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Mixture analysis on associations between semen quality and sperm DNA integrity and occupational exposure to polycyclic aromatic hydrocarbons

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ABSTRACT

The objective of this study was to assess relationships between exposure to PAHs at occupational levels and outcomes of human semen quality and sperm DNA integrity. Personal breathing zone air samples were collected to quantify exposure of 16 targeted PAHs to coke-oven workers at a steel company in southern Taiwan. Semen quality, including concentration, motility, morphology, and viability, were assessed. Sperm DNA fragmentation, 8-oxodGuo, bulky PAH adducts, and benzo[a]pyrene diol epoxide-DNA adducts served as biomarkers for assessment of sperm DNA integrity. The Bayesian Kernel Machine Regression modeling was employed to estimate mixture effects of the PAH mixture on the outcomes of semen quality and sperm DNA integrity and to identify individual compounds of PAH mixtures associated with the mixture effects. Exposure to the PAH mixture was inversely associated with sperm viability, while benzo(b)fluoranthene (B[b]F) was identified as the main predictor for sperm viability. Exposure to the PAH mixture also exhibited a positive trend with sperm DNA fragmentation. B[b]F and benzo(a)anthracene (B[a]A) were identified as individual PAH compounds associated with increased sperm DNA fragmentation.

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Bay regions; benzo(b)fluoranthene; mixture analysis;

Introduction

Research has shown a decline in human sperm counts over the last decade,[1,2] and the incidence of testicular cancer and other reproductive dysfunctions have progressively increased.[3–5] Environmental and occupational exposure to human-made or industrial chemicals have been implicated as causative factors in the overall decline of male reproductive health.[3,4] Increased literature has suggested that environmental and occupational exposure to human-made or industrial chemicals is implicated as causative factors in the decline of male reproductive health and semen quality.[5–8]

Polycyclic aromatic hydrocarbons (PAHs), a mixture of semi-volatile and lipophilic compounds, are generated from combustion and are widespread in the environment.[9] Human biomonitoring data show that essentially 100% of the U.S. population is exposed to PAHs in mixtures,[10] via grilled and barbecued food consumption, smoking, vehicle emissions and/or industrial emissions.[11] Upon entering the biological system, PAHs are metabolized to reactive intermediates by cytochrome P450 enzymes.[12] These active intermediates can covalently attach to DNA in tissues and lymphocytes and form PAH-like adducts, e.g. benzo[a]pyrene diol epoxide deoxyguanosine adducts (BPDE-dG).[13] In another metabolite pathway, they can undergo the redox cycling reaction that can generate excess reactive oxygen species (ROS) and form DNA oxidative base adducts, e.g. 7,8, dihydro-8-oxoguanie (8-oxodGuo).[13] Increasing epidemiological studies reported that exposure to PAHs was associated with decreased semen quality.[7,8,14] However, there is still lack epidemiologic data in mixture analysis on associations of PAHs and semen quality outcomes, particularly on sperm DNA integrity. Assessment of

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sperm DNA integrity is important, since it relates to male fertility potential and the risk of increased chromosomal abnormalities linking to minor or major birth defects of offspring.[15,16] Also, sperm DNA integrity may be a better predictor of male fertility potential than routine semen parameters.[17]

The objective of this study was to assess associations between both individual PAH compounds/PAH mixtures and semen quality and sperm DNA integrity. Specifically, sperm DNA adduct biomarkers, 8-oxodGuo, bulky PAH-like adducts, and BPDE-dG, were identified and quantified to increase our understanding of PAHs metabolic pathways in the testicular system and underlying mechanisms of the PAH reproductive toxicity. The Bayesian kernel machine regression (BKMR) modeling with Gaussian kernel function was used to determine the associations between exposure to PAH mixtures and the outcomes of semen quality and sperm DNA integrity. The BKMR can also assess interactions among PAH compounds, and evaluate a dose-response relationship between exposure to PAHs and the outcomes of semen quality and sperm DNA integrity, while controlling for confounding factors.

Methods

Human subjects

We selected coke-oven workers from a steel mill in southern Taiwan for PAH exposure assessment. We used the subjects because of the following factors: standard coking processes of the plant for over a decade; well-characterized demographics; long-term work relationships with plant managers for subject recruitment and retention. Increased concentrations of PAHs have been found in the work areas around and/or near coke ovens and blast furnaces.[7,8] A total of 201 topside-oven and side-oven workers participated in screenings to determine eligibility during their annual health examinations. Eligibility criteria included more than one year of employment in the plant; ages between 25 and 60 years old; no known reproductive dysfunction, and no presence of cancer. A total of 54 exposure subjects (31 topside-oven workers and 23 side-oven workers) were included in this study, since they met the criteria and provided all required biological and environmental samples. A power analysis was conducted to ensure that the sample size was sufficient to detect a 1.5-fold difference between the mean concentrations of sperm DNA fragmentation for the two exposure groups. A questionnaire was collected from each of the subject to characterize subjects' demographic information, including age, body mass index (BMI), education, smoking, alcohol consumption, and barbecued and grilled food consumption. The study was approved by the Institutes of Research Boards at both Old Dominion University and Kaohsiung Medical University. All participants were fully informed about the objective of the study and signed the consent form before screening and sampling took place.

Personal breathing zone sampling and quantification of PAHs

Personal breathing zone air samples were collected to determine the PAH intake of coke-oven workers. For personal intake assessment, each worker wore two personal air samplers (SKC, model 224PCXR7) for 7 hours on the first and sixth workdays. One sampler with glass fiber filters (diameter: 25 mm, pore size: 0.7 μm) at a flow rate of 2.0 L/min was used to collect particulate PAHs. The other sampler, coated with XAD-2 resin (SKC 226-30-04) at a flow rate of 0.5 L/min, was used to collect gaseous PAHs. After sampling, all filter and resin samples were stored at 4 °C before analysis.

The PAH mixture in this study included sixteen targeted PAH compounds, which were identified as priority pollutants by the U.S. Environmental Protection Agency.[18] The PAH compounds in the mixture include naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), pyrene (PRY), fluoranthene (FLA), benzo[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo(a)pyrene (B[a]P), indeno[1,2,3-cd]pyrene (IND), dibenzo[a,h]anthracene (D[ah]A), and benzo[ghi]perylene (B[ghi]P). The PAH compounds were analyzed using a gas chromatogram quadruple mass spectrometer (GC/MS, Agilent Technologies 6890 N) with an automatic sampler system. After the weights of PAHs were quantified, time weighted concentrations of PAHs were calculated. The detection limits were determined by conducting seven repeated analyses of the lowest standard for each PAH species. The detection limits of the 16 PAHs ranged from 6.1 ng for B[a]A to 9.8 ng for NAP. The relative standard deviation ranged from 2.32% for CHR to 19.2% for B[a]P. Measurements below the detection limit in each air sample were set at half of the detection limit.

Semen quality

The participants were instructed to abstain from ejaculation for at least three days before sperm collection. Each semen sample was collected by ejaculation. Routine semen analyses included semen volume, sperm concentration, total number of sperm per sample, percentage of motile sperm, and percentage of sperm with normal morphology (entire cell considered) and normal head morphology, and percentage of sperm with viability. These parameters were analyzed according to World Health Organization (WHO) guidelines.[19] All laboratory tests were done in a blind fashion. When sperm counts were less than 20×10^6/ml, that condition was recorded as oligospermia. Percentage of oligospermia was quantified as A/T, where A sperm count $< 20 \times 10^6$/ml and T equals total number of samples. For viability analysis, at least 300 sperm per sample were assessed from eosin-stained preparations. Percentage of sperm without being stained with eosin was counted to represent percentage of viable sperm. For the morphology assessment, two slide smears were prepared from each semen sample. Three hundred sperm per sample were evaluated from air-dried Papanicolaou-stained preparations and classified as either normal or abnormal according to the strict criteria recommended by the WHO guidelines.[19] When the percentage of motile sperm is less than 50, the condition was recorded as asthenosphermia.

Sperm DNA fragmentation

A sperm pellet was then collected from each semen sample for sperm DNA fragmentation analysis using the in-situ nick-end labeling (TUNEL) assay.[20] A sperm pellet was re-suspended, washed with 1% human serum albumin, and spread onto slides. Then, cells were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate at 4 °C for 2 min. A nucleotide labeling mixture prepared according to the Roche Diagnostic manufacture's instruction was deployed onto sperm cells. Fluorescence in sperm cells recorded as a positive for the TUNEL assay was assessed using an Olympus BX61 fluorescence microscope. At least 300 sperm cells from each sample were accounted for, and the percentage of TUNEL positive cells was calculated as the outcome of interest.

8-oxodguo adducts

Sperm DNA was isolated according to the procedure recommended by the European Standard Committee on Oxidative DNA Damage with several modifications to minimize DNA oxidization during DNA isolation procedures.[21] Briefly, a pellet from each 15 to 100×10^6 sperm sample was collected through centrifugation for DNA extraction using the DTT solution.[21] 8-oxodGuo adducts in sperm DNA were determined by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) with an on-line solid-phase extraction (SPE) as reported in recent studies.[22,23] After automatic sample cleanup, DNA samples were injected into an Agilent 1100 series HPLC system interfaced with a PE-SCIEX API 3000 triple quadrupole mass spectrometer with an electrospray ion source. Detection was performed in the positive ion multiple reaction monitoring mode for simultaneous quantification of 8-oxodGuo and dG. And transitions of the precursors to the product ions were as follows 8-oxodGuo (m/z 284→168), [$^{15}N_5$]-8-oxodGuo (m/z 289→173), dG (m/z 268→152), and [$^{15}N_5$]-dG (m/z 273→157). With the use of isotopic internal standards and on-line SPE, this method exhibited a low limit of detection of 1.8 fmol for 8-oxodGuo, which corresponds to 0.13 adducts/10^6 dG when using 20 μg of DNA per analysis.

Bulky PAH adducts

The presence of PAH-like DNA adducts was determined by the ^{32}P-Postlabeling method.[24,25] Briefly, DNA (10 µg). In brief, after treatment of the mixture with nuclease P1 to convert normal nucleotides to nucleosides, adducted nucleotides were converted to 5′-^{32}P-labeled deoxyribonucleoside 3′,5′-bisphosphates by incubation with carrier-free [γ-^{32}P]ATP and polynucleotide kinase.[24] Radioactive labeled modified nucleotides were mapped by multidirectional anion-exchange thin-layer chromatography (TLC) on polyethyleneimine -cellulose sheets.[25] ^{32}P-labeled I-compounds were visualized by screen-enhanced autoradiography at −80 °C using Kodak BioMax XAR film or with the aid of an InstantImager (Packard Instruments). The extent of covalent DNA adducts was estimated by calculating relative adduct labeling values from sample count rates, the amount of DNA assayed (expressed as pmol DNA monomer units or DNA-P), and the specific activity of [γ-^{32}P]ATP according to Zhou et al.[26]

Statistical analysis

Descriptive statistics were calculated for participant's demographics, outcome measurements of semen

Table 1. Grouping of PAHs for mixture analysis.

PAHs	Grouping	Bay regions (yes/no)	Molecular weight (low/high)
Anthracene (ANT)	1	No	Low
Acenaphthylene (ACY)	1	No	Low
Acenaphthene (ACE)	1	No	Low
Fluorene (FLE)	1	No	Low
Naphthalene (NAP)	1	No	Low
Benzo[g,h,i]perylene (B[ghi]p)	2	No	High
Indeno[1,2,3-cd]pyrene (IND)	2	No	High
Pyrene (PYR)	2	No	High
Chrysene (CHR)	3	Yes	High
Benzo[a]anthracene (B[a]A)	3	Yes	High
Benzo[b]fluoranthene (B[b]F)	3	Yes	High
Benzo[k]fluoranthene (B[k]F)	3	Yes	High
Benzo[a]pyrene (B[a]P)	3	Yes	High
Dibenzo[a,h]anthracene (D[ah]A)	3	Yes	High
Fluoranthene (FLA)	3	Yes	High
Phenanthrene (PHE)	4	Yes	Low

quality and sperm DNA integrity, and PAH concentrations. Distribution of the outcome variables and the participant's PAH concentrations were assessed, and all skewed variables were transformed to satisfy normality assumptions.

Mixture analysis was conducted to assess the associations between the PAH mixture and each outcome of semen quality and sperm DNA integrity using BKMR with Gaussian kernel function based on 10,000 Markov Chain Monte Carlo iterations.[27,28] The outcomes we considered for the analyses were semen quality (concentration, motility, viability, and morphology) and sperm DNA integrity (sperm DNA fragmentation, 8-oxodGuo, bulky PAHs-like adducts, BPDE-dG) The BKMR models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants. The mixture analyses were conducted using hierarchical variable selection process with BKMR, which requires grouping of the exposures. We performed the mixture analysis with grouping based on both "bay region" (yes vs. no) and "molecular weight" (low vs. high). The bay region grouping included both bay and bay-like regions. With the grouping approach, we yielded three groups as follows group 1 having PAHs without bay region and low molecular weight, group 2 with PAHs without bay region and high molecular weight, group 3 having PAHs with bay region and high molecular weight, and group 4 with PAHs with bay region and low molecular weight (Table 1).

We evaluated the overall joint effects (posterior mean estimates and 95% credible intervals) of the PAH mixture on each of the outcomes of semen quality and sperm DNA integrity when all PAHs were at a particular percentile (ranging from 25th percentile to 75th percentile) to when all were at their median values. Additionally, the individual effect (posterior mean estimates and 95% credible intervals) of each PAH exposure, were estimated by comparing the outcome levels when the individual PAH compound was at 75th vs. 25th percentile and all the other PAHs were fixed at a particular percentile (25th, 50th, and 75th percentiles).

BKMR also allows us to visualize the dose-response relationship of each PAH exposure with the outcomes of semen quality and sperm DNA integrity. We plotted a dose-response relationship of each PAH compound with semen quality (concentration, motility, viability, and normal morphology) and sperm DNA integrity (DNA fragmentation, 8-oxodGuo, and bulky PAH-like adducts) by fixing the other PAHs at their medians. Additionally, we evaluated the interaction effect of individual PAH compound with all other PAHs in the mixture by comparing the single PAH risk summary when all the other PAHs were held at the 75th percentile vs. when they were at the 25th percentile for each outcome of sperm DNA integrity.

Results

PAH exposure, study population characteristics, semen quality and sperm DNA integrity

All 16 PAH compounds were identified and quantified in the personal breathing zones of the subjects. Total concentrations of the 16 PAH compounds ranged from 19,887 to 41,620 ng/m^3 (Table 2). The participants were, on average, 39 years old. 28% of the subjects were asthenosphermia, while only 2.5% of the subjects were oligospermia. Only 8.9% of the subjects had sperm with normal morphology. More than 83% of the subjects had sperm with amphorous head. In the domain of sperm DNA integrity, both bulky PAH-like adducts and BPDE-dG were present in sperm (Table 3). All these subjects had DNA

Table 2. PAH Concentrations at the personal breathing zone of coke-oven workers.

PAH compounds (ng/m^3)	Topside-oven workers $n = 31$	Side-oven workers $n = 23$
Acenaphthene	341.05 ± 191.96	598.53 ± 656.06
Acenaphthylene	315.24 ± 218.96	193.09 ± 145.98
Anthracene	353.54 ± 93.33	340.98 ± 66.58
Benzo(a)anthracene	2062.98 ± 1147.33	918.42 ± 1035.69
Benzo(a)pyrene	2114.09 ± 352.18	1517.97 ± 571.15
Benzo(b)fluoranthene	1464.27 ± 354.40	913.00 ± 393.14
Benzo(g,h,i)perylene	3553.93 ± 1250.74	3001.95 ± 1367.16
Benzo(k)fluoranthene	436.16 ± 112.40	666.65 ± 719.16
Chrysene	920.50 ± 132.16	1036.86 ± 461.60
Dibenzo(a,h)anthracene	326.72 ± 124.28	358.64 ± 108.07
Fluoranthene	12336.00 ± 11444.69	2374.69 ± 2488.87
Fluorene	5043.05 ± 2281.81	3368.96 ± 1836.26
Indeno(1,2,3-cd)pyrene	746.87 ± 136.61	617.95 ± 231.16
Naphthalene	47.71 ± 55.14	7.61 ± 7.22
Phenanthrene	10651.44 ± 7908.62	3182.06 ± 2356.71
Pyrene	906.74 ± 95.09	790.23 ± 139.79
Total PAHs	41620.3 ± 17697.6	19887.6 ± 1378.1

Table 3. Demographics of human subjects, semen quality, and sperm DNA integrity.

Variables	**Coke-oven workers average ± SD**
Age (in years)	40 ± 10
BMI (kg/m^2)	23.1 ± 3.0
Education (%)	
Less than high school	47.4
At least high school	52.6
Smoking status (%)	
No	47.4
Yes	52.6
Drinking status (%)	
No	63.2
Yes	36.8
Semen quality	
Concentration (10^6/mL)	112.3 ± 96.4
Motility (% motile)	54.9 ± 19.6
Viability (% viable)	69.1 ± 18.2
Normal morphology (% normal)	8.9 ± 3.2
Amorphous head (%)	83.8 ± 10.1
Oligospermia (%)	2.5
Asthenospermia (%)	28
Sperm DNA fragmentation (%)	37.9 ± 20.3
8-oxodGuo (/10^6 dG)	24.4 ± 27.8
Bulky PAH like adducts (in10^9 nucleotides)	59.7 ± 19.3
BPDE-dG (in10^9 nucleotides)	2.6 ± 1.9

fragmentation and 8-oxodGuo in sperm with concentrations of 37.8% and 24.4 (/10^6 dG), respectively (Table 3).

Associations between PAHs and semen quality

The joint effects on associations between the 16 PAH compounds and each outcome of semen quality and sperm DNA integrity are shown in Figure 1(a)–(d). We observed an inverse association of the PAHs as a mixture on sperm viability (see Figure 1(c)). In particular, sperm viability decreased by 0.05 log units (95% CI = (-0.15, 0.05) log units), respectively, when all PAHs increased from median values to their 75th percentiles. The PAHs, as a mixture, had mostly null associations on, sperm concentration, sperm motility and sperm with normal morphology (see Figure 1(a), (b), (d)).

Associations between individual PAH compound and semen quality are shown in Figure 2(a)–(d). Although not statistically significant, IND had a positive effect on sperm concentration. B[b]F had an inverse effect on sperm viability and a slightly inverse effect on sperm motility. B[ghi]P had inverse effects on sperm motility and sperm with normal morphology. All other PAHs had mostly null effects on the outcomes. There was no evidence of any significant interaction effect between a PAH concentration with other PAHs in the mixture for any of the outcomes on sperm quality (supplementary Figure 1(a)–(d)).

Associations between PAHs and sperm DNA integrity

Figure 3(a) shows the joint effects of PAHs on sperm DNA fragmentation from the BKMR model. An

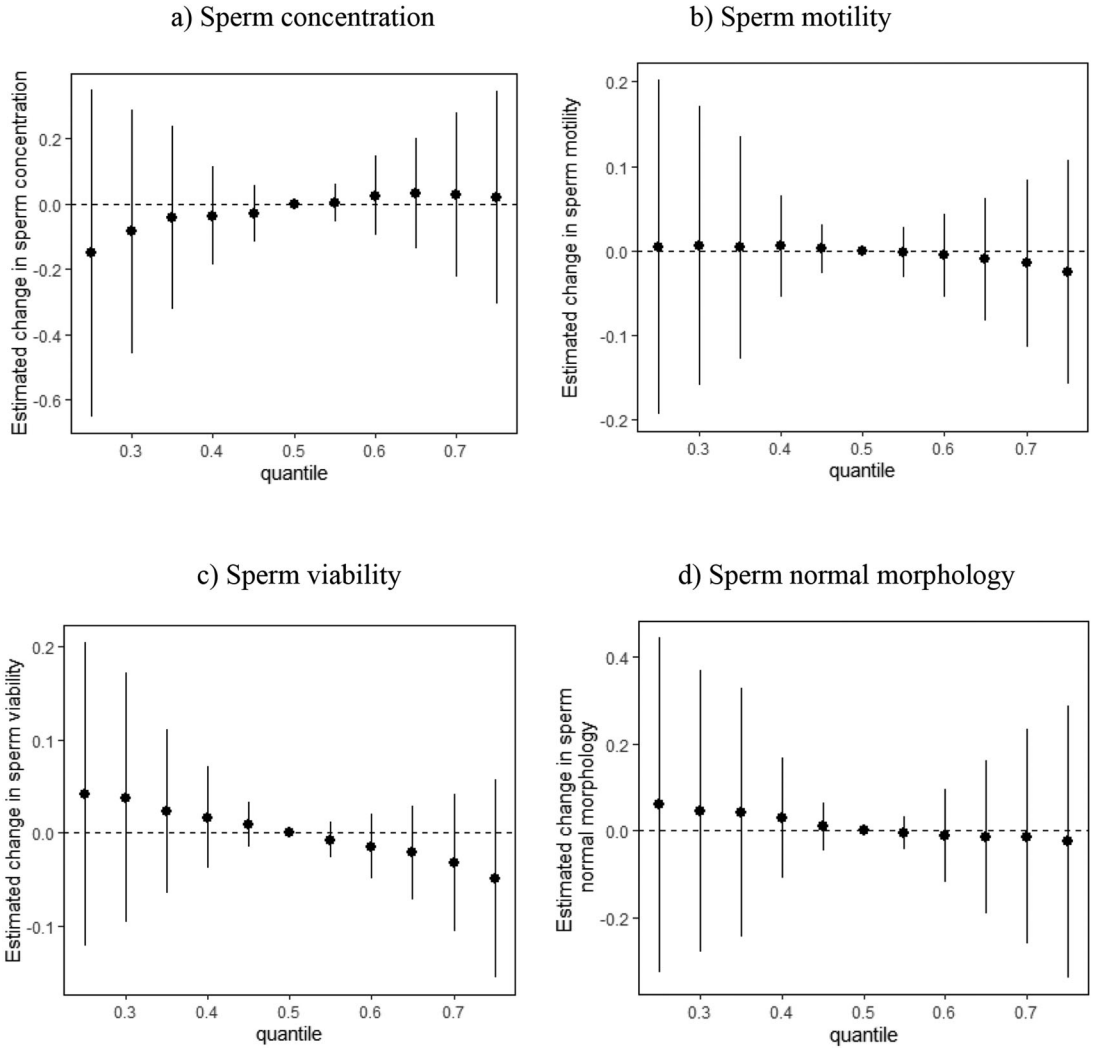

Figure 1. Predictor-response function for the joint effects (posterior mean estimates and 95% credible intervals) of the PAHs on semen quality. The estimates were obtained by comparing the outcome levels when all PAHs were at a particular percentile ranging from 25th percentile to 75th percentile to when all are at their median values. The models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants.

increase in the exposure of the PAH mixture was associated with an increase in sperm DNA fragmentation, although the 95% credible intervals contained the null values. The BKMR selected B[b]F and B[a]A as most predictive of sperm DNA fragmentation (see Figure 4(a)). No evidence of significant interaction effects between PAH individual compounds with the other PAHs was observed for sperm DNA fragmentation, since the effects of PAH individual compounds did not significantly change when the concentrations of the other PAHs changed from 25th to 75th percentiles (supplementary Figure 1(e)).

Figure 3(b) shows associations between the PAH mixture and 8-oxodGuo. A slightly positive trend was observed in the cumulative association between the PAH mixture and 8-oxodGuo, however, the trend became stable at higher percentiles, although all 95% credible intervals contained the null values. PHE and B[b]F showed positive associations with on 8-oxodGuo, although the 95% credible intervals contained the null values (see Figure 4(b)).

The PAHs mixture were not associated with bulky PAHs-like adducts (see Figure 3(c)). All PAHs individual compounds had mostly null association on the outcomes (see Figure 4(c)–(d)). No evidence of significant interaction effect between an individual compound of PAHs with the other PAHs (supplementary Figure 1(g)–(h)).

Dose-response relationship

The dose-response relationships of the PAH concentrations with sperm DNA fragmentation, 8-oxodGuo, and PAH-like adducts are shown in Figure 5(a)–(d).

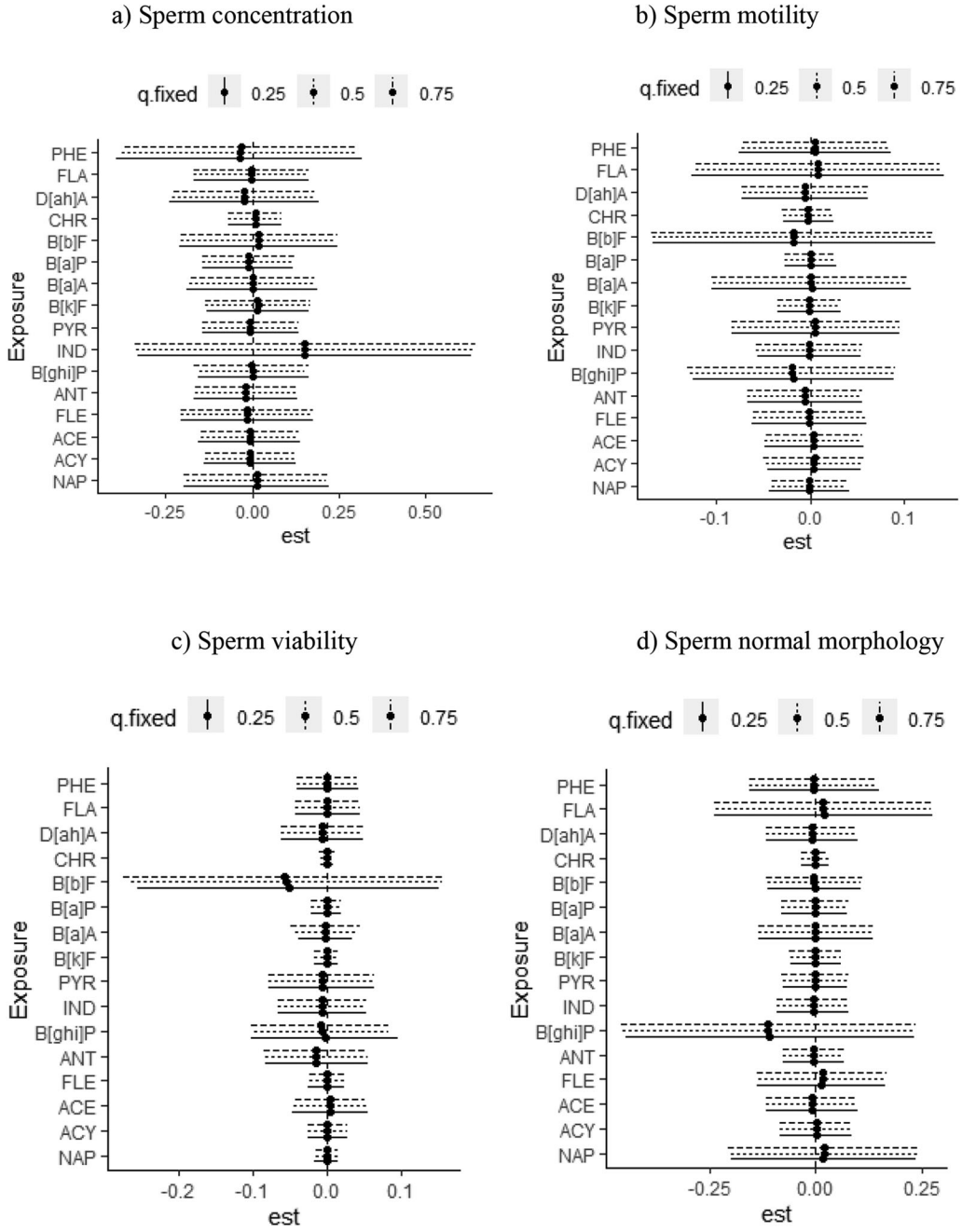

Figure 2. Change in semen quality associated with individual PAH concentration. The effect of each PAH concentration was estimated (posterior mean estimates and 95% credible intervals), when all other PAHs were fixed at their 25th, 50th, and 75th percentiles, for semen quality parameters. The models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants.

The PAH mixture exhibited a positive dose-response relationship with sperm DNA fragmentation. Sperm DNA fragmentation increased by 0.45 log units (95% CI = (−0.02 to 0.92) log units) when the concentrations of the PAH mixture increased from their 25th percentiles to their medians. Among the targeted PAHs compounds, sperm DNA fragmentation increased by 0.31 log units and 0.37 log units when B[a]A and B[b]F increased from their 25th percentiles to their 75th percentiles, respectively, when all other PAHs were fixed at their medians (see Figure 6(a)). There was no dose-response relationship between the PAH mixture and 8-oxodGuo. However, B[b]F and PHE showed slightly positive relationships with 8-oxodGuo (see Figure 4(b)). For the PAH-like adducts, the dose-response relationships of PAHs were mostly the flat as shown in Figure 3(c) and (d). None of the individual PAH compounds showed any evidence of non-linear relationship with any of the outcomes (see Figure 6(c) and (d)).

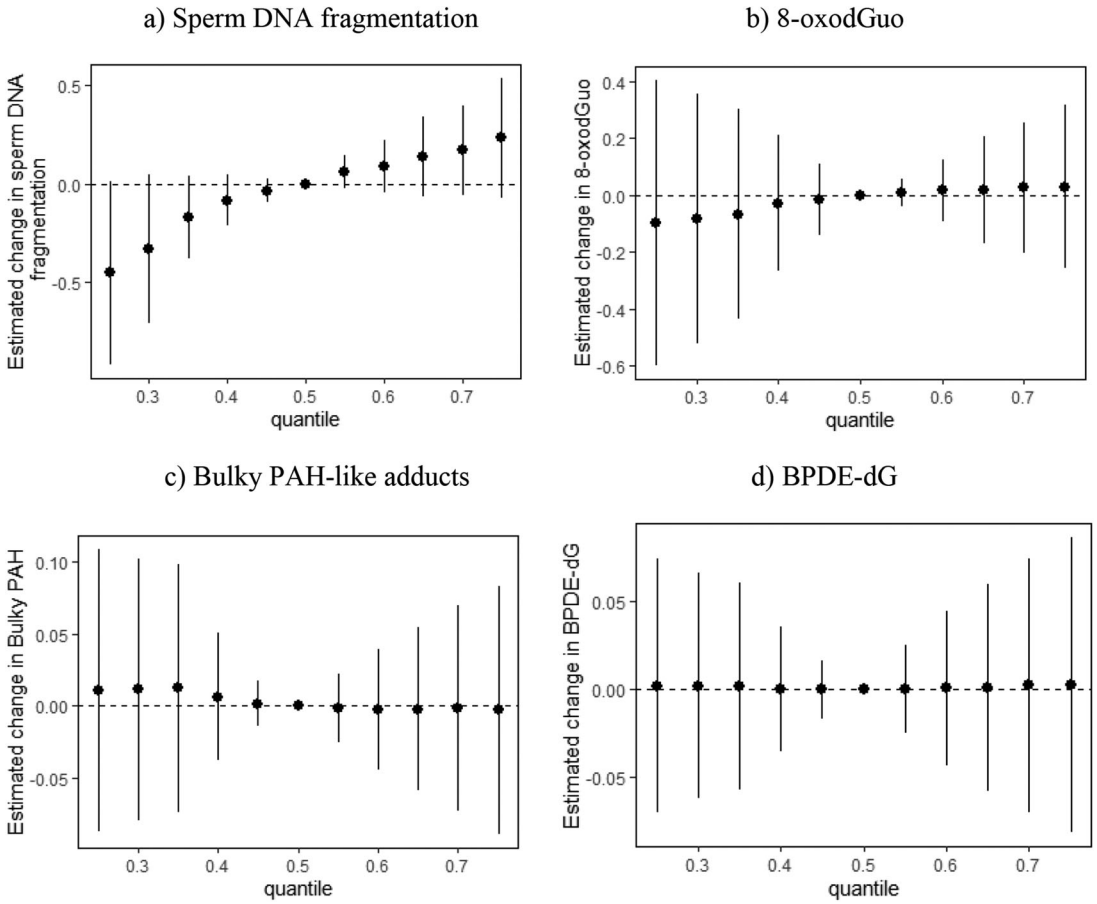

Figure 3. Predictor-response function for the joint effects (posterior mean estimates and 95% credible intervals) of the PAHs on sperm DNA integrity. The estimates were obtained by comparing the outcome levels when all PAHs were at a particular percentile ranging from 25th percentile to 75th percentile to when all are at their median values. The models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants.

Discussion

More than 97% of individuals tested have healthy sperm counts greater than 20×10^6/mL. However, we observed that 18.8% of the coke-oven workers had asthenospermia, of which the percentage of motile sperm was 40%. The percentage of asthenospermia of the coke-oven workers was close to those of the general population (16-18.7%) in a retrospective study.[29] A high percentage of teratozoospermia (91.1%), a condition in which human spermatozoa have abnormal morphology, was recorded in the coke-oven workers. We examined amorphous head, cytoplasmic droplet, duplicates, and coiled tails to characterize teratozoospermia. Amorphous head (88.2%) of sperm of the coke-oven workers attribute to 88% of teratozoospermia, while coiled tails only contribute less than 6% of abnormal morphology. We didn't quantify associations between teratozoospermia and asthenospermia. A retrospective study recorded teratozoospermia attributed 63.13% to asthenozoospermia.[29]

According to the U.S. EPA toxic priority list, 16 PAH constitutes were quantified in this study. It is common that PAHs occurs in mixtures in the environment. We conducted mixture analysis using the BMKR with Gaussian kernel function to examine joint associations of the PAH mixture with semen quality and sperm DNA integrity. For this mixture analysis, personal breathing zone samples were collected to quantify concentrations of 16 PAH compounds instead of measuring PAH metabolites in urine, since no reliable analytical methods and techniques are available to identify and quantify urinary PAH metabolites for the 16 PAH constitutes.[30] The study was the first to identify a mixture of PAHs and individual PAH compounds associated with semen quality and sperm DNA integrity.

The application of BKMR allows for possibility of nonlinearity and interactions between PAHs. We developed the novel grouping approach for the BMKR model by grouping PAH compounds based on bay

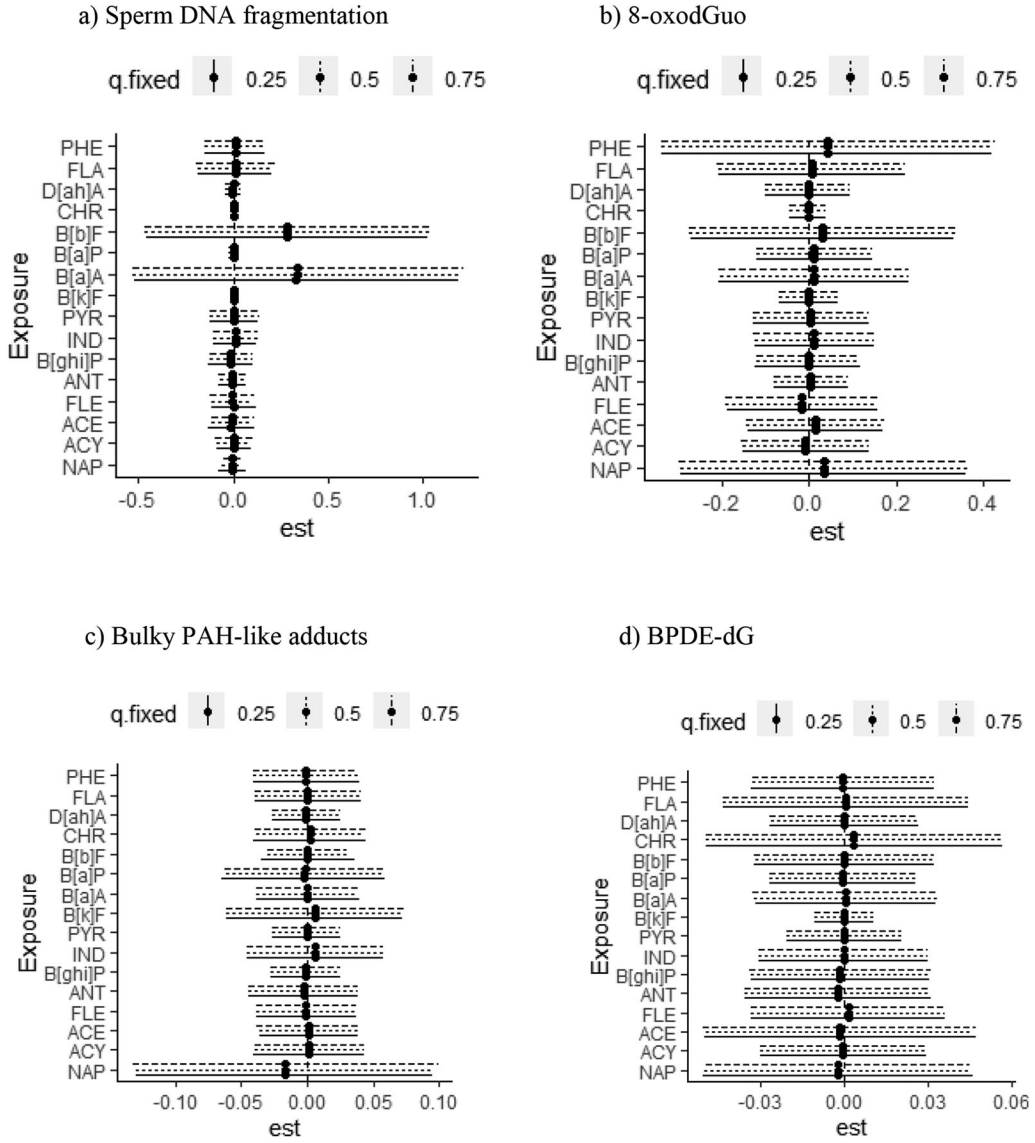

Figure 4. Change in sperm DNA integrity associated with individual PAH concentration. The effect of each PAH concentration was estimated (posterior mean estimates and 95% credible intervals), when all other PAHs were fixed at their 25th, 50th, and 75th percentiles, for sperm DNA integrity. The models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants.

region and molecular weight. We used this approach because their associations with chemical structure and metabolism of PAHs were reported by cellular, animal, and epidemiological studies.[13,31–33] This approach allows the BMKR model to address correlations among PAH mixture compounds with chemical and biological relevance.

We observed the associations between exposure to the PAH mixture and semen quality outcomes. Particularly, the PAH mixture had an inverse, linear relationship with sperm viability. The findings were slightly different from our prior studies using urinary PAH metabolites for exposure assessment and multiple regression analysis.[8,34] In our prior study, we observed that concentrations of single PAH urinary metabolite, 1-hydropyrene, were correlated with sperm motility.[34] Other studies also reported associations between independent urinary PAH metabolites with semen quality parameters, including motility and normal morphology.[14,35,36]

Expanding our understanding of the impact of individual PAH compounds, the BKMR modeling identified B[b]F was the dominant PAH compounds driving the associations between PAH mixture exposure and semen quality. The epidemiological study was the first to demonstrate that B[b]F was associated with sperm viability. Despite widespread PAH compounds, limited *in vivo* and *in vitro* studies are available for documenting toxicological evidence concerning the effects of individual PAH compounds

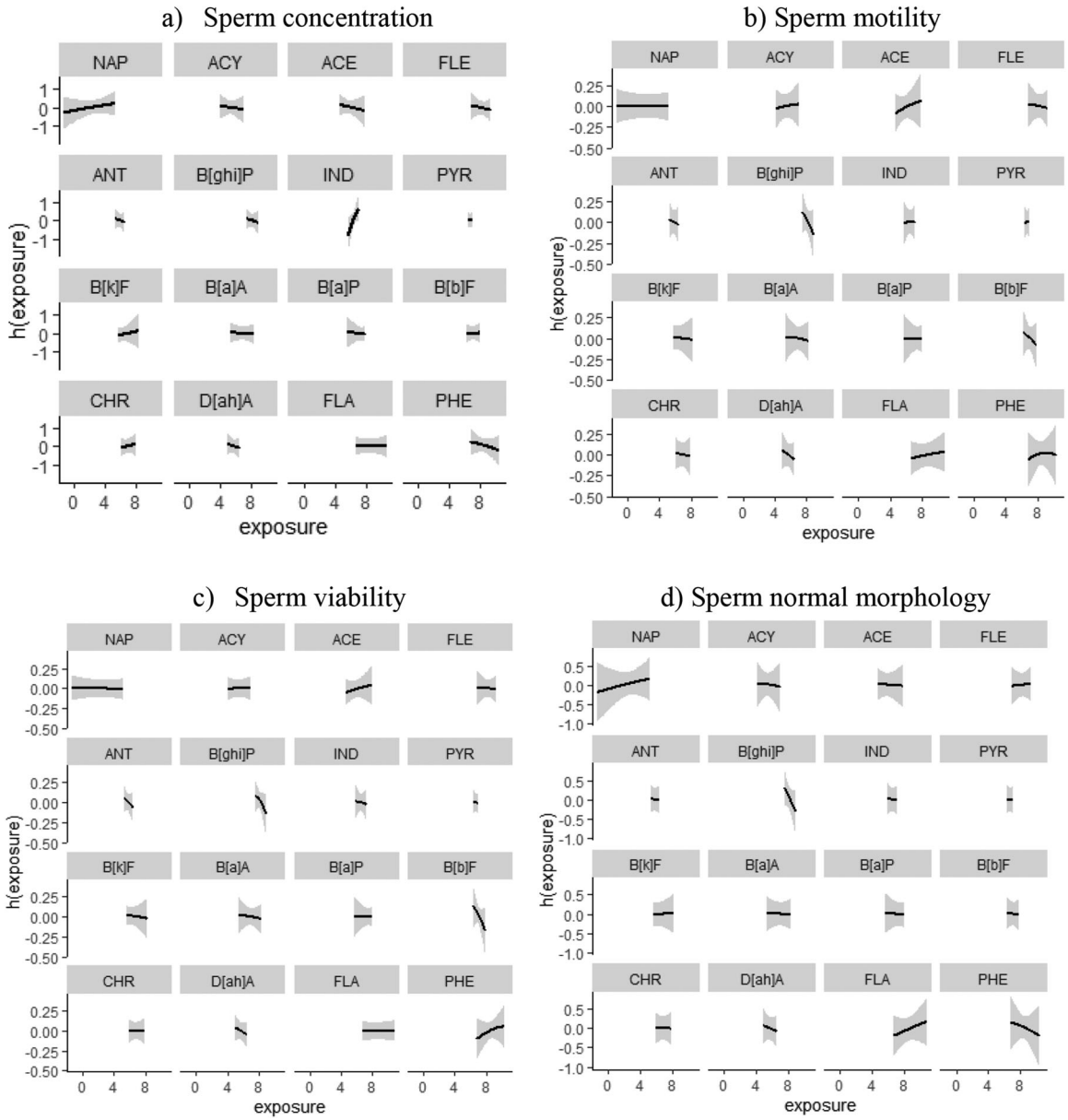

Figure 5. Dose-response relationships between PAH concentrations and function with the 95% credible intervals for the effect of a log unit increase in each PAH concentration on log units of semen quality.

on semen quality. An animal study showed that maternal B[b]F exposure decreased normal semen function in F1 offspring, due to significant upregulations of the steroidogenesis-related and testicular apoptosis mediators, aryl hydrocarbon receptor, and estrogen receptor α.[37]

In this study, we observed exposure to the PAH mixture was associated with sperm DNA fragmentation and 8-oxodGuo in coke-oven workers. Other studies, including a general population and patients from an infertility clinic, reported similar findings.[38,39] The BKMR models revealed that the PAH group containing bay and bay-like regions was the most important exposure responsible for the associations with sperm DNA fragmentation (Table 4). As compared to PAHs without bay regions, PAH compounds with bay and/or bay-like regions are more prone to undergo an oxidative process involving cytochrome P450 monooxygenase, and constitute planar structures to bind to guanine nucleotides and to form o-quinones, which can engage in the redox cycling processes.[32,33,40,41] Oxidative stress may not be the sole mechanism for sperm DNA fragmentation induced by PAHs exposure, since the positive relationship between PAHs and 8-oxodGuo reached plateaus at the high percentile of 8-oxodGuo concentrations. Other mechanisms may also be involved in inducing sperm DNA damage. Mechanistically, the group of PAHs explored in this

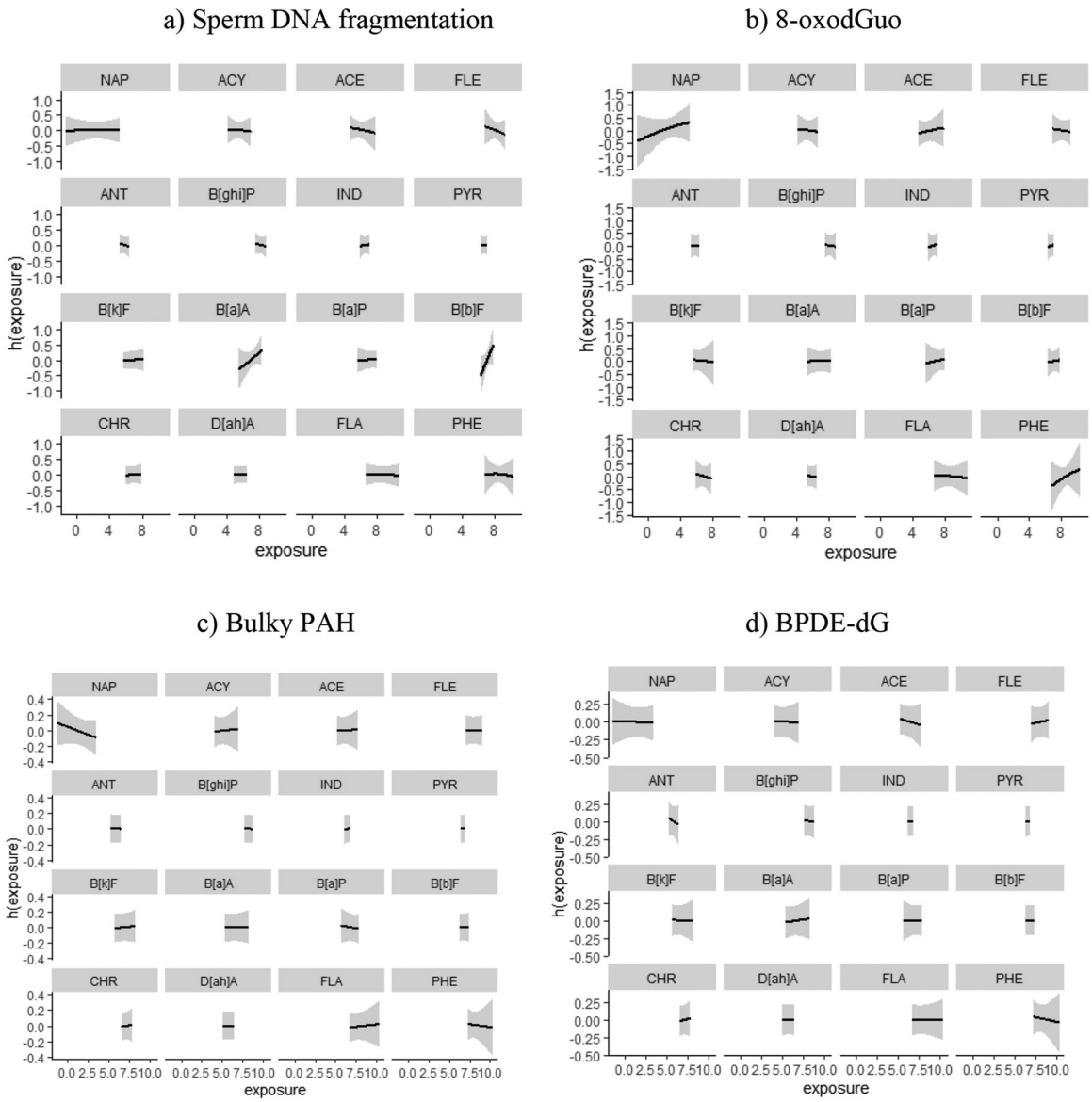

Figure 6. Dose-response relationships between PAH concentrations and function with the 95% credible intervals for the effect of a log unit increase in each PAH concentration on log units of semen quality (a) sperm DNA fragmentation, (b) 8-oxodGuo, (c) bulky PAHs, (d) BaP-like, and (e) BPDE-dG.

study will likely undergo various biological pathways associated with sperm DNA integrity.

Among the 16 PAH compounds, the study was the first to show that B[b]F and B[a]A were the key PAH compounds associated with sperm DNA fragmentation and they had a dose-dependent relationship with sperm DNA fragmentation. B[b]F and B[a]A contributed to an increase greater than 0.43 and 0.27 log units of sperm DNA fragmentation, respectively. Such results would be valuable for health risk assessment on PAH exposure scenarios in relation to increased risk to male reproductive health. Currently, scientific evidence on reproductive and developmental toxicity of B[a]A is scarce. Cellular models showed that B[b]F can easily be activated by cytochrome P450 to generate epoxides and diols in the bay regions.[42] These active metabolites can bind to DNA to form DNA adducts, which can initiate the processes of carcinogenesis.[43,44] Cellular studies demonstrated that the bay regions at 1 and 12 positions of B[a]A were prone to form biologically active metabolites with the diol epoxides.[45,46]

B[a]P has been one of PAH compounds studied extensively due to its mutagenicity and carcinogenicity. Similar to a small number of epidemiological studies,[47–49] low levels of BPDG-dG in sperm were detected in the study. The relatively low levels of BPDG-dG indicated relevant exposure to B[a]P from coking procedures to coke-oven workers. Although BPDG-dG and bulky PAH-like adducts occurred in

Table 4. The posterior inclusion probability (PIP) for each exposure group and individual exposure within each group from BKMR models for sperm quality parameters with grouping based on both chemical structure with bay region diol epoxide (yes vs. no) and molecular weights (low vs. high) of the PAHs.

	Sperm concentration		Sperm motility		Sperm viability		Sperm normal morphology	
Exposure	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP
NAP	0.42	0.13	0.30	0.01	0.30	0.01	0.46	0.18
ACY		0.16		0.14		0.05		0.16
ACE		0.15		0.19		0.11		0.18
FLE		0.24		0.21		0.18		0.23
ANT		0.32		0.44		0.65		0.25
B[ghi]P	0.65	0.17	0.58	0.47	0.60	0.53	0.66	0.55
IND		0.66		0.23		0.22		0.23
PYR		0.17		0.30		0.25		0.22
B[k]F	0.47	0.16	0.42	0.06	0.52	0.05	0.47	0.08
B[a]A		0.08		0.16		0.08		0.10
B[a]P		0.15		0.11		0.07		0.12
B[b]F		0.19		0.28		0.62		0.18
CHR		0.16		0.13		0.05		0.15
D[ah]A		0.20		0.22		0.12		0.24
FLA		0.07		0.04		0.003		0.14
PHE	0.46	1.00	0.20	1.00	0.13	1.00	0.34	1.00

The models were adjusted for age, BMI, education, smoking status, drinking status, and job site of the participants.
groupPIP indicates the posterior inclusion probability for a group.
condPIP indicates the posterior inclusion probability for a single exposure within a group.
Note: NAP = Naphthalene; ACY = Acenaphthylene; ACE = Acenaphthene; FLE = Fluorene; ANT = Anthracene; B[ghi]P = Benzo[g,h,i]perylene; IND = Indenol[1,2,3-cd]anthracene; PYR = Pyrene; B[k]F = Benzo[k]fluoranthene; B[a]A = Benzo[a]anthracene; B[a]P = Benzo[a]pyrene; B[b]F = Benzo[b]fluoranthene; CHR = Chrysene; D[ah]A = Dibenz[a,h]anthracene; FLA = Fluoranthene; and PHE = Phenanthrene.

sperm, the 16 PAH compounds were not associated with BPDG-dG and bulky PAH-like adducts.

There were two major limitations in this study. This study included a small sample size. However, the sample size sufficed for yielding a statistical power to detect a 1.5-fold difference between the mean concentrations of sperm DNA fragmentation and oxidative stress parameters for the topside workers and the side-oven workers. The concentrations of PAHs in the personal breathing zone of the coke-oven workers serve as markers for the intake doses of PAHs and may not reflect concentrations of urinary PAH metabolites that may be more biologically relevant. PAH urinary metabolites has been recommended for biomarkers to accurately reflect biological active doses of adsorption, metabolism, and excretion of PAHs. However, analytical capacity is lacking to quantify urinary metabolites representing a complete profile of PAH mixtures with a wide range of constituents. Despite the limitations, our study included the entire 16 PAH whole mixture and demonstrated a novel approach to group PAHs in the BKMR analysis, which allows us to examine nonlinearity and interactions among PAHs.

Conclusions

In conclusion, exposure to the PAH mixture was associated with altered semen quality. B[b]F and B[a]A were the PAH compounds which confirms the association. Findings from our study highlight the importance of assessing the joint effects of PAH mixtures on male reproductive health outcomes. Future studies with a larger sample size are recommended to confirm the findings of the study.

Disclosure statement

The authors declare that they have no conflict of interest.

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