al., 2003; Rappaport et al., 2004). These background combustion sources, along with moth repellents and cigarette smoke, are the primary sources of naphthalene exposure among individuals without specific occupational exposures. Unlike carbaryl, naphthalene is metabolized to both 1N and 2-naphthol (2N), both of which are excreted in urine (Waidyanatha et al., 2002; ATSDR, 2003). Thus, if levels of 1N and 2N are highly correlated in urine samples, it would be reasonable to infer that naphthalene and not carbaryl is the source of 1N. On the other hand, if levels of 1N and 2N are poorly correlated, then carbaryl is likely to be a major source of urinary 1N (Hill et al., 1995, Shealy et al., 1997). Studies of nonoccupational naphthalene exposure have used urinary 2N rather than 1N as a surrogate for exposure, to eliminate misclassification and/or confounding by coexposure to carbaryl (Kim et al., 1999, 2001, Nan et al., 2001; Preuss et al., 2003; Kato et al., 2004).

We recently reported associations between urinary 1N levels and intermediate measures of male reproductive health, including reduced sperm motility, increased sperm DNA damage, and reduced circulating testosterone (Meeker et al., 2004a, b, 2006). If either carbaryl or naphthalene (but not both) were associated with these outcomes, then use of urinary 1N as the sole surrogate for exposure could have introduced measurement errors, and the resulting attenuation biases, into those analyses. The present study was conducted to investigate this possibility by using urinary levels of both 1N and 2N to differentiate between carbaryl and naphthalene exposure in these subjects.

We used two previous studies to obtain the necessary data with which to differentiate urinary 1N arising from exposures to carbaryl and naphthalene. The first study reported levels of urinary 1N and 2N among carbaryl applicators and their families in the Agricultural Health Study (Shealy et al., 1997). In that investigation, carbaryl should have been a major contributor to urinary 1N. The second study reported levels of 1N and 2N data from a cohort of Air Force personnel occupationally exposed to jet fuel (JP-8) (Serdar et al., 2004, 2003a). In that investigation, the authors demonstrated high correlations between airborne levels of naphthalene vapors and the corresponding urinary levels of 1N and 2N.

Methods

Subject Recruitment

Subjects were men in subfertile couples seeking infertility diagnosis from the Massachusetts General Hospital Vincent Burnham Andrology lab between January 2000 and April 2003. Details of subject recruitment have been described previously (Hauser et al., 2003). Briefly, consecutive eligible men who presented to the Andrology Laboratory were recruited to participate. Of those approached, approximately 65% consented. Most men who declined to participate in the study cited lack of time on the day of their clinic visit as the reason for not participating.

Urine Sample Collection and Analysis

A single spot urine sample was collected from each subject. Urine samples were frozen at $-20^{\circ}C$ and sent to the US Centers for Disease Control and Prevention (CDC) where 1N and 2N were measured (Bravo et al., 2005). Samples were fortified with stable isotope analogues of the target analytes and glucuronide or sulfate-bound metabolites were liberated using enzymatic hydrolysis. Urinary 1N and 2N were isolated using liquid–liquid extraction, derivatized, and measured using gas chromatography–chemical ionization–tandem mass spectrometry. Specific gravity (SG) was used to adjust urine samples for dilution (Boeniger et al., 1993). Urine samples with a SG of greater than 1.03 or less than 1.01 were considered too concentrated or too dilute, respectively, to provide valid results and were excluded from the primary statistical analysis (Teass et al., 1998). SG was measured using a handheld refractometer (National Instrument Company Inc., Baltimore, MD, USA).

Intermediate Measures of Male Reproductive Health

Details of the outcome measures in the present study have been described (Meeker et al., 2004a, b, 2006). Briefly, a semen sample and a blood sample were collected from each subject on the same day that urine was collected. Semen samples were analyzed for sperm count and motility in the Andrology lab, by computer-aided semen analysis, using the Hamilton Thorne IVOS 10 Analyzer (Hamilton-Thorne Research, Beverly, MA, USA). An aliquot of semen was preserved in liquid nitrogen ($-196^{\circ}C$) and later analyzed for DNA damage using the neutral comet assay at the Harvard School of Public Health. Measures of DNA damage included comet extent, tail distributed moment, and percent DNA in comet tail. Blood samples were centrifuged and serum stored at $-80^{\circ}C$ until analysis. Testosterone was measured directly using the Coat-A-Count RIA kit (Diagnostics Products, Los Angeles, CA, USA).

Statistical Analysis

As urinary 1N levels were right skewed, the values were transformed using natural logarithms for statistical analyses. Nondetected samples were assigned a value of one-half the limit of detection. Multiple linear regression was used to assess associations between levels of 1N in urine and intermediate measures of male reproductive health. Outcome measures were chosen for this study based on our previous work, where we observed associations with urinary 1N. Details of the original analysis and results have been

described for sperm motility (Meeker et al., 2004a), sperm DNA damage (Meeker et al., 2004b), and serum testosterone levels (Meeker et al., 2006). Briefly, for sperm motility and DNA damage, subjects with a history of medical risk factors for infertility (e.g. varicocele, orchidopexy) were excluded from the analysis. For testosterone, subjects taking hormone medication (e.g. propecia, finasteride, cabergoline, clomid, GnRH, testosterone, or prednisone taper) were excluded. Age, body mass index, abstinence time, smoking, and season were considered as covariates, and were included or excluded from models based on biologic and statistical considerations (Kleinbaum et al., 1998). In the case of testosterone, sex hormone binding globulin and time of day that the blood was collected were also included in the models. The association between urinary 1N and sperm motility was also assessed by multiple logistic regression, where subjects were dichotomized as either above or below 50% motile sperm based on WHO reference levels (WHO, 1999).

Three approaches were used in an attempt to separate carbaryl and naphthalene exposure. The first method (Approach 1) utilized only data collected in the present study. Effect modification of the relationship between ln(1N) and reproductive outcomes by the ratio of urinary 1N to 2N (1N/2N ratio) was tested in multiple linear regression using an interaction term (ln(1N)*ln(1N/2N ratio)) in addition to the main effects of ln(1N), ln(1N/2N), and the other covariates from the final original models (e.g. age, smoking, etc.).

The second approach (Approach 2) stratified subjects based on their (natural scale) ratio of 1N to 2N. The method utilized repeated levels of 1N and 2N among six carbaryl applicators and their families in the Agricultural Health Study (Shealy et al., 1997) to estimate a ratio cutpoint with which to differentiate subjects for which 1N was primarily derived from either carbaryl or naphthalene. As the biologic half-lives of carbaryl metabolites are between 24 and 48 h (Maroni et al., 2000), only urine samples collected from applicators within 2 days of exposure were considered. The distribution of 1N levels among the applicators had a median of 7 μg/g creatinine and ranged from less than 1 μg/g creatinine to greater than 20,000 μg/g creatinine. The distribution was skewed right and appeared bimodal, with modes near 2 μg/g creatinine and 15 μg/g creatinine. Because background exposure to naphthalene is ubiquitous and a portion of samples had low 1N concentrations similar to those found among the general population (suggesting no specific carbaryl exposure), we only considered ratios from applicators having 1N levels that were above the median value. Among the 32 samples (out of 160 total samples from the applicators and their families) that fit these criteria, the ratio of 1N to 2N ranged from 0.75 to over 1000 with a median of 4.4. Twenty-seven (84%) samples had a 1N/2N ratio equal to or greater than 2.0. However, it was apparent that among the five samples with a ratio below 2.0, all had 2N levels greater than 20 μg/g creatinine, which was between five- and 10-fold greater than 2N levels from all other samples and indicative of high naphthalene exposure. Thus, a 1N/2N ratio above 2.0 was chosen as a cutoff to stratify individuals with 1N primarily from carbaryl.

The third method (Approach 3) for differentiating carbaryl and naphthalene exposure was evaluated using levels of urinary 1N and 2N from 323 Air Force personnel exposed to JP-8 (Serdar et al., 2004, 2003a). To estimate the portion of 1N that resulted from naphthalene exposure in the present study, the following linear relationship was used, based upon the 323 data pairs from the Air Force study: $\ln(1N) = -0.37 + 0.92\ln(2N)$ ($R^2 = 0.79$). From this relationship, each subject's observed level of 2N was used to predict the particular contribution of 1N from naphthalene exposure; this is designated as $1N_n = \exp[-0.37 + 0.92\ln(2N)]$. Then, each subject's particular contribution of 1N from carbaryl exposure was estimated as $1N_c = 1N_o - 1N_n$, where $1N_o$ refers to the subject's observed level of 1N. Logged values of $1N_c$ were used in multiple linear regression models to explore associations with intermediate male reproductive outcomes. Effect estimates were then compared with the original multiple regression models where $1N_o$ had been used to estimate exposures. Results were also compared to models using ln(2N) to estimate naphthalene exposures. When comparing effect estimates across exposure variables, coefficients were reported as a change in outcome associated with an interquartile range increase in the exposure variable of interest.

Results

Demographic characteristics of this study population have been described previously (Meeker et al., 2004a). Briefly, 370 men provided a urine sample analyzed for 1N and 2N. Of these, 330 men without a history of medical factors for infertility were primarily white (82%) nonsmokers (91%) with a mean (SD) age of 36 (5.5) years. Measurable levels of 1N and 2N were found in 99.7% and 74.5% of samples, respectively (Table 1). As expected, SG-adjusted 1N and 2N levels were higher among smokers (median = 8.13 and 9.84 μg/l, respectively) than among nonsmokers (median = 3.13 and 0.96 μg/l, respectively). The correlation between 1N and 2N levels was also higher among smokers (Spearman's correlation coefficient = 0.89 among smokers vs. 0.42 among nonsmokers), and the ratio of 1N over 2N was lower among smokers as anticipated (median ratio = 3.44 among nonsmokers, 0.93 among smokers).

In the multiple regression analyses, a single extreme 1N value (>150 μg/l) was found to influence parameter estimates (Cook's $D>4$) and was removed. When intermediate reproductive outcomes were modeled with 1N, the

Table 1. Distribution of 1N, 2N, SG-adjusted 1N, SG-adjusted 2N, and 1N/2N ratio ($N = 330$)

Insecticide metabolite[a]	Geometric mean	Selected percentiles						
		5th	10th	25th	50th	75th	90th	95th
Unadjusted (μg/l)								
1N	2.82	0.63	0.93	1.61	2.86	4.49	7.61	13.3
2N	0.93	<0.4	<0.4	<0.4	0.96	2.36	7.66	15.2
SG-adjusted (μg/l)								
1N	3.38	0.78	1.03	1.95	3.41	5.72	11.5	16.8
2N	1.11	0.16	0.17	0.34	1.16	2.82	7.05	14.6
Ratio								
1N/2N	3.04	0.43	0.64	1.29	3.09	6.67	13.0	25.7

Abbreviations: 1N = 1-naphthol; 2N = 2-naphthol; SG = specific gravity.
[a]Limit of detection (LOD) for 1N and 2N = 0.40 μg/l; 99.7% of 1N samples above LOD; 74.5% of 2N samples above LOD.

Table 2. Adjusted[a] regression coefficients and odds ratios for association between 1N and sperm motility, stratified by 1N/2N ratio ($N = 271$)[b,c]

1N/2N Ratio	Multiple linear regression		Multiple logistic regression	
	Estimate in (%) (95% CI)[d]	*P*-value	Odds ratio (95% CI)[e]	*P*-value
All ($n = 271$)	−4.58 (−8.03, −1.13)	0.01	2.41 (1.43, 4.07)	0.001
Ratio <2 ($n = 96$)	−2.29 (−8.32, 3.74)	0.45	1.27 (0.52, 3.06)	0.38
Ratio >2 ($n = 175$)	−5.48 (−9.74, −1.21)	0.01	3.52 (1.80, 6.91)	0.0003

[a]Adjusted for age and abstinence time.
[b]Forty subjects excluded with history of medical risk factors for infertility.
[c]Fifty-eight subjects excluded with SG above 1.03 or below 1.01.
[d]Estimated change in the percentage of motile sperm per unit increase in (ln) SG-adjusted 1N.
[e]Odds of having less than 50% motile sperm comparing SG-adjusted 1N levels above and below the median.

1N/2N ratio and a 1N*1N/2N interaction term (Approach 1), the interaction term in the testosterone and tail percent models were significantly different from zero ($P = 0.05$ and 0.01, respectively), whereas the interaction term in the motility model was not ($P = 0.7$). When the interaction term was removed and 1N and the 1N/2N ratio were included in the model, effect estimates for 1N were similar to original estimates. Inclusion of only the 1N*1N/2N interaction term, which by itself may represent a suitable measure of carbaryl exposure, was not associated with any of the outcome measures in multivariate models.

There were differences in the multiple linear regression coefficients for urinary 1N levels and percent motile sperm when subjects were separated based on their 1N/2N ratios (Approach 2; Table 2). The inverse association was stronger (coefficient farther from zero) among subjects with a 1N/2N ratio of above 2 (regression coefficient = −5.48%; 95% confidence interval (CI) −9.74, −1.21) compared to those below 2 (−2.29%; 95% CI −8.32, 3.74). The association between 1N and sperm motility was also stronger among subjects with a higher 1N/2N ratio in the logistic regression analysis (Table 2). For subjects with a 1N/2N ratio above 2, the odds of having less than 50% motile sperm when 1N level was above the median are greater (odds ratio (OR) = 3.52; 95% CI 1.80, 6.91) compared to subjects with a 1N/2N ratio below 2 (OR = 1.27; 95% CI 0.52, 3.06). A similar difference was found when comparing linear regression coefficients for 1N and testosterone among subjects with a high 1N/2N ratio (regression coefficient = −25.5 ng/dl; 95% CI −44.6, −6.43) to subjects with a low 1N/2N ratio (−10.4 ng/dl; 95% CI −46.4, 25.5) (Table 3). However, the regression coefficient from subjects with a high 1N/2N ratio was similar to that from analysis including all subjects (−25.2; 95% CI −42.3, −8.05).

The opposite trend was observed for 1N/2N ratio subgroups in linear regression models for urinary 1N and sperm DNA damage, estimated as the percentage of DNA in the tail measured by the neutral comet assay (Table 3). A stronger positive association was observed among subjects with a low 1N/2N ratio (regression coefficient = 6.99%; 95% CI 3.07, 10.9) compared to subjects with a high 1N/2N ratio (1.76%; 95% −0.34, 3.85).

Results from the carbaryl exposure calibration analyses were not as clear (Approach 3; Table 4). Effect estimates were attenuated when 1N levels estimated to be from carbaryl exposure ($1N_c$) were used in the models compared to the observed 1N levels ($1N_o$). Unexpectedly, effect estimates from $1N_c$ were also lower for sperm motility and

Table 3. Adjusted regression coefficients for change in serum testosterone (ng/dl) and percentage of total DNA in comet tail per unit increase in (ln) SG-adjusted 1N

1N/2N ratio	Serum testosterone[a]		DNA in Comet tail[b]	
	Estimate, in ng/dl (95% CI)[c]	*P*-value	Estimate, in (%) (95% CI)[d]	*P*-value
All	−25.2 (−42.3, −8.05)	0.004	3.16 (1.27, 5.06)	0.001
Ratio <2	−10.4 (−46.4, 25.5)	0.57	6.99 (3.07, 10.9)	0.0007
Ratio >2	−25.5 (−44.6, −6.43)	0.009	1.76 (−0.34, 3.85)	0.1

[a]$N = 267$ ($n = 96$ for ratio <2, $n = 171$ for ratio >2). Testosterone was not measured in 36 subjects, 12 subjects taking hormone medication were excluded, and 54 subjects were excluded with SG above 1.03 or below 1.01.
[b]$N = 213$ ($n = 65$ for ratio <2, $n = 148$ for ratio >2). Comet assay results were not available for 70 subjects, 40 subjects with a history of medical risk factors for infertility were excluded, and 46 subjects were excluded with SG above 1.03 or below 1.01.
[c]Adjusted for SHBG, age, BMI, smoking, and time of day at blood draw.
[d]Adjusted for age and smoking status.

Table 4. Adjusted linear regression coefficients for intermediate reproductive associated with an interquartile range increase in observed 1-naphthol ($1N_o$), estimated 1-naphthol from carbaryl ($1N_c$), and estimated naphthalene exposure using 2N concentration

	$1N_o$	$1N_c$[a]	2N
Sperm Motility (%)[b]	−4.70 (−8.24, −1.16)	−1.52 (−5.12, 2.07)	−3.45 (−7.87, 0.97)
Testosterone (ng/dl)[c]	−25.8 (−43.4, −8.26)	−14.2 (−31.4, 3.14)	−24.8 (−47.0, −2.54)
Tail%[d]	3.24 (1.30, 5.19)	1.29 (−0.61, 3.21)	4.15 (1.73, 6.58)

All metabolite levels adjusted for specific gravity.
[a]$1N_c$ calculated with 1N/2N linear equation from 322 JP-8 exposed workers. Negative $1N_c$ values were omitted from the analysis; $N = 248$, 242, and 195 for motility, testosterone, and tail percent models, respectively.
[b]Adjusted for age and abstinence time.
[c]Adjusted for SHBG, age, BMI, smoking, and time of day at blood draw.
[d]Adjusted for age and smoking status.

serum testosterone compared to those from naphthalene exposure (estimated as 2N levels). Consistent with results from the stratified analysis, naphthalene exposure (2N) was more strongly associated with tail percent than both $1N_o$ and $1N_c$.

Several linear equations with slightly different intercepts and slopes were considered from different strata and substrata in the JP-8 study (smokers vs. nonsmokers; high, medium, and low naphthalene exposure). However, results did not differ appreciably when using the various equations, and results from the full data set are presented here for robustness. Because some of the resulting $1N_c$ values (approximately 8%) were negative following calibration, analysis was conducted when excluding all negative values, and also when negative values were assigned a value equal to one-half of the lowest observed positive $1N_c$ value. Results were similar between the two methods for treating negative $1N_c$ values.

Discussion

A limitation of our previous findings, where we reported associations between urinary 1N levels and intermediate measures of male reproductive health, was the inability to distinguish between exposures to carbaryl and naphthalene in the population. In the present study, data were reanalyzed, using the ratio of 1N to 2N to differentiate between carbaryl and naphthalene as sources of 1N in each subject. We found stronger associations between 1N and sperm motility and serum testosterone when carbaryl was the likely source of 1N (high 1N/2N ratio compared to low 1N/2N ratio; Approach 2). The opposite was found for DNA damage (tail%), which was more strongly associated with 1N among men with low 1N/2N ratios, which is indicative of naphthalene exposure. Results for tail percent were consistent with effect modification by 1N/2N when an interaction term was included in multiple regression models (Approach 1). As the parameter estimate for 1N was positive while that for the interaction term was negative, the strength of the association between 1N and tail percent weakened with increasing 1N/2N ratio. Also, the association between naphthalene and tail percent observed with Approach 3, which used the level of 2N to estimate naphthalene exposure, was consistent with results from Approaches 1 and 2.

These results suggest that sperm DNA damage is associated with exposure to naphthalene or, perhaps, to other PAH if naphthalene is serving as a surrogate for PAH more generally (Rappaport et al., 2004). Although the genotoxic potential of naphthalene has not been extensively studied (ATSDR, 2003), there is ample evidence, from both *in vivo* and *in vitro* studies, that other PAHs are genotoxic in

somatic and sperm cells (Gaspari et al., 2003; DeMarini, 2004; ATSDR, 1995). Results from the present study indicate that sperm motility and circulating testosterone are associated with exposure to carbaryl. This leads to the hypothesis that carbaryl impacts sperm quality (i.e. motility) through the endocrine system rather than by direct action on sperm DNA as originally hypothesized (Meeker et al., 2004b). Limited animal and *in vitro* studies suggest that carbaryl alters endocrine activity (Ghosh and Bhattacharya, 1990; Klotz et al., 1997).

The distribution of unadjusted urinary 1N values in the present study is similar to the distributions among males recently reported by the Second and Third National Reports on Human Exposure to Environmental Chemicals (CDC, 2003, 2005). The median, 90th, and 95th percentile 1N levels in the present study were 2.86, 7.61, and 13.3 μg/l, respectively, compared to 1.40, 6.60, and 11.0 μg/l in the Second National Report and 1.98, 12.2, and 21.5 μg/l in the Third National Report. The 2N concentrations reported in the Third National Report were higher than in the present study. The median, 90th, and 95th, percentile levels for 2N in the Third National Report were 2.51, 16.4, and 28.1 μg/l, respectively, compared to 0.96, 7.66, and 15.2 μg/l in the present study. Levels of 1N and 2N representing the median, 90th, and 95th percentiles in our study are also somewhat lower than those indicated previously by Hill et al. (1995) in a nonrepresentative sample of adults, that is, 4.4, 26, and 43 μg/l for 1N, respectively, and 3.4, 21, and 30 μg/l for 2N.

As carbaryl and naphthalene are metabolized and excreted rapidly, urinary levels of 1N reflect exposure in the previous 24–48 h (reviewed by Maroni et al., 2000; Preuss et al., 2003). Nonetheless, we recently showed that a single urine sample was reasonably predictive of 3-month average urinary 1N levels (Meeker et al., 2005). The intraclass correlation coefficient (ICC) for 1N was 0.5 (the ICC increased to 0.7 when a single extreme value was removed), and a single urine sample correctly classified men in the highest 3-month 1N tertile with a sensitivity of 0.6 and specificity of 0.9.

Data on the relationship between urinary 1N and 2N from two occupational populations were applied in the present study. First, a 1N/2N ratio was chosen to separate subjects by primary exposure source, based on a study of carbaryl applicators (Shealy et al., 1997). Among applicators in that study, there was little correlation between 1N and 2N ($R^2 = 0.2$, $P > 0.050$), whereas the correlation was much greater among nonapplicators ($R^2 = 0.6$, $P = 0.0001$). This suggests that 1N was derived from exposure to carbaryl among applicators, whereas naphthalene was the likely source of 1N in nonapplicators. In occupational studies of naphthalene exposure (e.g. coke oven workers), much higher correlations between 1N and 2N have been reported, reflecting direct and specific naphthalene exposure (Preuss et al., 2005; Serdar et al., 2003b; Yang et al., 1999; Bieniek, 1997). This is consistent with the high correlation between 1N and 2N and the low 1N/2N ratios found among smokers in the present study, because cigarette smoke is a source of naphthalene.

We also investigated a calibration method (Approach 3) to adjust 1N levels for concurrent carbaryl and naphthalene exposure. This approach employed a calibration curve between levels of 1N and 2N derived from a group of workers exposed to naphthalene (from jet fuel). As Approach 3 resulted in weaker associations than those from Approaches 1 and 2, we conclude either that the calibration method did not reduce exposure measurement error or possibly that total 1N (from both carbaryl and naphthalene) is a better predictor of intermediate measures of male reproductive health than the portion of 1N derived from carbaryl alone. The latter possibility that 1N rather than carbaryl or naphthalene is the causative agent will require further data to consider more thoroughly.

In conclusion, we explored alternatives for teasing out the proportions of urinary 1N that could be attributed to exposures to either carbaryl or naphthalene. We found that stratification of subjects according to their 1N/2N ratios altered our understanding of associations between urinary 1N and various measures of male reproductive health. Men with high 1N/2N ratios had stronger associations between 1N and sperm motility and circulating testosterone levels than men with low 1N/2N ratios. This indicates that sperm motility and circulating testosterone levels may have been depressed owing to exposure to carbaryl among men with high 1N/2N ratios. Conversely, we found stronger associations between 1N and sperm DNA damage among men with lower 1N/2N ratios, suggesting that sperm DNA damage may be caused by exposure to naphthalene and perhaps to other PAHs. This was consistent when comparing associations of 1N and 2N with DNA damage, where the relationship was stronger for 2N. As has been previously proposed but untested to date, our results support that the 1N/2N ratio should be used to differentiate between groups of men with likely exposure to carbaryl or naphthalene. Also, our results further verify that it may be appropriate for studies of naphthalene-related effects to estimate exposure through levels of urinary 2N. Our attempt at adjusting urinary 1N levels for naphthalene exposures, based upon a calibration curve of 1N vs. 2N from a naphthalene-exposed population, was less conclusive but could suggest that 1N *per se* is responsible for the observed effects. This conjecture will require further confirmation.

References

ATSDR. *Toxicological Profile for Naphthalene, 1-Methylnapthalene, and 2-Methylnapthalene*. Agency for Toxic Substances and Disease Registry, Atlanta, GA, 2003.

ATSDR. *Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)*. Agency for Toxic Substances and Disease Registry, Atlanta, GA, 1995.

Barr D.B., Barr J.R., Driskell W.J., Hill Jr. R.H., Ashley D.L., and Needham L.L., et al. Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol Ind Health* 1999: 15: 168–179.

Bieniek G. Urinary naphthols as an indicator of exposure to naphthalene. *Scand J Work Environ Health* 1997: 23: 414–420.

Boeniger M.F., Lowry L.K., and Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am Ind Hyg Assoc J* 1993: 54: 615–627.

Bravo R., Caltabiano L.M., Fernandez C., Smith K.D., Gallegos M., and Whitehead Jr. R.D., et al. Quantification of phenolic metabolites of environmental chemicals in human urine using gas chromatography-tandem mass spectrometry and isotope dilution quantification. *J Chromatogr B* 2005: 820: 229–236.

CDC. *Second National Report on Human Exposure to Environmental Chemicals*. Centers for Disease Control and Prevention. Washington, DC, 2003.

CDC. *Third National Report on Human Exposure to Environmental Chemicals*. Centers for Disease Control and Prevention, Washington, DC, 2005: http://www.cdc.gov/exposurereport/3rd/results.htm February 2006.

Colt J.S., Lubin J., Camann D., Davis S., Cerhan J., and Severson R.K., *et al.* Comparison of pesticide levels in carpet dust and self-reported pest treatment practices in four US sites. *J Expo Anal Environ Epidemiol* 2004: 14: 74–83.

DeMarini D.M. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. *Mutat Res* 2004: 567: 447–474.

EPA. *Pesticide Industry Sales and Usage: 1998 and 1999 Market Estimates.*, Report # 773-R-02-001 United States Environmental Protection Agency, Washington, DC, 2002.

FDA. *Summary of Residues Found, Ordered by Food: Market Baskets 91-301-04*. United States Food and Drug Administration Total Diet Study, Rockville, MD, 2003: http://www.cfsan.fda.gov/~acrobat/TDS1byfd.pdf February 2006.

Gaspari L., Chang S.S., Santella R.M., Garte S., Pedotti P., and Taioli E. Polycyclic aromatic hydrocarbon–DNA adducts in human sperm as a marker of DNA damage and infertility. *Mutat Res* 2003: 535: 155–160.

Ghosh P., and Bhattacharya S. Impairment of the regulation of gonadal function in *Channa punctatus* by metacid-50 and carbaryl under laboratory and field conditions. *Biomed Environ Sci* 1990: 3: 106–112.

Hauser R., Chen Z., Pothier L., Ryan L., and Altshul L. The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and *p,p′*-DDE. *Environ Health Perspect* 2003: 111: 1505–1511.

Hill Jr. R.H., Head S.L., Baker S., Gregg M., Shealy D.B., and Bailey S.L., et al. Pesticide residues in urine of adults living in the United States: reference range concentrations. *Environ Res* 1995: 71: 99–108.

Kato M., Loomis D., Brooks L.M., Gattas G.F., Gomes L., and Carvalho A.B., et al. Urinary biomarkers in charcoal workers exposed to wood smoke in Bahia State, Brazil. *Cancer Epidemiol Biomarkers Prev* 2004: 13: 1005–1012.

Kim H., Cho S.H., Kang J.W., Kim Y.D., Nan H.M., and Lee C.H., et al. Urinary 1-hydroxypyrene and 2-naphthol concentrations in male Koreans. *Int Arch Occup Environ Health* 2001: 74: 59–62.

Kim H., Kim Y.D., Lee H., Kawamoto T., Yang M., and Katoh T. Assay of 2-naphthol in human urine by high-performance liquid chromatography. *J Chromatogr B* 1999: 734: 211–217.

Kleinbaum D.G., Kupper L.L., Muller K.E., and Nizam A. Selecting the best regression equation. In: Kugushev A (ed.) *Applied Regression Analysis and Other Multivariate Methods*, 3rd ed. Brooks/Cole Publishing Company, Pacific Grove, CA, 1998.

Klotz D.M., Arnold S.F., and McLachlan J.A. Inhibition of 17 beta-estradiol and progesterone activity in human breast and endometrial cancer cells by carbamate insecticides. *Life Sci* 1997: 60: 1467–1475.

Maroni M., Colosio C., Ferioli A., and Fait A. Biological monitoring of pesticide exposure: a review. *Toxicology* 2000: 143: 1–118.

Meeker J.D., Barr D.B., Ryan L., Herrick R.F., Bennett D.H., and Bravo R., et al. Temporal variability of urinary levels of nonpersistent insecticides in adult men. *J Expo Anal Environ Epidemiol* 2005: 15: 271–281.

Meeker J.D., Ryan L., Barr D.B., and Hauser R. Exposure to non-persistent insecticides and reproductive hormones in adult men. *Epidemiology* 2006: 17: 61–68.

Meeker J.D., Ryan L., Barr D.B., Herrick R.F., Bennett D.H., and Bravo R., et al. The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality. *Environ Health Perspect* 2004a: 112: 1665–1670.

Meeker J.D., Singh N.P., Ryan L., Duty S.M., Barr D.B., and Herrick R.F., et al. Urinary levels of insecticide metabolites and DNA damage in human sperm. *Hum Reprod* 2004b: 19: 2573–2580.

Nan H.M., Kim H., Lim H.S., Choi J.K., Kawamoto T., and Kang J.W., et al. Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. *Carcinogenesis* 2001: 22: 787–793.

Preuss R., Drexler H., Bottcher M., Wilhelm M., Bruning T., and Angerer J. Current external and internal exposure to naphthalene of workers occupationally exposed to polycyclic aromatic hydrocarbons in different industries. *Int Arch Occup Environ Health* 2005: 78: 355–362.

Preuss R., Angerer J., and Drexler H. Naphthalene — an environmental and occupational toxicant. *Int Arch Occup Environ Health* 2003: 76: 556–576.

Rappaport S.M., Waidyanatha S., and Serdar B. Naphthalene and its biomarkers as measures of occupational exposure to polycyclic aromatic hydrocarbons. *J Environ Monit* 2004: 6: 413–416.

Rudel R.A., Camann D.E., Spengler J.D., Korn L.R., and Brody J.G. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ Sci Technol* 2003: 37: 4543–4553.

Serdar B., Egeghy P.P., Gibson R., and Rappaport S.M. Dose-dependent production of urinary naphthols among workers exposed to jet fuel (JP-8). *Am J Ind Med* 2004: 46: 234–244.

Serdar B., Egeghy P.P., Waidyanatha S., Gibson R., and Rappaport S.M. Urinary biomarkers of exposure to jet fuel (JP-8). *Environ Health Perspect* 2003a: 111: 1760–1764.

Serdar B., Waidyanatha S., Zheng Y., and Rappaport S.M. Simultaneous determination of urinary 1- and 2-naphthols, 3- and 9-phenanthrols, and 1-pyrenol in coke oven workers. *Biomarkers* 2003b: 8: 93–109.

Shealy D.B., Barr J.R., Ashley D.L., Patterson Jr. D.G., Camann D.E., and Bond A.E. Correlation of environmental carbaryl measurements with serum and urinary 1-naphthol measurements in a farmer applicator and his family. *Environ Health Perspect* 1997: 105: 510–513.

Teass A.W., Biagini R.E., DeBord G., and Hull R.D. Application of biological monitoring methods. In: Eller PM (Ed.). *NIOSH Manual of Analytical Method,*, 4th edn. National Institute for Occupational Safety and Health, Cincinnati, OH, 1998 pp. 52–62.

USDA. *USDA Pesticide Data Program: PDP Databases Available for Downloading*. United States Department of Agriculture, Washington, DC, 2002: http://www.ams.usda.gov/science/pdp/Download.htm February 2006.

Waidyanatha S., Troester M.A., Lindstrom A.B., and Rappaport S.M. Measurement of hemoglobin and albumin adducts of naphthalene-1,2-oxide, 1,2-naphthoquinone and 1,4-naphthoquinone after administration of naphthalene to F344 rats. *Chem Biol Interact* 2002: 141: 189–210.

WHO. *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction,*4th edn. World Health Organization, Cambridge University Press, New York, NY, 1999.

Yang M., Koga M., Katoh T., and Kawamoto T. A study for the proper application of urinary naphthols, new biomarkers for airborne polycyclic aromatic hydrocarbons. *Arch Environ Contam Toxicol* 1999: 36: 99–108.