



The role of oxidative stress in association between disinfection by-products exposure and semen quality: A mediation analysis among men from an infertility clinic



Min Zhang^{a, b}, Chong Liu^{a, b}, Fei-Peng Cui^{a, b}, Pan-Pan Chen^{a, b}, Yan-Ling Deng^{a, b}, Qiong Luo^{a, b}, Yu Miao^{a, b}, Sheng-Zhi Sun^c, Yu-Feng Li^d, Wen-Qing Lu^{a, b}, Qiang Zeng^{a, b, e, *}

^a Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China

^b Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China

^c Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA

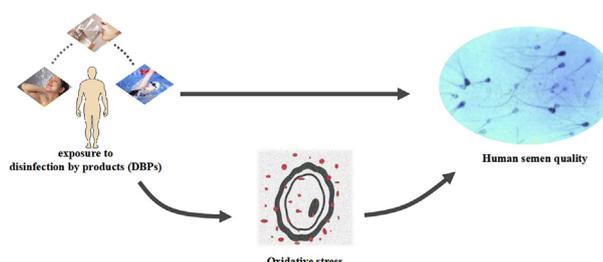
^d Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China

^e Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA

HIGHLIGHTS

- The mediating role of oxidative stress in association between DBP exposure and semen quality was firstly examined.
- Urinary TCAA was positively associated with urinary 8-OHdG and 8-isoPGF_{2α}.
- Urinary 8-isoPGF_{2α} was negatively associated with sperm concentration.
- Urinary 8-OHdG was negatively associated with sperm motility.
- Urinary 8-isoPGF_{2α} mediated the effect of urinary TCAA on decreased sperm concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxicological and epidemiologic evidence has suggested that exposure to disinfection by-products (DBPs) impairs semen quality, while the underlying biological mechanisms remain unclear. This study aimed to examine the mediating role of oxidative stress in association between DBP exposure and semen quality. We measured a urinary biomarker of DBP exposure [trichloroacetic acid (TCAA)] and three urinary biomarkers of oxidative stress [8-hydroxy-2-deoxyguanosine (8-OHdG), 8-iso-prostaglandin F_{2α} (8-isoPGF_{2α}) and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)] among men from an infertility clinic (n = 299). The associations of oxidative stress biomarkers with urinary TCAA and semen quality were evaluated using multivariable linear regression models, and the mediating role of oxidative stress biomarkers was assessed by a mediation analysis. Urinary TCAA was positively associated with urinary 8-OHdG and 8-isoPGF_{2α} in a dose-response manner (both *P* for trend < 0.001). Significantly inverse dose-response associations were observed between urinary 8-isoPGF_{2α} and sperm concentration and

* Corresponding author. Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China.

E-mail addresses: zengqiang506@163.com, zengqiang506@hust.edu.cn (Q. Zeng).

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Mediation

between urinary 8-OHdG and sperm motility (both P for trend < 0.05). The mediation analysis indicated a significant indirect effect of urinary 8-isoPGF_{2α} in the association between urinary TCAA and decreased sperm concentration ($P = 0.01$). Our results suggest that lipid peroxidation may be an intermediate mechanism by which DBP exposure impairs semen quality.

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1. Introduction

Chlorinated disinfection is widely used to reduce the incidence of waterborne diseases via killing the pathogenic microorganisms in raw water. However, disinfection by-products (DBPs) are inevitably produced from the reaction between chlorine and naturally organic and inorganic matter in raw water (Krasner et al., 2006; Weinberg et al., 2006). Among the identified > 700 DBPs, trihalomethanes (THMs) and haloacetic acids (HAAs) are the most prevalent and dominant in chlorinated drinking water. Human exposure to DBPs occurs during various routine water-use activities by ingestion, inhalation and dermal absorption (Nieuwenhuijsen et al., 2009). The presence of DBPs in drinking water have raised a public health concern because of their constant human exposure and adverse health effects such as increased risk of cancers (Evlampidou et al., 2020), adverse pregnancy outcomes (Wright et al., 2017) and impaired male reproductive health (Grellier et al., 2015; Villanueva et al., 2015).

Toxicological studies in rodent species have revealed that exposure to DBPs can induce histopathologic abnormalities in testis and epididymis, reduce serum testosterone levels and impair spermatogenesis, as well as decrease semen quality parameters including sperm count, motility and morphology (Kaydos et al., 2004; Klinefelter et al., 2002, 2004; Linder et al., 1997; Narotsky et al., 2015; Veeramachaneni et al., 2007), suggesting that testis and epididymis are the potential targets for DBP insult (Ahmed et al., 1991). Furthermore, increasing epidemiological evidence has suggested inverse associations of DBP exposure with sperm concentration, count, motility and morphology (Chen et al., 2020; Fenster et al., 2003; Xie et al., 2011; Zeng et al., 2014a). However, some studies have also reported null associations between DBP exposure and semen quality (Iszatt et al., 2013; Luben et al., 2007). The inconsistency may result from differential characteristics of study populations and varied methods of evaluating DBP exposure. Recently, we conducted a large cross-sectional study using urinary trichloroacetic acid (TCAA), a valid biomarker of exposure to chlorinated drinking water DBPs through ingestion (Froese et al., 2002; Smith et al., 2013), to improve exposure assessment, and found elevated urinary TCAA concentrations were associated with decreased sperm concentration, count and motility (Zeng et al., 2014b). However, the underlying mechanisms remain poorly characterized.

Oxidative stress has been proposed to be involved in male reproductive damage (Bisht et al., 2017; Tremellen, 2008). Specifically, spermatozoa are vulnerable to damage caused by oxygen free radicals due to their limited antioxidant capacity and lacking DNA repair mechanisms (Tremellen, 2008). Moreover, oxidative stress can impair semen quality by disrupting the production of proteins and lipids in the sperm plasma membrane and damaging the integrity of sperm DNA (Aitken et al., 2014; Wong and Cheng, 2011). Exposure to DBPs may cause detrimental effects on semen quality by a mechanism involving in induced oxidative stress (Nieuwenhuijsen et al., 2009; Zeng et al., 2013). Both *in vivo* (Abbassi et al., 2010; Gong et al., 2019; Hassoun et al., 2014; Wang et al., 2014a) and *in vitro* (Hassoun and Ray, 2003; Stalter et al.,

2016; Xie et al., 2010) studies have reported that exposure to DBPs can lead to oxidative stress. Limited epidemiological studies have also found positive associations between exposure to DBPs and oxidative stress biomarkers (Liu et al., 2020; Morissette et al., 2016; Varraso et al., 2002). Nevertheless, no study has explored the potential mediation effect of oxidative stress in the association between DBP exposure and semen quality.

In this study, we measured urinary 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-iso-prostaglandin F_{2α} (8-isoPGF_{2α}) and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA) as biomarkers of oxidative stress among men from an infertility clinic. We examined the mediating role of oxidative stress in the association between DBP exposure as reflected by urinary TCAA and semen quality.

2. Materials and methods

2.1. Study population

Our participants were recruited from Reproductive Center of Tongji Hospital in Wuhan, China between March and May 2012, which has been illustrated in our previous study (Zeng et al., 2014b). After informing the purpose of our study, all volunteers signed written consent and completed a questionnaire to provide detailed information including demographic characteristics, occupational exposure, medical history, lifestyle factors and routine water-use habits. A total of 1262 men were recruited and provided a spot urine and semen sample on the day of their clinic visit. After excluding subjects with occupational exposure to synthetic materials that can be metabolized into TCAA (e.g., trichloroethylene, 1,1,1-trichloroethane and perchloroethylene) and self-reported diseases that may affect semen quality (e.g., azoospermia, vasectomy, varicocele, orchiditis, epididymitis), 1189 men were finally retained. Of them, 299 men had the sufficient urine samples for the analyses of oxidative stress biomarkers and thus were ultimately included in the current analysis. The research was approved by the Ethics Committee of Tongji Medical College.

2.2. Measurement of urinary TCAA

Each participant provided a urine sample in a sterile polyethylene cup during their clinic visit during 08:30 and 11:30 a.m. The urine specimens were transported with an ice pack and stored at -40 °C until laboratory analysis. Concentrations of urinary TCAA were measured on the basis of the method has been developed previously (Xie et al., 2011). In brief, a 10 mL of urine sample was pre-treated by liquid-liquid extraction with methyl-*tert*-butyl-ether and the TCAA underwent esterification reaction with acidic methanol. The target analyte was quantified by gas chromatography (GC) coupled with an electron capture detector. The limit of detection (LOD) for urinary TCAA was 2.00 μg/L. Concentrations of urinary creatinine were measured by picric acid assay with commercial test kits.

2.3. Measurements of oxidative stress biomarkers

The method used to measure urinary concentrations of 8-OHdG, HNE-MA and 8-isoPGF_{2α} has been described in detail previously (Wang et al., 2019b). Briefly, a 100 μL of urine sample was diluted with 2.4 mL of deionized water. After the addition of 50 μL of internal standards, the samples underwent solid-phase extraction (SPE). The collected eluent was evaporated to dryness and re-dissolved with 200 μL of 5% methanol/water. The target compounds were analyzed by an Agilent 6460 triple quadrupole mass spectrometer. Each batch including two quality controls and one blank sample was also analyzed to monitor the performance of the analytical method. The recoveries for the target compounds were in the range of 87%–102%. The average intra- and inter-day variation were 6.9% and 8.4%, respectively. The concentrations of target compounds in the blank sample were below their LOQs. The limits of qualification (LOQs) were 0.08 ng/mL for 8-OHdG, 0.06 ng/mL for 8-isoPGF_{2α} and 0.03 ng/mL for HNE-MA.

2.4. Semen collection and analysis

Semen collection and analysis has been described previously (Zeng et al., 2014b). In short, the semen sample of participant was collected in a specialized semen collection room by masturbating into a sterile plastic specimen cup. Semen volume was determined by a serologic pipette after the liquefaction of semen sample. Sperm concentration (million/mL), motility (% a + b motile sperm) and motion parameters were assessed using a microcell slide and computer-aided semen analysis. Furthermore, we calculated sperm count (semen volume × sperm concentration). Percent of sperm normal morphology and percent of sperm abnormal heads were evaluated using an optical microscope on fixed and stained smears. Three sperm motion parameters including straight-line velocity (VSL), curvilinear velocity (VCL) and linearity (LIN = VSL/VCL × 100) were also reported.

2.5. Statistical analysis

Descriptive statistics were conducted on participants' characteristics and distribution of urinary TCAA, oxidative stress biomarkers and semen quality parameters. Concentrations under the LOD or LOQ were assigned using the LOD or LOQ/√2 in the analyses. Creatinine-adjusted concentrations (μg/g) of urinary TCAA and oxidative stress biomarkers were calculated by dividing the crude target compound concentrations (μg/L) by creatinine concentrations (g/L) to correct urine dilution. Ln-transformed values were used in the analyses to normalize the skewed distribution of urinary TCAA, oxidative stress biomarkers, sperm concentration, sperm count, semen morphology parameters and sperm LIN. We conducted Chi-square test to compare the differences of categorical characteristics between the study population and the total population.

Multivariate linear regression models were used to evaluate the associations between urinary TCAA with oxidative stress biomarkers and between oxidative stress biomarkers and semen quality parameters. The regression coefficients and 95% confidence intervals were back-transformed $\{100 \times [\exp(\beta) - 1]\}$ to obtain percent changes. Urinary TCAA and oxidative stress biomarkers were categorized into tertiles based on their distribution. Tests for trend were performed by converting the tertile of urinary TCAA and oxidative stress biomarkers into integer values (1–3).

We conducted a mediation analysis to assess the role of oxidative stress biomarkers in the association between TCAA exposure and semen quality using mediation package in R software. To get a stable result, we fitted mediation model with 1000 simulations

according to normal approximation using the quasi-Bayesian Monte Carlo approach. The total effect of TCAA exposure on semen quality was composed of the average causal mediation effects (ACME) and the average direct effects (ADE). The ACME describes the indirect effect of TCAA exposure on semen quality through the mediator (e.g., oxidative stress biomarkers). The ADE describes the effect of TCAA exposure on semen quality that is not mediated by a mediator. The proportion of mediation by oxidative stress biomarkers was calculated using ACME divided by the total effect (Valeri and Vanderweele, 2013).

The approach of change-in-estimate ($\geq 10\%$ change for the regression coefficient) (Greenland, 1989) and biological consideration were used to identify potential confounders. The following covariates were included in the final multivariate models: age (continuous), body mass index (BMI, continuous), education (\geq high school vs. < high school), abstinence time (<3 days, 3–5 days or >5 days), income (<2,000, 2000–6000 or >6,000yuan/month), smoking status (current and former vs. never-smoker) and alcohol use (yes vs. no). The mediation model adjusted for the same covariates to the regression models.

We conducted all the data analyses with IBM SPSS Statistics (version 22.0, IBM Corp., Armonk, NY, USA) and R software (version 3.1.2, R Foundation for Statistical Computing, Austria). Results were considered as statistically significant as a *p*-value below 0.05.

3. Results

3.1. Characteristics of study population

Our final study population consisted of 299 men and their characteristics were summarized in Table 1. The mean (\pm SD) age and BMI of subjects were 32.1 (\pm 5.4) years and 23.7 (\pm 4.0) kg/m², respectively. Among the study population, 97.0% were Han ethnicity, 42.6% had never smoked, 60.4% had educational background as above high school and only 27.8% had ever drunk. Nearly half of the man reported their abstinence time more than 5 days (48.8%) and income less than 2000 yuan/month (50.7%). No significant differences in categorical characteristics were found between the study population and the total population except for abstinence time, income and use of boiled water.

3.2. Distribution of urinary TCAA, oxidative stress biomarkers and semen parameters

The distribution of urinary TCAA, urinary biomarkers of oxidative stress and semen parameters among the study population is presented in Table 2. The detection rates for the all measured biomarkers (TCAA, 8-OHdG, HNE-MA and 8-isoPGF_{2α}) in urine samples were > 98%. The median of urinary TCAA concentrations was 4.86 μg/g. For the three urinary biomarkers of oxidative stress, the HNE-MA in urine samples was the dominant, followed by 8-isoPGF_{2α} and 8-OHdG, with the median concentration values of 74.27 μg/g, 5.83 μg/g and 4.68 μg/g, respectively. The median values of sperm concentration, motility and count were 50.28 million/mL, 43.64% and 154.05 million, respectively.

3.3. Associations between urinary TCAA and oxidative stress biomarkers

Fig. 1 reveals the associations between urinary TCAA and urinary biomarkers of oxidative stress. The results from adjusted models were similar to those from crude models. In the adjusted models, urinary TCAA as a continuous exposure was positively associated with urinary 8-OHdG and 8-isoPGF_{2α} (both *P* < 0.001) but not with HNE-MA (*P* = 0.25). For the exposure biomarker modeled as

Table 1
Characteristics of the study population comparing to the total population.

Characteristic	Study population ^a (n = 299)	Total population ^b (n = 1189)	P value ^c
Ethnicity			
Han	290 (97.0)	1159 (97.6)	0.52
Other	9 (3.0)	28 (2.4)	
Age (years)			
≤30	123 (42.0)	504 (43.4)	0.67
>30	170 (58.0)	658 (56.6)	
Body mass index (kg/m ²)			
<18.5	12 (4.0)	47 (4.0)	0.81
18.5–25	201 (67.2)	775 (65.3)	
>25	86 (28.8)	364 (30.7)	
Abstinence time (days)			
<3	26 (8.7)	112 (9.4)	<0.0001
3–5	127 (42.5)	720 (60.6)	
>5	146 (48.8)	357 (30.0)	
Smoking status			
Never-smoker	127 (42.6)	458 (38.7)	0.37
Former smoker	55 (18.5)	214 (18.1)	
Current smoker	116 (38.9)	512 (43.2)	
Alcohol use			
Yes	83 (27.8)	275 (23.2)	0.10
No	216 (72.2)	908 (76.8)	
Education			
< High school	118 (39.6)	480 (40.6)	0.75
≥ High school	180 (60.4)	702 (59.4)	
Income (RMB yuan/month)			
<2000	151 (50.7)	325 (27.5)	<0.0001
2000–6000	108 (36.2)	690 (58.4)	
≥6000	39 (13.1)	166 (14.1)	
Use of boiled water			
Yes	284 (96.3)	973 (83.4)	<0.0001
No	11 (3.7)	193 (16.5)	
Total tap-water consumption (mL/day)			
<1000	208 (70.0)	809 (68.2)	0.55
≥1000	89 (30.0)	377 (31.8)	
Showering/bathing time (min/week)			
<70	175 (58.7)	684 (58.2)	0.86
≥70	123 (41.3)	492 (41.8)	
Swimming in chlorinated pool			
Yes	293 (98.3)	1167 (98.3)	0.99
No	5 (1.7)	20 (1.7)	

^a In study population, 6 missing age, 4 missing use of boiled water, 2 missing total tap-water consumption, 1 missing smoking status, alcohol use, education, income, showering/bathing time and swimming in chlorinated pool.

^b In total population, 2 missing ethnicity, 27 missing age, 3 missing body mass index, 5 missing smoking status, 6 missing alcohol use, 7 missing education, 8 missing income, 23 missing use of boiled water, 3 missing total tap-water consumption, 13 missing showering/bathing time and 2 missing swimming in chlorinated pool.

^c For categorical variables, a Chi-square test was used to compare the differences of characteristics across tertiles of urinary TCAA concentrations.

Table 2
Distribution of urinary TCAA, urinary biomarkers of oxidative stress and semen parameters among study participants (n = 299).

Measures	Arithmetic mean ± SD	Geometric mean	Median	Percentile				Range
				10th	25th	75th	90th	
DBP exposure								
TCAA (µg/g)	6.25 ± 4.39	5.30	4.86	2.74	3.99	7.19	10.85	0.93–42.33
Oxidative stress								
8-OHdG (µg/g)	5.23 ± 3.33	4.29	4.68	2.11	2.97	6.60	8.58	0.02–23.90
HNE-MA (µg/g)	147.50 ± 209.73	67.71	74.27	17.32	31.81	185.06	352.21	0.01–1547.77
8-isoPGF _{2α} (µg/g)	8.21 ± 9.88	5.65	5.83	1.98	3.72	10.01	14.64	0.02–124.16
Semen quality								
Sperm concentration (million/mL)	65.39 ± 50.19	49.17	50.28	16.86	28.36	88.13	134.70	5.93–134.70
Sperm motility (% motile)	44.02 ± 17.51	39.75	43.64	21.88	33.06	56.56	68.30	0.00–83.41
Sperm count (millions)	198.98 ± 182.10	138.57	154.05	43.06	77.09	248.29	390.58	6.64–1196.78
Semen morphology								
Normal morphology (%)	23.88 ± 7.26	22.62	24.00	15.00	21.00	27.00	31.50	0.00–51.00
Abnormal head (%)	60.79 ± 8.80	60.45	60.00	52.00	58.00	65.50	71.50	0.00–91.00
Sperm motion								
VSL (µm/sec)	26.66 ± 5.56	26.32	26.65	20.02	23.48	30.09	33.32	0.00–44.10
VCL (µm/sec)	42.55 ± 9.63	41.85	42.32	32.14	36.39	48.83	55.05	0.00–66.99
LIN (%)	63.24 ± 8.57	63.31	63.03	55.02	58.83	67.69	73.21	0.00–89.31

tertiles, significantly positive dose-response associations of urinary TCAA with urinary 8-OHdG, HNE-MA and 8-isoPGF_{2α} were

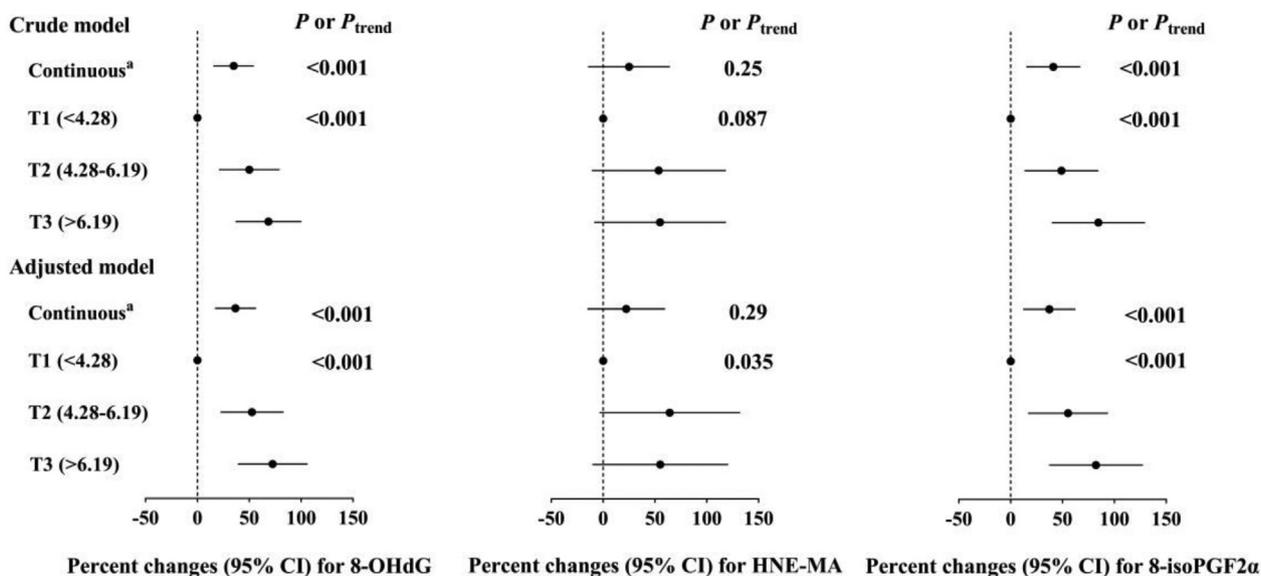


Fig. 1. Associations between urinary TCAA concentrations and urinary biomarkers of oxidative stress. ^aLn-transformed values. Percent changes were transformed by the natural logarithm and back-transformed. Adjusted model was adjusted for age, BMI, education, abstinence time, income, smoking status and alcohol use.

observed (all $P_{trend} < 0.05$). Men in the highest tertiles of urinary TCAA had increases of 69.89% (95% CI: 40.49%, 107.50%), 46.23% (95% CI: 3.63%, 122.55%) and 78.60% (95% CI: 39.10%, 129.33%) in urinary 8-OHdG, HNE-MA and 8-isoPGF2 α compared those in the lowest tertiles, respectively.

3.4. Associations between oxidative stress biomarkers and semen parameters

Fig. 2 presents the adjusted associations between urinary biomarkers of oxidative stress and semen quality parameters, which were similar to crude results (Table S1). After adjusting for various confounders, urinary 8-isoPGF2 α as a continuous variable was negatively associated with sperm concentration and sperm count (both $P < 0.05$). For the oxidative stress biomarkers modeled as tertiles, we found significant dose-dependent relationships between urinary 8-OHdG and decreasing sperm motility ($P_{trend} = 0.046$) and between urinary 8-isoPGF2 α and decreasing sperm concentration ($P_{trend} = 0.049$). Compared with men in the lowest tertiles of urinary 8-OHdG and 8-isoPGF2 α , those in the highest tertiles had decreases of 4.99 (95% CI: 9.90, -0.09) in sperm motility and 19.75% (95% CI: 36.24%, 0.05%) in sperm concentration, respectively. We did not find any significant associations of urinary biomarkers of oxidative stress with semen morphology parameters and sperm motion parameters (Table S2 to Table S5).

3.5. Mediating effects

Given that a mediator is associated with both the exposure and the outcome (Valeri and Vanderweele, 2013), we conducted a mediation analysis to investigate whether urinary 8-OHdG and 8-isoPGF2 α mediated the associations between urinary TCAA and semen quality (Fig. 3). The analysis indicated a significantly indirect effect of the association between urinary TCAA and sperm concentration by urinary 8-isoPGF2 α ($\beta = -0.039$, 95%CI: 0.087, -0.01) and the proportion of mediation was 87.3%. We did not find significantly indirect effects of urinary TCAA on sperm motility through urinary 8-OHdG ($\beta = -0.19$, 95%CI: 1.54, 0.94).

4. Discussion

In our previous study among men from an infertility clinic, exposure to drinking water DBPs as reflected by urinary TCAA was related with declined semen quality (Zeng et al., 2014b). In this study, we further investigated the mediation effect of oxidative stress to uncover the potential mechanisms underlying the associations. The findings showed that urinary TCAA was positively associated with 8-OHdG and 8-isoPGF2 α , which in turn, were inversely associated with sperm motility and sperm concentration, respectively. In the analysis of mediation effects, we observed a significant mediating effect of 8-isoPGF2 α in the association between urinary TCAA and reduced sperm concentration.

Experimental studies have well demonstrated the ability of high levels of DBPs to induce oxidative stress through acceleration of free radical production (Hassoun et al., 2014) or inhibition of glutathione S-transferase zeta (GSTZ) and superoxide dismutase (SOD) activities (Cornett et al., 1999; Schultz et al., 2002). In this study, we found that DBP exposure was associated with increased 8-OHdG and 8-isoPGF2 α , which represents oxidative DNA damage and lipid peroxidation, respectively (Il'Yasova et al., 2012). Our results were consistent with previous those experimental studies. To date, only limited population-based studies have estimated the association between DBP exposure and oxidative stress (Liu et al., 2020; Morissette et al., 2016; Varraso et al., 2002). In agreement with our results, Liu et al. found positive dose-response relationships between tertiles of urinary TCAA and oxidative stress biomarkers (e.g., 8-OHdG, HNE-MA and 8-isoPGF2 α) among pregnant women (Liu et al., 2020). Morissette et al. also showed that competitive swimmers who had never asthma had higher level of 8-isoprostane in exhaled breath condensate after a swimming session in an indoor chlorinated pool (Morissette et al., 2016). However, no significant increases in oxidative stress parameters such as plasma MDA were observed in healthy swimmers after 40 min swimming in chlorinated pool (Font-Ribera et al., 2010; Llana-Belloch et al., 2016). More human studies are warranted to examine the effect of DBP exposure and oxidative stress.

Oxidative stress is a major cause of defective sperm function, which has observed in approximately half of all infertile men (Agarwal et al., 2018; Deng et al., 2017; Tremellen, 2008).

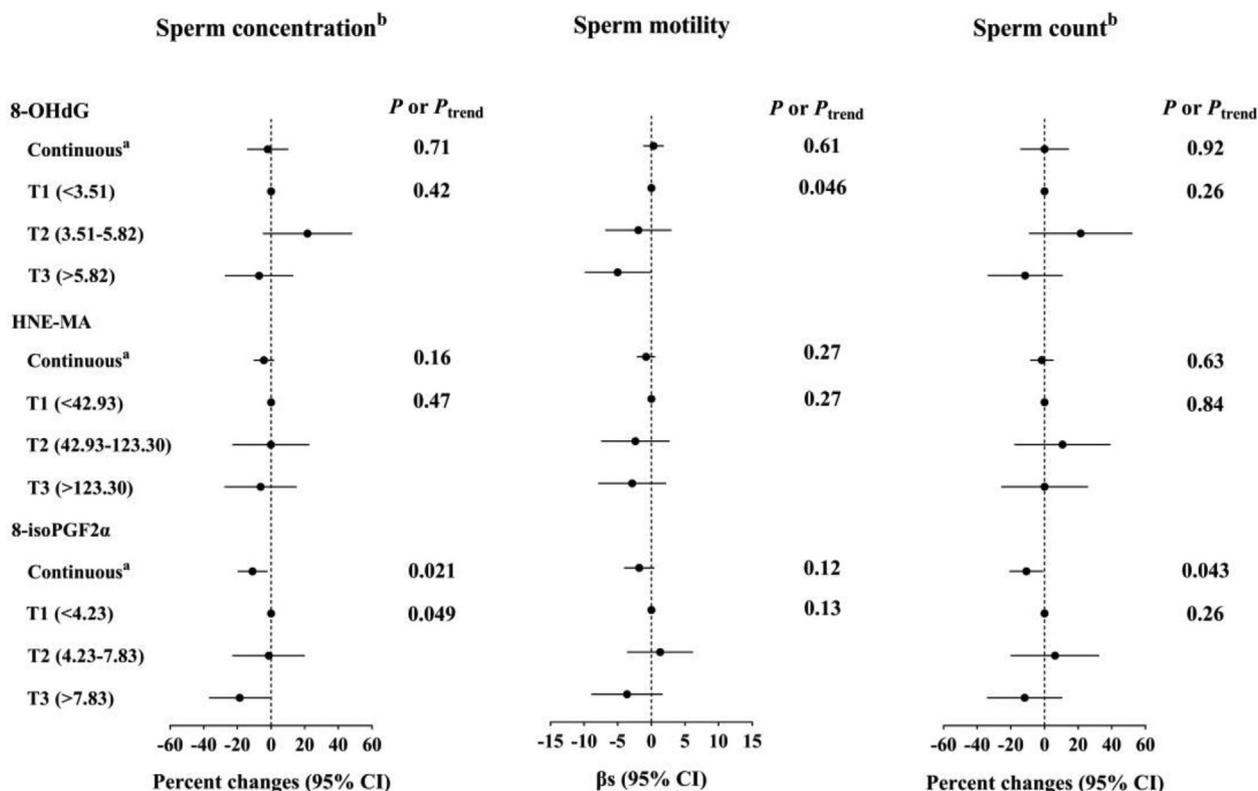


Fig. 2. Associations between urinary biomarkers of oxidative stress and semen quality parameters. ^a Ln-transformed values. ^b The values were transformed by the natural logarithm and back-transformed to obtain percent change. Adjusted model was adjusted for age, BMI, education, abstinence time, income, smoking status and alcohol use.

Accumulating evidence has reported significant results of higher reactive oxygen species (ROS) (Agarwal et al., 2014; Chen et al., 2012) and MDA levels (Benedetti et al., 2012; Hosen et al., 2015; Taken et al., 2016), lower SOD (Dorostghoal et al., 2017; Murawski et al., 2007) and glutathione peroxidase (GPx) activities (Dorostghoal et al., 2017; Giannattasio et al., 2002) in the seminal plasma of infertile men compared to those of proven fertile men. Specially, Agarwal et al. found that ROS levels in the seminal ejaculates were negatively correlated with sperm concentration, motility and morphology (Agarwal et al., 2014); Kao et al. revealed a negative correlation between sperm progressive motility and 8-OHdG in spermatozoa (Kao et al., 2008). In our data, lower sperm motility was associated with higher urinary 8-OHdG levels, which was consistent with the majority of previous studies. However, by contrast with several studies (Cambì et al., 2013; He et al., 2019; Liu et al., 2019), we did not find significant associations between urinary 8-OHdG and sperm concentration, count and percent of normal morphology. One possible explanation was that oxidative stress biomarkers in our study were detected in urine rather than spermatozoa or seminal plasma. Posteriorly, varied sample sizes and study populations may also contribute to this discrepancy.

In agreement with prior studies (He et al., 2019; Liu et al., 2019), our results also showed that 8-isoPGF2_α was inversely correlated with sperm concentration and sperm count, indicating the adverse effect of lipid peroxidation on semen quality. Further, Tartibian and his colleagues observed a significantly positive correlation between seminal 8-isoprostane with sperm DNA fragmentation (Tartibian and Maleki, 2012). Interestingly, Bejarano et al. found that long-term supplementation with melatonin to infertile men improved sperm quality through reducing oxidative damage to sperm DNA among 30 men suffered from primary infertility (Bejarano et al., 2014). These findings bring up a hint that some interventions can

substantially improve the probability of infertile couples conceiving naturally by regulate oxidative stress beyond assisted reproduction technology (Alizadeh et al., 2018; Bisht et al., 2017).

In the further mediation analysis, we found that the association between urinary TCAA and reduced sperm concentration was mediated by urinary 8-isoPGF2_α, though no significant direct effect was observed between urinary TCAA and decreased sperm concentration. Actually, earlier study has suggested that the significant direct effect of exposure on outcome is not necessary for the mediation analysis (Valeri and Vanderweele, 2013). Nevertheless, an inverse association between urinary TCAA and sperm concentration was observed in our previous study with a large sample size (Zeng et al., 2014b). Taken together, these results suggest that exposure to DBPs may exert its damage to semen quality though induced lipid peroxidation. However, our results did not reveal significant evidence of mediation effect through 8-OHdG in the associations between urinary TCAA and reduced sperm motility. Given the limited sample size in this study, further studies with more statistical power are warranted to explore the mediation effect of oxidative DNA damage. In addition, inflammation alteration as an underlying mechanism involved in the effects of DBPs-induced oxidative stress and semen quality should be further explored (Prochazka et al., 2019).

We compared the urinary TCAA concentrations measured in our participants to those reported in other populations. The median urinary TCAA concentrations in our study (4.86 μg/g) were relatively higher than adults from the United States (3.20 and 2.50 μg/g) (Calafat et al., 2003; Parvez et al., 2019) and Shijiazhuang, China (4.29 μg/g) (Zhang et al., 2019), but were lower than pregnant women from Wuhan, China (9.50 μg/g) (Zhou et al., 2012), Bradford, UK (5.07 μg/g) (Smith et al., 2013) and Brittany, France (20 μg/g) (Costet et al., 2012). The differences in exposure levels across

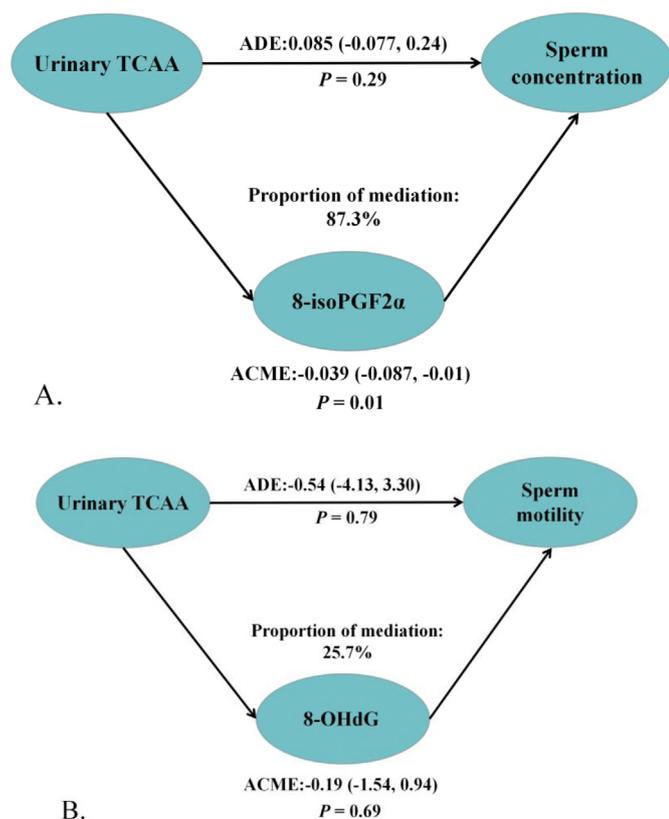


Fig. 3. Mediation effects investigating whether urinary biomarkers of oxidative stress mediated the associations between urinary TCAA and semen quality. The figure presents urinary 8-isoPGF2 α and 8-OHdG as mediator, the estimate of average causal mediation effects (ACME), the estimate of the average direct effects (ADE) and the proportion of mediation (ACME/ADE + ACME). Urinary concentrations of TCAA and biomarkers of oxidative stress were ln-transformed. The mediation model was adjusted for age, BMI, education, abstinence time, income, smoking status and alcohol use.

different populations may be attributed to different water sources, disinfection methods, routine water-use habits, and socio-demographic characteristics (Zeng et al., 2014c).

Our study has some limitations. Firstly, as a cross-sectional study, we were not able to demonstrate the causal relationships between DBP exposure and oxidative stress and between oxidative stress and semen quality. Secondly, as our study population consisted of most infertile or sub-infertile men from a fertility clinic and certain characteristics such as abstinence time, use of boiled water and income were substantially different from the total population, the results may not be generalizable to other populations. Moreover, this study was limited by its sample size, possibly leading to null associations or bias estimation. Thirdly, although urinary TCAA has been considered as a valid biomarker of ingestion of chlorinated DBPs such as THMs and HAAs (Costet et al., 2012; Froese et al., 2002), it is not clear whether the biomarker reflects other bromo- and iodo-DBPs. Experimental studies have shown that the bromo- and iodo-DBPs are more potent testicular toxicants than chlorinated DBPs (Linder et al., 1995). Moreover, there are still some residual and unmeasured confounding factors such as other environmental exposures (e.g. air pollution and phthalates) that we do not take into account, which may have confounded the observed results. Lastly, we relied on single spot urine to assess the exposure and oxidative stress status, which can result in misclassification. Some studies have observed substantial intra-individual variations of urinary TCAA (Wang et al., 2014b, 2019a) and oxidative stress

biomarkers (Martinez-Moral and Kannan, 2019; Wang et al., 2019b) across different populations. Therefore, further confirmation studies with better design, preferably in a large general population, are warranted.

5. Conclusion

To the best of our knowledge, this is the first study evaluating the mediation role of oxidative stress in the association between exposure to DBPs and semen quality. Our analysis provides the evidence on positive associations between DBP exposure and 8-OHdG and 8-isoPGF2 α , which in turn were negatively related to semen quality. Mediation analysis further indicated that 8-isoPGF2 α exhibited a mediating role of the association between urinary TCAA and decreased sperm concentration, suggesting that lipid peroxidation may be an intermediate mechanism by which DBP exposure impairs semen quality. However, confirmation of the finding is warranted.

Credit author statement

Zhang Min: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization. Liu Chong: Conceptualization, Methodology, Formal analysis. Cui Fei-Peng: Investigation, Software. Chen Pan-Pan: Software, Methodology. Deng Yan-Ling: Methodology. Luo Qiong: Investigation. Miao Yu: Investigation. Sun Sheng-Zhi: Investigation. Li Yu-Feng: Resources. Lu Wen-Qing: Resources. Zeng Qiang: Project administration, Conceptualization, Supervision, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.128856>.

References

- Abbassi, R., Chamkhia, N., Sakly, M., 2010. Chloroform-induced oxidative stress in rat liver: implication of metallothionein. *Toxicol. Ind. Health* 26 (8), 487–496. <https://doi.org/10.1177/0748233710373088>.
- Agarwal, A., Sharma, R.K., Sharma, R., Assidi, M., Abuzenadah, A.M., Alshahrani, S., et al., 2014. Characterizing semen parameters and their association with reactive oxygen species in infertile men. *Reprod. Biol. Endocrinol.* 12 (1), 33. <https://doi.org/10.1186/1477-7827-12-33>.
- Agarwal, A., Rana, M., Qiu, E., AlBunni, H., Bui, A.D., Henkel, R., 2018. Role of oxidative stress, infection and inflammation in male infertility. *Andrologia* 50 (11), e13126. <https://doi.org/10.1111/and.13126>.
- Ahmed, A.E., Jacob, S., Loh, J.P., 1991. Studies on the mechanism of haloacetonitriles toxicity: quantitative whole body autoradiographic distribution of [2-¹⁴C] chloroacetonitrile in rats. *Toxicology* 67 (3), 279–302. [https://doi.org/10.1016/0300-483x\(91\)90029-z](https://doi.org/10.1016/0300-483x(91)90029-z).

- Aitken, R.J., Smith, T.B., Jobling, M.S., Baker, M.A., De Iuliis, G.N., 2014. Oxidative stress and male reproductive health. *Asian J. Androl.* 16 (1), 31–38. <https://doi.org/10.4103/1008-682X.122203>.
- Alizadeh, F., Javadi, M., Karami, A.A., Gholaminejad, F., Kavianpour, M., Haghghighian, H.K., 2018. Curcumin nanomicelle improves semen parameters, oxidative stress, inflammatory biomarkers, and reproductive hormones in infertile men: a randomized clinical trial. *Phytother. Res.* 32 (3), 514–521. <https://doi.org/10.1002/ptr.5998>.
- Bejarano, I., Monllor, F., Marchena, A.M., Ortiz, A., Lozano, G., Jiménez, M.I., et al., 2014. Exogenous melatonin supplementation prevents oxidative stress-evoked DNA damage in human spermatozoa. *J. Pineal Res.* 57 (3), 333–339. <https://doi.org/10.1111/jpi.12172>.
- Benedetti, S., Tagliamonte, M.C., Catalani, S., Primiterra, M., Canestrari, F., De Stefani, S., et al., 2012. Differences in blood and semen oxidative status in fertile and infertile men, and their relationship with sperm quality. *Reprod. Biomed. Online* 25 (3), 300–306. <https://doi.org/10.1016/j.rbmo.2012.05.011>.
- Bisht, S., Faiq, M., Tolahunase, M., Dada, R., 2017. Oxidative stress and male infertility. *Nat. Rev. Urol.* 14 (8), 470–485. <https://doi.org/10.1038/nrurol.2017.69>.
- Calafat, A.M., Kuklennyik, Z., Caudill, S.P., Ashley, D.L., 2003. Urinary levels of trichloroacetic acid, a disinfection by-product in chlorinated drinking water, in a human reference population. *Environ. Health Perspect.* 111 (2), 151–154.
- Cambi, M., Tamburrino, L., Marchiani, S., Olivito, B., Azzari, C., Forti, G., et al., 2013. Development of a specific method to evaluate 8-hydroxy, 2-deoxyguanosine in sperm nuclei: relationship with semen quality in a cohort of 94 subjects. *Reproduction* 145 (3), 227–235. <https://doi.org/10.1530/rep-12-0404>.
- Chen, H., Zhao, H.X., Huang, X.F., Chen, G.W., Yang, Z.X., Sun, W.J., et al., 2012. Does high load of oxidants in human semen contribute to male factor infertility? *Antioxidants Redox Signal.* 16 (8), 754–759. <https://doi.org/10.1089/ars.2011.4461>.
- Chen, Y.J., Duan, P., Meng, T.Q., Chen, H.G., Chavarro, J.E., Xiong, C.L., et al., 2020. Associations of blood trihalomethanes with semen quality among 1199 healthy Chinese men screened as potential sperm donors. *Environ. Int.* 134, 105335. <https://doi.org/10.1016/j.envint.2019.105335>.
- Cornett, R., James, M.O., Henderson, G.N., Cheung, J., Shroods, A.L., Stacpoole, P.W., 1999. Inhibition of glutathione S-transferase zeta and tyrosine metabolism by dichloroacetate: a potential unifying mechanism for its altered biotransformation and toxicity. *Biochem. Biophys. Res. Commun.* 262 (3), 752–756. <https://doi.org/10.1006/bbrc.1999.1287>.
- Costet, N., Garlandezec, R., Monfort, C., Rouget, F., Gagnière, B., Chevrier, C., et al., 2012. Environmental and urinary markers of prenatal exposure to drinking water disinfection by-products, fetal growth, and duration of gestation in the PELAGIE birth cohort (Brittany, France, 2002–2006). *Am. J. Epidemiol.* 175 (4), 263–275. <https://doi.org/10.1093/aje/kwr419>.
- Deng, Y., Zhang, Y., Lu, Y., Lu, K., Bai, H., Ren, H., 2017. Metabolomics evaluation of the in vivo toxicity of bromoacetonitriles: one class of high-risk nitrogenous disinfection byproducts. *Environ. Sci. Technol.* 51, 107–114. <https://doi.org/10.1021/acs.est.6b01003>.
- Dorostghoal, M., Kazeminejad, S.R., Shahbazian, N., Pourmehdi, M., Jabbari, A., 2017. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia* 49, e12762. <https://doi.org/10.1111/and.12762>.
- Evlampidou, I., Font-Ribera, L., Rojas-Rueda, D., Gracia-Lavedan, E., Costet, N., Pearce, N., et al., 2020. Trihalomethanes in drinking water and bladder cancer burden in the European union. *Environ. Health Perspect.* 128 (1), 17001. <https://doi.org/10.1289/ehp4495>.
- Fenster, L., Waller, K., Windham, G., Henneman, T., Anderson, M., Mendola, P., et al., 2003. Trihalomethane levels in home tap water and semen quality. *Epidemiology* 14 (6), 650–658. <https://doi.org/10.1097/01.ede.0000077980.52697.01>.
- Font-Ribera, L., Kogevinas, M., Zock, J.P., Gomez, F.P., Barreiro, E., Nieuwenhuijsen, M.J., et al., 2010. Short-term changes in respiratory biomarkers after swimming in a chlorinated pool. *Environ. Health Perspect.* 118 (11), 1538–1544. <https://doi.org/10.1289/ehp.1001961>.
- Froese, K.L., Sinclair, M.I., Hruyey, S.E., 2002. Trichloroacetic acid as a biomarker of exposure to disinfection by-products in drinking water: a human exposure trial in Adelaide, Australia. *Environ. Health Perspect.* 110 (7), 679–687. <https://doi.org/10.1289/ehp.02110679>.
- Giannattasio, A., De Rosa, M., Smeraglia, R., Zarrilli, S., Cimmino, A., Di Rosario, B., et al., 2002. Glutathione peroxidase (GPX) activity in seminal plasma of healthy and infertile males. *J. Endocrinol. Invest.* 25 (11), 983–986. <https://doi.org/10.1007/bf03344072>.
- Gong, T., Jiang, W., Gao, Z., Chen, Y., Gao, S., 2019. Dibromoacetic acid induced hepatotoxicity in mice through oxidative stress and toll-like receptor 4 signaling pathway activation. *Oxid. Med. Cell. Longev.* 5637235. <https://doi.org/10.1155/2019/5637235>, 2019.
- Greenland, S., 1989. Modeling and variable selection in epidemiologic analysis. *Am. J. Publ. Health* 79 (3), 340–349. <https://doi.org/10.2105/ajph.79.3.340>.
- Grellier, J., Rushton, L., Briggs, D.J., Nieuwenhuijsen, M., 2015. Assessing the human health impacts of exposure to disinfection by-products—A critical review of concepts and methods. *Environ. Int.* 78, 61–81. <https://doi.org/10.1016/j.envint.2015.02.003>.
- Hassoun, E.A., Ray, S., 2003. The induction of oxidative stress and cellular death by the drinking water disinfection by-products, dichloroacetate and trichloroacetate in J774.A1 cells. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 135 (2), 119–128. [https://doi.org/10.1016/s1532-0456\(03\)0082-6](https://doi.org/10.1016/s1532-0456(03)0082-6).
- Hassoun, E., Cearfoss, J., Mamada, S., Al-Hassan, N., Brown, M., Heimberger, K., et al., 2014. The effects of mixtures of dichloroacetate and trichloroacetate on induction of oxidative stress in livers of mice after subchronic exposure. *J. Toxicol. Environ. Health* 77 (6), 313–323. <https://doi.org/10.1080/15287394.2013.864576>.
- He, Y., Zou, L., Luo, W., Yi, Z., Yang, P., Yu, S., et al., 2019. Heavy metal exposure, oxidative stress and semen quality: exploring associations and mediation effects in reproductive-aged men. *Chemosphere* 244, 125498. <https://doi.org/10.1016/j.chemosphere.2019.125498>.
- Hosen, M.B., Islam, M.R., Begum, F., Kabir, Y., Howlader, M.Z., 2015. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. *Iran. J. Reprod. Med.* 13 (9), 525–532. <https://doi.org/10.1007/s00192-007-0454-1>.
- Il'Yasova, D., Scarbrough, P., Spasojevic, I., 2012. Urinary biomarkers of oxidative status. *Clin. Chim. Acta* 413 (19–20), 1446–1453. <https://doi.org/10.1016/j.cca.2012.06.012>.
- Iszatt, N., Nieuwenhuijsen, M.J., Bennett, J., Best, N., Povey, A.C., Pacey, A.A., et al., 2013. Chlorination by-products in tap water and semen quality in England and Wales. *Occup. Environ. Med.* 70 (11), 754–760. <https://doi.org/10.1136/oemed-2012-101339>.
- Kao, S.H., Chao, H.T., Chen, H.W., Hwang, T.I., Liao, T.L., Wei, Y.H., 2008. Increase of oxidative stress in human sperm with lower motility. *Fertil. Steril.* 89 (5), 1183–1190. <https://doi.org/10.1016/j.fertnstert.2007.05.029>.
- Kaydos, E.H., Suarez, J.D., Roberts, N.L., Bobseine, K., Zucker, R., Laskey, J., et al., 2004. Haloacid induced alterations in fertility and the sperm biomarker SP22 in the rat are additive: validation of an ELISA. *Toxicol. Sci.* 81 (2), 430–442. <https://doi.org/10.1093/toxsci/kfh218>.
- Klinefelter, G.R., Strader, L.F., Suarez, J.D., Roberts, N.L., 2002. Bromochloroacetic acid exerts qualitative effects on rat sperm: implications for a novel biomarker. *Toxicol. Sci.* 68 (1), 164–173. <https://doi.org/10.1093/toxsci/68.1.164>.
- Klinefelter, G.R., Strader, L.F., Suarez, J.D., Roberts, N.L., Goldman, J.M., Murr, A.S., 2004. Continuous exposure to dibromoacetic acid delays pubertal development and compromises sperm quality in the rat. *Toxicol. Sci.* 81 (2), 419–429. <https://doi.org/10.1093/toxsci/kfh217>.
- Krasner, S.W., Howard, S.W., Susan, D.R., Salvador, J.P., Russell, C., Michael, J.S., et al., 2006. Occurrence of a new generation of disinfection byproducts. *Environ. Sci. Technol.* 40 (23), 7175–7185. <https://doi.org/10.1021/es060353j>.
- Linder, R.E., Klinefelter, G.R., Strader, L.F., Narotsky, M.G., Suarez, J.D., Roberts, N.L., et al., 1995. Dibromoacetic acid affects reproductive competence and sperm quality in the male rat. *Fund. Appl. Toxicol.* 28 (1), 9–17. <https://doi.org/10.1006/faat.1995.1140>.
- Linder, R.E., Klinefelter, G.R., Strader, L.F., Veeramachaneni, D.N.R., Roberts, N.L., Toxicology, J.D.S.J.R., 1997. Histopathologic changes in the testes of rats exposed to dibromoacetic acid. *Reprod. Toxicol.* 11 (1), 47–56. [https://doi.org/10.1016/s0890-6238\(96\)00196-7](https://doi.org/10.1016/s0890-6238(96)00196-7).
- Liu, C., Duan, P., Chen, Y.J., Deng, Y.L., Luo, Q., Miao, Y., et al., 2019. Mediation of the relationship between phthalate exposure and semen quality by oxidative stress among 1034 reproductive-aged Chinese men. *Environ. Res.* 179 (Pt A), 108778. <https://doi.org/10.1016/j.envres.2019.108778>.
- Liu, C., Wang, Y.X., Chen, Y.J., Sun, Y., Huang, L.L., Cheng, Y.H., et al., 2020. Blood and urinary biomarkers of prenatal exposure to disinfection byproducts and oxidative stress: a repeated measurement analysis. *Environ. Int.* 137, 105518. <https://doi.org/10.1016/j.envint.2020.105518>.
- Llana-Belloch, S., Priego Quesada, J.I., Perez-Soriano, P., Lucas-Cuevas, A.G., Salvador-Pascual, A., Olaso-Gonzalez, G., et al., 2016. Disinfection by-products effect on swimmers oxidative stress and respiratory damage. *Eur. J. Sport Sci.* 16 (5), 609–617. <https://doi.org/10.1080/17461391.2015.1080306>.
- Luben, T.J., Olshan, A.F., Herring, A.H., Jeffay, S., Strader, L., Buus, R.M., et al., 2007. The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. *Environ. Health Perspect.* 115 (8), 1169–1176. <https://doi.org/10.1289/ehp.10120>.
- Martinez-Moral, M.P., Kannan, K., 2019. How stable is oxidative stress level? An observational study of intra- and inter-individual variability in urinary oxidative stress biomarkers of DNA, proteins, and lipids in healthy individuals. *Environ. Int.* 123, 382–389. <https://doi.org/10.1016/j.envint.2018.12.009>.
- Morissette, M.C., Murray, N., Turmel, J., Milot, J., Boulet, L.P., Bougault, V., 2016. Increased exhaled breath condensate 8-isoprostane after a swimming session in competitive swimmers. *Eur. J. Sport Sci.* 16 (5), 569–576. <https://doi.org/10.1080/17461391.2015.1063702>.
- Murawski, M., Saczko, J., Marcinkowska, A., Chwilkowska, A., Grybos, M., Banas, T., 2007. Evaluation of superoxide dismutase activity and its impact on semen quality parameters of infertile men. *Folia Histochem. Cytobiol.* 45 (Suppl. 1), S123–S126. <https://doi.org/10.5603/4471>.
- Narotsky, M.G., Klinefelter, G.R., Goldman, J.M., DeAngelo, A.B., Best, D.S., McDonald, A., et al., 2015. Reproductive toxicity of a mixture of regulated drinking-water disinfection by-products in a multigenerational rat bioassay. *Environ. Health Perspect.* 123 (6), 564–570. <https://doi.org/10.1289/ehp.1408579>.
- Nieuwenhuijsen, M.J., Grellier, J., Smith, R., Iszatt, N., Bennett, J., Best, N., et al., 2009. The epidemiology and possible mechanisms of disinfection by-products in drinking water. *Philos. Trans. A Math. Phys. Eng. Sci.* 367 (1904), 4043–4076. <https://doi.org/10.1098/rsta.2009.0116>.
- Parvez, S., Ashby, J.L., Kimura, S.Y., Richardson, S.D., 2019. Exposure characterization of haloacetic acids in humans for exposure and risk assessment applications: an exploratory study. *Int. J. Environ. Res. Publ. Health* 16 (3). <https://doi.org/10.3390/ijerph16030471>.
- Prochazka, E., Melvin, S.D., Escher, B.I., Plewa, M.J., Leusch, F.D.L., 2019. Global transcriptional analysis of nontransformed human intestinal epithelial cells

- (FHs 74 int) after exposure to selected drinking water disinfection by-products. *Environ. Health Perspect.* 127 (11), 117006. <https://doi.org/10.1289/ehp4945>.
- Schultz, I.R., Merdink, J.L., Gonzalez-Leon, A., Bull, R.J., 2002. Dichloroacetate toxicokinetics and disruption of tyrosine catabolism in B6C3F1 mice: dose-response relationships and age as a modifying factor. *Toxicology* 173 (3), 229–247. [https://doi.org/10.1016/s0300-483x\(02\)00034-3](https://doi.org/10.1016/s0300-483x(02)00034-3).
- Smith, R.B., Nieuwenhuijsen, M.J., Wright, J., Raynor, P., Cocker, J., Jones, K., et al., 2013. Validation of trichloroacetic acid exposure via drinking water during pregnancy using a urinary TCAA biomarker. *Environ. Res.* 126, 145–151. <https://doi.org/10.1016/j.envres.2013.05.004>.
- Stalter, D., O'Malley, E., von Gunten, U., Escher, B.I., 2016. Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products. *Water Res.* 91, 19–30. <https://doi.org/10.1016/j.watres.2015.12.047>.
- Taken, K., Alp, H.H., Eryilmaz, R., Donmez, M.I., Demir, M., Gunes, M., et al., 2016. Oxidative DNA damage to sperm cells and peripheral blood leukocytes in infertile men. *Med. Sci. Monit.* 22, 4289–4296. <https://doi.org/10.12659/msm.898631>.
- Tartibian, B., Maleki, B.H., 2012. Correlation between seminal oxidative stress biomarkers and antioxidants with sperm DNA damage in elite athletes and recreationally active men. *Clin. J. Sport Med.* 22 (2), 132–139. <https://doi.org/10.1097/JSM.0b013e31823f310a>.
- Tremellen, K., 2008. Oxidative stress and male infertility—a clinical perspective. *Hum. Reprod. Update* 14 (3), 243–258. <https://doi.org/10.1093/humupd/dmn004>.
- Valeri, L., Vanderweele, T.J., 2013. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol. Methods* 18 (2), 137–150. <https://doi.org/10.1037/a0031034>.
- Varraso, R., Massin, N., Hery, M., Fradier-Dusch, M., Michaely, J.P., Fournier, M., et al., 2002. Not only training but also exposure to chlorinated compounds generates a response to oxidative stimuli in swimmers. *Toxicol. Ind. Health* 18 (6), 269–278. <https://doi.org/10.1191/0748233702th1500a>.
- Veeramachaneni, D.N.R., Palmer, J.S., Klinefelter, G.R., 2007. Chronic exposure to low levels of dibromoacetic acid, a water disinfection by-product, adversely affects reproductive function in male rabbits. *J. Androl.* 28 (4), 565–577. <https://doi.org/10.2164/jandrol.107.002550>.
- Villanueva, C.M., Cordier, S., Font-Ribera, L., Salas, L.A., Levallois, P., 2015. Overview of disinfection by-products and associated health effects. *Curr. Environ. Health Rep.* 2 (1), 107–115. <https://doi.org/10.1007/s40572-014-0032-x>.
- Wang, S., Zheng, W., Liu, X., Xue, P., Jiang, S., Lu, D., et al., 2014a. Iodoacetic acid activates Nrf2-mediated antioxidant response in vitro and in vivo. *Environ. Sci. Technol.* 48 (22), 13478–13488. <https://doi.org/10.1021/es502855x>.
- Wang, Y.X., Zeng, Q., Wang, L., Huang, Y.H., Lu, Z.W., Wang, P., et al., 2014b. Temporal variability in urinary levels of drinking water disinfection byproducts dichloroacetic acid and trichloroacetic acid among men. *Environ. Res.* 135, 126–132. <https://doi.org/10.1016/j.envres.2014.05.035>.
- Wang, Y.X., Liu, C., Chen, Y.J., Duan, P., Wang, Q., Chen, C., et al., 2019a. Profiles, variability and predictors of concentrations of blood trihalomethanes and urinary haloacetic acids along pregnancy among 1760 Chinese women. *Environ. Res.* 172, 665–674. <https://doi.org/10.1016/j.envres.2019.03.017>.
- Wang, Y.X., Liu, C., Shen, Y., Wang, Q., Pan, A., Yang, P., et al., 2019b. Urinary levels of bisphenol A, F and S and markers of oxidative stress among healthy adult men: variability and association analysis. *Environ. Int.* 123, 301–309. <https://doi.org/10.1016/j.envint.2018.11.071>.
- Weinberg, H.S., Pereira, V.R.P.J., Singer, P.C., Savitz, D.A., 2006. Considerations for improving the accuracy of exposure to disinfection by-products by ingestion in epidemiologic studies. *Environ. Sci. Technol.* 354 (1), 35–42. <https://doi.org/10.1016/j.scitotenv.2004.12.016>.
- Wong, E.W., Cheng, C.Y., 2011. Impacts of environmental toxicants on male reproductive dysfunction. *Trends Pharmacol. Sci.* 32 (5), 290–299. <https://doi.org/10.1016/j.tips.2011.01.001>.
- Wright, J.M., Evans, A., Kaufman, J.A., Rivera-Nunez, Z., Narotsky, M.G., 2017. Disinfection by-product exposures and the risk of specific cardiac birth defects. *Environ. Health Perspect.* 125 (2), 269–277. <https://doi.org/10.1289/EHP103>.
- Xie, S.H., Liu, A.L., Chen, Y.Y., Zhang, L., Zhang, H.J., Jin, B.X., et al., 2010. DNA damage and oxidative stress in human liver cell L-02 caused by surface water extracts during drinking water treatment in a waterworks in China. *Environ. Mol. Mutagen.* 51 (3), 229–235. <https://doi.org/10.1002/em.20537>.
- Xie, S.H., Li, Y.F., Tan, Y.F., Zheng, D., Liu, A.L., Xie, H., et al., 2011. Urinary trichloroacetic acid levels and semen quality: a hospital-based cross-sectional study in Wuhan, China. *Environ. Res.* 111 (2), 295–300. <https://doi.org/10.1016/j.envres.2010.12.010>.
- Zeng, Q., Li, M., Xie, S.H., Gu, L.J., Yue, J., Cao, W.C., et al., 2013. Baseline blood trihalomethanes, semen parameters and serum total testosterone: a cross-sectional study in China. *Environ. Int.* 54, 134–140. <https://doi.org/10.1016/j.envint.2013.01.016>.
- Zeng, Q., Chen, Y.Z., Xu, L., Chen, H.X., Luo, Y., Li, M., 2014a. Evaluation of exposure to trihalomethanes in tap water and semen quality: a prospective study in Wuhan, China. *Reprod. Toxicol.* 46, 56–63. <https://doi.org/10.1016/j.reprotox.2014.03.005>.
- Zeng, Q., Wang, Y.X., Xie, S.H., Xu, L., Chen, Y.Z., Li, M., et al., 2014b. Drinking-water disinfection by-products and semen quality: a cross-sectional study in China. *Environ. Health Perspect.* 122 (7), 741–746. <https://doi.org/10.1289/ehp.1307067>.
- Zeng, Q., Zhou, B., Cao, W.C., Wang, Y.X., You, L., Huang, Y.H., et al., 2014c. Predictors of urinary trichloroacetic acid and baseline blood trihalomethanes concentrations among men in China. *Sci. Total Environ.* 493, 806–811. <https://doi.org/10.1016/j.scitotenv.2014.06.067>.
- Zhang, S.H., Guo, A.J., Zhao, W.X., Gu, J.L., Zhang, R., Wei, N., 2019. Urinary trichloroacetic acid and high blood pressure: a cross-sectional study of general adults in Shijiazhuang, China. *Environ. Res.* 177, 108640. <https://doi.org/10.1016/j.envres.2019.108640>.
- Zhou, W.S., Xu, L., Xie, S.H., Li, Y.L., Li, L., Zeng, Q., et al., 2012. Decreased birth weight in relation to maternal urinary trichloroacetic acid levels. *Sci. Total Environ.* 416, 105–110. <https://doi.org/10.1016/j.scitotenv.2011.10.073>.