Annals of Work Exposures and Health, 2022, Vol. 66, No. 2, 203–215
https://doi.org/10.1093/annweh/wxab072
Advance Access publication 18 September 2021
Original Article

Original Article

Mixture Analysis of Associations between Occupational Exposure to Polycyclic Aromatic Hydrocarbons and Sperm Oxidative DNA Damage

Hueiwang Anna Jeng[1,*], Sinjini Sikdar[2], Chih-Hong Pan[3,4] and Guo-Ping Chang-Chien[5]

[1]School of Community and Environmental Health, College of Health Sciences, Old Dominion University, 4608 Hampton Boulevard, Norfolk, VA 23509, USA; [2]Department of Mathematics and Statistics, College of Sciences, Old Dominion University, 4608 Hampton Boulevard, Norfolk, VA 23509, USA; [3]Institute of Labor, Occupational Safety and Health, Ministry of Labor, No. 99, Lane 407, Hengke Rd., Sijhih District, New Taipei City 22143, Taiwan; [4]School of Public Health, National Defense Medical Center, No.161, Sec. 6, Minquan E. Rd., Neihu Dist., Taipei 11490, Taiwan; [5]Center for Environmental Toxin and Emerging Contaminant Research, Cheng Shiu University, No. 840, Chengqing Rd., Niaosong District, Kaohsiung City 833, Taiwan

*Author to whom correspondence should be addressed. Tel: 757-683-4594; e-mail: HJeng@odu.edu

Submitted 28 January 2021; revised 7 June 2021; editorial decision 26 July 2021; revised version accepted 18 August 2021.

Abstract

Objective: This study aimed to determine (i) associations between levels of the polycyclic aromatic hydrocarbon (PAH) mixture with 16 targeted PAH compounds in the personal breathing zone area and sperm oxidative DNA damage, (ii) associations between levels of individual PAH compounds and sperm oxidative DNA damage, (iii) oxidative stress as the mode of action for the genotoxic effects on sperm, and (iv) any dose–response relationship between exposure to the PAH mixture and/or individual PAH compounds and sperm oxidative DNA damage.

Methods: Sixteen targeted PAH compounds in the personal breathing zone area of 38 coke-oven workers and 24 control subjects were quantified using gas chromatography–mass spectrometry. Sperm oxidative damage and status were evaluated by measuring levels of sperm 7,8-dihydro-8-oxoguanie (8-oxodGuo), seminal malondialdehyde (MDA) and seminal reactive oxygen species (ROS). Bayesian kernel machine regression with hierarchical variable selection process was employed to determine associations of the PAH mixture and the biomarkers of sperm oxidative damage. A novel grouping approach needed for the hierarchical variable selection process was developed based on PAH bay region and molecular weight.

Results: The PAH mixture exhibited a positive trend with increased sperm 8-oxodGuo levels at their lower percentiles (25th–50th). The exposure of the PAH mixture was associated with increased MDA levels in sperm. Bay and bay-like regions of the PAH mixture were the most important group for estimating the associations between the PAH mixture and sperm oxidative

What's Important About This Paper?

This study employed a novel statistical method, Bayesian kernel regression with hierarchical variable selection, to assess mixture effects of exposures to polycyclic aromatic hydrocarbons (PAHs) from coke-oven emissions on sperm oxidative damage among coke-oven workers. The study found epidemiological evidence that oxidative stress my link exposures to PAH mixtures with sperm DNA damage. The study seeks to shift current research practice by applying novel statistical methods and concepts on advancing our understanding of reproductive toxicology of PAHs.

stress status. Benzo[a]anthracene was the main individual PAH compound that was associated with increased MDA levels.

Conclusion: Sperm oxidative DNA damage induced by occupational exposure to the PAH mixture had a suggestive association with increased MDA levels in coke-oven workers. Finally, the study identified that the individual PAH compound, benzo[a]anthracene, was the primary driver for the suggestive association between the PAH mixture and sperm oxidative damage.

Keywords: polycyclic aromatic hydrocarbons; bay regions; 8-oxodGuo; Bayesian kernel machine regression

Introduction

Polycyclic aromatic hydrocarbons (PAHs), a mixture of toxic and lipophilic chemicals, are widespread in the environment. PAHs are ubiquitous pollutants generated primarily during the incomplete combustion of organic materials and are the main pollutants of coke-oven emissions and other industrial processes (Qiu *et al.*, 2007; Stella *et al.*, 2012). Upon entering the biological system in the human body, PAHs are metabolized to reactive intermediates by cytochrome P450 enzymes. That can lead to production of reactive oxygen species (ROS) via redox cycling and consequently form DNA oxidative base adducts, e.g. 7,8, dihydro-8-oxoguanie (8-oxodGuo) (Penning, 2014; Martín Muñoz *et al.*, 2018; Liu *et al.*, 2019). Epidemiological studies have demonstrated that PAHs could induce oxidative stress in the biological system, as evident by increased levels of urinary malondialdehyde (MDA) and 8-oxodGuo in humans (Singh *et al.*, 2008; Bae *et al.*, 2010; Yoon *et al.*, 2012; Agarwal *et al.*, 2018a).

In vivo studies have linked the involvement of PAHs in the development of sperm cells. PAHs and their metabolites were found to pass through the blood–testis barrier of the testes of mic and interact directly with germ cells and sperm during spermatogenesis (Ramesh *et al.*, 2001a). Epidemiologic studies showed that exposure of PAHs was associated with decreased semen quality, increased sperm DNA fragmentation and increased sperm 8-oxodGuo adducts of coke-oven workers (Han *et al.*, 2011; Jeng *et al.*, 2015; Jeng *et al.*, 2016). Since sperm lacks repair mechanisms, sperm oxidative damage could accumulate and eventually pass onto embryos. That could affect embryonic development and increase health risks of offspring (Gunes *et al.*, 2015).

Regardless of exposure routes, humans are exposed to mixtures of PAHs. Health risk assessment and toxicological analysis on single PAH compounds may fall short on yielding a full aspect of health impact from the exposure of PAHs. Currently, mixture effects of PAHs on semen quality and reproductive health are scarce. Primary reasons for that are lack of appropriate statistical methods for mixture analysis and a well-defined exposure assessment for determining chemical composition profiles of PAH mixtures in the personal breathing zone area. Like other epidemiologic studies, our prior cross-sectional study used the multivariable parameter regression method to analyze the effects of PAH exposure on semen quality and reproductive health (Xia *et al.*, 2009; Jeng *et al.*, 2011; Jeng *et al.*, 2016). Since PAHs do act as a mixture, this statistical approach might be limited by multicollinearity and might not account for non-linearity and interactions among individual PAH compounds. In this present study, we employed a new, flexible statistical approach, Bayesian kernel machine regression (BKMR), for estimating effects of PAH mixtures on sperm oxidative damage (Bobb *et al.*, 2015; Bobb *et al.*, 2018). Specifically, the hierarchical variable section approach was selected in the BKMR model to incorporate the chemical structure of PAH mixtures and to consider correlations among individual PAH compounds in the statistical analysis (Bobb *et al.*, 2015; Bobb *et al.*, 2018). BKMR has been successfully used to evaluate health effects of environmental mixtures, e.g. metals and endocrine disruptors (Valeri *et al.*, 2017; Liu *et al.*, 2018; Kupsco *et al.*, 2019; Signes-Pastor *et al.*, 2019).

Urinary PAH hydroxylated metabolites, such as urinary 1-hydroxpyrene, 1-hydroxybenzo[a]pyrene, and 2-hydroxyfluorene, have commonly been used as biomarkers in epidemiological studies for assessing health effects of PAH mixtures (Hsu *et al.*, 2006; Jeng *et al.*, 2016; Lou *et al.*, 2019). The use of the biomarker is mainly based on correlations between levels of the hydroxylated metabolites and levels of PAH mixtures. Recent analytical methods have been developed to detect and quantify more PAH hydroxylated metabolites (Yang *et al.*, 2017; Guan *et al.*, 2020). However, the present PAH hydroxylated metabolites still have limits to represent four to five parent PAH compounds and do not fully depict the profile of PAH mixtures (Han *et al.*, 2011; Li *et al.*, 2016). The limitation impedes the use of the biomarker on determining contributions of any individual compounds in PAH mixtures to decreased semen quality and a dose-response relationship between PAH individual compounds and semen quality. The information gap also existed in determining underlying mechanisms of the impact of PAH exposure on semen quality. So far, limited epidemiological studies were designed to identify specific individual PAH compounds that contribute to increased sperm oxidative damage. To address the information gaps, this study aimed to determine (i) associations between levels of joint and individual PAH compounds in the personal breathing zone area and sperm oxidative DNA damage and (ii) any dose–response relationship between exposure to PAH mixture and/or individual PAH compounds and sperm oxidative DNA damage.

Methods

Human subjects

Human subjects include coke-oven workers for the exposure group and control subjects for the control group. Coke-oven workers, who served as the exposure group, were recruited from a steel plant in southern Taiwan. The coking processes in the steel plant have been well established and operated for more than five decades. The long-term occupational monitoring from the Taiwan Department of Labor reported that increased PAH levels have been found in the coking areas, particularly near coke ovens and blast furnaces (Wu *et al.*, 2003; Jeng *et al.*, 2016; Wu *et al.*, 2019). We selected the coke-oven workers as the subjects because they were a high risk of population due to PAH exposure from coke-oven emissions. Their demographics and occupational work history were well characterized. Also, exposure levels of PAHs can accurately quantified because they worked in the same operational area and their work rotation and work shift are standardized (Wu *et al.*, 2003; Jeng *et al.*, 2013). Finally, high cooperation and retention rates of the subjects made personal breathing zone and semen sampling possible. More than 200 workers participated in screening 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 reproductive dysfunction, no infection during sampling, and no cancer. More than 90% of the subjects met the inclusion criteria and were eligible for providing biological samples. However, based on working schedule during the time for the personal breathing zone sampling, 38 subjects (18 of topside workers for high exposure and 20 of side-oven workers for low exposure) were randomly selected and included in the study. Also, 22 office workers were recruited to serve as the reference group. Based on our prior study, the reference subjects in the office were exposed to minimal PAH levels (Wu *et al.*, 2003). A two-sided ANOVA test with α 0.05 margin of error was conducted to determine the number of participants that met a minimum yield of over 90% power. The statistical power was sufficient to detect a 1.5-fold difference between the mean levels of sperm DNA adducts and oxidative stress parameters for the two exposure groups. 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 the human subjects. Coke-oven workers worked 8 h day^{-1} for six continuous days in the steel processes and had 2 days off. Each worker wore two personal air samplers (SKC, model 224PCXR7) for 7 h 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^{-1} 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^{-1}, was used to collect gaseous PAHs. After sampling, all filter and resin samples were stored at 4°C before analysis.

We quantified 16 targeted PAH compounds, including 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) by using a gas chromatogram squadruple mass spectrometer (GC/MS; Agilent Technologies 6890N) with an automatic sampler system (Jeng *et al.*, 2013). After the weights of PAHs were quantified, time weighted PAH levels 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 D[ah]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.

Analysis of 8-oxodGuo adducts

Each semen sample was collected by ejaculation. Sperm DNA was isolated by using the DTT digestion-based method. The method was slightly modified by procedures recommended by the European Standard Committee on Oxidative DNA Damage (ESCODD) to minimize DNA oxidization during DNA isolation procedures (Gedik and Collins, 2005). 8-OxodGuo adducts in sperm DNA were determined by a liquid chromatography–mass spectrometry (LC–MS/MS) with an on-line solid-phase extraction as reported in recent studies (Hu *et al.*, 2004; Chao *et al.*, 2008; Hu *et al.*, 2018). 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 (ESI). Detection was performed in the positive ion multiple reaction monitoring mode for simultaneous quantification of 8-oxodG 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.

ROS measurement

ROS levels in sperm were measured by the chemiluminescence assay method using luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Sigma Chemical Co., St. Louis, MO) as the probe.(Agarwal *et al.*, 2004) Then, 5 mM of luminol prepared in dimethyl sulfoxide was added to 400 μl of the washed sperm suspension by phosphate-buffered saline. The ROS levels were determined by measuring chemiluminescence with a luminometer in the integrate mode for 15 min, and results were expressed as counted photons per minute × 10^6 per sperm.

MDA measurement

MDA levels were analyzed according to the thiobarbituric acid (TBA) method (Janero, 1990; Tsikas, 2017). Briefly, 0.1 ml of seminal plasma was added to 0.5 ml of TBA reagent (0.67 g of 2-thiobarbituric acid dissolved in 100 ml of distilled water with 0.5 g of NaOH and 100 ml of glacial acetic acid). The mixture was then heated for 1 h in a boiling water bath. After cooling, the tube was centrifuged for 10 min at 4000 rpm, and the supernatant absorbance was read on a spectrophotometer at 534 nm.

Statistical analysis

Descriptive statistics were calculated for all exposure and outcome variables. Data were log-transformed to conform any skewed data to normality. We employed BKMR to evaluate the joint effect of multiple PAH compounds, interactions between PAH compounds, and potential non-linear relationships between PAHs and outcomes of interest. BKMR was fitted using a Gaussian kernel function (Bobb *et al.*, 2018). Results were obtained based on 10 000 Markov Chain Monte Carlo iterations (Bobb *et al.*, 2015). All BKMR models were adjusted for age, education, smoking status, drinking status, and job site of the participants. Because exposures in our study are correlated, we employed BKMR with hierarchical variable selection. This approach requires grouping of the exposures based on correlations between exposures, chemical structures, and similar mechanisms of action. Therefore, we grouped the PAH mixtures based on bay region (no versus yes) and molecular weight (low versus high). The bay region grouping includes both bay and bay-like regions. NAP, ACY, ACE, FLE, ANT were grouped into group 1 without bay region and low molecular weight, B[ghi]P, IND, PYR into group 2 without bay region and high molecular weight, and B[k]F, B[a]A, B[a]P, B[b]F, CHR, D[ah]A, FLA, and PHE into group 3 with bay region and high molecular weight (Table 1).

BKMR provides posterior inclusion probabilities (PIPs), which give a measure of variable importance for each exposure group and how each exposure in that group is driving the association between the group and the biomarkers. We estimated the overall mixture effects of the PAHs (posterior mean estimates and 95% credible intervals) on each biomarker for sperm oxidative DNA damage and oxidative stress biomarkers. The overall effects were estimated by comparing the biomarker levels when all PAHs were at a particular percentile (ranging from 25th percentile to 75th percentile) to when all were

Table 1. Grouping of PAHs for the BKMR analysis

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

at their median values. We also evaluated the effect of each individual PAH exposure on the outcomes (posterior mean estimates and 95% credible intervals), when all the other PAHs are fixed at a particular percentile. Additionally, we plotted a dose-response relationship of each PAH level on the biomarkers, fixing the other PAHs at their medians. The PAH levels were natural log-transformed for the analyses. All analyses were done in R version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria), and we used the R package 'bkmr' to perform the BMKR analyses.

In sensitivity analysis, we performed the mixture analysis using BKMR with grouping based on bay region (yes versus no). In total, we got two groups as follows: group 1 having PAHs without bay region (NAP, ACY, ACE, FLE, ANT B[ghi]P, IND, and PYR), and group 2 having PAHs with bay region (B[k]F, B[a]A, B[a]P, B[b]F, CHR, D[ah]A, FLA, and PHE).

Results

Study population characteristics and PAH levels

The characteristics of the participants are shown in Table 2. The participants were, on average, 39 years old. A majority of the participants were nondrinkers. About 53% of the participants had at least high school degree. (Table 2). Comparing to the control subjects, the coke-oven workers had significantly higher levels of sperm 8-oxodGuo, seminal MDA, and seminal ROSs. The summary statistics (median, 25th percentile, and 75th percentile) of the 16 natural log-transformed PAH levels for the exposure group are presented in Table 3.

Table 2. Characteristics of the human participants and oxidative stress biomarkers

Variables	Exposure group (N = 38)	
	Topside oven	Side oven
Age (in years)	40.4 ± 10.4	38.4 ± 9.1
Body mass index (kg m^{-2})	25.8 ± 3.3	23.6 ± 3.7
Education (%)		
Less than high school	47.4	46.2
At least high school	52.6	53.8
Smoking status (%)		
No	42.6	59.1
Yes	58.4	40.9
Drinking status (%)		
No	43.7	49.8
Yes	56.3	50.2
MDA (nmol ml^{-1})	0.48 ± 0.13	0.43 ± 0.11
ROS ($\times 10^6$ per million sperm)	0.39 ± 0.26	0.26 ± 0.12
8-oxodGuo (per 10^6dG)	29.4 ± 27.8	33.6 ± 20.1

*$P < 0.05$.

Bayesian kernel machine regression analyses

The estimated PIP for each exposure group (groupPIP) and individual exposure in each group (condPIP) from the BKMR model with grouping based on bay region and molecular weight is shown in Table 4. The importance of each exposure group or individual exposure for a biomarker was determined using a common threshold of PIP > 0.5 (Coker *et al.*, 2018; Zhang *et al.*, 2019). For 8-oxodGuo and MDA, the groupPIPs for all three

Table 3. Descriptive statistics for all the 16 natural log-transformed PAHs for the exposure group

PAH	Median	25th percentile–75th percentile
Naphthalene (NAP)	2.40	1.20–3.47
Acenaphthylene (ACY)	5.30	4.98–5.72
Acenaphthene (ACE)	5.78	5.34–6.05
Fluorene (FLE)	8.21	7.88–8.61
Phenanthrene (PHE)	8.68	7.58–9.06
Anthracene (ANT)	5.82	5.69–5.94
Benzo[g,h,i]perylene (B[ghi]P)	8.08	7.81–8.26
Benzo[k]fluoranthene (B[k]F)	6.18	5.93–6.37
Indenol[1,2,3-cd]anthracene (IND)	6.57	6.34–6.68
Pyrene (PYR)	6.73	6.60–6.84
Benzo[a]anthracene (B[a]A)	6.88	5.87–7.53
Benzo[a]pyrene (B[a]P)	7.53	7.32–7.73
Benzo[b]fluoranthene (B[b]F)	7.12	6.65–7.33
Chrysene (CHR)	6.81	6.69–6.89
Dibenz[a,h]anthracene (D[ah]A)	5.70	5.54–6.03
Fluoranthene (FLA)	8.38	6.96–9.16

Table 4. The PIP for each exposure group and individual exposure within each group from BKMR models

Exposure	8-OxodGuo		ROS		MDA	
	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP
NAP	0.59	0.18	0.53	0.05	0.57	0.07
ACY		0.19		0.12		0.09
ACE		0.18		0.43		0.27
FLE		0.20		0.18		0.26
ANT		0.25		0.23		0.31
B[ghi]P	0.57	0.29	0.58	0.20	0.63	0.22
IND		0.38		0.27		0.24
PYR		0.33		0.53		0.53
B[k]F	0.59	0.13	0.44	0.12	0.98	0.001
B[a]A		0.09		0.15		0.87
B[a]P		0.15		0.20		0.003
B[b]F		0.19		0.14		0.10
CHR		0.11		0.19		0.002
D[ah]A		0.11		0.09		0.002
FLA		0.06		0.05		0.005
PHE		0.16		0.06		0.01

groupPIP indicates the posterior inclusion probability for a group. condPIP indicates the posterior inclusion probability for a single exposure within a group. The models were adjusted for age, education, smoking status, drinking status, and job site of the participants. 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; PHE, phenanthrene.

groups were >0.5, suggesting that all were important for the associations with the biomarkers. However, group 3 was particularly driving the association for MDA (groupPIP = 0.98). Within the groups, B[a]A was mostly driving the association between group 3 and MDA (condPIP = 0.87) and PYR was the most important driver for the association between group 2 and MDA (condPIP = 0.53). No exposure was particularly driving the association between the groups and 8-oxodGuo (all condPIPs < 0.5). For ROS, groups 1 and 2 were important for the association (groupPIPs > 0.5). Within the groups, PYR was the most important driver for group

2 and ROS (condPIP = 0.53), and ACE was the most important driver for group 1 and ROS (condPIP = 0.43) although its PIP did not pass the threshold of 0.5.

Figure 1a–c show the overall effects of all the PAHs as a mixture on the biomarkers for sperm oxidative DNA damage. An increase in the exposure of all the PAHs was significantly associated with an increase in MDA (Figure 1c). In particular, MDA increased by 0.20 log units [95% CI = (0.02, 0.38) log units] when all PAH levels increased to their medians compared to when all were at their 25th percentiles. A slightly positive trend was observed in the mixture effect of PAHs on 8-oxodGuo when they were at their lower percentiles, but the effect became stable at higher percentiles, although all 95% credible intervals contained the null values (Figure 1a). The PAHs did not have any significant trend in the mixture effect on ROS in our data (Figure 1b).

Figure 2a–c show the effects of individual PAH level in the mixture on each biomarker by comparing the biomarker levels when the individual PAH was at 75th versus 25th percentile, when all other PAHs were fixed

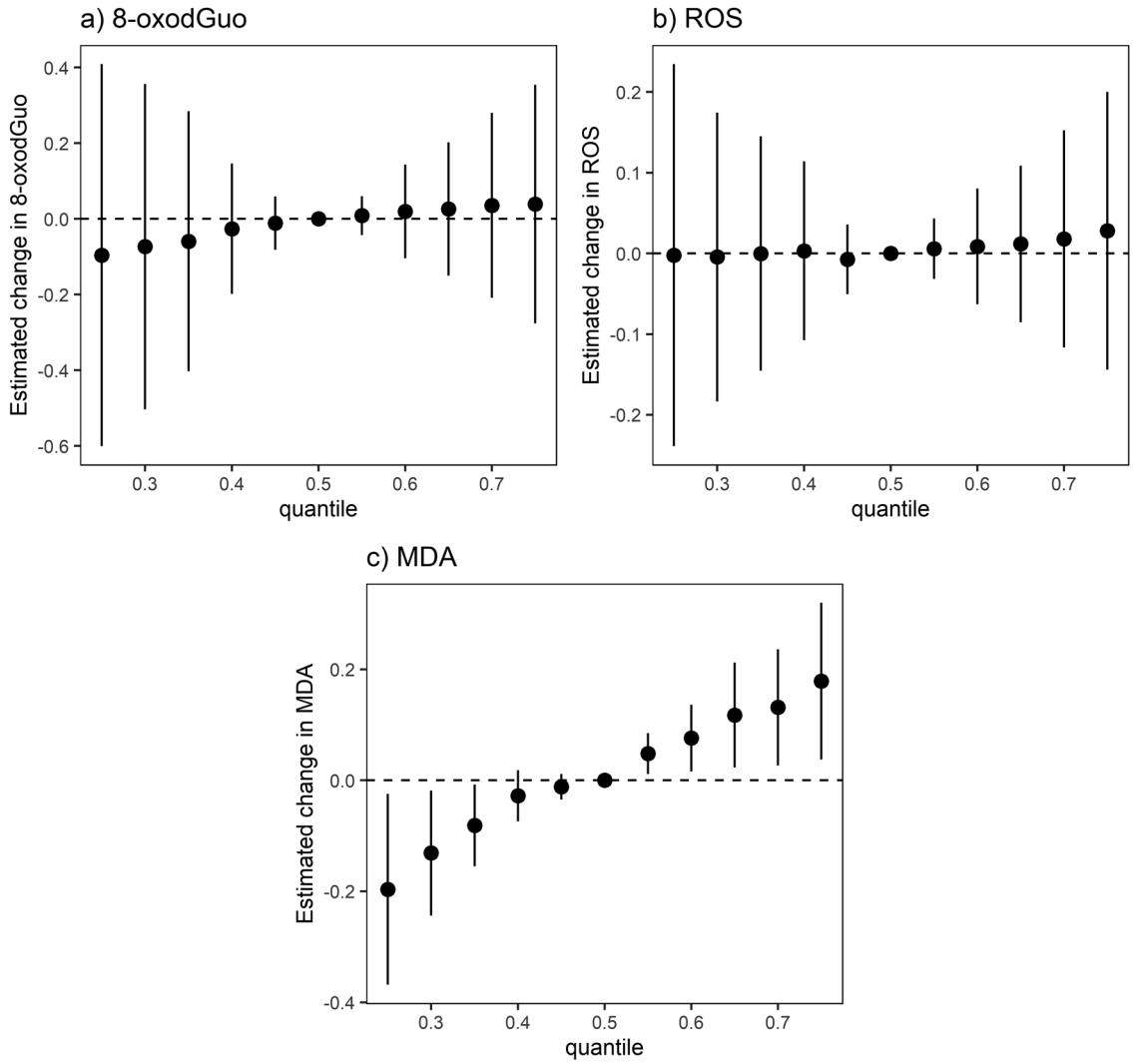

Figure 1. The mixture effects (posterior mean estimates and 95% credible intervals) of the PAHs on the biomarkers for sperm oxidative DNA damage from BKMR models with grouping based on bay region and molecular weight: (a) 8-oxodGuo; (b) ROS; and (c) MDA. The estimates were obtained by comparing the biomarker levels when all PAHs are at a particular percentile (ranging from 25th percentile to 75th percentile) to when all were at their median values, adjusting for age, education, smoking status, drinking status, body mass index, and job site of the participants.

at their 25th, 50th and 75th percentiles. NAP, B[b]F, and PHE had positive effects on 8-oxodGuo, although not statistically significant (Figure 2a). For ROS, B[a]A and PYR showed positive effects, while ACE showed an inverse effect, none being statistically significant (Figure 2b). B[a]A and B[b]F showed positive effects on MDA, although the 95% credible intervals contained the null values (Figure 2c). In particular, MDA increased by 0.33 log units [95% CI = (−0.003, 0.66) log units] when B[a]A increased from 25th percentiles to 75th percentiles, when all other PAHs were fixed at their medians. All other PAHs had mostly null effect on the biomarkers. There was no evidence of significant interaction effect between an individual PAH with the other PAHs for any of the biomarkers since the individual effects of the PAHs did not significantly change when the levels of the other PAHs changed from 25th to 75th percentiles (Figure 2a–c).

The dose–response relationship between each PAH level and the biomarkers for sperm oxidative DNA damage are shown in Figure 3a–c. There was no evidence of significant non-linear relationships between the PAHs and the biomarkers. NAP and ACE showed slightly positive relationships with 8-oxodGuo (Figure 3a) For ROS,

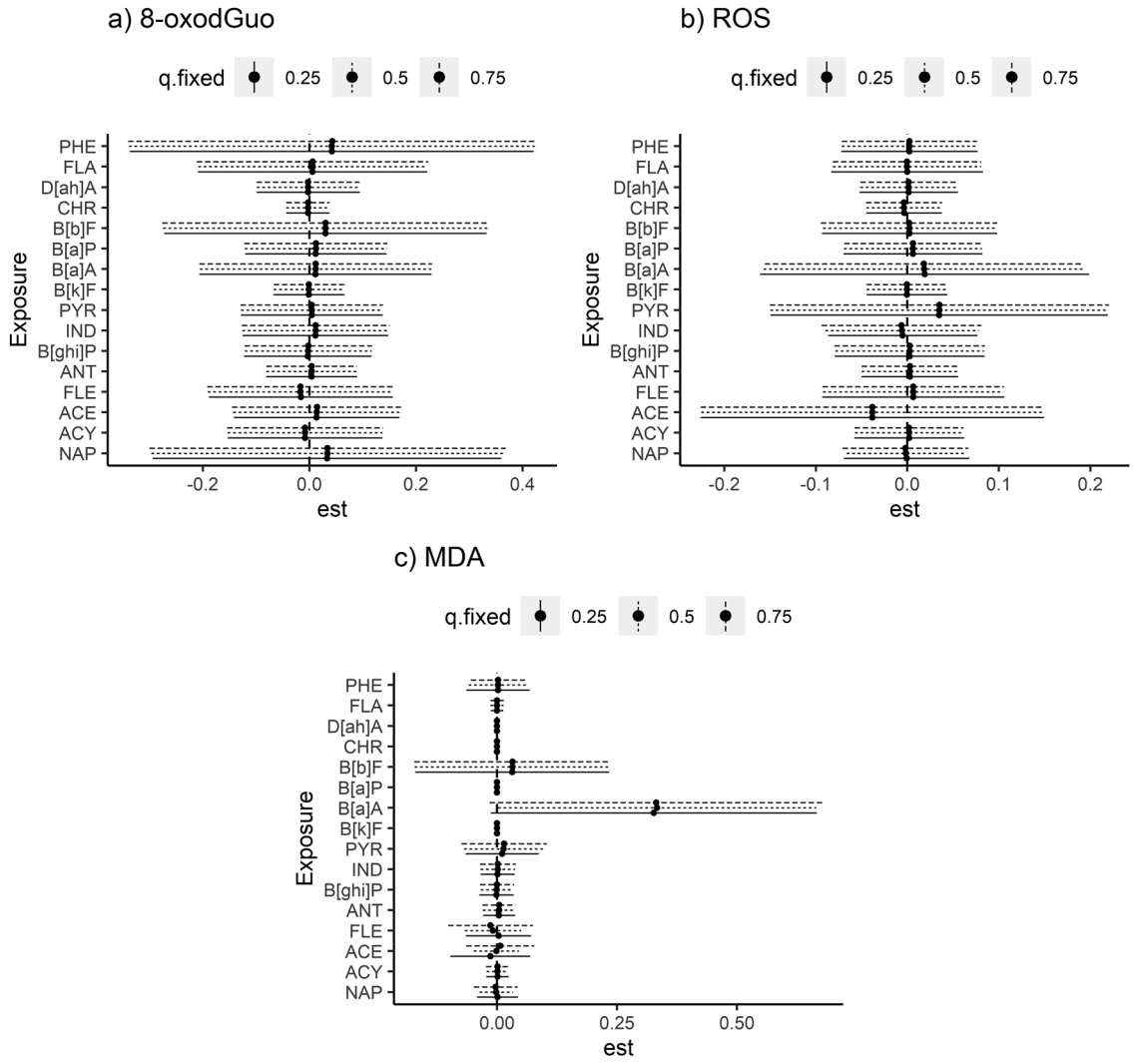

Figure 2. The individual effects (posterior mean estimates and 95% credible intervals) of each PAH level, when all others were fixed at their 25th, 50th, and 75th percentiles, on the biomarkers for sperm oxidative DNA damage: (a) 8-oxodGuo; (b) ROS; and (c) MDA; for BKMR models with grouping based on bay region and molecular weight, adjusting for age, education, smoking status, drinking status, body mass index, and job site of the participants.

NAP and ACE showed inverse relationships, while PYR showed positive relationship (Figure 3b). B[a]A, B[b]F, and PYR showed positive relationships, while FLE showed an inverse relationship with MDA.

In sensitivity analysis with PAH exposure groups based only on bay region, results from the main analysis persisted where the overall mixture effects on the biomarkers had significantly positive trend for MDA and slightly positive trend for 8-oxodGuo, while the effect was null for ROS. Group 2 was driving the association for MDA (groupPIP = 0.99), and B[a]A was driving the association between group 2 and MDA (condPIP = 0.85). However, no other exposure was particularly driving the association between the groups and the biomarkers (all condPIPs < 0.5).

Discussion

In this study, we characterized exposure of the 16 targeted PAHs to coke-oven workers who are commonly exposed to coke-oven emissions. We selected the 16 PAH compounds based on the PAH guidelines and our prior study on pollution profiles of coke-oven emissions.(Wu *et al.*, 2003) Our study is the first to use BKMR to assess mixture effects of PAHs on sperm oxidative DNA damage in the occupational setting. The

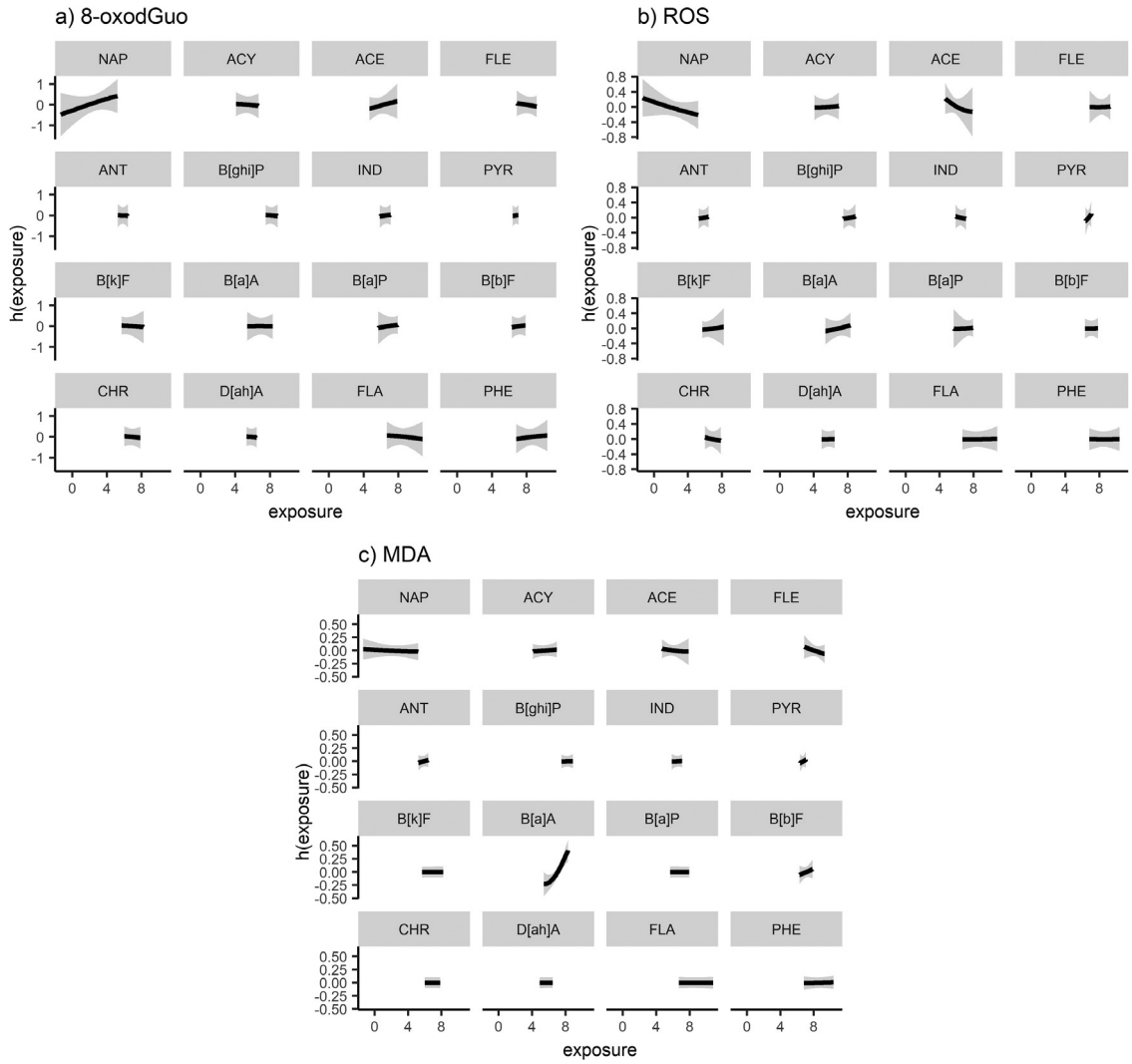

Figure 3. Univariate dose–response function with the 95% credible intervals for the effect of a log unit increase in each PAH level on log units of (a) 8-oxodGuo, (b) ROS, and (c) MDA. BKMR was used with grouping based on bay region and molecular weight, adjusting for age, education, smoking status, drinking status, body mass index, and job site of the participants.

strengths of BKMR include its capacity to (i) evaluate associations of multiple PAH mixtures with multiple biomarker endpoints of sperm oxidative damage, (ii) address multicollinearity by considering highly correlated exposures into non-overlapping groups, and (iii) succinctly summarize the magnitude or direction of the association. Since some PAH mixture compounds are highly correlated due to chemical structures and properties, we used the hierarchical variable selection in the kernel machine regression model (Bobb *et al.*, 2015; Bobb *et al.*, 2018). The hierarchical variable selection approach is affective to systematically handle highly correlated PAH compounds and can take into account for chemical structures of the PAH mixture (Bobb *et al.*, 2015; Bobb *et al.*, 2018). We have developed the novel grouping approach to incorporate chemical structures of PAHs in the statistical analysis with biological relevance. We conducted the sensitivity analysis, showing that statistical outputs were independent of the grouping approach. That confirms that any significant difference was due to the exposure to the PAH mixture rather than the grouping approach. The advantage of this grouping approach is that the chemical structure has biological relevance. The outputs from the statistical analysis could reflect toxicological pathways and modes of action of PAH mixtures. Also, the approach can enhance our understanding of toxicological effects of PAH mixtures on sperm DNA integrity.

The BMKR analysis showed that both the bay region and molecular weight groupings were important for predicting the associations between the levels of the PAH mixture and the biomarker levels of sperm oxidative DNA damage. Specifically, the bay region of the PAH group served the most important variable for estimating the increased levels of MDA in relation to exposure to the PAH mixture. Both cellular and animal models demonstrated that the bay and bay-like regions could increase biological activity of PAHs (Yan, 1985; Rummel *et al.*, 1999; Vijayalakshmi and Suresh, 2008). PAHs compounds with the bay region tend to form reactive region diol epoxides, which are prone to start the redox cycle to yield ROS, and easily bind to guanine nucleotides when they react with DNA (Vijayalakshmi and Suresh, 2008; Zhang *et al.*, 2012; Penning, 2014).

Based on their structure and volatility, PAHs can be classified into low-molecular-weight PAHs (two to four rings PAHs) and high-molecular-weight PAHs (five to six rings PAHs). Like the bay region grouping, the molecular weight grouping was also important for predicting the associations of exposure of PAH mixtures and overall oxidative status of sperm. Our results showed that the PAH group, which was most important for the association between exposure to the PAH mixture and MDA levels, include several high-molecular-weight PAH compounds. Coincidentally, these high-molecular-weight PAHs also contain bay and bay-like regions. Comparing to the molecular weight grouping, the bay region grouping was the primary driver responsible for estimating the association between exposure of the PAH mixture and increased oxidative stress status in sperm. The understanding of the role of molecular weight in reproductive toxicity of PAHs remains elusive. A few studies did report that some high-molecular-weight PAH compounds were associated with reproductive and developmental outcomes and sperm quality (Jeng *et al.*, 2013). High-molecular-weight PAHs could induce a higher magnitude of CYP1A expression in developmental tissues than low-molecular-weight PAHs (Geier *et al.*, 2018). On the contrary, two studies reported that urinary metabolites from low-molecular-weight PAHs were correlated with decreased sperm velocity and DNA fragmentation of Chinese men (Han *et al.*, 2011; Yang *et al.*, 2017). Genetic toxicity of PAH mixtures is complex and may not be elucidated by simple, generalized descriptors of physical properties. Physical, chemical, and biological properties should be considered for health risk assessments of PAHs.

As sperm lacks the downstream DNA repair protein of the base excision repair pathway, sperm DNA is vulnerable for direct oxidative insult (Bisht and Dada, 2017; Bisht *et al.*, 2017). Our study observed that exposure of the PAH mixture was associated with increased oxidative stress in sperm. Also, the exposure of the PAH mixture exhibited a positive trend with increased sperm 8-oxodGuo levels. However, the positive trend only existed at the lower percentile of 8-oxodGuo levels. This may suggest a possible threshold for the formation of the oxidative adducts in sperm. It is well-known that PAHs has distinct bioactivities, which is complex and dynamic. Beside oxidative stress, other modes of action could be involved in sperm DNA damage (Ramesh *et al.*, 2001b; Geier *et al.*, 2018). Also, although PAH compounds with the bay regions likely interact with phase I metabolism, the compounds may lack significantly toxicological responses, because they may be successfully detoxified to reach below a level threshold for overt toxicity (Ramesh *et al.*, 2001b; Geier *et al.*, 2018).

Oxidative stress can be defined by unbalance between ROS and antioxidants. Excessive production of ROS could profoundly affect sperm DNA by forming oxidized DNA base adducts within DNA strands. Urinary MDA assay, which is the stable lipid peroxidation product, is a simple method to evaluate the effect of lipid peroxidation on sperm (Janero, 1990; Ajina *et al.*, 2017). Our study observed the PAH mixture was associated

with increased MDA levels. Lipid peroxidation can trigger the loss of membrane and lead to structural damage to DNA. The association observed in this study may provide reasoning for the observed relationship between PAH exposure and decreased motility and increased morphology damage observed in our prior studies and others (Hsu *et al.*, 2006; Jeng *et al.*, 2011; Jeng *et al.*, 2016; Yang *et al.*, 2017).

Contrary with other studies' findings, we did not observe an association between exposure to the PAH mixture and ROS levels. One explanation for this finding is that the ROS levels may reduce or change in the analysis procedures. Semen samples were washed prior to ROS measurements using the chemiluminescence assay. Other studies showed that ROS could decline with time after ejaculation and during the washing procedure (Kobayashi *et al.*, 2001; Vessey *et al.*, 2014). To detect more accurate ROS levels in seminal plasma, we recommend that future studies measure ROS in whole semen soon after liquefaction without washing (Agarwal *et al.*, 2018b). Also, we also recommend a direct, real-time ROS measurement method using non-chemical based mechanical measurement based on oxidation and reduction potential (Agarwal *et al.*, 2018b).

The single-exposure estimates showed that the increase in exposure to B[a]A was associated with the higher levels of MDA. Currently, no epidemiological evidence is available on the reproductivity toxicity of B[a]A. Limited *in vitro* studies showed that the toxicological effect of B[a]A on sperm function. B[a]A is involved in apoptotic responses of testicular tissues and Sertoli cells (Raychoudhury and Kubinski, 2003). However, the mode of action for the response was unclear. There is a need to investigate associations of B[a]A with sperm DNA integrity and underlying mechanisms to enhance our understanding of toxicological effects of the individual PAH compound.

The major limitation of this study was the small sample size. Our power analysis showed that the sample size was sufficient to yield a statistical power of 95% and detect a 1.5-fold difference between the mean levels of sperm oxidative stress parameters for the exposure group and the reference group. With the small sample size, we carefully interpret our results by indicating suggestive associations rather than significant associations. We recommend future studies with a larger sample size to confirm the findings.

Conclusions

The study developed a novel grouping method for the BKMR model. Both bay region and molecular weight groupings played an important role in estimating the sperm oxidative DNA damage of the PAH mixture. Sperm oxidative DNA damage induced by occupational exposure to the PAH mixture had a suggestive association with the increased levels of MDA, a biomarker of lipid peroxidation. Finally, B[a]A, an individual PAH compound, was the primary driver for the suggestive association between PAH mixture exposure and increased MDA levels. The study highlights the need for future studies to incorporate structure and biological activity-based assessments on the mechanisms driving toxic effects of both individual PAH compounds and PAH mixtures on sperm DNA damage.

Supplementary data

Supplementary data are available at *Annals of Work Exposures and Health* online.

Acknowledgements

This work was partially supported by grants from the US National Institute of Environmental Health Sciences (1R15ES018952-01) and the National Institute for Occupational Safety & Health, USA (1R03OH009504-01).
The authors declare that they have no financial interests or personal relationships that could influence the work reported in this paper.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

Agarwal A, Allamaneni SS, Said TM. (2004) Chemiluminescence technique for measuring reactive oxygen species. *Reprod Biomed Online*; **9**: 466–8.

Agarwal P, Singh L, Anand M *et al.* (2018a) Association between placental polycyclic aromatic hydrocarbons (PAHS), oxidative stress, and preterm delivery: a case-control study. *Arch Environ Contam Toxicol*; **74**: 218–27.

Agarwal A, Qiu E, Sharma R. (2018b) Laboratory assessment of oxidative stress in semen. *Arab J Urol*; **16**: 77–86.

Ajina T, Sallem A, Haouas Z, Mehdi M. (2017) Total antioxidant status and lipid peroxidation with and without in vitro zinc supplementation in infertile men. *Andrologia*; **49**: e12703. doi:10.1111/and.12703.

Bae S, Pan XC, Kim SY *et al.* (2010) Exposures to particulate matter and polycyclic aromatic hydrocarbons and oxidative stress in schoolchildren. *Environ Health Perspect*; **118**: 579–83.

Bisht S, Dada R. (2017) Oxidative stress: major executioner in disease pathology, role in sperm DNA damage and preventive strategies. *Front Biosci (Schol Ed)*; **9**: 420–47.

Bisht S, Faiq M, Tolahunase M *et al*. (2017) Oxidative stress and male infertility. *Nat Rev Urol*; **14**: 470–85.

Bobb JF, Claus Henn B, Valeri L *et al*. (2018) Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ Health*; **17**: 67.

Bobb JF, Valeri L, Claus Henn B *et al*. (2015) Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics*; **16**: 493–508.

Chao MR, Yen CC, Hu CW. (2008) Prevention of artifactual oxidation in determination of cellular 8-oxo-7,8-dihydro-2'-deoxyguanosine by isotope-dilution LC–MS/MS with automated solid-phase extraction. *Free Radic Biol Med*; **44**: 464–73.

Coker E, Chevrier J, Rauch S *et al*. (2018) Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. *Environ Int*; **113**: 122–32.

Gedik CM, Collins A; ESCODD (European Standards Committee on Oxidative DNA Damage). (2005) Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. *FASEB J*; **19**: 82–4.

Geier MC, Chlebowski AC, Truong L *et al*. (2018) Comparative developmental toxicity of a comprehensive suite of polycyclic aromatic hydrocarbons. *Arch Toxicol*; **92**: 571–86.

Guan X, Fu W, Wei W *et al*. (2020) Mediation of the association between polycyclic aromatic hydrocarbons exposure and telomere attrition by oxidative stress: a prospective cohort study. *J Hazard Mater*; **399**: 123058.

Gunes S, Al-Sadaan M, Agarwal A. (2015) Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. *Reprod Biomed Online*; **31**: 309–19.

Han X, Zhou N, Cui Z *et al*. (2011) Association between urinary polycyclic aromatic hydrocarbon metabolites and sperm DNA damage: a population study in Chongqing, China. *Environ Health Perspect*; **119**: 652–7.

Hsu PC, Chen IY, Pan CH *et al*. (2006) Sperm DNA damage correlates with polycyclic aromatic hydrocarbons biomarker in coke-oven workers. *Int Arch Occup Environ Health*; **79**: 349–56.

Hu CW, Chang YJ, Chen JL *et al*. (2018) Sensitive detection of 8-nitroguanine in DNA by chemical derivatization coupled with online solid-phase extraction LC-MS/MS. *Molecules*; **23**: 605. doi:10.3390/molecules23030605.

Hu CW, Wu MT, Chao MR *et al*. (2004) Comparison of analyses of urinary 8-hydroxy-2'-deoxyguanosine by isotope-dilution liquid chromatography with electrospray tandem mass spectrometry and by enzyme-linked immunosorbent assay. *Rapid Commun Mass Spectrom*; **18**: 505–10.

Janero DR. (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med*; **9**: 515–40.

Jeng HA, Pan CH, Chao MR *et al*. (2015) Sperm DNA oxidative damage and DNA adducts. *Mutat Res Genet Toxicol Environ Mutagen*; **794**: 75–82.

Jeng HA, Pan CH, Chao MR *et al*. (2016) Sperm quality and DNA integrity of coke oven workers exposed to polycyclic aromatic hydrocarbons. *Int J Occup Med Environ Health*; **29**: 915–26.

Jeng HA, Pan CH, Diawara N *et al*. (2011) Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration. *Occup Environ Med*; **68**: 653–8.

Jeng HA, Pan CH, Lin WY *et al*. (2013) Biomonitoring of polycyclic aromatic hydrocarbons from coke oven emissions and reproductive toxicity in nonsmoking workers. *J Hazard Mater*; **244–245**: 436–43.

Kobayashi H, Gil-Guzman E, Mahran AM *et al*. (2001) Quality control of reactive oxygen species measurement by luminol-dependent chemiluminescence assay. *J Androl*; **22**: 568–74.

Kupsco A, Kioumourtzoglou MA, Just AC *et al*. (2019) Prenatal metal concentrations and childhood cardiometabolic risk using bayesian kernel machine regression to assess mixture and interaction effects. *Epidemiology*; **30**: 263–73.

Li Y, Wu J, Zhou W *et al*. (2016) Association between environmental exposure to cadmium and human semen quality. *Int J Environ Health Res*; **26**: 175–86.

Liu SH, Bobb JF, Claus Henn B *et al*. (2018) Bayesian varying coefficient kernel machine regression to assess neurodevelopmental trajectories associated with exposure to complex mixtures. *Stat Med*; **37**: 4680–94.

Liu Y, Li X, Zhang B *et al*. (2019) CYP1A1 methylation mediates the effect of smoking and occupational polycyclic aromatic hydrocarbons co-exposure on oxidative DNA damage among Chinese coke-oven workers. *Environ Health*; **18**: 69.

Lou XY, Wu PR, Guo Y. (2019) Urinary metabolites of polycyclic aromatic hydrocarbons in pregnant women and their association with a biomarker of oxidative stress. *Environ Sci Pollut Res Int*; **26**: 27281–90.

Martín Muñoz P, Anel-López L, Ortiz-Rodríguez JM *et al*. (2018) Redox cycling induces spermptosis and necrosis in stallion spermatozoa while the hydroxyl radical (OH•) only induces spermptosis. *Reprod Domest Anim*; **53**: 54–67.

Penning TM. (2014) Human aldo-keto reductases and the metabolic activation of polycyclic aromatic hydrocarbons. *Chem Res Toxicol*; **27**: 1901–17.

Qiu L, Leng S, Wang Z *et al*. (2007) Path analysis of biomarkers of exposure and early biological effects among coke-oven workers exposed to polycyclic aromatic hydrocarbons. *Cancer Epidemiol Biomarkers Prev*; **16**: 1193–9.

Ramesh A, Greenwood M, Inyang F *et al*. (2001a) Toxicokinetics of inhaled benzo[a]pyrene: plasma and lung bioavailability. *Inhal Toxicol*; **13**: 533–55.

Ramesh A, Inyang F, Hood DB *et al*. (2001b) Metabolism, bioavailability, and toxicokinetics of benzo(alpha)pyrene in F-344 rats following oral administration. *Exp Toxicol Pathol*; **53**: 275–90.

Raychoudhury SS, Kubinski D. (2003) Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. *Environ Health Perspect*; **111**: 33–8.

Rummel AM, Trosko JE, Wilson MR *et al*. (1999) Polycyclic aromatic hydrocarbons with bay-like regions inhibited gap junctional intercellular communication and stimulated MAPK activity. *Toxicol Sci*; **49**: 232–40.

Signes-Pastor AJ, Doherty BT, Romano ME *et al*. (2019) Prenatal exposure to metal mixture and sex-specific birth outcomes in the New Hampshire Birth Cohort Study. *Environ Epidemiol*; **3**: e068. doi:10.1097/EE9.0000000000000068.

Singh VK, Patel DK, Jyoti et al. (2008) Blood levels of polycyclic aromatic hydrocarbons in children and their association with oxidative stress indices: an Indian perspective. *Clin Biochem*; **41**: 152–61.

Stella A, Piccardo MT, Pala M *et al*. (2012) Temporal and spatial variations of polycyclic aromatic hydrocarbon concentrations around a coke oven plant. *J Air Waste Manag Assoc*; **62**: 1003–11.

Tsikas D. (2017) Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges. *Anal Biochem*; **524**: 13–30.

Valeri L, Mazumdar MM, Bobb JF *et al*. (2017) The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: evidence from Rural Bangladesh. *Environ Health Perspect*; **125**: 067015.

Vessey W, Perez-Miranda A, Macfarquhar R *et al*. (2014) Reactive oxygen species in human semen: validation and qualification of a chemiluminescence assay. *Fertil Steril*; **102**: 1576–83.e4.

Vijayalakshmi KP, Suresh CH. (2008) Theoretical studies on the carcinogenicity of polycyclic aromatic hydrocarbons. *J Comput Chem*; **29**: 1808–17.

Wu MT, Lin PC, Pan CH *et al*. (2019) Risk assessment of personal exposure to polycyclic aromatic hydrocarbons and aldehydes in three commercial cooking workplaces. *Sci Rep*; **9**: 1661.

Wu MT, Pan CH, Huang YL *et al*. (2003) Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. *Environ Mol Mutagen*; **42**: 98–105.

Xia Y, Han Y, Zhu P *et al*. (2009) Relation between urinary metabolites of polycyclic aromatic hydrocarbons and human semen quality. *Environ Sci Technol*; **43**: 4567–73.

Yan LS. (1985) Study of the carcinogenic mechanism for polycyclic aromatic hydrocarbons–extended bay region theory and its quantitative model. *Carcinogenesis*; **6**: 1–6.

Yang P, Wang YX, Chen YJ *et al*. (2017) Urinary polycyclic aromatic hydrocarbon metabolites and human semen quality in China. *Environ Sci Technol*; **51**: 958–67.

Yoon HS, Lee KM, Lee KH *et al*. (2012) Polycyclic aromatic hydrocarbon (1-OHPG and 2-naphthol) and oxidative stress (malondialdehyde) biomarkers in urine among Korean adults and children. *Int J Hyg Environ Health*; **215**: 458–64.

Zhang Y, Dong T, Hu W *et al*. (2019) Association between exposure to a mixture of phenols, pesticides, and phthalates and obesity: Comparison of three statistical models. *Environ Int*; **123**: 325–36.

Zhang L, Jin Y, Huang M *et al*. (2012) The role of human aldo-keto reductases in the metabolic activation and detoxication of polycyclic aromatic hydrocarbons: interconversion of PAH catechols and PAH o-quinones. *Front Pharmacol*; **3**: 193.