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Urinary concentrations of 3-(diethylcarbamoyl)benzoic acid (DCBA), a major metabolite of N,N-diethyl-m-toluamide (DEET) and semen parameters among men attending a fertility center

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Abstract

STUDY QUESTION—Are specific gravity (SG)-adjusted urinary concentrations of 3-(diethylcarbamoyl)benzoic acid (DCBA) associated with semen parameters among men attending an academic fertility center?

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Authors' contributions

R.H. was involved in the study concept and design, and critical revision for important intellectual content of the manuscript. P.L.W. was involved in the study concept and design, and critical revision for important intellectual content of the manuscript and provided statistical expertise. T.R.S. was involved in the study concept, drafted the manuscript, and had a primary responsibility for final content. L.M.-A. analyzed data, drafted the manuscript and had a primary responsibility for final content. T.R.S., L.M.A., Y-H.C., P.L.W., F.L.N. and R.H. interpreted the data. Y.-H.C. reviewed the statistical analysis. R.D., M.O. and A.M.C. were involved in acquisition of the data. All authors were involved in the critical revision of the manuscript and approved the final manuscript.

Conflict of interest

None of the authors has any conflicts of interest to declare. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

SUMMARY ANSWER—Our study did not demonstrate any association between SG-adjusted urinary DCBA concentrations and semen parameters among men attending an academic fertility center.

WHAT IS KNOWN ALREADY—N,N-Diethyl-*m*-toluamide (DEET) is the most common active ingredient in consumer insect repellents. The recent rise in public health concerns regarding mosquito-borne diseases such as Zika, have led to an increased use of DEET insect repellents, especially among couples planning pregnancy. Animal studies have observed reproductive toxicity from DEET exposure. However, the reproductive health effects of DEET and its metabolites on human reproduction are unknown.

STUDY DESIGN, SIZE, DURATION—Between 2007 and 2015, 90 men participating in a prospective cohort study at the Massachusetts General Hospital Fertility Center provided 171 urine samples and 250 semen samples for analysis.

PARTICIPANTS/MATERIALS, SETTING, METHODS—The urinary concentrations of DEET, N,N-diethyl-3-hydroxymethylbenzamide (DHMB) and DCBA were quantified by isotope-dilution tandem mass spectrometry and adjusted by SG. We used linear mixed models to evaluate the association between tertiles of SG-adjusted urinary DCBA concentrations and semen parameters (semen volume, sperm concentration, total sperm count, progressive motility, total progressive motility count, normal morphology and total normal morphology count), adjusting for covariates. DEET and DHMB were not considered for analysis because of the low percentage of detectable concentrations (<7%). Effect modification by BMI and smoking status was explored.

MAIN RESULTS AND THE ROLE OF CHANCE—Participants had a median age of 36 years and BMI of 27 kg/m^2, and 68% had never smoked. The SG-adjusted geometric mean DCBA urinary concentration was 2.20 μg/l, with 85% detection frequency. The majority of semen parameters fell within the normal range with the exception of progressive motility, where 64% of the men had values below the WHO 2010 lower reference limits. SG-adjusted urinary DCBA concentrations were not associated with semen parameters in unadjusted or adjusted models. Men in the highest tertile of SG-adjusted urinary DCBA concentrations had comparable semen parameters to men in the lowest tertile (2.59 vs. 2.88 ml for semen volume, 47.9 vs. 45.8 million/ml for sperm concentration, 116 vs. 118 million for total sperm count, 25 vs. 24% for progressive sperm motility, and 6.1 vs. 5.8% for morphologically normal sperm). In addition, BMI and smoking status did not modify the associations.

LIMITATIONS REASONS FOR CAUTION—We had a relatively small sample size with similar socioeconomic backgrounds and with overall relatively low urinary concentrations of DEET biomarkers. However, our sample size was enough to detect moderate differences with at least 80% statistical power, between the first and third tertiles of urinary DCBA concentrations. Limitations also include possible misclassification of DCBA exposure and difficulties in extrapolating the findings to the general population.

WIDER IMPLICATIONS OF THE FINDINGS—Our study found no associations between urinary concentrations of DCBA, a major metabolite of the insect repellent DEET, and semen parameters in men presenting for infertility treatment. While these results are reassuring, further studies including larger sample sizes and higher exposures are warranted.

STUDY FUNDING/COMPETING INTEREST(S)—The project was financed by the National Institute of Health grants R01ES022955 and R01ES009718 and by grant P30ES000002 from the National Institute of Environmental Health Sciences (NIEHS). None of the authors has any conflicts of interest to declare. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Keywords

DEET; DCBA; insect repellent; male fertility; semen parameters

Introduction

N,N-Diethyl-*m*-toluamide (DEET), in use since the 1940s, is the most widely used ingredient in insect repellents in the USA (Chen-Hussey *et al.*, 2014). The Centers for Disease Control and Prevention (CDC) recommends insecticides with at least 20% DEET as a first line preventive measure against mosquitos carrying Zika, malaria, Dengue fever and West Nile viruses (CDC, 2016). Most significantly, American men and women of reproductive age who are contemplating pregnancy are advised to use DEET by the American College of Obstetricians & Gynecologist to prevent the risks of prenatal exposure to Zika (Wylie *et al.*, 2016).

There are 505 products registered on the US Environmental Protection Agency (EPA) website as containing DEET at greater than 5% concentrations (EPA, 2016). DEET can be absorbed topically or through consumption of contaminated water. Trace levels of DEET have been detected in drinking water globally (Sandstrom *et al.*, 2005; Costanzo *et al.*, 2007; Merel and Snyder, 2016). DEET is metabolized by cytochrome P450 enzymes into oxidative metabolites, including N,N-diethyl-3-hydroxymethylbenzamide (DHMB) and 3-(diethylcarbamoyl) benzoic acid (DCBA) (Selim *et al.*, 1995); then DEET and its metabolites are eliminated primarily in urine (Selim *et al.*, 1995). According to the National Health and Nutrition Examination Survey (NHANES), an estimated 84% of the US population has detectable concentrations of DCBA, a major DEET metabolite, in their urine (Calafat *et al.*, 2016). However, only 3% of NHANES participants had detectable DEET in urine, thus suggesting that DEET itself is an inadequate biomarker for background environmental exposures. When DEET is given intravenously, the half-life of DEET in humans is estimated to be 4 h with 52% of the dose recovered in urine (Feldmann and Maibach, 1970). Selim *et al.* administered 20 and 100% topical DEET to male volunteers and detected radiolabeled DEET in plasma within two hours of application and undetectable levels 4 h after the last dose (Selim *et al.*, 1995).

Animal studies have demonstrated reproductive toxicity from DEET exposure. Studies in 35-day-old hamsters fed ≥624 mg/kg/day of DEET for 90 days had histopathologic evidence of tubular degeneration in the testes (EPA, 1998). Another study on rats treated with the insecticide permethrin and DEET (40 mg/kg/day) for 28 days found increased apoptosis in testicular germ cells (Abou-Donia *et al.*, 2003). Glieberman *et al.* (1976) found that male

rats treated with DEET had abnormal morphology and reduced sperm motility. Several case reports in humans have highlighted the toxic effects from >4 g per week of topical DEET including skin reactions, neurotoxicity, encephalopathy, seizures and even death (Robbins and Cherniack, 1986; ATSDR, 2015). In children, products containing <20% DEET have been linked to several cases of respiratory distress and seizures (Briassoulis *et al.*, 2001). Despite, the widespread use of DEET in individuals of all ages, there is limited data on DEET exposure and human reproduction. To date, there has been only one study on urinary DEET concentrations and semen quality among men, which excluded key metabolites in their analysis and was limited to fertile men (Swan *et al.*, 2003). Since, it has been shown that DEET is not the best biomarker of exposure (Calafat *et al.*, 2016), the study of metabolites is essential. This is the first study to investigate whether urinary DEET biomarkers are associated with semen parameter values in men from a fertility center.

Methods

Study population

Study participants were male partners of couples enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort that recruits couples seeking infertility treatment from the Massachusetts General Hospital (MGH) Fertility Center. EARTH Study was established in 2004 to evaluate environmental and dietary determinants of fertility (Hauser *et al.*, 2006). Men between the ages of 18–56 years were eligible to participate. Men who had a vasectomy were ineligible. Approximately 40% of those contacted by the research nurses were enrolled. Men included in this analysis were those selected as a convenient sample for the purpose of a previous pesticide validation study (unpublished). Of 164 men who had completed a food frequency questionnaire (FFQ) and provided at least two urine samples between April 2007 and July 2015, we selected 90 men with urine samples collected within nine months before or after FFQ completion, and had their stored urine samples analyzed for urinary pesticide metabolites. Of the 180 urine samples, 3 had record errors and 6 were collected before the semen sample, leaving 171 samples available for analysis. Those 90 men provided a total of 250 semen samples. The urine samples were collected the same day as (29%) or before the semen samples were collected. Men included in this analysis had similar demographic, reproductive and semen parameter characteristics compared to the rest of men included in the EARTH Study (data not shown). After the study procedures were explained and all questions were answered, participants signed an informed consent form. The participant's date of birth was collected at entry, and weight and height were measured by trained study staff. BMI was calculated as weight (in kilograms) per height (in meters) squared. The participants provided health information and completed a nurse-administered questionnaire that contained additional questions on lifestyle factors, reproductive health and medical history.

Ethical approval

The study was approved by the Human Studies Institutional Review Boards of the MGH, the Harvard T.H. Chan School of Public Health, University Hospitals Cleveland Medical Center, and the Centers for Disease Control and Prevention (CDC).

Quantification of DEET and its metabolites

Men provided a spot urine sample at study entry and at other routine clinical visits during the infertility treatment of the couple. The present analysis included 171 urine samples obtained before the collection of the semen sample (one to two urine samples per man). Urine was collected in a sterile, clean polypropylene specimen cup at the MGH Fertility Center. Specific gravity (SG) was used to adjust DEET concentrations for urinary dilution. SG was measured at room temperature and within several hours (typically within one hour) of the urine collection with a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA) that was calibrated with deionized water before each measurement. The urine was then divided into aliquots, frozen and stored at −80°C. Samples were shipped on dry ice overnight to the CDC where they were stored at or below −40°C until analysis. CDC staff quantified DEET, DCBA and DHMB in 100 μl of urine after enzymatic hydrolysis of the conjugated species of the target analytes, followed by on-line solid-phase extraction, separation with high-performance liquid chromatography, and detection by isotope-dilution positive ion atmospheric pressure chemical ionization tandem mass spectrometry (Kuklenyik *et al.*, 2013). The limits of detection (LOD) were DCBA (0.475 μg/l), DEET (0.083 μg/l) and DHMB (0.089 μg/l). In addition to study samples, each analytical run included low- and high-concentration quality control urine pools and reagent blanks to assure the accuracy and reliability of the data (Kuklenyik *et al.*, 2013). Concentrations of DEET and its metabolites were adjusted for dilution by using the following formula: $Pc = P[(1.015 - 1)/SG - 1]$, where Pc is the SG-corrected biomarker concentration (μg/l), P is the measured bio-marker concentration (μg/l) and 1.015 is the mean SG in the study population (Pearson *et al.*, 2009; Smith *et al.*, 2012). For men with only one urine sample (35%), the biomarker concentration for that single sample was used as the man-specific urinary concentration. For men with two samples (65%), we estimated the biomarker geometric mean (GM) of those two samples collected up to one year before the semen analysis. Biomarker concentrations below the LOD were assigned a value equal to the LOD divided by the square root of 2 before SG adjustment (Hornung and Reed, 1990).

Assessment of semen parameters

Semen was collected on site at MGH in a sterile plastic specimen cup following a recommended 48 h abstinence period. Some men provided multiple samples because their female partner underwent more than one cycle of infertility treatment. There were 23 men (26%) who provided one semen sample and 67 men (74%) who provided more than one (ranging from one to seven semen samples). Semen volume (ml) was measured by an andrologist using a graduated serological pipet. Sperm concentration (mill/ml) and motility (% motile) were assessed using a computer-aided semen analyzer (CASA; 10HTM-IVOS, Hamilton-Thorne Research, Beverly, MA, USA). To measure semen concentration and motility, 5 μl of semen was placed into a pre-warmed (37°C) and disposable Leja Slide (Spectrum Technologies, CA, USA). A minimum of 200 sperm cells from at least four different fields were analyzed from each specimen. Motile spermatozoa were defined as according to the World Health Organization (WHO) classification and included progressive spermatozoa (moving actively, either linearly or in a large circle, regardless of speed) and non-progressive sperm cells (all other patterns of motility with an absence of progression)

(World Health Organization, 2010). Total sperm count (mill/ejaculate) was calculated by multiplying sperm concentration by semen volume. Total progressive motile sperm count (mill/ejaculate) was calculated by multiplying total sperm count by progressive motility. Sperm morphology (% normal) was assessed on two slides per specimen (with a minimum of 200 cells assessed per slide) via a microscope with an oil-immersion 100× objective (Nikon, Tokyo, Japan). Strict Kruger scoring criteria was used to classify men as having normal or below normal morphology (Kruger *et al.*, 1988). Total normal morphology sperm count (mill/ejaculate) was calculated by multiplying total sperm count by the percentage of normal morphology. Andrologists were blinded to the participants' urinary DEET biomarkers concentrations as well as their fertility treatment status, prior semen quality results and related clinical outcome data. For quality assurance/quality control purposes, the laboratory staff conducted weekly monitoring of sperm morphology smears, which were repeated until results differed by <0.5 SD of the mean. In addition, the laboratory performed quarterly competency evaluations of all technicians and proficiency testing by an outside evaluator every six months.

Statistical analysis

Demographic and baseline reproductive characteristics of the men were presented as median ± interquartile ranges (IQRs) or number and percentages. Men's exposure to DCBA was categorized in tertiles of urinary DCBA concentrations, to provide a conservative approach which does not require the strong assumption of linear trends between DCBA and semen parameters. DEET and DHMB were not considered for analysis because of the low percentage of detectable concentrations (5 and 6%, respectively). Sperm concentration and the sperm counts showed non-normal distributions and were transformed using the natural log (ln) before analysis. Associations of SG-adjusted urinary DCBA concentrations with demographic characteristics and baseline reproductive characteristics were evaluated by using Kruskal–Wallis tests for continuous variables and chi-squared tests for categorical variables (or Fisher's exact test where appropriate). Linear mixed-effects models with random subject effects, to account for repeated semen measurements from the same man, were used to estimate the association of SG-adjusted urinary DCBA concentrations with semen parameter values. Tests for linear trends were conducted using the SG-adjusted urinary DCBA concentrations as an ordinal level indicator variable of each concentration tertile, simulating a continuous variable. To allow for better interpretation of the results, population marginal means (Searle *et al.*, 1980) are presented adjusting for all the covariates in the model (at the mean level for continuous variables and at a value weighted according to their frequencies for categorical variables).

Confounding was assessed from prior knowledge of biological relevance and descriptive statistics from our study population through the use of directed acyclic graphs (Weng *et al.*, 2009). The variables considered as potential confounders included factors previously related to reproductive outcomes in this and other studies, and factors associated with DEET exposure and reproductive outcomes in this study (Sharma *et al.*, 2013; Rooney and Domar, 2014). Final models were adjusted for age (continuous), BMI (continuous), smoking status (ever or never smoked), season [warm (June–October) and cooler (November–May)], abstinence time (continuous), varicocele (yes or no) and previous infertility exam (yes or

no). Effect modification by BMI (<25 kg/m^2 vs. ≥25 kg/m^2) and smoking status (ever vs. never smoked) was tested by adding a cross-product term to the final multivariate model. Sensitivity analyses were conducted only including up to two semen samples per man, to reduce any bias that could result if men who provided more semen samples had poorer semen quality and thus had female partners with more infertility treatment cycles, and also only including the urine sample closest to the collection of the semen sample given the sensitive window of spermatogenesis (70 days approx.). Statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

The 90 participating men had a median (IQR) age of 36 (34, 40) years, were predominantly Caucasian (89%), and 68% had never smoked (Table I). The median (IQR) BMI was 27.0 (23.7, 28.9) kg/m^2. Approximately 4% of men had a history of cryptorchidism, and 11% had a varicocele. The majority of men (76%) had undergone a previous fertility exam and unexplained infertility was the primary infertility diagnosis at enrollment (42%). The percentage of non-smokers decreased across tertiles of urinary DCBA concentrations (T1 = 80% and T3 = 63%), however, this trend was not statistically significant (*P*-value = 0.16). No other baseline characteristics differed substantially among tertiles of DCBA concentration (Table I).

We detected DCBA in 85% of the 171 urine samples collected from the 90 men; the unadjusted and SG-adjusted median DCBA urinary concentrations were 1.26 and 1.91 μg/l, respectively (Table II). DEET and DHMB were detected in only 5 and 6% of samples, respectively. For the 250 semen samples contributed by the 90 men, the median (IQR) values were 59 (29, 108) mill/ml for sperm concentration; 137 (68, 257) mill for total sperm count; 25 (14, 38) % for progressive sperm motility; and 6 (4, 9) % for morphologically normal spermatozoa (Table III). Of all the semen samples, 161 (64%) were below the WHO 2010 lower reference limit for progressive sperm motility (> 32%) (World Health Organization, 2010).

In unadjusted and adjusted models, SG-adjusted urinary DCBA concentrations were not associated with sperm parameter values (Table IV). In models adjusted for age, BMI, smoking status, abstinence time, varicocele and previous infertility exam, men in the highest tertile of SG-adjusted urinary DCBA concentrations had comparable semen parameters to men in the lowest tertile (2.59 vs. 2.88 ml for semen volume, 47.9 vs. 45.8 million/ml for sperm concentration, 116 vs. 118 million for total sperm count, 25 vs. 24% for progressive sperm motility, and 6.1 vs. 5.8% for morphologically normal sperm cells) (Table IV). Although season was not associated with SG-adjusted urinary DCBA concentrations, it was accounted for in the models because the use of insect repellent for preventing mosquito bites is more frequent in warm weather as suggested by seasonal variation in DCBA urinary concentration in the NHANES study (Calafat *et al.*, 2016). However, further adjustment for season had little impact on estimated associations (data not shown). The results also remained the same when year of sample collection was included as a covariate in the models (data not shown).

In addition, there was no evidence of significant heterogeneity on the null relationship between SG-adjusted urinary DCBA concentrations and semen parameters by BMI (<25 kg/m^2 vs. = 25 kg/m^2) and smoking status (ever vs. never smoked) (data not shown). We also observed no significant associations (and similar effect estimates) when analyses were restricted to up to two semen samples per man (n = 90 men contributing 157 semen samples) and also when analyses included only the urine sample closest to the collection of the semen sample (data not shown).

Discussion

This study investigated the association between the urinary concentrations of DCBA, a metabolite of the insect repellent DEET, and semen parameter values in men presenting with their partner at a fertility center. We found no association between SG-adjusted urinary DCBA concentrations and semen quality in unadjusted and adjusted models for smoking status, age, BMI, days of abstinence, varicocele, and prior infertility. Effect modification for BMI and smoking status, known predictors of semen quality, did not modify the null results. An explanation for the lack of observed association could be the homogeneous sample of 90 men who were mostly urban, educated and Caucasian, seeking infertility treatment at MGH. These were patients who were more likely to be conscientious of their lifestyle and behavior and to monitor their daily use of products with synthetic chemicals. It is also possible that DEET exposure among the men in our population, who provided samples before the emergence of the Zika epidemic in 2015, was relatively low compared with US background exposures. The participant with the highest urinary DCBA, DEET and DHMB concentrations (SG-adjusted = 12 887, 7.43 and 69.5 μg/L, respectively) reported recent insect repellent use and had a normal semen analysis result.

In 5348 2007–2010 NHANES participants for whom urine was analyzed for DEET and its metabolites, DEET was detected in 3%, DHMB in 15.5%, and DCBA in 84% of urine samples (Calafat *et al.*, 2016), similar to our detection frequencies. In men, the GM concentration of DCBA was 5.6 μg/L in NHANES, which was more than double our GM concentration of 2.2 μg/L. Among NHANES participants, concentrations of DCBA were higher in warmer months than in colder weather which is consistent with a higher detection of DEET in surface water and waste water samples during the summer compared to winter (Aronson *et al.*, 2012). In our study, we did not find this seasonal variation.

Only one prior study has explored the association between environmental DEET exposure and semen quality (Swan *et al.*, 2003). As part of the Study for Future Families (SFF), DEET was measured in urine collected between 2000 and 2001 from 86 fertile men from agricultural communities in Missouri and Minnesota, and the authors reported no association between urinary DEET concentrations and semen parameter values (Swan *et al.*, 2003). The study reported 37% of men had detectable urinary DEET, which is much higher than the detection reported by NHANES or our own study (3% and 5%, respectively) even though the LODs are comparable. The authors did not measure DCBA, the most reliable biomarker of DEET exposure (Calafat *et al.*, 2016) and the primary metabolite detected in our study and NHANES. Swan *et al.* assigned the men, all with proven fertility, to ‘cases’ and ‘controls’ on the basis of sperm concentration above or below the population median.

However, the reported values for mean sperm concentrations, total motile sperm, percentage motility and morphology were all above the normal WHO 2010 reference values for both cases and controls (Cooper *et al.*, 2010). Finally, the study recruited men with proven fertility, which also limits the interpretation of their results.

Recent animal studies have demonstrated mixed results of DEET exposure on male fertility. Dermal application of DEET at 100, 300 and 1000 mg/kg dose, five times per week for nine weeks did not affect sperm count, morphology, or viability in rats (Lebowitz *et al.*, 1983). However, this study did not assess whether the rats were able to breed after exposure. A study in rats showed that exposure to a mixture of DEET (40 mg/kg/day) and permethrin during the time of gonadal sex determination caused pubertal abnormalities, azoospermia, seminiferous tubule defects, and sloughed spermatogenic cells, all of which persisted to the next generation (Manikkam *et al.*, 2012). The transgenerational inheritance of these phenotypes were mediated by pesticide-induced changes in the DNA methylation regions of the sperm epigenome (Manikkam *et al.*, 2012). Collectively, these findings suggest that DEET could have in-utero developmental effects on the fetus and postnatal life. It is possible that permethrin alone or its synergistic action in combination with DEET caused the gonadal defects. There is a scarcity of data on the additive actions of multiple insect repellents and the EPA reports only the individual chemical profiles (Kepner, 2004). Additional studies of insect repellent mixtures may help better assess fertility affects.

This study has some limitations. We had a relatively small sample size with similar socioeconomic backgrounds and with overall relatively low urinary concentrations of DEET biomarkers. However, the study had a sufficient sample size to provide 80% power for detecting, between the first and third tertiles of urinary DCBA concentrations, a 49% decrease in sperm concentration, a 50% decrease in total sperm count, a 65% decrease in total progressive motile count, a 55% decrease in total normal morphology sperm count, an absolute mean difference of 1 mL fewer semen volume, an absolute mean difference of 11% fewer progressive motility and an absolute mean difference of 3% fewer normal morphology; which is reasonable for a study of this design. In addition, the infertility diagnosis among our patients consisted of 42% with unexplained infertility, 30% male factor and 28% female factor, which may account for the majority having normal semen quality. Notably, progressive motility was abnormal in 64% of the men. Even so, urinary DCBA concentrations were not associated with progressive motility. Future studies could focus on DCBA concentrations among men with only male-factor infertility. Also, mis-classification of DEET exposure based on urinary concentrations of DCBA from spot samples is possible because DEET metabolites have relatively short elimination half-lives (Feldmann and Maibach, 1970) and exposures to DEET are likely to be episodic in nature. However, we collected two urine samples in most of the participants and this will reduce but not prevent exposure misclassification. Strengths of this study include its prospective design and detailed assessment of lifestyle factors and reproductive disorders which allowed the control of potential confounders in the analyses. The andrologists analyzing the semen samples were blind to the participants' urinary DCBA concentrations as well as their fertility treatment and prior semen analysis results. The urine samples were shipped to the CDC and tested with validated, high precision techniques, identical to the protocols used to analyze the 2007–2010 NHANES samples (Calafat *et al.*, 2016).

In conclusion, our study found no associations between urinary concentrations of DCBA, a major metabolite of the insect repellent DEET, and semen parameters in men presenting for infertility treatment at a fertility center. While this is the first study to examine the relationship between urinary concentrations of DCBA and semen quality, it only included 90 men with relatively low DEET exposure. Therefore, while these findings are reassuring, further studies including larger sample sizes and higher exposures are warranted along with studying the concomitant use of DEET with other chemicals in insect repellents.

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Table I

Demographic and reproductive characteristics[a] by tertiles of urinary DCBA concentrations (μg/l) among 90 men in the EARTH Study.

	Total cohort	Urinary DCBA concentrations		
	(*n* = 90)	T1 (*n* = 30)	T3 (*n* = 30)	*P*-value[b]
Baseline characteristics				
Age, years	36.1 (33.8, 40.4)	35.9 (31.2, 40.8)	36.8 (34.3, 40.4)	0.52
Race/Ethnic group, *n* (%)				
White/Caucasian	80 (89)	28 (94)	27 (90)	0.36
Black	1 (1)	0 (0)	0 (0)	
Asian	6 (7)	1 (3)	1 (3)	
Other	3 (3)	1 (3)	2 (7)	
BMI, kg/m^2	27.0 (23.7, 28.9)	26.9 (23.7, 29.1)	27.5 (23.8, 29.1)	0.74
Smoking status, *n*[c] (%)				
Never smoked	61 (68)	24 (80)	19 (63)	0.16
Former smoker	26 (29)	5 (17)	9 (30)	
Current smoker	3 (3)	1 (3)	2 (7)	
Education[c], *n* (%)				
<College graduate	15 (17)	2 (7)	8 (27)	0.50
College graduate	23 (26)	8 (27)	7 (23)	
Graduate degree	49 (54)	19 (63)	14 (47)	
Reproductive characteristics, *n* (%)				
Undescended testes	4 (4)	2 (7)	0 (0)	0.48
Varicocele	10 (11)	3 (10)	2 (7)	0.59
Epididymitis	2 (2)	1 (3)	0 (0)	0.99
Prostatitis	1 (1)	0 (0)	1 (3)	0.99
Previous infertility exam	68 (76)	25 (83)	22 (73)	0.49
Infertility diagnosis at enrollment				
Male factor	27 (30)	8 (26)	11 (37)	0.88
Female factor	25 (28)	11 (37)	6 (20)	
Unexplained	38 (42)	11 (37)	13 (43)	

[a]Values are presented as median (IQR) unless otherwise noted.

[b]From Kruskal–Wallis test for continuous variables and chi-squared tests (or Fisher's exact test where appropriate) for categorical variables.

[c]These variables have missing dataes. Abbreviations: DCBA, 3-(diethylcarbamoyl)benzoic acid; EARTH (Environment and Reproductive Health); IQR, interquartile range; *N*, number; T, tertile.

Table II

Distribution of man-specific urinary concentrations (μg/l) of DEET and its metabolites among 90 men (n = 171 urines) in the EARTH Study.

	Detection frequency %	GM (SD)	Min	25th	50th	75th	Max.
Urinary DCBA concentrations	85	2.2 (0.26)	<LOD	0.61	1.26	4.75	18 900
SG-adj urinary DCBA concentrations	–	2.2 (0.21)	<LOD	0.78	1.91	4.53	12 887
Urinary DEET concentrations	5	–	<LOD	<LOD	<LOD	<LOD	10.9
SG-adj urinary DEET concentrations	–	–	<LOD	<LOD	<LOD	<LOD	7.43
Urinary DHMB concentrations	6	–	<LOD	<LOD	<LOD	<LOD	102
SG-adj urinary DHMB concentrations	–	–	<LOD	<LOD	<LOD	<LOD	69.5

DEET, N,N-Diethyl-m-toluamide; EARTH, Environment and Reproductive Health; DCBA, 3-(diethylcarbamoyl)benzoic acid; DHMB, N,N-diethyl-3-hydroxymethylbenzamide; GM, geometric mean; <LOD, limit of detection (in μg/l) for DCBA (0.475), for DEET (0.083) and for DHMB (0.089); Min, minimum; Max, maximum; SG-adj, specific gravity adjusted.

Table III

Distribution of semen parameter values among 90 men contributing 250 semen samples in the EARTH Study.

	Mean (SD)	25th	50th	75th	WHO 2010 lower ref limits	N (%) below WHO 2010 lower ref limits
Semen volume, mL	2.7 (1.3)	1.7	2.5	3.5	1.5	39 (16)
Sperm concentration, mill/mL	72.3 (61.2)	29.0	59.0	108	15	30 (12)
Total sperm count, mill/ejaculate	173 (134)	68.0	137	257	39	28 (11)
Progressive motility,%	26.1 (14.3)	14.0	25.0	38.0	32	161 (64)
Total progressive motile sperm count, mill/ejaculate	54.0 (55)	9.2	36.4	79.3	–	–
Normal morphology[c], %	6.5 (3.2)	4.0	6.0	9.0	4	36 (14)
Total normal morphology sperm count[c], mill/ejaculate	12.1 (11)	3.5	8.8	18.4	–	–
Abstinence time, days	4.5 (17)	2.0	2.4	3.0	–	–

ml, milliliters; mill, millions; ref, reference.

Thirteen semen samples had missing data for normal morphology and therefore total normal morphology sperm count.

Table IV

Semen parameters by tertiles of urinary DCBA concentrations (μg/l) among 90 men contributing 250 semen samples in the Environment and Reproductive Health (EARTH) Study.

Urinary DCBA concentrations (μg/l) (range)	Semen volume (ml)	Sperm concentration (mill/ml)	Total sperm count (mill)	Progressive motility (%)	Total progressive motile sperm count (mill)	Normal morphology (%)	Total normal morphology sperm count (mill)
Unadjusted							
T1 (<LOD-0.97)	2.90 (2.56, 3.25)	45.3 (35.6, 57.7)	116 (92.2, 144)	24.1 (20.2, 28.1)	23.0 (15.4, 34.4)	5.8 (5.0, 6.6)	6.1 (4.4, 8.60)
T2 (0.98–2.86)	2.73 (2.40, 3.06)	51.0 (42.2, 61.7)	117 (94.9, 145)	25.5 (21.7, 29.3)	24.8 (17.3, 35.3)	7.0 (6.2, 7.9) *	7.4 (5.7, 9.6)
T3 (2.87–12887)	2.57 (2.26, 2.88)	47.9 (38.0, 60.4)	116 (89.8, 150)	25.3 (21.1, 29.6)	24.1 (16.5, 35.3)	6.0 (5.2, 6.9)	6.2 (4.5, 8.4)
p-trend	0.10	0.59	0.96	0.63	0.83	0.51	0.87
Adjusted[a]							
T1 (<LOD-0.97)	2.88 (2.56, 3.22)	45.8 (36.1, 58.1)	118 (94.9, 146)	24.0 (20.2, 27.8)	23.6 (16.2, 34.2)	5.8 (5.1, 6.6)	6.2 (4.5, 8.6)
T2 (0.98–2.86)	2.75 (2.40, 3.10)	51.4 (42.3, 62.5)	118 (95.0, 147)	25.6 (21.7, 29.4)	25.6 (18.1, 36.0)	7.3 (6.4, 8.1) *	7.6 (5.8, 10.0)
T3 (2.87–12 887)	2.59 (2.28, 2.89)	47.9 (39.0, 58.8)	116 (92.1, 147)	25.3 (21.1, 29.4)	24.2 (16.6, 35.3)	6.1 (5.2, 7.0)	6.2 (4.7, 8.3)
p-trend	0.14	0.72	0.95	0.62	0.88	0.50	0.87

[a]Data are presented as predicted marginal means (95% CI) adjusted for age (continuous), BMI (continuous), smoking status (ever and never smoked), abstinence time (continuous), varicocele (yes and no) and previous infertility exam (yes and no).

*Indicates a *P*-value < 0.05 when compared that tertile with the lowest tertile of exposure.

Thirteen semen samples had missing data for normal morphology and therefore total normal morphology sperm count. Tests for linear trends were conducted using the SG-adjusted urinary DCBA concentrations as an ordinal level indicator variable of each tertile of exposure. Abbreviations: DCBA, 3-(diethylcarbamoyl)benzoic acid; LOD, limit of detection (0.475 μg/l).