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Environmental exposures and male reproductive outcomes.

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ENVIRONMENTAL EXPOSURES AND MALE REPRODUCTIVE OUTCOMES

By

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ABSTRACT

ENVIRONMENTAL EXPOSURES AND MALE REPRODUCTIVE OUTCOMES

Damilola R. Owoade

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Male sexual dysfunction comprises various conditions, including erectile dysfunction (ED) and ejaculatory dysfunction. Testosterone deficiency (TD), which has been linked to some of these sexual dysfunctions, is characterized by low production of testosterone by the testes. These male reproductive outcomes may develop due to exposure to various risk factors, including environmental exposures. These environmental exposures may include volatile organic compounds (VOCs) and tear gas. This dissertation aims to examine the effects of tear gas exposure and VOCs on male reproductive outcomes.

The association between tear gas exposure and male reproductive outcomes was assessed using data obtained from an online anonymous questionnaire. Tear gas exposure was estimated using acute tear gas symptoms including eye, lung, skin, and heart effects, and were summed into composite scores ranging from 0 to 14. Male reproductive outcomes that were assessed include ED and ejaculation dysfunction. Odds ratios (OR) and 95% confidence intervals (CI) were obtained using logistic regression, controlling for potential confounders. Approximately 46% of the men (N=92) exposed to tear gas reported at least one reproductive issue. There was a significant association between

acute tear gas exposure score and higher odds of erectile dysfunction (OR 6.57; 95% CI 1.28-40.08).

Weighted logistic regression and Bayesian kernel machine regression (BKMR) were used to assess the association between a selection of VOCs and ED or TD using NHANES data. The analysis was also performed among non-smokers. One of the seven analyzed VOCs, 1,4-dichlorobenzene, was associated with increased odds of ED; however, some VOCs, including benzene, toluene, and ethylene, were inversely associated with TD. No significant association was observed when the data was restricted to only non-smokers, pointing to possible confounding by smoking or related characteristics.

In conclusion, this dissertation provides novel evidence for the potential effects of tear gas on male reproductive outcomes, and further elucidates the effects of VOCs on these outcomes. Educating the public, particularly health professionals and policymakers, on the health impacts of these environmental exposures may help reduce the burden of adverse male reproductive outcomes in the population.

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CHAPTER ONE

BACKGROUND AND RATIONALE

Exposure to various chemical compounds may occur in indoor and outdoor settings. Indoors, many households utilize products, such as disinfectants, building supplies, fuels, and cleaning supplies, or engage in behaviors like cigarette smoking. These products may contain chemical compounds, such as volatile organic compounds (VOCs), that may potentially have an impact on human health (1-3). Aside from indoor spaces, VOCs may also be present outdoors. In addition, people may be exposed to other chemical agents, mostly outdoors, such as tear gas, which, upon exposure, may potentially result in various health outcomes (4-6). Therefore, to consider the holistic impact of environmental exposures on population health, this dissertation will explore the effect of VOCs, a chemical compound present indoors and outdoors, and tear gas, a commonly used riot control agent, on male reproductive health.

In the last decade, law enforcement officers' use of tear gas has increased globally (4). Although this irritant has been linked to various acute effects, such as eye, nose, throat, skin, and heart effects, little is known about its impact on long-term effects, such as reproductive health (4). Although there is preliminary evidence of its effect on female reproductive health (5), no study has explored its association with male reproductive outcomes. This study will examine the effect of tear gas, as measured by acute tear gas

exposure symptoms, on male reproductive outcomes (erectile dysfunction, ejaculatory dysfunction, and trouble conceiving).

VOCs are also known sources of environmental exposure and are present in our daily lives. VOCs are emitted through indoor and outdoor sources, with a high proportion in indoor settings. VOCs have also been linked to many acute (7-9) and chronic effects, (10) including reproductive health. Increasing evidence showed that VOCs may have an adverse effect on the production of hormones responsible for stimulating testosterone, such as luteinizing hormone (LH) or testosterone itself, which plays a pivotal role in sexual dysfunction, including erectile dysfunction, ejaculatory dysfunction, and testosterone deficiency (11, 12). However, there are limited studies on the effect of VOCs on sexual dysfunction using a nationally representative sample. Therefore, this dissertation investigated the association between VOCs and adverse male reproductive outcomes (erectile dysfunction and testosterone deficiency) using National Health and Nutrition Examination Survey (NHANES) data. In addition, the effect of tear gas exposure on male reproductive outcomes was explored.

MALE REPRODUCTIVE OUTCOMES

Erectile dysfunction

Erectile dysfunction (ED) is the inability to achieve and maintain the erection required for sexual pleasure (13). Estimates of the prevalence of ED ranges from approximately 3-76.5% across the globe (14), and it is estimated that by 2025, over 300 million men will have ED worldwide, significantly affecting the quality of life of men

globally (15). Studies have shown that ED is predominant among older men, and as reported by the Massachusetts male aging study, the prevalence of mild to severe ED was 52% among men aged 40 to 70 years (16).

Previously, ED was considered a psychological disorder; however, recent studies have found that it results from a combination of different processes, including vascular, neurological, and psychological processes (17). ED has gone beyond being just a sexual problem but is now an indicator of chronic conditions such as cardiovascular disease (18). Also, with the increase in life expectancy, the risk of ED may continue to rise in the coming years.

Mechanisms of penile erection

There are several pathways to erection, one of which is the nitric oxide (NO) pathway. This process begins with sexual arousal, followed by the release of NO, which is synthesized by the enzyme nitric oxide synthase (19). This emission results in an increase in cyclic GMP (cGMP) concentration and smooth muscle cell relaxation. Then the vascular relaxation causes blood to fill the erectile tissues, leading to the compression of the vein, thereby tapping the blood in the penis and sustaining erection. Any alteration in the NO pathway, such as an imbalance in the smooth muscle contraction and relaxation, may result in ED (20, 21).

Pathophysiology of Erectile Dysfunction

The mechanism that underlies ED is mainly divided into two: organic and psychogenic ED.

Psychogenic Erectile Dysfunction

Psychological disorders like stress, anxiety, depression, and lack of sexual arousal have been linked to the inability to achieve and maintain an erection (22). These conditions can interfere with the functioning of the brain (18). One of the pathways that may lead to ED is the effect of these mental disorders on noradrenaline, a primary erectolytic (anti-erectile) neurotransmitter (23). High levels of noradrenaline can cause ejaculation in men. However, when there is a continuous increase in noradrenaline, the man may experience anxiety, fatigue, or adrenal exhaustion, subsequently leading to loss of libido and ED (24). Another possible psychogenic ED mechanism may occur due to conditions that affect the brain. Once there is damage to the brain, including the hypothalamus and cerebral cortex, that controls penile erection, ED can occur (25). Last, too much sympathetic outflow can affect the smooth muscle relaxation/contraction process, leading to ED.

Organic Erectile Dysfunction

The organic ED pathway is divided into three: neurogenic, vasculogenic, and endocrinologic.

Neurogenic

Maintaining an erection often requires nerves to function correctly, which may be one reason ED has been linked to various neurological conditions, such as multiple sclerosis, Parkinson's disease, and Alzheimer's (26).

Neurological conditions like diabetes may affect endothelium-related relaxation, resulting in NO decline. The inadequate release of NO interferes with smooth muscle

relaxation, leading to ED. Trauma, which may be associated with spinal cord injury, may result in ED, depending on the location and nature of the spinal cord damage (25). These surgeries may result in impotence because the cavernous nerves (responsible for penile erection) pass through the pelvis on their way to supply the corpus cavernosum (27).

Vasculogenic

The majority of organic ED occurs due to conditions that affect the normal functioning of the veins or arteries (18). Endothelial dysfunction, regarded as a predictor of CVD and other heart problems (28), may interfere with the normal flow of blood to the penis, leading to atherosclerosis and then the development of ED (28).

Endocrinologic

Various endocrine disorders have been linked to ED, including hypogonadism (29), hyperprolactinemia, and thyroid disease. Testosterone controls various processes required for erection, such as smooth muscle relaxation, NO synthesis, and cavernosal nerve function. Therefore, when testosterone levels are affected due to these endocrine disorders, it may lead to ED (26).

Risk factors

Several risk factors have been found to increase the risk of ED. Some of the non-modifiable factors associated with ED are age (16, 30), race/ethnicity (31-34), and genetics (35, 36). Lifestyle factors that have been linked to ED are smoking (16, 37, 38), alcohol (16, 39, 40), diets (41-43), and physical activities (44, 45). Other comorbidities

that may result in ED include diabetes (16, 46, 47), cardiovascular diseases (48), hypertension (49), obesity (50, 51), lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH) (52), and psychological disorders (16, 22).

Aside from the well-established risk factors of ED, some researchers have reported the effect of environmental exposures on ED (53). Many of these environmental exposures are known to have antiandrogenic or estrogenic properties, which may interfere with the body's hormones (endocrine disruptors) (54-56). Some of these environmental factors are lead (57), agricultural exposures (pesticides, herbicides, insecticides, and fumigants) (53, 58), bisphenol A (59), stilbene (60-62), and arsenic (63).

Ejaculatory dysfunction

Ejaculatory dysfunction is one of the most common types of sexual dysfunction in men. There are four types of ejaculatory dysfunction, including premature ejaculation (PE), delayed ejaculation, retrograde ejaculation, and anejaculation (64).

Premature ejaculation (PE)

The most prevalent type of ejaculatory dysfunction is premature ejaculation, which happens when a male ejaculates prior to or soon after initiating sexual intercourse (65, 66). The global prevalence of PE is about 30% (67). In the US, the prevalence of PE ranges from 30 to 70% (68).

Among the risk factors of PE are genetic disposition (69), diabetes (70), metabolic syndrome (71), neurological disorders (72), recreational drugs (73), alcohol (74, 75),

chronic Prostatitis/chronic pelvic pain syndrome (76, 77), thyroid disorder (78), psychological conditions (79, 80), and erectile dysfunction (81, 82).

Retrograde ejaculation

Retrograde ejaculation occurs when the semen meant to be expelled during ejaculation goes back to the bladder (83). Retrograde ejaculation may be as a result of: (84-87) a) neurological conditions- multiple sclerosis, diabetes mellitus, b) pharmacological- use of medications such as anti-depressants, antihypertensives, and antipsychotics; c) anatomic- due to bladder surgeries.

Anejaculation

Anejaculation refers to the absence of ejaculation during sexual intercourse despite the occurrence of an erection (88). Anejaculation may be due to several causes: organic (comorbidities-diabetes, transverse myelitis, multiple sclerosis), surgery (spinal cord injury and retroperitoneal lymph node dissection); and pharmacological or psychogenic causes (83, 89).

Delayed ejaculation

Delayed ejaculation (DE) occurs when a man takes a long period during sexual intercourse to release semen or reach a sexual climax. It is characterized by absent or reduced seminal release, diminished ejaculatory contractions, and little to no organism. Approximately 1 to 4% of sexually active men experience DE (90).

Testosterone deficiency

Like other male reproductive outcomes examined in this dissertation, testosterone deficiency (TD) is a condition of older age, with a global prevalence ranging from 10 to 40% (91-94). In the US, the prevalence of TD in men above 40 years is higher and varies between 24 to 39% (95). Studies in Asia (94, 96-98) and Europe (96, 99-101) reported TD prevalence of 17-33% and 8-20%, respectively. Although age may be an established risk factor for TD, other studies have reported that the conditions predominant in aged men, such as diabetes, cardiovascular diseases, diabetes, and osteoporosis, may be responsible for the decline in testosterone levels, and age may be a proxy for these risk factors (102).

Risk factors

Prior studies have shown that TD may be associated with comorbidities, such as obesity (103), metabolic syndrome (103), stroke, diabetes (103), and dyslipidaemia (103). Exposure to environmental factors may also result in testosterone deficiency. These environmental exposures include radiation (104), phthalates (105), polyfluoroalkyl substances (106), polychlorinated biphenyls (PCBs) (107), and heavy air pollutants such as lead (108, 109), cadmium (110), and PM 2.5 (111).

Tear gas

Tear gas refers to a group of chemical compounds that, when exposed to it, may cause irritation to the eyes, nose, lungs, heart, and skin (112). The most common compounds used as tear gas are chloroacetophenone (CN), chlorobenzylidenemalononitrile (CS), and dibenz[b,f]-1,4-oxazepine (CR). Chloroacetophenone (CN) is the most toxic lacrimator, with a maximum safe dose of short-term inhalation of 500 mg/m^3 (113). CS is more potent but less toxic than CN. CR is the most potent and least toxic form of tear gas.

Around the world, there has been increased use of tear gas, especially by law enforcement officers who rely on tear gas to disperse crowds during protests, demonstrations, or civil unrest (4). Some of the countries with heightened use of this toxic lacrimator are Turkey (114), the United States (115, 116), Hong Kong (117), Greece (118), Brazil (119), Egypt, and Bahrain (120, 121). In the US, especially between 2020 and 2021, there were numerous protests due to racial injustice and the Black Lives Matter Movement; and this was what prompted this tear gas research (122). Another recent evidence of the detrimental effect of tear gas on population health was the over 100 deaths that occurred in Indonesia after the release of tear gas at a soccer game. These deaths resulted from suffocation and trampling after the firing of tear gas (123). With the heightened use of tear gas, some areas in the US are beginning to put in place measures to curtail its spread. For example, Madison city council in Wisconsin is considering banning the use of tear gas in their city (124).

Although some medical studies have reported that tear gas has no significant clinical effects on humans (125), others have attributed this conclusion to the limited epidemiologic data available (4).

Physical and chemical properties

Tear gas, including CS and CR, are not gases but solids, usually dispersed as aerosols using canisters, grenades, spray tanks, and larger weapons. These riot control agents are less likely to dissolve in water but may be soluble in organic solvents (112). Under high temperatures, tear gas like CS may change its properties, therefore undergoing thermal degradation (126). Among the most common forms of tear gas, CS is the one that can easily undergo hydrolysis with water/alkaline solution, and this is the reason why it can be rendered inactive with water and soap. However, CN and CR are less likely to be made inactive with water or alkaline solution. Exposure to CN or CR may cause irritation (112).

Adverse effects of tear gas exposure

Eye effects

The eye is the most sensitive body part to tear gas. Exposure to tear gas may cause watery eyes, pain, burning eyes, redness, and swollen eyes. However, these symptoms may last for a few hours (112, 127). Prolonged exposure to this lachrymatory gas may result in glaucoma, cataracts, conjunctival injection, and pseudopterygium (128, 129). In a study by Holopainen et al., all four patients who were exposed to pepper spray reported that they developed corneal erosion after exposure (130).

Respiratory effects

Exposure to CS and CR may result in acute respiratory effects such as cough, nasal congestion, sore throat, and sneezing. These effects may fade within a couple of minutes (112). Coughing occurs when tear gas activates vagal sensory nerve endings in the larynx, which in turn expresses TRPV1 and TRPA1. The resulting effect of tear gas exposure, coughing, may further obstruct regular breathing and possibly elicit the fear of suffocation (4).

In cases of prolonged exposure to tear gas, especially in a confined space, the exposure to CS and CR may lead to acute laryngotracheobronchitis (131), reactive airway dysfunction syndrome (RADS) (132-134), paroxysmal cough, feeling of tightness, and burning chest. These effects may last for several weeks. In the case of CS, the symptoms may last up to 12 weeks in cases of extreme exposures (4).

Although tear gas exposure may result in various acute respiratory effects, no evidence of permanent lung effects has been reported. A study on nine military men exposed to heavy CS reported that five developed hemoptysis and the remaining four had hypoxia. However, after a week, these men recovered and were confirmed to have normal functioning lungs (135).

A Turkish study of 148 male participants, of which 93 were exposed and 55 were unexposed to tear gas, reported that those exposed were more likely to report chronic bronchitis (136). On the other hand, a UK study reported no evidence of long-term respiratory effects after 34 individuals were exposed to CS (137). Aside from individuals directly exposed to tear gas, research has shown that people residing in the area where

tear gas was released may also experience respiratory effects, suggesting that tear gas is an environmental health hazard (138).

Gastrointestinal effects and cardiovascular effects

Exposure to prolonged tear gas and ingestion of compounds may result in vomiting, abdominal cramping pain, and diarrhea (112). Also, a Portland study reported delayed gastrointestinal tract problems among those exposed to tear gas (5).

Several cardiovascular effects, such as tachycardia and transient hypertension, have been reported in individuals exposed to tear gas (113).

Skin effects

Exposure to tear gas may result in skin effects such as burning sensation, tingling, and erythema, which may last for about an hour (112). Prolonged exposure to tear gas, especially when damp clothes are worn or petroleum jelly is applied to the affected areas, may lead to second-degree burns. The exposure to CN may result in allergic contact dermatitis, which may last up to 72 hours (112, 131, 139).

A report on an incarcerated man exposed to CS showed that he developed erythroderma, wheezing, pneumonitis with hypoxemia, hepatitis with jaundice, and eosinophilia eight days after exposure (140). The dermatitis he experienced lasted for 6 to 7 months. Another study on three police officers exposed to CN showed that they developed localized dermatitis after exposure, with one experiencing widespread lesions after four days (141). CS has been reported to act as a contact sensitizer, which was

evident in a study on CS plant manufacturing workers. Ninety percent of these workers reported a history of dermatitis (142).

Reproductive effects

Limited studies have examined the effect of tear gas on reproductive health. In a recent cross-sectional study conducted in Oregon, 54.5% of 1650 female respondents reported that they saw menstrual changes after exposure to tear gas (5). Another study reported that women exposed to tear gas experienced certain menstrual disorders, such as early and late menstruation (6).

Although there has been preliminary evidence on the effect of tear gas on female reproductive health, little is known about its effect on male reproductive health. There is currently no study on the effect of tear gas on male reproductive outcomes.

Volatile organic compounds

According to the EPA, volatile organic compounds (VOCs) are "compounds that have a high vapor pressure and low water solubility" (143). Typically, exposure to VOCs occurs indoors and outdoors, although indoor exposure is the most common due to the frequent use of products containing VOCs (144). The level of indoor VOCs is based on factors such as the amount of ventilation, house age, and renovations (145-149). VOCs are present in many household products, including paints, varnishes, waxes, cleaning

products, disinfecting products, cosmetics, degreasing products, aerosol sprays, cleansers, moth repellents, air fresheners, tobacco smoke, solvents, and automotive products (2, 3).

VOCs are released in the form of a gas from solids or liquids, making inhalation the most common route of exposure. Other means of exposure are ingestion and skin contact (2). As the name implies, VOCs have a volatile character due to their ubiquitous nature and have a boiling point between 50°C and 260 °C (3). VOCs are poorly soluble in water; however, through the metabolism process, these compounds may be converted into water-soluble metabolites. The resulting metabolites is inactive (detoxification), e.g., toluene metabolized to inactive hydroxyl and carboxyl (2).

VOCs are grouped based on their molecular structure or functional group, and they include: aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, ethers, esters, and aldehydes (2).

VOCs may also be classified based on their environmental harmfulness. The first category is the most harmful. These VOCs are known to be carcinogenic, mutagenic, and very toxic. The means of exposure are via inhalation or ingestion. Examples include benzene, vinyl chloride, and 1,2-dichloroethane. The second category is the Class A compounds. These organic compounds are less toxic but may pose harm to the environment. Examples are acetaldehyde, aniline, benzyl chloride, carbon tetrachloride, CFCs, ethyl acrylate, halons, maleic anhydride, 1,1,1-trichloroethane, and trichloroethylene, trichlorotoluene. The third category is Class B compounds. These VOCs cause less environmental harm compared to the other two classes. Examples include butane and ethyl acetate (150).

VOCs examined in this dissertation

Benzene

Benzene can be found in products such as paints, varnishes, lacquer thinners, and gasoline (151, 152). Benzene is one of the most common environmental pollutants, with inhalation being the typical mode of exposure (153). Other routes of exposure are through ingestion (drinking water, beverages). Other routes of exposure may be through ingestion. Benzene is one of the major indoor sources of VOCs, and exposure may occur through smoking, in-house burning, and household products (3). Other external sources of benzene are vehicle exhaust emissions and gas engines (154). Also, exposure to benzene may be through occupational sources--such settings include factories, rubber production companies, shoe factories, and printing factories (155, 156). According to the International Agency for Research on Cancer (IARC), benzene is regarded as a human carcinogen (Group 1 carcinogen) (157, 158).

Exposure to benzene has been linked to various acute effects such as eye and skin irritation, nausea, headaches, and respiratory effects. Chronic effects of benzene exposure include hematotoxicity (159), lymphoma (160), aplastic anemia and leukemia (161, 162). The mode of toxicity of benzene is through its metabolism, which occurs in the liver. The P4502E1 cytochrome catalyzes the single oxygen atom, forming benzene oxide (163).

Toluene

Toluene can be found in paints, thinners, cleaning agents, nail polish, and automobile emissions (164-167). Also, toluene is present in crude oil. The routes of exposure are ingestion (foods and drinking water) and inhalation (occupational exposure

and cigarette smoke) (168). Toluene exposure may result in acute symptoms such as headache and dizziness. In contrast, long-term exposure to toluene may result in liver, kidney, lung problems, spontaneous abortion, premature delivery, and congenital malformations (168, 169). According to IARC, there is inadequate evidence to classify toluene as a human carcinogen (Group 3 carcinogen) (157, 170).

Xylene

Xylene is mainly a human-generated chemical that naturally exists in petroleum and coal tar. This VOC also serves as a solvent in the printing and leather industries. The route of exposure is inhalation from sources such as contaminated air and automobile exhaust. Exposure to xylene may result in acute effects such as eye, nose, throat, skin irritation, and lung effects. Chronic effects may include neurological disorders; lung, liver, and kidney problems (171). Xylene exposure may also result in reproductive effects such as irregular menstrual flow and spontaneous abortion (172). Xylene is classified as a group 3 carcinogen (157, 170).

There are three forms of xylene, and they differ based on the position of methyl groups on the benzene ring. They include meta-xylene, ortho-xylene, and para-xylene (173). Meta-xylene has two methyl substituents bonded to positions 1 and 3 of the benzene ring (174). Ortho-xylene has two methyl groups attached to adjacent carbon atoms of a benzene ring (175). Para-xylene is a xylene with methyl groups in positions 1 and 4 of the benzene ring (176).

Styrene

Styrene is also a human-made chemical. A minimal level can be found in certain foods such as fruits, vegetables, nuts, and beverages. One of the routes of styrene exposure is inhalation through soil and water surface emissions, automobile exhaust, and cigarette smoking. Another means of contact is ingestion, e.g., drinking water and food wraps (polystyrene packaging material). Some acute symptoms from exposure to styrene are nose, mouth and throat irritations and neurological effects (dizziness, headaches). Other chronic effects are depression, anxiety, leukemia, and lymphoma (177). Styrene exposure, especially among industry workers, has been linked to reproductive outcomes such as spontaneous abortions, irregular menstruation, and sperm abnormalities (178). According to IARC, styrene is classified as a possible carcinogen (Group 2B carcinogen) (157, 170).

Ethylbenzene

Ethylbenzene may be found in vehicles, petroleum and industrial emissions, and foods such as orange peel, parsley leaves, and legumes (179, 180). Resulting acute effects of ethylbenzene are eye irritation and chest constriction. Occupational exposure to ethylbenzene may result in hearing loss, hematological effects, and chromosomal aberrations (181). Ethylbenzene is grouped as a probable carcinogen (Group 2B carcinogen) (157, 170).

1,4-dichlorobenzene

1,4-dichlorobenzene is commonly found in indoor environment and are present in products such as air freshener and moth repellents (182). An animal study has reported that higher exposure to 1,4-dichlorobenzene may be associated with kidney damage (183, 184). According to IARC, 1,4-dichlorobenzene is classified as a possible carcinogen (Group 2B carcinogen) (185).

In summary, there are limited studies that have examined the effect of tear gas exposure on reproductive health. The few studies available explored the association between tear gas and female reproductive conditions (5, 6). However, there is currently no study that has examined the association between tear gas and male reproductive outcomes.

There is also a need for more research on the effect of VOCs on male reproductive outcomes. The majority of the previous studies were either animal studies or occupational studies (186), but only a few epidemiologic studies using a nationally representative sample (187).

Therefore, this study will address a gap in the literature by investigating the effects of tear gas and VOCs on male reproductive outcomes, including erectile dysfunction, ejaculatory dysfunction, and testosterone deficiency.

SPECIFIC AIMS

AIM 1: To investigate the association between tear gas exposure, as estimated by acute symptoms of exposure, and male reproductive outcomes.

- SUBAIM 1A: To investigate the association between tear gas exposure and erectile dysfunction.
- SUBAIM 1B: To investigate the association between tear gas exposure and ejaculatory dysfunction.
    - *Hypothesis: A higher number of tear gas exposure symptoms is associated with higher odds of male reproductive outcomes.*

AIM 2: To determine the association between VOC exposure and erectile dysfunction.

- SUBAIM 2A: To determine the association of VOC exposure and erectile dysfunction through single-pollutant models.
    - *Hypothesis: There is a significant positive association between some VOCs and ED.*
- SUBAIM 2B: To determine the association of VOC exposure and erectile dysfunction through multi-pollutant models.
    - *Hypothesis: There is a significant positive association between VOCs, considered jointly, and ED.*
- SUBAIM 2C: To determine the association of VOC exposure and erectile dysfunction among nonsmokers through single and multi-pollutant models.
    - *Hypothesis: There is a significant positive association between VOCs, considered jointly, and ED among non-smokers.*

AIM 3: To examine the association between VOC exposure and testosterone deficiency.

- SUBAIM 3A: To examine the association between VOC exposure and testosterone deficiency through single-pollutant models.
    - *Hypothesis: There is a significant positive association between some VOCs and testosterone deficiency.*

- SUBAIM 3B: To examine the association between VOC exposure and testosterone deficiency through multi-pollutant models.
    - *Hypothesis: There is a significant positive association between VOCs, considered jointly, and testosterone deficiency.*
- SUBAIM 3C: To examine the association between VOC exposure on testosterone deficiency among non-smokers through single and multi-pollutant models.
    - *Hypothesis: There is a significant positive association between VOCs, considered jointly, and testosterone deficiency.*

CHAPTER TWO

AIM 1: ACUTE TEAR GAS EXPOSURE SYMPTOMS AND ADVERSE MALE REPRODUCTIVE OUTCOMES

INTRODUCTION

Tear gas refers to a group of chemical compounds that when exposed to, may cause irritation to the eyes, nose, lungs, heart, and skin (112). The most common compounds used as tear gas are chloroacetophenone (CN), chlorobenzylidenemalononitrile (CS), and dibenz[b,f]-1,4-oxazepine (CR) (113). Around the world, there has been increased use of tear gas, especially by law enforcement officers who rely on tear gas to disperse crowds during protests, demonstrations, or civil unrest (4). In the US, especially between 2020 and 2021, there were numerous protests due to racial injustice and the black lives matter movement; and this was what prompted this tear gas research (122). Although there has been increased usage of this lachrymator agent, little is known about its long-term effects such as reproductive health, on the population (4).

The most common sexual dysfunctions in men are erectile dysfunction (ED) and ejaculatory dysfunction (premature ejaculation) (188). ED refers to the inability to achieve and maintain the erection required for sexual pleasure (13). Estimates of the prevalence of ED ranges from 3-76.5% across the globe (14), and it is estimated that by 2025, about 322 million men will have ED worldwide, significantly affecting the quality

of life of men globally (15). Although organic and psychological factors have been linked to ED, recent studies have implicated environmental factors in the global trend of ED (53). Like ED, the prevalence of premature ejaculation is relatively high, affecting 30 to 50% of men in the world (189).

Although exposure to tear gas is known to result in acute effects, only a few studies have reported its impact on long-term outcomes, such as reproductive health. In a recent study by Torgrimson-Ojerio (5), approximately 54.5% of women exposed to tear gas saw changes in their menstrual cycle, indicating that tear gas may affect female reproduction. Exposure to tear gas may increase the risk of cardiovascular (190, 191), respiratory (136), and psychological (5) conditions that are linked to the pathogenesis of ED. In addition, tear gas has been hypothesized to be an endocrine disruptor (192); these are known to have antiandrogenic effects (56). There are currently no studies that have investigated whether exposure to tear gas may have an adverse impact on male reproductive health; therefore, this dissertation will investigate the effects of tear gas, as estimated by acute tear gas exposure symptoms, on male reproductive outcomes, including erectile dysfunction, ejaculatory dysfunction, and trouble conceiving.

METHODS

Study design

The Louisville tear gas study was a cross-sectional study of individuals reporting exposure to tear gas in 2020 or 2021. An online survey was conducted between March 2021 to September 2021, including subjects from various parts of the United States. The questionnaire was composed of questions related to demographics, acute tear gas effects, chronic diseases, reproductive health, and protest attendance (Appendix A).

Study setting and subjects

The participants were aged 18 years and older who reported being exposed to tear gas during the 2020 to 2021 protests and demonstrations held across the country. These subjects were recruited using online marketing strategies (Facebook and Twitter ads) and a gift card was offered upon completion of the questionnaire. Also, community recruitment among protestors or demonstrators in the Louisville area occurred through word-of-mouth, which was promoted by one of the investigators (M. Unseld). The questionnaire was created and maintained on Research Electronic Data Capture (REDCap). This questionnaire included screening questions, such as state, age, sex, race, ethnicity, education, income, occupation, and smoking, and a preamble introducing the purpose of the study. Each completed questionnaire was assigned a unique identifier. The study was approved by the University of Louisville Institutional Review Board.

Measures

Exposure assessment

Tear gas exposure was measured by acute tear gas exposure symptoms, as reported on the questionnaire (Appendix A). These symptoms include eye effects (watery eyes, burning, stingy eyes, and other eye effects), lung effects (coughing, burning lungs, shortness of breath, and other lung effects), skin effects (burning sensation, blistering, and other skin effects), and heart effects (increased heart rate, irregular heart rate, chest pain, and other heart effects). The acute tear gas exposure symptoms for each participant were summed up into a composite score, ranging from 0-14, where 0 represents not having any of the symptoms and 14 means having all the symptoms. The scores were further divided into approximate tertiles, low (0-5), medium (6-8), and high (9-14) acute symptom scores.

Outcome assessment

The male reproductive outcomes were assessed using the question: “What reproductive or hormonal problems have you had since you started attending protests? Check all that apply:” The response options were: erectile dysfunction; ejaculatory dysfunction; trouble conceiving; and don't know/don't wish to answer. Participants could select more than one. Those who selected don’t know/don't wish to answer were excluded from the study. Male reproductive outcomes were analyzed using three methods: having at least one male reproductive outcome (erectile dysfunction, ejaculatory dysfunction, trouble conceiving) vs. none; erectile dysfunction vs. none, and ejaculatory dysfunction vs. none.

Covariates

Demographic data collected from the participants included the following: age, sex, race, ethnicity, income, education, occupation, and state. Sex was categorized as female, male, non-binary or transgender and other. Race was categorized as White, Black, or African American, Asian, American Indian, or Alaska Native, Native Hawaiian or Other Pacific Islander, and other. Ethnicity was grouped as Non-Hispanic, Hispanic, and don't know. The self-reported income was categorized as Less than $10,000, $10,000-$19,999, $20,000-$29,999, $30,000-$39,999, $40,000-$49,999, $50,000-$59,999, $60,000-$69,999, More than $70,000, and don't know/don't wish to answer. Education was grouped as less than high school, high school diploma, some college, bachelor's degree, graduate degree, and don't know/don't wish to answer. Participants were also classified into non-smokers and smokers, though this question was added in a later version of the questions and therefore only a subset of participants had the opportunity to respond.

Statistical analysis

Descriptive statistics was used to compare the variables by exposure status. Because of the small sample sizes in some categories, race was categorized into white, black, and other. Education was categorized as less than or some college, and bachelor's degree or more. Income was grouped into less than 39,999, $40,000 to 59,999, and greater than $60,0000. Categorical variables were presented using frequencies and percentages and compared using Chi-square test or Fisher's exact test. The continuous variable, age, was compared using the Kruskal Wallis test since it was not normally distributed.

To investigate the association between acute tear gas symptoms and male reproductive outcomes, logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI). Two models for each outcome were built to determine the association between acute tear gas exposure score and male reproductive outcomes (any male outcome/ erectile dysfunction/ ejaculatory dysfunction). Model I did not adjust for covariates; model II was adjusted for age, race, education, and income. Prior literature and the descriptive table were used to identify possible confounders; covariates that were significantly associated with the exposure score in Table 1 ($P<0.05$) were included in the adjusted model.

Additional analyses were conducted. First, multivariable logistic regression was conducted to examine the effect of heart-related symptoms (increased heart rate, irregular heartbeat, chest pain, and other heart effects) on male reproductive outcomes because of the known link between cardiovascular diseases and ED (48, 193). Second, the effect of seeking medical care after exposure to tear gas on male reproductive outcomes was explored using multivariable logistic regression. Seeking medical care may be another indication of the intensity of exposure.

All statistical analyses were performed using R software (version 4.1.0).

RESULTS

The baseline characteristics of 92 male participants, classified by acute tear gas exposure scores were reported in (Table 1). The result showed no significant difference in the distribution of ages across the three groups (p-value=0.92). The participants in the high exposure score group were more likely to be black, non-Hispanic, and have some college degree or less. Seventy-five percent of the high exposure group earned between $40,000 to $59,999 per year compared to those with low exposure score which was 18.8%. Also, half of the men in the high exposure group sought medical care after exposure to tear gas. Approximately 52% of participants with acute tear gas exposure score of greater than eight had erectile dysfunction, 54.2% had ejaculatory dysfunction, and 8.3% had trouble conceiving; among participants in the lowest category of exposure score, these outcomes were only experienced by 15.6%, 15.6%, and none, respectively.

Multivariable logistic regression analyses were conducted to determine the effect of tear gas exposure, as estimated by acute tear gas score, on male reproductive outcomes, including erectile dysfunction and ejaculatory dysfunction, adjusting for age, race (white/black/other), education (some college or less/graduate or more), and income (less than $39,999/ $40,000 to $59,999/ greater than $60,000) (Tables 2, 3 & 4).

There was a significant positive association between tear gas exposure scores and any male reproductive outcome when comparing those with high exposure scores to those with low exposure scores in both the unadjusted (OR 7.7; 95% CI 2.4-27.4) and adjusted models (OR 5.1; 95% CI 1.1–26.2). However, this association was not significant when comparing medium to low (Table 2). The effect of the acute tear gas exposure score on each male reproductive outcome was then examined. The result showed that compared to

those with low scores, those with high scores had 6.6 times the odds of having erectile dysfunction, after adjusting for age, race, education, and income (OR=6.6; 95% CI 1.3-40.1, Table 3). The result also indicated a significant positive association between high tear gas exposure score and ejaculatory dysfunction (OR 6.4; 95% CI 1.9-24.1). However, this association was no longer significant after adjusting for covariates (OR 2.0; 95% CI 0.4-11.4, Table 4). After a great decline in OR was observed, the covariates were included individually, and the result showed that income was responsible for the change in the effect of acute tear gas exposure on ejaculatory dysfunction.

Because of the similar unadjusted odds ratios observed for ejaculatory dysfunction and erectile dysfunction, the extent of overlap between these two outcomes was examined (X^2= 5.33, P=0.02). Of those with erectile dysfunction, only half also had ejaculatory dysfunction (Table 5). In addition, two participants reported that they had all three male reproductive outcomes, including ED, ejaculatory dysfunction and trouble conveying (Tables 6 & 7).

When specifically examining the association between heart effects and reproductive outcomes, men who reported one or two heart effects (OR 6.89; 95% CI 1.67-37.82) and those who reported more than two heart-related symptoms after exposure to tear gas (OR 14.19; 95% CI 2.27-110.95) had increased odds of having at least one of the male reproductive outcomes compared to those without any heart effects after adjusting for age, race, education, and income. Also, the effect of other tear gas symptoms, including eye, lung, and skin effects on male sexual dysfunction were examined. The result showed no significant association between eye (OR 1.34; 95% CI 0.47-3.85) or lung effects (2 vs 0-1 lung effects: OR 2.90; 95% CI 0.73-12.17 & 3-4 vs 0-

1 lung effects: OR 0.98; 95% CI 0.26-3.59) and any male reproductive outcome, after adjusting for age, race, education, and income. However, there was a positive association between skin effects and any male reproductive outcome, after adjusting for age, race, education, and income (OR 9.33; 95% CI 2.22-48.24, Table 8).

Table 9 presents the results of the association between seeking medical care after exposure to tear gas and any male reproductive outcomes. Those who sought medical care after exposure to tear gas had 6.01 times the odds (95% CI: 1.86-22.56) of having at least one of the male reproductive outcomes compared to those who did not seek medical care after controlling for age, race, education, and income.

In summary, the result showed that high exposure scores were significantly associated with male reproductive outcomes.

DISCUSSION

In this cross-sectional study, there was a significant association between high acute tear gas symptoms and male reproductive outcomes. This effect was observed when the sexual dysfunctions were analyzed as a group and individually (ED). Similar results were recorded when only heart-related symptoms were examined. The effect of tear gas on trouble conceiving was not able to be evaluated, due to the small number of men who reported trouble conceiving.

This is the first study to investigate the effect of tear gas exposure on male reproductive outcomes. Although a study reported changes in menstrual cycle among women exposed to tear gas (5), little is known on its impact on male sexual dysfunction.

An animal study reported that CS gas metabolized into cyanide after mice were exposed to the gas (194). The resulting cyanide formation may result in a decline in oxygen levels in different parts of the body (195), and possibly leading to ED. Also, according to Shivanoor et al., rats exposed to high level of cyanide experienced a decline in LH and testosterone levels (196).

The occurrence of erection involves multiple events, including vascular, neurological, endocrine and/or psychological systems (25). When these systems are affected, it may lead to ED. A common condition related to vascular ED is CVD. ED is regarded as a harbinger of CVD (48, 197, 198); therefore, it is not surprising that these conditions may share similar risk factors, including environmental exposures like tear gas. Some studies have reported that exposure to tear gas may result in cardiovascular conditions such as transient hypertension (113), and high blood pressure in those with pre-existing heart conditions (199). A case report documented the onset of acute myocardial infarction after the exposure to pepper spray (200). The current study also corroborates this evidence, where those with more heart-related tear gas symptoms were more likely to develop ED. However, this study did not control for prior heart-related conditions due to the small sample size. The questionnaire did ask whether the participant had any pre-existing heart conditions, and only one participant responded affirmatively.

Psychological factors, such as stress, depression, and anxiety, are known predictors of ED (16, 22). Stress may increase the cortisol level in the body, causing the constriction of blood vessels and when there is an improper flow of blood to the penis, erection may be affected (201). These psychological conditions may be related to quality of life (202), chronic health conditions (203), or environmental exposures (204). Studies

have reported a relationship between tear gas exposure and psychological disorders (5). In the Portland-based study, the result showed that approximately 72% of participants had various psychological conditions, including anxiety and depression, after exposure to tear gas (5). Since there is a strong link between psychological factors and ED, there is a possibility of tear gas exposure resulting in psychogenic erectile dysfunction.

Another possible biological mechanism linking tear gas exposure to ED may be the relationship between tear gas exposure and respiratory illnesses. A Turkish study reported an increased risk of chronic bronchitis, a type of COPD, among subjects exposed to tear gas (136). The progression of COPD may result in inflammatory responses of the airways, and with the involvement of phagocytes, reactive oxygen species (ROS) is increased. The heightened ROS may further induce oxidative stress causing detrimental effects such as DNA damage (205). The affected DNA may lower the levels of testosterone produced by Leydig cells, subsequently resulting in ED (206). Also, high ROS (superoxide) may react with penile nitrous oxide (NO) and, in turn decrease NO concentration through the induction of peroxynitrite, leading to ED (207). NO is important in the mechanism of penile erection, as it is responsible for the relaxation of the smooth muscles that allows blood to flow to the penis (20, 21). Therefore, there is a possibility that exposure to tear gas may result in ED of respiratory-related origin.

Recent reports on the effect of tear gas on menstrual cycle may support the claim that tear gas may be an endocrine disruptor. Although there is no established scientific basis for this, the Torgrimson-Ojerio study reported that 54.5% of the 899 study participants experienced menstrual disorders after exposure to tear gas (5). Endocrine disruptors, such as bisphenol A (BPA), tend to be anti-androgenic (106) and have been

linked to ED and ejaculatory dysfunction (59, 208). A study on factory workers showed a strong relationship between BPA (median TWA_8 levels of BPA in air samples $=4.57mg/m^3$) and male sexual dysfunctions. Compared to workers not exposed to BPA, those exposed to BPA had 4.5 times the odds of having ED, after adjusting for age, education, marital status, current smoking status, presence of chronic diseases, exposure to other chemicals and occupational history (OR = 4.5, 95% CI 2.1-9.8). Similarly, there was a strong association between high level of BPA exposure and ejaculatory dysfunction, after adjusting for possible confounders (OR = 7.1, 95% CI 2.9-17.6) (59). A possible mechanism in which endocrine disruptors may lead to ED is through ROS induction, which may lower testosterone levels, subsequently resulting in ED (107, 206, 209). In summary, the exposure to tear gas may increase the risk of conditions that may result in ED.

A strength of this study is that it addresses novel and timely research question. Also, multiple aspects of the exposure (eye, skin, lung, and heart effects) and outcome (ED and ejaculatory dysfunction) were assessed, permitting the examination of various definitions of exposure and outcome.

The first limitation of this study is the small sample size, which limits the statistical power of the study. The effect of tear gas exposure on trouble conceiving was not assessed due to the small number of men who reported trouble conceiving. Second, the data used for this analysis was self-reported using an online questionnaire. This may be prone to information bias such as misclassification bias. Third, it was a cross-sectional study, making it difficult to assure temporal relationships between acute tear gas exposure symptoms and male reproductive outcomes. Another limitation of this study is the

potential for residual confounding. This bias may be minimized by methodically considering potential confounders through use of a DAG, and by using appropriate categories for each variable. There are some potential confounders, such as pre-existing health conditions, smoking, and stress, that we were unable to include in the multivariable models. Lastly, the duration of the adverse male outcomes was not assessed. This may be partially addressed by examining the amount of time elapsed between the date of protest attended (if available) to when the questionnaire was completed. However, participants were not asked when they first began experiencing symptoms of ED or ejaculatory dysfunction—the questions only asked whether they had experienced these symptoms since being exposed to tear gas.

CONCLUSION

In summary, this study observed a strong association between tear gas exposure, as measured by acute tear gas exposure symptoms, and sexual dysfunctions in men. Since this is the first study that has examine the effects of these irritants on male reproductive outcomes, further studies using a larger sample size and a more thorough examination of medical history is recommended to understand the association between tear gas exposure and incident ED. Based on this result, a recommendation will be to educate the public, including protestors, law enforcement, and passersby on the health impact of tear gas on reproductive health and on the immediate actions they need to take when exposed. Also, this research can help support policymakers in their efforts to reduce or eliminate the use of tear gas in protests and demonstrations.

Table 1: Participant characteristics by low, medium, and high tear gas exposure scores

	Low exposure score (0-5 acute symptoms) N = 32	Medium exposure score (6-8 acute symptoms) N = 36	High exposure score (9-14 acute symptoms) N = 24	P-values
Age				0.916
Median (IQR)	35 (9.0)	35 (8.3)	32 (8.0)	
Race, n (%)				0.001
White	20 (62.5)	21 (58.3)	4 (16.7)	
Black	11 (34.4)	9 (25.0)	16 (66.7)	
Others	1 (3.1)	4 (11.1)	4 (16.7)	
Missing	0 (0.0)	2 (5.6)	0 (0.0)	
Ethnicity, n (%)				0.960
Non-Hispanic	27 (84.4)	31 (86.1)	22 (91.7)	
Hispanic	2 (6.2)	3 (8.3)	2 (8.3)	
Missing	3 (9.4)	2 (5.6)	0 (0.0)	
Education, n (%)				<0.001
Some college or less	9 (28.1)	18 (50.0)	21 (87.5)	
Bachelor's & Graduate degree	23 (71.9)	18 (50.0)	3 (12.5)	
Income, n (%)				<0.001
Less than $39,999	7 (21.9)	12 (33.3)	4 (16.7)	
$40,000 – $59,999	6 (18.8)	13 (36.1)	18 (75.0)	
Greater than $60,000	19 (59.4)	11 (30.6)	1 (4.2)	
Missing	0 (0.0)	0 (0.0)	1 (4.2)	
Smoking, n (%)				0.778
No	6 (18.8)	10 (27.8)	3 (12.5)	
Yes	1 (3.1)	1 (2.8)	0 (0.0)	
Missing	25 (78.1)	25 (69.4)	21 (87.5)	
Medical care, n (%)				0.042
No	14 (43.8)	24 (66.7)	10 (41.7)	

	Low exposure score (0-5 acute symptoms) N = 32	Medium exposure score (6-8 acute symptoms) N = 36	High exposure score (9-14 acute symptoms) N = 24	P-values
Yes	15 (46.9)	8 (22.2)	12 (50.0)	
Missing	3 (9.4)	4 (11.1)	2 (8.3)	
Any Male Outcome, n (%)				0.002
No	23 (71.9)	21 (58.3)	6 (25.0)	
Yes	9 (28.1)	15 (41.7)	18 (75.0)	
Erectile dysfunction, n (%)				0.007
No	27 (84.4)	26 (72.2)	11 (45.8)	
Yes	5 (15.6)	10 (27.8)	13 (54.2)	
Ejaculatory dysfunction, n (%)				0.004
No	27 (84.4)	28 (77.8)	11 (45.8)	
Yes	5 (15.6)	8 (22.2)	13 (54.2)	
Trouble conceiving, n (%)				0.262
No	32 (100.0)	35 (97.2)	22 (91.7)	
Yes	0 (0.0)	1 (2.8)	2 (8.3)	

Table 2: Univariate & multivariable model for acute tear gas symptoms and any male reproductive outcome

	Any male outcomes		Unadjusted		Adjusted**	
	No	Yes	OR	95% CI	OR	95% CI
Acute exposure score						
Low	23	9	1.00		1.00	
Medium	21	15	1.83	0.67-5.19	1.59	0.50-5.08
High	6	18	7.67	2.42-27.43	5.10	1.11-26.16

** Adjusted for age, race (White/Black/other), education (some college or less/college degree), income (>$39k/$40k-$59k/>$60k)

Table 3: Univariate & multivariable model for acute tear gas symptoms and erectile dysfunction

	Erectile dysfunction		Unadjusted		Adjusted**	
	No	Yes	OR	95% CI	OR	95% CI
Acute exposure score						
Low	27	5	1.00		1.00	
Medium	26	10	2.08	0.65-7.44	1.95	0.52-8.02
High	11	13	6.38	1.93-24.13	6.57	1.28-40.08

** Adjusted for age, race (White/Black/other), education (some college or less/college degree), income (>$39k/$40k-$59k/>$60k)

Table 4: Univariate & multivariable model for acute tear gas symptoms and ejaculatory dysfunction

	Ejaculatory dysfunction		Unadjusted		Adjusted**	
	No	Yes	OR	95% CI	OR	95% CI
Acute exposure score						
Low	27	5	1.00		1.00	
Medium	28	8	1.54	0.46-5.66	0.93	0.20-4.35
High	11	13	6.38	1.93-24.13	1.96	0.35-11.36

** Adjusted for age, race (White/Black/other), education (some college or less/college degree), income (>$39k/$40k-$59k/>$60k)

Table 5: Cross tabulation of erectile dysfunction and ejaculatory dysfunction

		Ejaculatory dysfunction		
		No (%)	**Yes (%)**	
Erectile dysfunction	**No**	51 (77.3)	13 (50)	64 (69.6)
	Yes	15 (22.7)	13 (50)	28 (30.4)
		66 (71.7)	26 (28.3)	92 (100)

Table 6: Cross tabulation of erectile dysfunction and trouble conceiving

		Trouble conceiving		
		No (%)	**Yes (%)**	
Erectile dysfunction	**No**	63 (70.8)	1 (33.3)	64 (69.6)
	Yes	26 (29.2)	2 (66.7)	28 (30.4)
		89 (96.7)	3 (3.3)	92 (100)

Table 7: Cross tabulation of ejaculatory dysfunction and trouble conceiving

		Trouble conceiving		
		No (%)	**Yes (%)**	
Ejaculatory dysfunction	**No**	65 (73.0)	1 (33.3)	66 (71.7)
	Yes	24 (27.0)	2 (66.7)	26 (28.3)
		89 (96.7)	3 (3.3)	92 (100)

Table 8: Univariate & multivariable model for each acute effects category and any male reproductive outcome

	Any male outcomes		Unadjusted		Adjusted**	
	No	**Yes**	**OR**	**95% CI**	**OR**	**95% CI**
Heart effects						
No heart effect	23	3	1.00		1.00	
1 -2 heart effects	21	22	8.03	2.35-37.49	6.89	1.67-37.82
3-4 heart effects	6	17	21.72	5.55-118.81	14.19	2.27-110.95
Eye effects						
0-1 eye effects	20	13	1.00		1.00	
2-3 eye effects	30	29	1.49	0.63-3.59	1.34	0.47-3.85
Lung effects						
0-1 lung effects	18	8	1.00		1.00	
2 lung effects	7	14	4.50	1.36-16.31	2.90	0.73-12.17
3-4 lung effects	25	20	1.80	0.66-5.18	0.98	0.26-3.59
Skin effects						
0-1 skin effects	45	20	1.00		1.00	
2-3 skin effects	5	22	9.90	3.50-33.04	9.33	2.22-48.24

** Adjusted for age, race (White/Black/other), education (some college or less/college degree), income (>$39k/$40k-$59k/>$60k)

Table 9: Univariate & multivariable model for medical care and any male reproductive outcome

	Any male outcomes		Unadjusted		Adjusted**	
	No	Yes	OR	95% CI	OR	95% CI
Medical care						
No	35	13	1.00		1.00	
Yes	12	23	5.16	2.05-13.72	6.01	1.86-22.56

** Adjusted for age, race (White/Black/other), education (some college or less/college degree), income (>$39k/$40k-$59k/>$60k)

CHAPTER THREE

AIM 2: BLOOD VOCs AND ERECTILE DYSFUNCTION

INTRODUCTION

Erectile dysfunction (ED), defined as the ability to achieve or maintain erection required for sexual pleasure, is a common condition among men ages 40 and older (13, 210). As reported in a 2007 study conducted using NHANES data, the prevalence of ED in the US was 18.4% (211). ED may result from underlying vascular, endocrinological, neurological, or psychological causes (18). Aside from these common risk factors, research has shown that environmental factors may also be associated with ED (53). Numerous environmental factors have been implicated in the development of ED, and they include cigarette smoke, BPA, pesticides, lead, radiation, and air pollution (18, 206, 212). Although ED is known to affect the sexual life of men, it may also have an enormous impact on their quality of life and work productivity (213, 214).

Volatile organic compounds (VOCs) are chemical compounds that result from various sources, including automobile exhaust, industrial processes, cigarette smoke, paints, solvents, and cleaning supplies (215, 216). VOCs are present in both indoor and outdoor settings, with a higher concentration in enclosed spaces (217, 218). Since a

majority of people spend more time indoors (219, 220), they may be more likely to be exposed to VOCs, resulting in various adverse health effects, including reproductive health outcomes (186, 221). In humans, VOCs can be measured via blood, urine, breath, or sweat (222). Detecting VOCs from the blood may be a more reliable method because the VOCs reach the blood before the organs in the body after exposure. However, blood VOCs is more invasive than the other methods (223-226).

Currently, there are no established mechanisms that link VOC exposure to ED. However, VOCs like benzene, toluene, xylene, and ethylbenzene have been identified as endocrine disruptors and may affect male reproductive hormones, possibly leading to ED (227). In addition, VOCs have been found to be associated with CVDs, such as heart failure (228), stroke (229), ischemic heart disease (229), and endothelial injury (230). Since ED is a known predictor of CVD and shares certain risk factors with CVD, there is a possibility that this sexual dysfunction may be associated with a CVD risk factor such as VOCs. Smoking, a common source of VOCs, has also been linked to ED (231, 232). A plausible mechanism in which smoking may result in ED is through the decline in the production of nitric oxide (NO) required for erection (233). Also, exposure to smoking may lead to the development of penile endothelial dysfunction, potentially resulting in ED (231).

There are limited studies that have examined the effect of VOCs on ED. An occupational study reported that lacquerers exposed to toluene and xylene were more likely to be impotent compared to non-lacquerers after adjusting for age, smoking, and dust exposure (186). In another study on veterans, those exposed to VOCs in water had higher odds of developing ED compared to the unexposed (221). These two studies,

implicated impairment of the nervous system as the basis for ED. Given the limited population-based studies on the effect of VOCs on ED, this dissertation aims to investigate the effect of VOCs (individually and jointly) on erectile dysfunction using data from the 2001–2004 National Health and Nutrition Examination Survey (NHANES).

METHODS

Study Population

The data was obtained from the 2001-2004 National Health and Nutrition Examination Survey (NHANES). NHANES is a cross-sectional survey created to monitor the health and nutrition of the civilian noninstitutionalized US population. The survey, conducted by the CDC and the National Center for Health Statistics (NCHS), ensures representativeness using multistage-cluster probability sampling. The data was collected from participants via in-person interviews, physical examinations, and blood samples (234, 235). There were 4116 males >=20 years old with data on ED in the 2001-2004 surveys. After excluding 120 males with prostate cancer, the final sample size eligible for VOC analysis was 1224.

Assessment of ED

The ED-related question was included in the prostate condition section of the questionnaire in the 2001-2004 NHANES year cycles. ED was assessed using the question: “Many men experience problems with sexual intercourse. How would you

describe your ability to get and keep an erection adequate for satisfactory intercourse?" The responses are "Always or almost always able," "Usually able," "Sometimes able," and "Never able." For analysis, ED was defined as "sometimes able" or "never able" to keep an erection adequate for satisfactory intercourse. Participants who responded "almost always able" or "usually able" to maintain an erection were defined as not having ED (211, 236).

Measurement of VOCs

Blood specimens were collected at NHANES Mobile Examination Centers (MECs) during the participants' scheduled appointments. Blood VOCs were measured from participants aged 12 years and older. The common VOCs measured in both NHANES 2001-2002 & 2003-2004 year cycles were benzene, toluene, m-/p xylene, o-xylene, 1,4-dichlorobenzene, ethylbenzene, styrene, trichloroethane, tetrachloroethane, carbon tetrachloride, bromoform, chloroform, bromodichloromethane, dibromochloromethane, and MTBE. This study excluded waterborne VOCs (bromoform, chloroform, bromodichloromethane, dibromochloromethane, and MTBE). Also, VOCs in which the percentage below the detection limit and/or missing was ≥90% were removed from the study (trichloroethane, tetrachloroethane, and carbon tetrachloride). Ultimately, seven VOCs were used for the analysis: benzene, toluene, m-/p xylene, o-xylene, 1,4-dichlorobenzene, ethylbenzene, and styrene. Each of the VOCs was further categorized into three groups (E0-E2). For all VOCs except toluene and m-/p-xylene, the first group (E0) represented below the detection limit, while the remaining values were split into two

equal groups (E1 & E2). Furthermore, toluene and m-/p-xylene were divided into tertiles because of the low proportion that were below the limit of detection.

The sample size available for analysis was different for each VOC. The sample sizes for each VOCs are benzene (N=966), toluene (N=1008), m-/p xylene (N=1015), o-xylene (N=1036), ethylbenzene (N=952), 1,4-dichlorobenzene (N=948), and styrene (N=968) (Figure 1).

Covariates

Based on prior literature, covariates related to ED were identified and included in the multivariable models. ED is commonly diagnosed in older men Age is a known risk factor of ED, as previous studies have reported that ED increases with age (16, 210). A California Men's Health Study reported that Asians and Blacks were less likely to develop ED compared to Whites (237). Also, previous studies have shown that smoking is an independent risk factor of ED (38, 231, 238). Unlike smoking, some studies have reported a protective effect of alcohol on ED. Two meta-analyses reported that regular consumption of alcohol was inversely associated with ED (40, 239). BMI is also a predictor of ED. A 2006 prospective study reported higher odds of having ED among obese men compared to normal weight men (51).

These covariates are age, race/ethnicity, education, marital status, family income-to-poverty ratio, BMI, smoking, and alcohol. Race/ethnicity was categorized as non-Hispanic White; non-Hispanic Black; Hispanic (Mexican American and other Hispanics); and other. Family income-to-poverty ratio was classified as <1 (less than poverty level) and >=1 (poverty level /above poverty level). BMI was classified as <18.5, 18.5 to <25,

25 to <30, and ≥30 kg/m^2. Men who smoked <100 cigarettes during their lifetime were grouped as Non-smokers. Those who smoked more than 100 cigarettes in their entire life but were not smoking at the time of the interview were grouped as former smokers. Current smokers were defined as men who smoked more than 100 cigarettes in their entire life and reported smoking every day or some days at the time of the interview or men having serum cotinine level above 10 ng/ml. Alcohol intake was defined as none (<1 drink per week), light (1–3 drinks per week), and heavy (≥4 drinks per week) (240).

Statistical analysis

Continuous variables were presented as means and standard deviation and assessed for association with ED using t-tests. Categorical variables were presented as counts and proportions and analyzed using Chi-square test or Fisher's exact test. Weighted logistic regression was used to calculate the odds ratios and 95% confidence interval (CI) for the relationship between each of the VOCs (log-transformed continuous VOCs and categorized VOCs) and ED. The subsample weight for the blood VOCs present in the 2001-2004 NHANES laboratory data (WTSVOC2Y) was used in estimating the svydesign function required for the weighted logistic regression analysis. The LLOD for each blood VOC was reported in the NHANES documentation (https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L04VOC_C.htm). The below the limit values for each VOC were imputed using the formula: lower limit of detection (LLOD) divided by square root of 2 (LLOD/sqrt [2]).

Four models were used: model 1 was adjusted for the NHANES year cycle; model 2 was adjusted for age, race/ethnicity, education level, poverty income ratio,

marital status, and NHANES year cycle; model 3 was adjusted for model 2 covariates + body mass index (kg/m2), alcohol and smoking; and model 4 was adjusted for model 2 covariates + body mass index (kg/m2), alcohol and cotinine (ng/mL). In addition, the effects of each VOC on ED were examined among non-smokers. All analyses were performed using R version 4.1.0.

Bayesian kernel machine regression (BKMR) was used to estimate the overall effect of the VOCs mixture on ED. BKMR is a statistical method used in assessing the overall effect of a mixture on a health outcome, identifying the subset of the pollutants responsible for the effect, and detecting interactions among the environmental exposures (241). Since the outcome is a categorical variable, the probit-BKMR model was used for the analysis. The model is fit using 10,000 iterations of the Markov Chain Monte Carlo sampling algorithm (242). Each of the VOCs included in the model was log-transformed to ensure all exposures have the same scale. The BKMR formula is:

$Yi^{*}=h(Ei1,\ldots,Ei7) + \beta Xi+\epsilon i$

Where:

Yi*is the latent continuous outcome, ED (Yi*> 0 equal to have ED (Y=1); Yi* ≤ 0 equal to No ED (Y=0)); E is the VOC mixture (benzene, toluene, 1,4, -dichlorobenzene, ethylbenzene, xylene, o-xylene, and styrene); Xi represents the covariates (age, race/ethnicity, education, marital status, family income to poverty ratio, BMI smoking, alcohol, and NHANES cycle); ϵi is the error; and h is the exposure-response function.

The hierarchical variable selection procedure was used to address the problem of multicollinearity in the probit-BKMR model. The first step of the process was to classify the highly correlated VOCs into the same group and non-correlated VOCs into their

individual groups based on the Pearson correlation plot. The posterior inclusion probabilities (PIP), representing the level of importance of each VOC in the exposure mixture-outcome association (243), were then estimated for each group (group PIP). Simultaneously, the within-group variable selection was reported (Conditional PIP) (242, 244). The relative importance of the VOCs within the groups relies on the conditional PIP. As reported in a previous study, the threshold value for PIP was set as 0.5 to determine the pollutant's influence level (243). Graphical representations were used to show the individual and the overall effect of VOCs on ED. Furthermore, the BKMR model was used to estimate the presence of interactions of any two VOCs on ED when the level of another VOC was held at the 10th, 50th, or 90th percentile, with all other VOCs simultaneously fixed at the 50th percentile, respectively. Statistical analyses were performed using R (version 4.1.0) with the package "bkmr" (242).

RESULTS

Of the 1224 eligible participants in the 2001-2004 NHANES year cycles, 10.7% (n =131) had erectile dysfunction. Compared to participants without ED, those with ED were more likely to be other races besides non-Hispanic white, have lower educational level (38.9%), lower family income to poverty ratio (26.6%), and current (50.4%) or former smokers (21.4%). However, there were no significant differences in marital status, BMI, and alcohol intake between the two groups ($p > 0.05$). Also, comparing men in the non-ED group to those in the ED group, there were no statistically significant ($p>0.05$) differences in any of the seven VOCs (Table 10).

Weighted multivariable logistic regression was performed to estimate the effect of VOC exposure on ED. Based on the results, benzene, toluene, xylene, o-xylene, ethylbenzene, and styrene were not significantly associated with ED after adjusting for age, race, education, marital status, body mass index (kg/m^2), family income to poverty ratio, alcohol, smoking/cotinine (ng/mL), and NHANES year cycle (Table 11).
For 1,4-dichlorobenzene, those in the E1 group had significantly increased odds of ED after adjusting for NHANES year cycle (OR 1.89; 95% CI 1.15-3.09, Table 11).

The probit-BKMR analysis was used to estimate the joint effect of the VOCs (log-transformed) on ED and detect interaction among the VOCs. The probit BKMR model considers correlations between pollutants when estimating these overall effects, which is evident in the posterior inclusion probabilities (PIPs). The posterior inclusion probabilities (PIPs), estimated from the BKMR model, were used to assess variable importance in the exposure mixture-outcome association. The higher the PIP, the more influential the variable is in the association or relationship. In Table 12, the highly correlated (r>70) VOCs were placed in the same group. The result from the correlation analysis showed that m-/p-Xylene, ethylbenzene, and o-xylene were highly correlated and placed in group 5 (Figure 2). The non-correlated VOCs (benzene, toluene, 1,4, -dichlorobenzene, and styrene) were placed in individual groups (groups 1-4). According to Table 12, group 5 (group PIP= 0.54), including m-/p-xylene, o-xylene, and ethylbenzene, had the highest group PIP, with o-xylene being the highest in the group (conditional PIP= 0.60). This implies that o-xylene was the most vital contributor in group 5, in relation to ED.

Based on Figure 3, there was no significant association between the overall VOC mixture and ED risk when all the VOCs at particular percentiles (25th to 75th by 0.05) were compared to all the VOCs at their 50th percentile (reference point), after adjusting for age, race, education, marital status, body mass index (kg/m^2), family income to poverty ratio, alcohol, smoking, and NHANES year cycle. Also, the relationship between each VOC and ED was explored, with the other VOCs fixed at the median values (50th percentile). The result showed no significant effects for any VOC on ED after adjusting for all covariates (Figure 4**)**. This BKMR result further confirms the output from the multivariable logistic regression. In addition, there was no evidence of two-way interactions between pairs of VOCs when the second VOC was fixed at 10%, 50%, and 90% percentile, respectively, and all other VOCs were fixed at their median levels, adjusting for all covariates **(**Figure 5).

A supplementary analysis was performed to explore the effects of VOCs on ED among non-smokers. A total of 684 men were nonsmokers (never and former smokers) and were included in the analysis. In addition, the overall effect of VOCs on ED among non-smokers was explored, and no significant relationship was observed, either in the regression models (Table 13) or in the BKMR analysis (Figure 6).

DISCUSSION

This is the first study to examine the effect of VOCs on ED using a nationally representative sample. The result showed no significant relationship between all the VOCs, except 1,4-dichlorobenzene and ED, after full adjustment. Furthermore, the

relationship between the seven VOCs and ED among non-smokers was not significant. Similar to the results from the single pollutant models, the probit-BKMR result demonstrated no significant association between the overall VOCs and ED after adjusting for all covariates.

Household product products are the largest source of 1,4-dichlorobenzene, which is mainly found in air fresheners and moth repellent, and as such, humans mostly get exposed via indoor spaces (245). Several studies, including animal studies, have linked 1,4-dichlorobenzene to female reproductive outcomes, including low birth weight (245, 246). A previous animal study reported that compared to the control rats, the rats exposed to 1,4-dichlorobenzene and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p′-DDE), saw a 80% decrease in the ovaries weight of their female offspring (245). Similarly, another study on rats reported lower birth weight and increased mortality rate among the offspring of mothers exposed to 1,4-dichlorobenzene (247). Ding et al. found that women who use vaginal douche tend to have high levels of 1,4-dichlorobenzene compared to non-users (246). Although there are a few reproductive studies on women, little is known about its effect on male sexual function. The result from the current study indicates that exposure to 1,4-dichlorobenzene is associated with higher odds of ED (OR 1.89; 95% CI 1.15-3.09), comparing those with moderate exposure vs. low exposure. The effect was similar in the highest exposure group (OR=1.86), though not statistically significant. These effects were slightly attenuated after adjusting for potential confounders (adjusted ORs = 1.82 and 1.77, respectively).

Exposure to VOCs may have detrimental effects on human health. Like some other VOCs, 1,4-dichlorobenzene has been identified to cause central nervous system

tissue and neurological damages (248), which may be a possible reason for its association with ED (neurogenic ED). Other studies have reported that acute or prolonged exposure to toluene and xylene may result in neurological disorders (249, 250). The nervous system, including central and peripheral, plays a vital role in achieving and maintaining erection during sexual activities (251). The nerves in the brain and pelvic area send signals to the corpora cavernosa in the penis, activating the blood vessels to dilate and allowing blood flow into the penis, subsequently leading to an erection (252, 253). Once this process is interrupted due to factors such as neurological conditions or toxins, it may result in ED. A few occupational studies have supported the association between VOCs and ED. A Denmark-based study on lacquerers exposed to toluene and xylene explored the effect of these pollutants on impotence. They observed that those exposed to these organic solvents were more likely to have ED compared to non-lacquerers (186). This paper reported that toxic effects on the nervous system may be responsible for this association. The current result showed no association between toluene or xylene and ED. A possible reason for the contrasting result may be that the Denmark study was focused on occupationally exposed subjects. In contrast, the participants in the current study were exposed to VOCs in a natural condition. Additional evidence for the impact of VOCs' neurological effects on ED was observed in a study on veterans who resided at Camp Lejeune, North Carolina. These men exposed to contaminated water containing water-borne VOCs (such as trichloroethylene, tetrachloroethylene, and benzene) reported an increased odds of ED among the exposed. This study identified ED as a condition that is associated with Parkinson's disease, a neurodegenerative disorder (221). Other studies have linked Parkinson's disease to ED due to the effect of Parkinson's disease on the

nervous system, penile blood flow, and dopamine levels (254, 255). Another probable reason for the difference in results between the two settings, the Denmark study and Camp Lejeune study, and this dissertation is the magnitude of exposure to VOCs. The participants (lacquerers and veterans) in the former are more likely to be exposed to high levels of VOCs from their workplace compared to the participants in the NHANES study.

Smoking is a known source of VOCs, including benzene, toluene, styrene, xylene, and ethylbenzene (256). The VOCs found in cigarette smoke have been linked to various adverse health outcomes, such as cardiovascular diseases (257), neurological conditions, (258) and respiratory outcomes (259). Although this study found no association between the majority of VOCs and ED, a few studies have implicated smoking in the development of ED (231, 232). These studies highlighted the vascular effect of smoking as the cause of this relationship. To date, there has not been a study that that has identified the VOC content of cigarette smoke as a direct cause of this sexual dysfunction.

This study has a number of strengths. This is the first study to examine the effect of VOCs on ED using a nationally representative sample such as NHANES using a multipollutant model. The NHANES data encompassed VOC exposures from the general population and not from occupational sources, therefore the results are more generalizable. The effect among nonsmokers was also examined, where nonsmokers were defined both by their questionnaire answers and by blood cotinine levels, to eliminate the possibility of residual confounding by smoking.

The first limitation of this study is the cross-sectional nature of the data, which cannot establish temporality and does not prove causation. Second, ED was assessed using a self-reported questionnaire. However, the NHANES ED questionnaire has been

validated previously (236). Third, VOCs are known to have short half-lives in the blood. Therefore, the VOC levels assessed during the examination may not depict the participants' lifetime exposure. Fourth, although some covariates were adjusted for, there are other factors that were not adjusted for in the model, possibly leading to residual confounding. For example, occupation, urbanicity, and other prostate or bladder surgeries.

CONCLUSION

In conclusion, most of the VOCs were not associated with erectile dysfunction. 1,4-dichlorobenzene was significantly associated with ED; however, after adjusting for the covariates, the effect estimate became attenuated and lost its significance. The primary source of 1,4-dichlorobenzene is through household supplies, meaning there is a possibility of high dose exposure at home, making it a public health concern. Therefore, further investigation, specifically a prospective study, is needed to establish whether 1,4-dichlorobenzene exposure could increase the risk of ED and associated health outcomes.

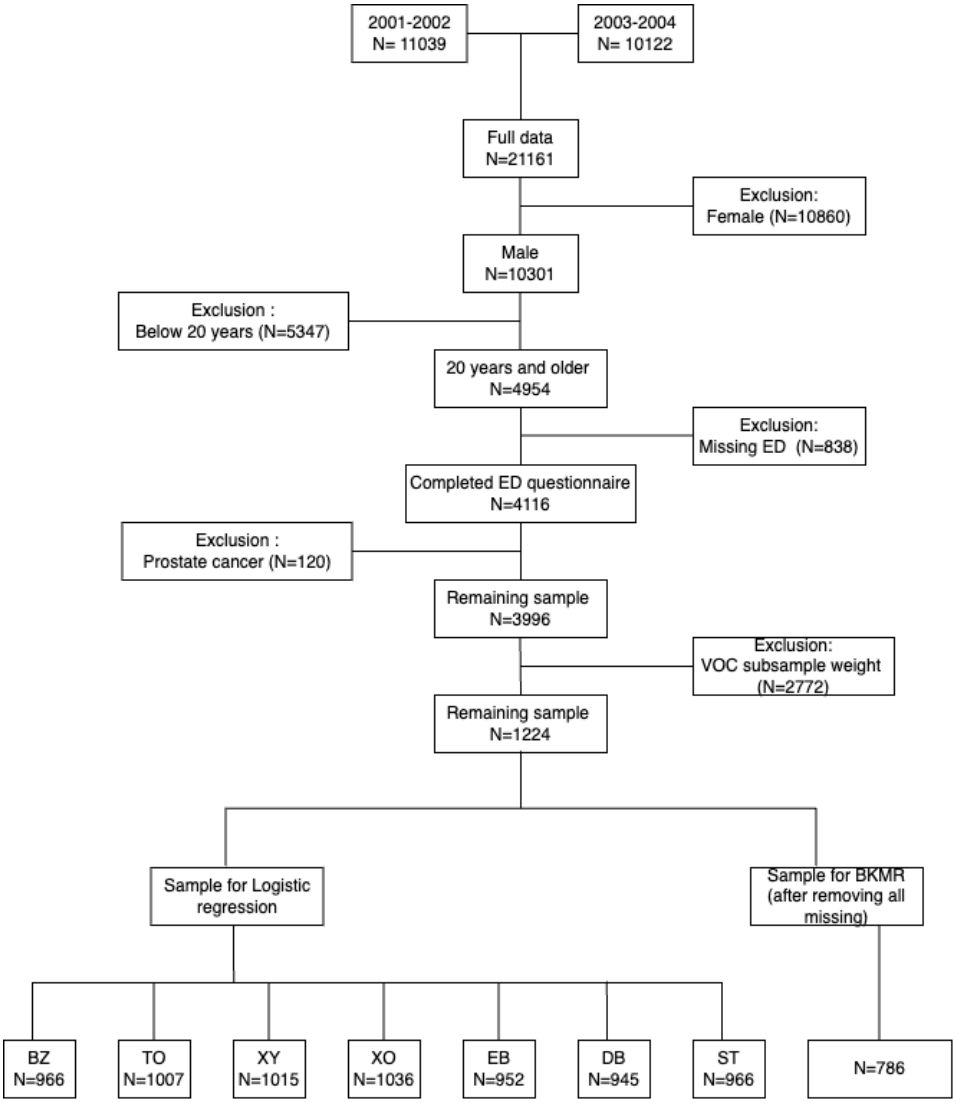

Figure 1. Flow chart of selected study participants for the association of VOCs and ED

BZ = Benzene; TO= Toluene; XY= m-/p xylene; XO=o-xylene; EB= ethylbenzene;

DB= 1,4-dichlorobenzene

Table 10: Participant characteristics by erectile dysfunction and no erectile dysfunction (2001-2004)

	No Erectile dysfunction N=1093	Erectile dysfunction N=131	P value
Age, Mean (SD)	37.9 (10.9)	43.6 (11.9)	<0.001
Race/Ethnicity, n (%)			0.029
Non-Hispanic white	562 (51.4)	52 (39.7)	
Non-Hispanic Black	224 (20.5)	27 (20.6)	
Hispanic	263 (24.1)	43 (32.8)	
Other	44 (4.0)	9 (6.9)	
Education, n (%)			<0.001
Less than high school	225 (20.6)	51 (38.9)	
High school	287 (26.3)	28 (21.4)	
Above high school	581 (53.2)	52 (39.7)	
Marital status, n (%)			0.098
Never married	286 (26.2)	23 (17.6)	
Married/Living with Partner	696 (63.7)	94 (71.8)	
Widowed/Divorced/Separated	111 (10.2)	14 (10.7)	
BMI (kg/m^2), n (%)			0.582
Normal/Underweight	355 (32.9)	38 (29.2)	
Overweight	418 (38.7)	50 (38.5)	
Obese	307 (28.4)	42 (32.3)	
Missing	13	1	
Smoking, n (%)			0.015
Never	448 (41.0)	37 (28.2)	
Current	474 (43.4)	66 (50.4)	
Former	171 (15.6)	28 (21.4)	
Alcohol, n (%)			0.142
None (<1 drink per week)	416 (40.8)	58 (48.7)	
Light (1–3 drinks per week)	194 (19.0)	24 (20.2)	
Heavy (>=4 drinks per week)	409 (40.1)	37 (31.1)	
Missing	74	12	

	No Erectile dysfunction N=1093	Erectile dysfunction N=131	P value
Ratio of family income to poverty, n (%)			<0.001
<1 (less than poverty level)	145 (14.0)	33 (26.6)	
≥1 (at or above poverty level; more affluent)	888 (86.0)	91 (73.4)	
Missing	60	7	
Benzene (ng/ml), n (%)			0.388
E0 (Below detectable limit)	345 (39.8)	40 (40.0)	
E1	266 (30.7)	25 (25.0)	
E2	255 (29.4)	35 (35.0)	
Missing	227	31	
Toluene (ng/ml), n (%)			0.301
E0 (Below detectable limit)	35 (3.9)	7 (6.8)	
E1	438 (48.5)	45 (43.7)	
E2	431 (47.7)	51 (49.5)	
Missing	189	28	
m-/p-Xylene (ng/ml), n (%)			0.333
E0 (Below detectable limit)	19 (2.1)	0 (0.0)	
E1	447 (49.0)	51 (49.5)	
E2	446 (48.9)	52 (50.5)	
Missing	181	28	
o-xylene (ng/ml), n (%)			0.998
E0 (Below detectable limit)	539 (58.1)	63 (57.8)	
E1	194 (20.9)	23 (21.1)	
E2	194 (20.9)	23 (21.1)	
Missing	166	22	
1, 4-dichlorobenzene (ng/ml), n (%)			0.095
E0 (Below detectable limit)	396 (47.0)	37 (36.3)	
E1	226 (26.8)	30 (29.4)	
E2	221 (26.2)	35 (34.3)	
Missing	250	29	

	No Erectile dysfunction N=1093	Erectile dysfunction N=131	P value
Ethylbenzene (ng/ml), n (%)			0.601
E0 (Below detectable limit)	266 (31.2)	28 (28.0)	
E1	290 (34.0)	39 (39.0)	
E2	296 (34.7)	33 (33.0)	
Missing	241	31	
Styrene (ng/ml), n (%)			0.822
E0 (Below detectable limit)	431 (49.8)	47 (46.5)	
E1	217 (25.1)	27 (26.7)	
E2	217 (25.1)	27 (26.7)	
Missing	228	30	

Table 11: Odds ratios (95% confidence intervals) for each VOCs and ED

VOCs	No ED	ED	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 3[c] OR (95% CI)	Model 4[d] OR (95% CI)
Benzene (ng/mL) (N=966)	**N=866**	**N=100**				
Log (Continuous)			1.16 (0.85-1.57)	1.08 (0.80-1.46)	0.99 (0.65-1.50)	1.01 (0.68-1.50)
E0 (BLD*)	345	40	1.00	1.00	1.00	1.00
E1 (0.024-0.064)	266	25	0.70 (0.35-1.43)	0.65 (0.29-1.44)	0.61 (0.21-1.80)	0.67 (0.26-1.71)
E2 (0.064-1.300)	255	35	1.17 (0.64-2.14)	0.99 (0.51-1.91)	0.74 (0.29-1.92)	0.82 (0.33-2.03)
Toluene (ng/mL) (N=1007)	**N=904**	**N=103**				
Log (Continuous)			1.09 (0.87-1.37)	1.02 (0.82-1.27)	0.95 (0.73-1.24)	0.98 (0.76-1.27)
E0 (BLD)	302	34	1.00	1.00	1.00	1.00
E1 (0.082-0.216)	306	30	1.37 (0.78-2.40)	1.36 (0.74-2.50)	1.31 (0.66-2.60)	1.42 (0.73-2.77)
E2 (0.216-9.000)	296	39	1.36 (0.73-2.51)	1.24 (0.68-2.25)	1.06 (0.50-2.22)	1.17 (0.56-2.45)
m-/p-Xylene (ng/mL) (N=1015)	**N=912**	**N=103**				
Log (Continuous)			1.16 (0.79-1.70)	1.07 (0.74-1.54)	0.99 (0.65-1.51)	1.04 (0.70-1.53)
E0 (BLD)	308	31	1.00	1.00	1.00	1.00
E1 (0.110-0.200)	299	39	1.35 (0.76-2.38)	1.34 (0.73-2.46)	1.24 (0.61-2.50)	1.32 (0.68-2.54)
E2 (0.200-5.600)	305	33	1.18 (0.54-2.62)	1.05 (0.47-2.34)	0.90 (0.35-2.28)	0.97 (0.40-2.37)
o-xylene (ng/mL) (N=1036)	**N=927**	**N=109**				
Log (Continuous)			1.09 (0.62-1.89)	0.92 (0.51-1.68)	0.87 (0.44-1.71)	0.88 (0.46-1.71)
E0 (BLD)	539	63	1.00	1.00	1.00	1.00
E1 (0.024-0.063)	194	23	1.08 (0.58-1.99)	0.90 (0.47-1.72)	0.91 (0.44-1.90)	0.92 (0.46-1.84)
E2 (0.064-1.500)	194	23	1.16 (0.63-2.17)	0.91 (0.47-1.76)	0.84 (0.41-1.70)	0.85 (0.42-1.70)
Ethylbenzene (ng/mL) (N=952)	**N=852**	**N=100**				
Log (Continuous)			1.13 (0.73-1.73)	1.03 (0.68-1.57)	0.92 (0.55-1.55)	0.99 (0.61-1.59)

E0 (BLD)	266	28	1.00	1.00	1.00	1.00
E1 (0.019-0.046)	290	39	1.40 (0.73-2.68)	1.43 (0.71-2.88)	1.38 (0.62-3.06)	1.49 (0.69-3.21)
E2 (0.047-1.600)	296	33	1.22 (0.59-2.53)	1.05 (0.49-2.23)	0.83 (0.34-2.06)	0.96 (0.42-2.22)
Styrene (ng/mL) (N=966)	**N=865**	**N=101**				
Log (Continuous)			1.19 (0.93-1.52)	1.13 (0.90-1.42)	1.09 (0.83-1.44)	1.10 (0.86-1.41)
E0 (BLD)	431	47	1.00	1.00	1.00	1.00
E1 (0.015-0.065)	217	27	1.15 (0.67-1.99)	1.03 (0.59-1.78)	0.95 (0.48-1.86)	0.99 (0.53-1.85)
E2 (0.065-4.220)	217	27	1.28 (0.66-2.51)	1.20 (0.61-2.38)	1.08 (0.49-2.40)	1.11 (0.51-2.40)
1, 4-dichlorobenzene (ng/mL) (N=945)	**N=843**	**N=102**				
Log (Continuous)			1.17 (0.95-1.44)	1.10 (0.84-1.44)	1.08 (0.78-1.51)	1.09 (0.80-1.48)
E0 (BLD)	396	37	1.00	1.00	1.00	1.00
E1 (0.055-0.330)	226	30	1.89 (1.15-3.09)**	1.82 (0.95-3.46)	1.75 (0.78-3.94)	1.71 (0.83-3.51)
E2 (0.330-33.000)	221	35	1.86 (0.88-3.95)	1.77 (0.69-4.51)	1.69 (0.51-5.65)	1.74 (0.60-5.03)

[a]Adjusted for NHANES year cycle

[b]Adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle

[c]Adjusted for the same covariates as model 2 and alcohol, BMI (kg/m^2), and smoking

[d]Adjusted for the same covariates as model 2 and alcohol, BMI (kg/m^2), and cotinine (ng/mL)

*BLD = Below the limit of detection

** Significant association

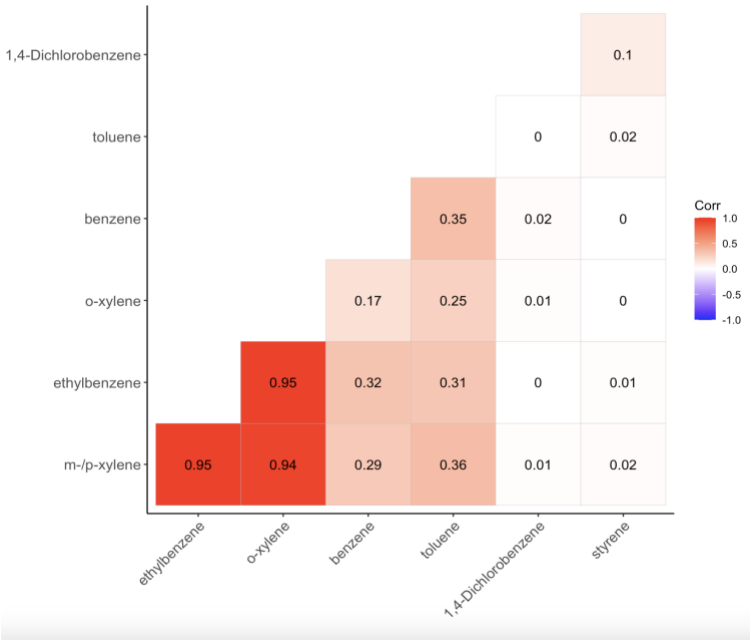

Figure 2. Pearson correlation analysis among specific VOCs

Table 12: Posterior probabilities of inclusion (PIPs) of VOCs in the probit-BKMR model

VOCs	group	groupPIP	conditional PIP[a]
Benzene	1	0.18	
Toluene	2	0.18	
1,4-dichlorobenzene	3	0.09	
Styrene	4	0.19	
Ethylbenzene	5	0.54	0.26
m-/p-Xylene	5	0.54	0.16
o-xylene	5	0.54	0.60

[a]Conditional PIPs are only estimated for multi-exposure groups.

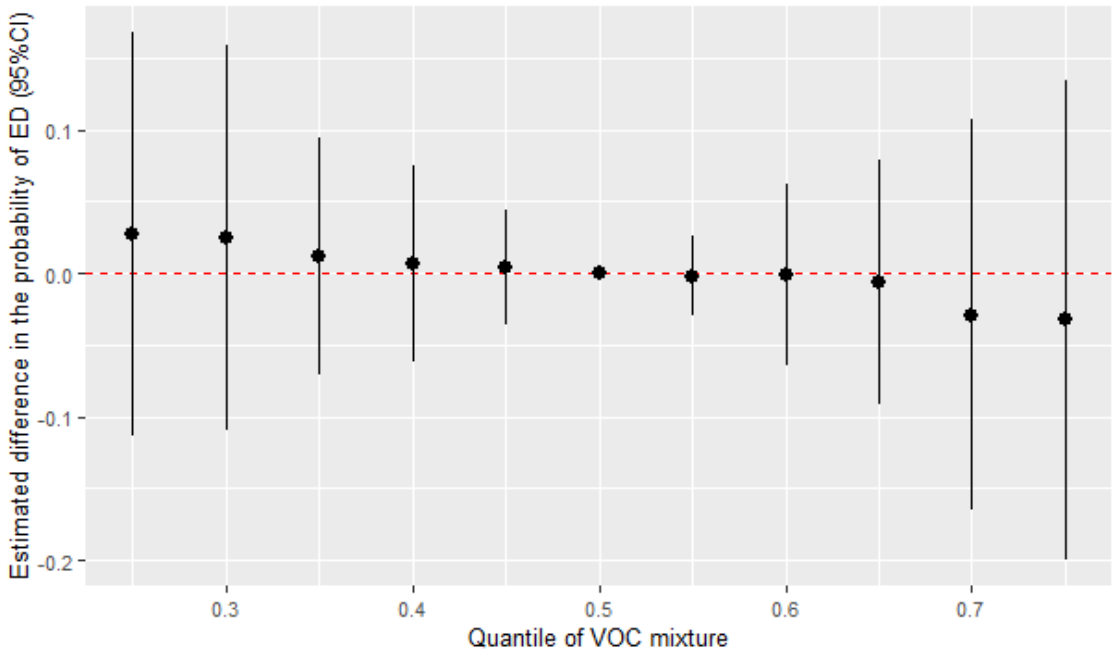

Figure 3. Overall effect of the VOCs mixture on ED

Overall effect (95% CI) of the VOCs mixture on ED when all the VOCs at particular percentiles (x axis) were compared to all the VOCs at their 50th percentile (median). The probit-BKMR model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle.

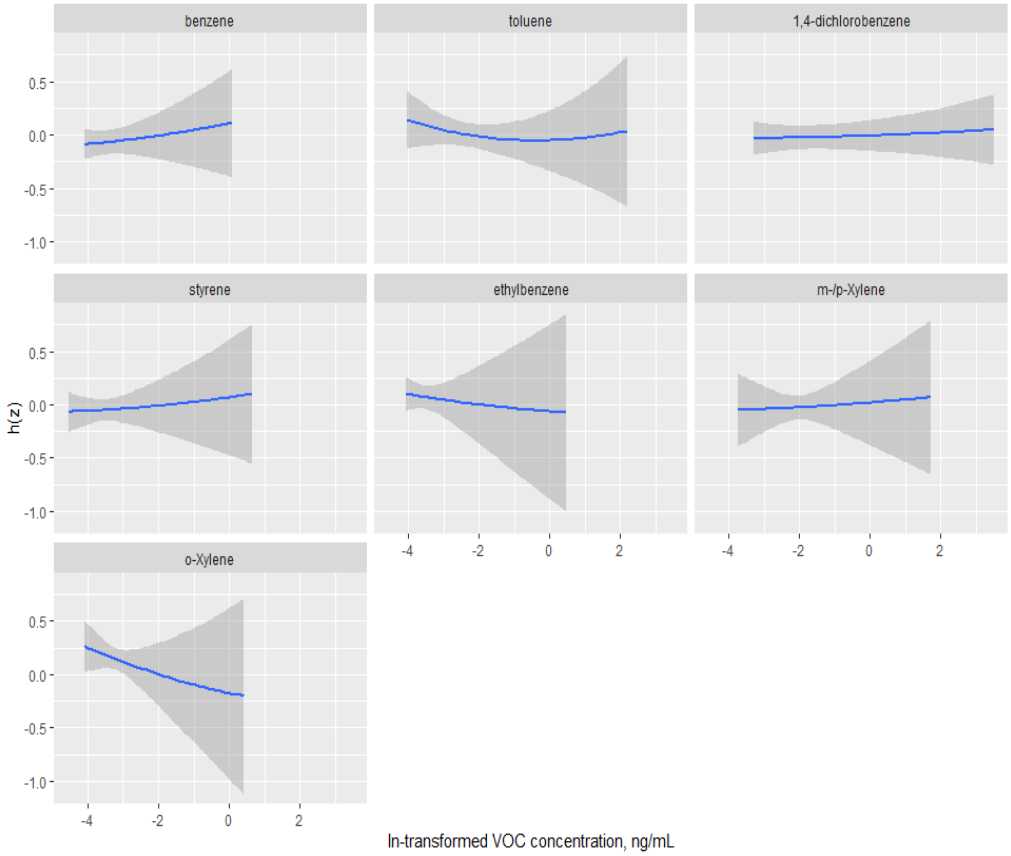

Figure 4. Univariate exposure–response function

Univariate exposure–response function with 95% CI (shaded areas) for each VOCs with the other VOCs fixed at the median values (50th percentile). h(z) can be interpreted as the relationship between each VOCs and latent continuous ED (continuous marker of the binary ED outcome). The probit-BKMR model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle.

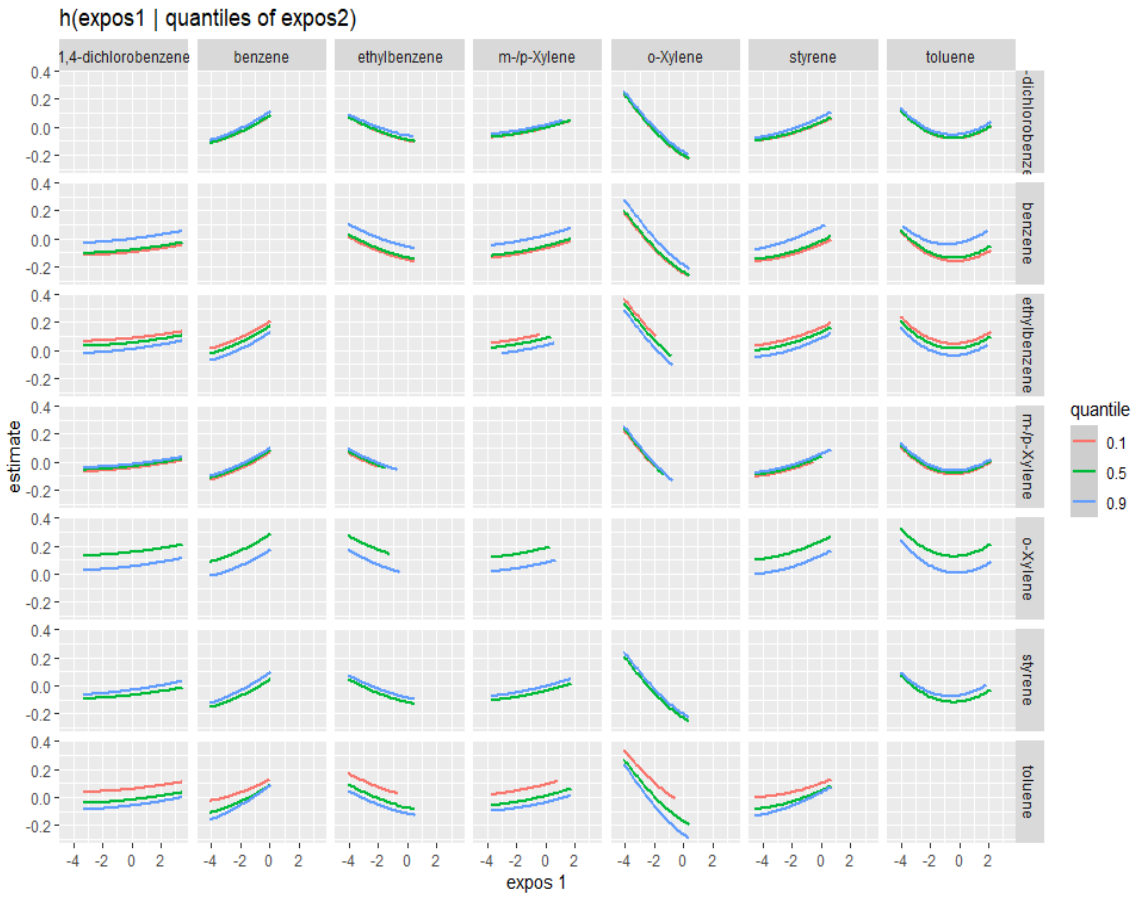

Figure 5. Bivariate exposure response functions

Bivariate exposure response functions for each VOCs presented on x-axis when VOCs on the y-axis were fixed at 10% (red line), 50% (green line), and 90% (blue line) percentile respectively, and other VOCs were fixed at their median levels. The probit-BKMR model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle.

Table 13: Odds ratios (95% confidence intervals) for each VOCs and ED among non-smokers

VOCs	No ED	ED	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 3[c] OR (95% CI)
Benzene (ng/mL) (N=549)	**N=499**	**N=50**			
Log (Continuous)			0.65 (0.32-1.33)	0.60 (0.28-1.31)	0.54 (0.21-1.40)
E0 (BLD*)	287	33	1.00	1.00	1.00
E1 (0.024-0.038)	109	6	0.56 (0.20-1.60)	0.50 (0.15-1.74)	0.49 (0.11-2.21)
E2 (0.038-0.860)	103	11	0.80 (0.29-2.20)	0.69 (0.24-1.97)	0.62 (0.16-2.47)
Toluene (ng/mL) (N=560)	**N=511**	**N=49**			
Log (Continuous)			0.81 (0.50-1.32)	0.77 (0.49-1.19)	0.75 (0.46-1.21)
E0 (BLD)	167	20	1.00	1.00	1.00
E1 (0.061-0.130)	171	16	0.90 (0.36-2.27)	0.92 (0.33-2.55)	0.98 (0.33-2.87)
E2 (0.130-6.700)	173	13	0.96 (0.33-2.76)	0.89 (0.32-2.45)	0.87 (0.29-2.64)
m-/p-Xylene (ng/mL) (N=565)	**N=517**	**N=48**			
Log (Continuous)			0.68 (0.40-1.14)	0.62 (0.36-1.08)	0.61 (0.32-1.15)
E0 (BLD)	171	18	1.00	1.00	1.00
E1 (0.095-0.16)	167	21	1.51 (0.74-3.08)	1.40 (0.62-3.14)	1.50 (0.63-3.55)
E2 (0.16-3.6)	179	9	0.49 (0.13-1.85)	0.42 (0.11-1.66)	0.41 (0.08-2.05)
o-xylene (ng/mL) (N=581)	**N=528**	**N=53**			
Log (Continuous)			0.48 (0.21-1.05)	0.46 (0.21-1.03)	0.42 (0.16-1.05)
E0 (BLD)	350	38	1.00	1.00	1.00
E1 (0.024-0.063)	87	10	1.35 (0.57-3.23)	1.11 (0.46-2.71)	1.18 (0.49-2.85)
E2 (0.064-1.2)	91	5	0.50 (0.15-1.70)	0.42 (0.11-1.66)	0.36 (0.08-1.74)
Ethylbenzene (ng/mL) (N=535)	**N=487**	**N=48**			
Log (Continuous)			0.56 (0.25-1.24)	0.50 (0.22-1.15)	0.47 (0.18-1.18)

E0 (BLD)	207	22	1.00	1.00	1.00
E1 (0.0185-0.038)	132	21	1.81 (0.87-3.78)	1.80 (0.79-4.12)	1.98 (0.76-5.13)
E2 (0.038-0.87)	148	5	0.38 (0.07-2.02)	0.30 (0.05-1.80)	0.28 (0.04-2.12)
Styrene (ng/mL) (N=535)	**N=486**	**N=49**			
Log (Continuous)			0.73 (0.34-1.55)	0.74 (0.35-1.57)	0.65 (0.26-1.62)
E0 (BLD)	326	36	1.00	1.00	1.00
E1 (0.015-0.065)	80	7	0.91 (0.37-2.23)	0.79 (0.32-1.91)	0.74 (0.25-2.15)
E2 (0.0512-1.9)	80	6	0.69 (0.15-3.17)	0.73 (0.18-3.03)	0.60 (0.11-3.12)
1, 4-dichlorobenzene (ng/mL) (N=536)	**N=485**	**N=51**			
Log (Continuous)			1.21 (0.90-1.62)	1.23 (0.87-1.74)	1.23 (0.79-1.90)
E0 (BLD)	241	19	1.00	1.00	1.00
E1 (0.0546-0.3)	119	19	2.41 (0.94-6.16)	2.63 (0.93-7.42)	2.57 (0.74-8.92)
E2 (0.3-32.0)	125	13	1.65 (0.52-5.30)	1.88 (0.53-6.75)	1.88 (0.40-8.88)

[a]Adjusted for NHANES year cycle

[b]Adjusted for age, race/ethnicity, education level, family income to poverty ratio, marital status, and NHANES year cycle

[c]Adjusted for the same covariates as model 2 and alcohol, and BMI (kg/m^2)

*BLD = Below the limit of detection

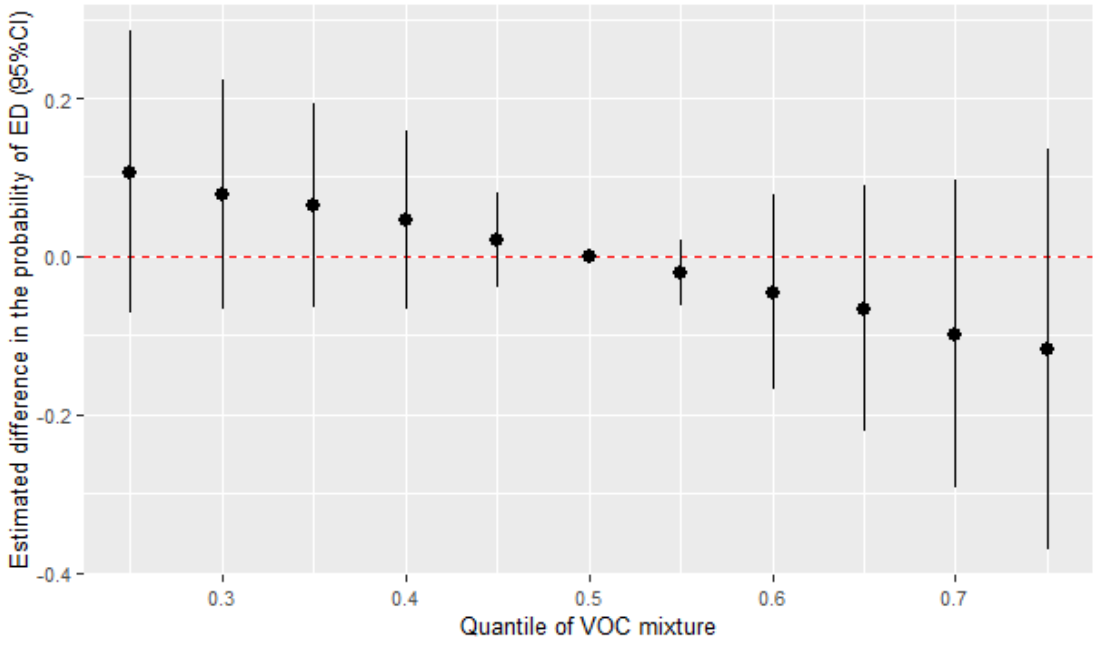

Figure 6. Overall effect of the VOCs mixture on ED among non-smokers

Overall effect (95% CI) of the VOCs mixture on ED (among non-smokers) when all the VOCs at particular percentiles (x axis) were compared to all the VOCs at their 50th percentile (median). The probit-BKMR model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle.

CHAPTER FOUR

AIM 3: BLOOD VOCs AND TESTOSTERONE DEFICIENCY

INTRODUCTION

Testosterone, an important hormone for reproduction, is secreted from the Leydig cells of the testes in men (260). When there is insufficient production of this hormone, it may result in testosterone deficiency (TD) (261). TD is a common condition among older men, especially those above 45 years, and its prevalence ranges from 10 to 40% globally (95). In the US, about 38.7% of males at least 45 years old have been reported to have TD (91). Aside from the known risk factors such as age (262), lifestyle factors (263, 264), and comorbidities (91, 264), such as obesity and diabetes, TD may also be influenced by environmental factors. A 2020 study examined the effect of some environmental factors, including smoking/cotinine, lead, and cadmium, on TD using the NHANES dataset. However, none of these exposures were significantly associated with TD (265). Volatile organic compounds (VOCs) are ubiquitous in nature, with the majority of human exposure observed in indoor spaces (266). The sources of VOCs may be natural or anthropogenic (267). Tobacco smoke, a predominant source of VOCs in humans, contains VOCs like benzene, toluene, xylenes, styrene, and 2,5-dimethylfuran [2,5-DMF] (268).

The prolonged exposure to VOCs may be detrimental to human health, possibly affecting prostate function and sex hormones (187, 269).

VOCs have been linked to various chronic conditions, including reproductive outcomes (187, 270, 271). Some studies have identified VOCs like benzene, toluene, xylene, and ethylbenzene as endocrine disruptors (227, 271). Previous studies have linked VOCs to both female and male sex hormone levels (187, 270). The result from an animal study on rats showed that parental exposure to toluene may reduce testosterone levels in their offspring (272). A recent study using NHANES data observed a significant positive association between blood Furan/ blood 2,5-Dimethylfuran and testosterone after adjusting for potential confounders (187). Similarly, occupational exposure to toluene has been linked to testosterone levels (273). Some other studies have observed contrasting results with no significant effects of VOCs on testosterone. Luderer et al. (274) study reported no significant association between toluene and testosterone.

Some studies have reported that the levels of VOCs are higher in smokers than non-smokers (275). In addition, there are several studies on the effect of smoking on testosterone levels. Many of these studies reported higher testosterone levels among smokers compared to non-smokers (276-279). Therefore, it may be important to understand the impact of smoking on the association between VOCs and TD. To date, there is currently no study that explored the effect of VOCs on TD among non-smokers. This study aimed to examine the effect of VOCs (individually and jointly) on TD using the National Health and Nutrition Examination Survey (NHANES) data. In addition, a supplementary analysis was performed to investigate the association between VOCs and TD among non-smokers.

METHODS

Study Population

The National Health and Nutrition Examination Survey (NHANES) data was used to examine the effect of VOCs on testosterone deficiency. NHANES is a study designed by the National Center for Health Statistics (NCHS) unit of the Centers for Disease Control and Prevention to assess the health and nutrition of Americans using interviews, physical examinations, and laboratory tests. NHANES utilizes the probability sampling technique to oversample the underrepresented groups in the survey (234, 235). The present study included adult male participants from the 2001-2002, 2003-2004, 2011-2012, 2013-2014, and 2015-2016 NHANES cycles (https://www.cdc.gov/nchs/nhanes/index.htm). Participants who were females (N=26,011), below 20 years (N=11,853), and with missing testosterone levels (N=5098) were excluded from the study. After these exclusions, a total of 4013 participants were eligible for the study (Figure 7).

Assessment of TD

Data on testosterone was obtained from the laboratory data file in the 2001-2004 and 2011-2016 NHANES cycles. During the laboratory examination, blood samples were collected and sent to the laboratory for analysis. Total testosterone (TT) was measured in the serum using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) after dissociating binding proteins and other interfering substances, such as the lipid, phospholipid, and polar lipid fractions. For the purpose of the current study, TD

was defined according to the American Urological Association definition, which is TT<300ng/dL (280).

Measurement of Blood VOCs

The common VOCs in the five NHANES year cycle were examined, which were 15 in total. After the removal of water-based VOCs and the VOCs in which the addition of the percentage below the limit of detection and missing was greater than 90%, six VOCs were used in the analysis. They include benzene, toluene, m-/p xylene, o-xylene, 1,4-dichlorobenzene, and ethylbenzene. Each of the VOCs was split into four categories (T0-T3). The T0 group represented the below the detection group, while the remaining values were split into three equal groups (T1-T3). Only a few participants were exposed to toluene below the limit of detection. Therefore, toluene was split into four equal groups (**Figure 7**).

The selected VOCs were measured in human blood using a combination of the stratospheric solid phase microextraction (SPME) headspace vial, gas chromatography, and isotope dilution mass spectrometry (281). These processes help reduce unwanted substances or chemicals in the blood. Each VOC has a lower limit of detection (LLOD), as indicated on the NHANES website (https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/VOCWB_I.htm). When a participant's VOC level is examined, and it is below the LLOD, the actual results are replaced with an imputed value using the formula: lower limit of detection divided by the square root of 2 (LLOD/sqrt [2]).

Of the 4013 eligible participants, VOC levels were available on a subset. The sample sizes for VOCs are benzene (N= 3694), toluene (N= 2662), m-/p xylene (N=

3731), o-xylene (N= 3800), ethylbenzene (N= 3698), and 1,4-dichlorobenzene (N=3752) (Figure 7).

Covariates

The covariates included in the model were identified using previous research and included in the model to minimize confounding (187, 261, 276). The demographic variables include age (continuous); race/ethnicity (categorized as: non-Hispanic White; non-Hispanic Black; Hispanic; and other); education (categized as: less than high school, high school, and above high school); marital status (categorized as: never married, married/living with partner, and widowed/divorced/separated); and family income-to-poverty ratio (categorized: less than poverty level (<1) and poverty level /above poverty level (>=1)). The lifestyle factors comprised of: BMI (categorized as : underweight (<18.5), normal weight (18.5 to <25), overweight (25 to <30), and obese (≥30 kg/m^2)); smoking (categorized as: never smoker (less than100 cigarettes during their lifetime represented never); former smoker (100 or more cigarettes in their entire life but not smoking at the time of the interview); and current smoker (100 or more cigarettes in their entire life and smoking every day or some days at the time of the interview or serum cotinine level above 10 ng/ml)); cotinine (continuous); and alcohol (categorized as: none (<1 drink per week), light (1–3 drinks per week), and heavy (≥4 drinks per week)) (240).

Statistical analysis

The descriptive table compared participants with and without TD. The data was presented as mean and standard deviation or count and percentages for continuous and

categorical variables respectively. Student's t-test was used for continuous variables, while Chi-square tests or Fisher's exact tests were used for categorical variables. Weighted multivariable logistic regression was used to evaluate the association between each VOC and TD. Svyglm in R was used to run the weighted logistic regression. Among the parameters or inputs of the Svyglm is the survey design, which includes variables such as sample weights, which are present in the NHANES dataset. The purpose of the sample weight is to make the sample representative of the US population by minimizing issues such as oversampling and non-response from participants. As stated on the NHANES website, the weight of the smallest subpopulation should be used for analysis. Therefore, the VOC subsample 2-year mobile examination center (MEC) weight (WTSVOC2Y) was used in this dissertation. In the weighted logistic regression, Model 1 was adjusted for NHANES year. For Model 2, adjustments were made for age, race/ethnicity, education level, poverty income ratio, marital status, and NHANES year cycle. Model 3 was adjusted for Model 2 covariates and body mass index (kg/m^2), alcohol, and smoking. Model 4 was adjusted for Model 2 covariates and body mass index (kg/m^2), alcohol, and cotinine (ng/mL). Furthermore, sub-analyses were performed to investigate the effects of each VOC and TD among non-smokers. The analyses were conducted using R version 4.1.0.

Bayesian kernel machine regression (BKMR) was used to explore the joint effect of VOCs on TD and detect interactions when present (241, 242). Specifically, probit-BKMR was used since the outcome is a binary variable. Markov chain Monte Carlo algorithm with 10,000 iterations was used for this analysis.

Following the test for correlations among the VOCs and the assignment of groups based on the Pearson correlation result, the hierarchical variable selection was used to select VOCs into the probit-bkmr model. The selection process involved two steps: the group selection and the individual VOC selection from each group. The hierarchical variable selection process at each step utilizes a parameter known as posterior inclusion probabilities (PIPs), which shows the significance of each group or individual VOCs in relation to TD. The higher the PIP, the better (PIP>0.5) (243). The PIP at the group selection stage, group PIP, indicates the group with the most contribution, and the conditional PIPs show the most important VOCs in each group in relation to TD.

The BKMR analysis provides three outputs. First, a visual representation of the overall effect of VOCs on TD, adjusting for all covariates. Second, the univariate effect of each VOC on TD when the other VOCs are fixed at their 50^{th} percentile. Third, the bivariate association of each pair of VOCs with TD when the VOCs in the rows are fixed at their 10th, 50th, and 90th percentile, and the rest of the VOC are fixed at their 50th percentiles. Statistical analysis was performed using R (version 4.1.0).

RESULTS

The baseline characteristics of the study participants by TD status are shown in (Table 14). Approximately 26.4% (N=1059) of the men in this study had testosterone deficiency. The result showed that race/ethnicity, education, and ratio of family income to poverty were not statistically different in the two groups with a p-value>0.05. Compared to males without TD, those with TD were more likely to be older, with an average age of

52.4 years; married/living with a partner; obese; former smokers; and non-drinkers. Also, participants in the TD groups were exposed to lower levels of benzene, toluene, m-/p-Xylene, o-xylene, and ethylbenzene than those in the no-TD group. There was no significant difference in 1,4-dichlorobenzene between the TD categories ($p>0.05$).

Weighted logistic regression was used to estimate the effect of each of the 6 VOCs on TD. Based on the result, there was a significant inverse association between benzene, measured continuously (ng/ml) and TD after adjusting for age, race/ethnicity, education level, poverty income ratio, marital status, alcohol, BMI, smoking, and NHANES year cycle (OR 0.82; 95% CI 0.69-0.98). Also, higher toluene levels were inversely associated with TD after adjusting for all covariates (T3 vs T0: OR 0.60; 95% CI 0.38-0.94 & continuous: OR 0.82; 95% CI 0.68-0.98). Compared to those exposed to ethylbenzene that was below the limit of detection (T0), those exposed to ethylbenzene between 0.041 and 0.079 ng/mL have 24% lower odds of having TD, after adjusting for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle (T2 vs T0: OR 0.76; 95% CI 0.60-0.96). No significant association was observed between m-/p-Xylene, o-xylene, or 1,4-dichlorobenzene and TD before and after adjusting for covariates (Table 15).

The Pearson correlation plot showed that the xylene group (m/p-xylene and o-xylene) and ethylbenzene were highly correlated (Figure 8). These VOCs were, therefore, placed in the same group (group 4). Based on the PIP result table, group 3 had the highest PIP (group PIP=0.84), which implies that 1,4-dichlorobenzene was the most important VOC in the VOC mixture-TD association (Table 16). Figure 9 shows the overall effect of the VOC mixture on TD after adjusting age, race/ethnicity, education level, poverty

income ratio, marital status, alcohol, BMI (kg/m^2), smoking, and NHANES year cycle. As shown in Figure 9, a higher exposure to the overall VOC mixture was associated with a lower probability of TD. Specifically, when all six VOCs were at 65th percentile and above, the probability of developing TD significantly decreased compared to when they were all fixed at 50th percentile (reference) after adjusting for all covariates.

The univariate plot in Figure 10 highlights the relationship between each VOC and TD when all the other VOCs are fixed at 50th percentile, adjusting for all covariates. There was a negative nonlinear relationship between 1,4-dichlorobenzene and TD when the remaining 5 VOCs were fixed at the 50th percentile. For benzene, toluene, m-/p-Xylene, and ethylbenzene, all the 95% CI (shaded area) included 0, indicating no significant relationship. Also, interactions between the VOCs were examined using the bivariate plot, where the VOCs on the y-axis were fixed at the 10th, 50th, and 90th percentile. There may be potential interactions between toluene and 1,4-dichlorobenzene in relation to TD, although statistical significance could not be evaluated (Figure 11).

The individual and joint effects of each VOC among nonsmokers were investigated using weighted logistic regression and probit-BKMR, respectively. The results of both the single (Table 17) and multi-pollutant model (Figure 12) showed no significant association after adjusting for all confounders.

DISCUSSION

The result from the current study, which utilized NHANES data for analyses, showed that benzene, toluene, and ethylbenzene were inversely associated with TD after adjusting for covariates; in other words, those with higher levels of these VOC's were less likely to have TD. However, when the data were restricted to non-smokers alone, these associations were no longer observed, and the effect estimates became positive in some cases. Similar results were observed in the multipollutant model, where the joint VOC mixture was negatively related to TD after adjusting for all covariates. In addition, no significant association was observed between the VOC mixture and TD among non-smokers using the probit-BKMR model.

Although a few studies have examined the relationship between VOCs and testosterone levels/TD, some of these studies investigated the VOC effect on the hormone (testosterone), not the clinical condition (TD). Lower rates of TD in a population would imply higher overall testosterone levels in that population. A recent study explored the effect of multiple VOCs on sex hormones among men in NHANES and observed a significant positive association between benzene and toluene with testosterone levels after adjusting for age, race/ethnicity, education level, poverty income ratio, and marital status (187). This study corroborates the results from the current study, in the population that included smokers.

Other studies examined individual VOC's such as toluene. Toluene is one of the most common chemical solvents used in industries. Therefore, it is not surprising that this VOC has been linked to various occupationally-related outcomes (282). Toluene is regarded as a neurotoxin, with high human exposure causing neurological conditions like

cognitive impairments, dizziness, and dementia (283). Toluene has been reported to inhibit hormones such as LH (284), which is produced by the pituitary gland and responsible for the simulation of the testes to secrete testosterone (285, 286). This may suggest that toluene is an endocrine disruptor. Aside from its neurotoxic effects, some studies have reported that exposure to toluene may result in direct reproductive organ damage, including DNA damage to the testes (287, 288). This effect was evident in an animal study, where the toluene-exposed rats observed oxidative damage to the testes, potentially leading to TD (287). Several occupational studies have reported varying results on the effect of toluene on testosterone levels. A study on men working in a printing company suggested a negative association between toluene and testosterone levels (273). Another study on those exposed to toluene-containing rotogravure printers observed lower median testosterone levels among the exposed men (289). Other studies have shown no significant effect on testosterone levels (274). Contrasting results were observed in the current study, with lower odds of TD when toluene was increased. A possible reason for the varying results may be that this research is not occupationally related. Also, there may be possible differences in the magnitude of exposure versus exposure due to smoking and everyday chemicals, as in the current study. As previously mentioned, the NHANES analysis examining multiple VOC's including toluene, observed similar results as this research (187).

Benzene is one of the VOCs that is carcinogenic to humans (290). Studies have shown that this pollutant may have reproductive toxicity (291, 292). A 2023 animal study reported a significant decline in testosterone levels when the adult male mice were exposed to 50 ppm of benzene compared to the controls. Exposure to benzene may result

in testicular damage, which may affect testosterone secretion (293). In addition to the direct reproductive organ damage, benzene has been identified as an endocrine disruptor, capable of interfering with the production of hormones, thus affecting sex hormones like testosterone (187, 294). Little is known about the effect of ethylbenzene on TD among men, although an animal study reported an increase in testosterone levels when female rats were exposed to ethylbenzene levels of 2000 and 8000 ppm (295).

There are several explanations for the inverse association observed between the VOCs, benzene, toluene, or ethylbenzene, and TD in this study. Smoking, a known source of many VOCs such as benzene, toluene, and ethylbenzene, has been suggested to be associated with higher testosterone levels. Several studies have found that those who smoke tend to have higher testosterone levels compared to non-smokers (276-278, 296, 297). A possible mechanism for this association is that the nicotine content of cigarette smoke and its metabolites, such as cotinine, exit the body via a similar route with androgens like testosterone. Specifically, nicotine, cotinine, and testosterone are removed from the body through glucuronidation, which utilizes enzymes to remove substances from the body. So, in the process of removal from the body, nicotine or cotinine may competitively prevent testosterone glucuronidation, thereby increasing the testosterone levels in the body (297-299). Another mechanism is through the increase in LH after exposure to cigarette smoke. Some studies have reported that smoking may stimulate the release of LH. Since LH is responsible for stimulating the Leydig cell in the testes to produce testosterone, it may, in turn, increase testosterone levels (300). In the current study, participants who smoked tended to have higher VOC levels (Table 18). The high

nicotine/cotinine levels among those exposed to more VOCs may thus be partially responsible for the negative association.

Another possible explanation for the inverse association is residual confounding. There may be other factors that have distorted the association between these VOCs and TD. Some characteristics associated with smoking may be driving this lower rate of TD. Individuals with higher levels of testosterone may be more likely to partake in unhealthy behaviors like smoking than men with low testosterone levels (301). Other confounding factors may be the occupations of these male participants.

A notable strength of the study is that this is the first study to explore the effect of VOC exposure on TD, a clinically important outcome, among non-smokers. Second, the large dataset (NHANES cycle 2001-2004 and 2011-2016) used also adds credibility to the result; the NHANES paper that examined testosterone levels only used data from (2013-2016). Another strength of this study was the use of the probit-BKMR model to detect interactions among the VOCs.

Several limitations were also present in the study. The first limitation is the cross-sectional nature of the study; temporality between VOC exposure and TD could not be established. A prospective study may be required to ascertain causality. Blood VOCs have a short half-life and may only reflect recent exposure. For example, the half-life of benzene may be up to 1.2 hours (302). Another limitation of the study is that the NHANES data measured testosterone in the eligible participants only once. However, based on the American Urological Association guideline, the diagnosis of low testosterone should be reported only after two measurements (280). This is to minimize individual testosterone variations. However, the blood collection occurred at the allocated

time, even though the times were not mentioned. Finally, there is a possibility of residual confounding from unadjusted variables such as occupation and personality differences. However, the restriction to nonsmokers at least partially addressed the residual confounding by smoking and characteristics observed among smokers.

CONCLUSION

In summary, this research showed that increased exposure to benzene, toluene, or ethylbenzene levels are associated with lower odds of having testosterone deficiency. However, after restricting the data to non-smokers only, these effects were no longer observed. This result further validated and expanded upon the results from previous studies using nationally representative data. Additional studies among nonsmokers are needed to confirm the result. Prospective studies may address the temporarily and further confirm the study findings.

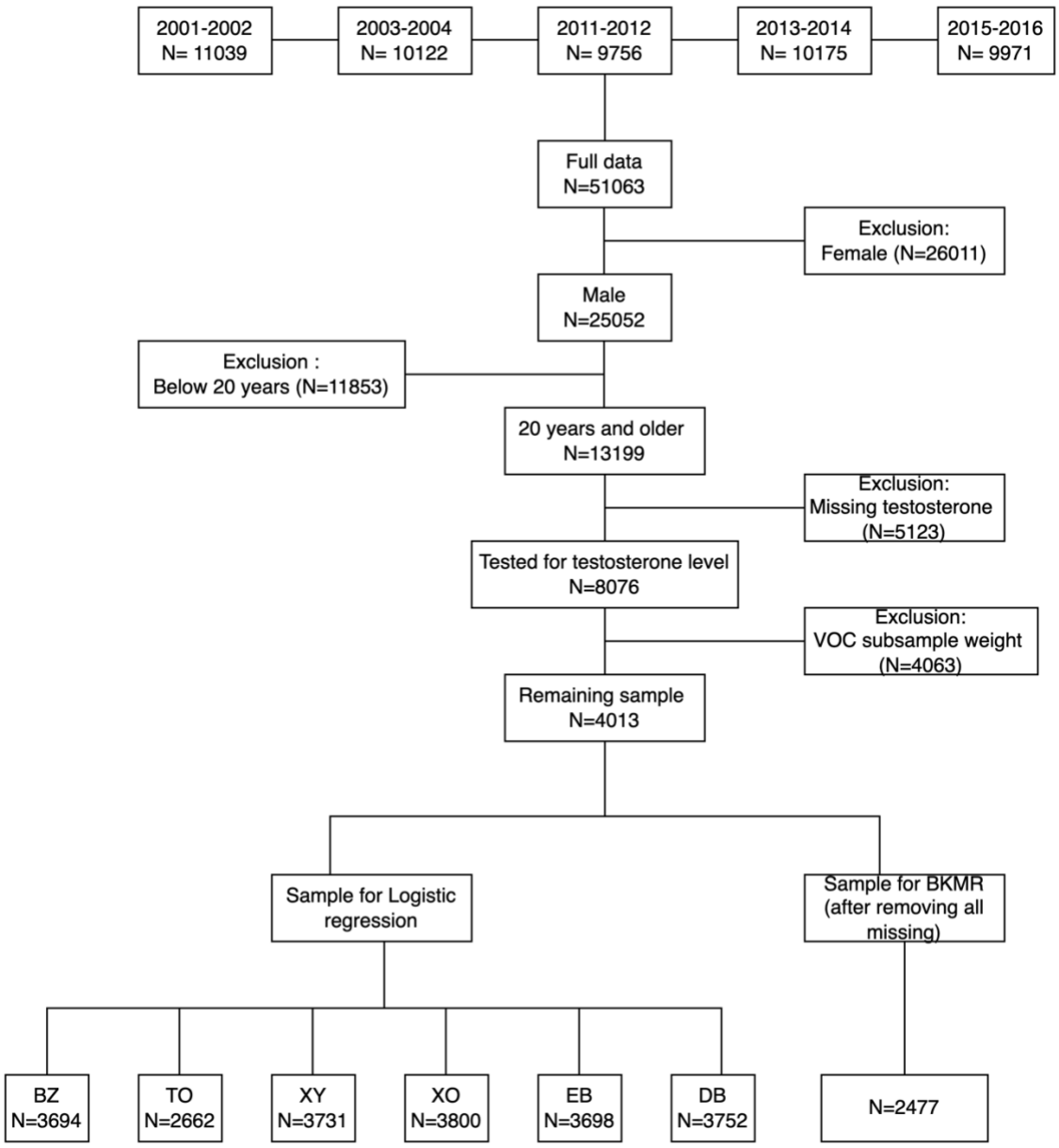

Figure 7. Flow chart of selected study participants for the association of VOCs and TD

BZ = Benzene; TO= Toluene; XY= m-/p xylene; XO=o-xylene; EB= ethylbenzene;

DB= 1,4-dichlorobenzene

Table 14: Participant characteristics by testosterone deficiency and no testosterone deficiency (2001-2004 & 2011-2016)

	No Testosterone deficiency (Testosterone >=300ng/dl) N=2954	Testosterone deficiency (Testosterone <300ng/dl) N=1059	P value
Age, Mean (SD)	46.9 (17.3)	52.4 (17.1)	<0.001
Race/Ethnicity, n (%)			0.137
Non-Hispanic white	1146 (38.8)	444 (41.9)	
Non-Hispanic Black	663 (22.4)	205 (19.4)	
Hispanic	700 (23.7)	246 (23.2)	
Other	445 (15.1)	164 (15.5)	
Education, n (%)			0.834
Less than high school	704 (23.8)	262 (24.7)	
High school	685 (23.2)	241 (22.8)	
Above high school	1565 (53.0)	556 (52.5)	
Marital status, n (%)			<0.001
Never married	689 (23.3)	150 (14.2)	
Married/Living with Partner	1838 (62.2)	757 (71.5)	
Widowed/Divorced/Separated	427 (14.5)	152 (14.4)	
BMI, n (%)			<0.001
Normal/Underweight	1048 (35.5)	147 (13.9)	
Overweight	1121 (37.9)	351 (33.1)	
Obese	760 (25.7)	539 (50.9)	
Missing	25 (0.8)	22 (2.1)	
Smoking, n (%)			<0.001
Never	1306 (44.2)	442 (41.7)	
Current	999 (33.8)	290 (27.4)	
Former	649 (22.0)	327 (30.9)	
Alcohol, n (%)			<0.001
None (<1 drink per week)	1130 (38.3)	489 (46.2)	
Light (1–3 drinks per week)	475 (16.1)	146 (13.8)	
Heavy (>=4 drinks per week)	892 (30.2)	247 (23.3)	

	No Testosterone deficiency (Testosterone >=300ng/dl) N=2954	Testosterone deficiency (Testosterone <300ng/dl) N=1059	P value
Missing	457 (15.5)	177 (16.7)	
Ratio of family income to poverty, n (%)			0.311
<1 (less than poverty level)	566 (19.2)	185 (17.5)	
≥1 (at or above poverty level; more affluent)	2147 (72.7)	776 (73.3)	
Missing	241 (8.2)	98 (9.3)	
Benzene (ng/ml), n (%)			<0.001
T0 (Below detectable limit)	1599 (59.2)	685 (69.1)	
T1	343 (12.7)	127 (12.8)	
T2	369 (13.7)	101 (10.2)	
T3	392 (14.5)	78 (7.9)	
Missing	251	68	
Toluene (ng/ml), n (%)			<0.001
T0 (Below detectable limit)	50 (2.5)	22 (3.3)	
T1	606 (30.4)	257 (38.6)	
T2	641 (32.1)	222 (33.4)	
T3	699 (35.0)	164 (24.7)	
Missing	958	394	
m-/p-Xylene (ng/ml), n (%)			<0.001
T0 (Below detectable limit)	637 (23.3)	259 (26.1)	
T1	658 (24.0)	287 (28.9)	
T2	690 (25.2)	255 (25.7)	
T3	754 (27.5)	191 (19.3)	
Missing	215	67	
o-xylene (ng/ml), n (%)			<0.001
T0 (Below detectable limit)	1747 (62.7)	703 (69.5)	
T1	334 (12.0)	116 (11.5)	
T2	350 (12.6)	100 (9.9)	
T3	357 (12.8)	93 (9.2)	

	No Testosterone deficiency (Testosterone >=300ng/dl) N=2954	Testosterone deficiency (Testosterone <300ng/dl) N=1059	P value
Missing	166	47	
1, 4-dichlorobenzene (ng/ml), n (%)			0.604
T0 (Below detectable limit)	1301 (47.4)	496 (49.5)	
T1	486 (17.7)	165 (16.5)	
T2	485 (17.7)	166 (16.6)	
T3	475 (17.3)	175 (17.5)	
Missing	207	57	
Ethylbenzene (ng/ml), n (%)			<0.001
T0 (Below detectable limit)	1639 (60.4)	692 (70.3)	
T1	345 (12.7)	111 (11.3)	
T2	356 (13.1)	100 (10.2)	
T3	373 (13.7)	82 (8.3)	
Missing	241	74	

Table 15: Odds ratios (95% confidence intervals) for each VOCs and TD

VOCs	No ED	ED	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 3[c] OR (95% CI)	Model 4[d] OR (95% CI)
Benzene (ng/mL) (N=3694)	**N=2703**	**N=991**				
Log (Continuous)			0.82 (0.73-0.92)**	0.83 (0.73-0.95)**	0.82 (0.69-0.98)**	0.86 (0.73-1.00)
T0 (BLD*)	1599	685	1.00	1.00	1.00	1.00
T1 (0.024-0.056)	343	127	0.96 (0.72-1.26)	0.98 (0.73-1.32)	0.97 (0.70-1.35)	1.01 (0.72-1.40)
T2 (0.056-0.164)	369	101	0.74 (0.57-0.97)**	0.78 (0.60-1.02)	0.74 (0.53-1.03)	0.81 (0.59-1.10)
T3 (0.166-2.750)	392	78	0.58 (0.37-0.92)**	0.60 (0.36-0.99)**	0.60 (0.33-1.10)	0.66 (0.38-1.15)
Toluene (ng/mL) (N=2661)	**N=1996**	**N=665**				
Log (Continuous)			0.87 (0.75-1.00)	0.86 (0.74-1.00)	0.82 (0.68-0.98)**	0.85 (0.72-1.00)
T0 (BLD)	468	198	1.00	1.00	1.00	1.00
T1 (0.051-0.083)	475	190	0.97 (0.69-1.38)	0.98 (0.70-1.38)	0.94 (0.64-1.40)	0.96 (0.65-1.41)
T2 (0.083-0.227)	509	156	0.88 (0.65 -1.18)	0.88 (0.66-1.19)	0.80 (0.57-1.11)	0.86 (0.63-1.17)
T3 (0.227-14.70)	544	121	0.69 (0.49-0.98)**	0.69 (0.48-1.01)	0.60 (0.38-0.94)**	0.67 (0.45-1.00)
m-/p-Xylene (ng/mL) (N=3731)	**N=2739**	**N=992**				
Log (Continuous)			0.90 (0.81-1.00)	0.89 (0.80-1.00)	0.90 (0.79-1.02)	0.90 (0.80-1.02)
T0 (BLD)	637	259	1.00	1.00	1.00	1.00
T1 (0.034-0.062)	658	287	1.26 (0.95-1.66)	1.22 (0.92-1.62)	1.17 (0.88-1.56)	1.17 (0.88-1.56)
T2 (0.062-0.133)	690	255	1.10 (0.87-1.40)	1.06 (0.82-1.35)	0.97 (0.75-1.26)	0.99 (0.76-1.28)
T3 (0.133-22.00)	754	191	0.84 (0.66-1.07)	0.81 (0.62-1.06)	0.80 (0.58-1.09)	0.82 (0.60-1.11)
o-xylene (ng/mL) (N=1036)	**N=2788**	**N=1012**				
Log (Continuous)			0.87 (0.74-1.03)	0.86 (0.72-1.02)	0.86 (0.72-1.02)	0.86 (0.72-1.02)
T0 (BLD)	1747	703	1.00	1.00	1.00	1.00
T1 (0.024-0.034)	334	116	0.89 (0.65-1.21)	0.86 (0.63-1.17)	0.79 (0.54-1.14)	0.80 (0.56-1.16)
T2 (0.034-0.055)	350	100	0.83 (0.64-1.08)	0.79 (0.60-1.04)	0.75 (0.55-1.01)	0.76 (0.56-1.02)

T3 (0.055-4.61)	357	93	0.75 (0.53-1.07)	0.74 (0.51-1.08)	0.74 (0.51-1.08)	0.75 (0.52-1.10)
Ethylbenzene (ng/mL) (N=3698)	**N=2713**	**N=985**				
Log (Continuous)			0.82 (0.71-0.95)**	0.81 (0.69-0.96)**	0.83 (0.68-1.02)	0.85 (0.71-1.02)
T0 (BLD)	1639	692	1.00	1.00	1.00	1.00
T1 (0.0185-0.041)	345	111	0.95 (0.75-1.21)	0.91 (0.70-1.17)	0.85 (0.64-1.13)	0.88 (0.68-1.15)
T2 (0.041-0.079)	356	100	0.77 (0.61-0.97)**	0.76 (0.60-0.96)**	0.75 (0.55-1.02)	0.80 (0.60-1.05)
T3 (0.079-8.180)	373	82	0.63(0.40-1.02)	0.63 (0.38-1.04)	0.67 (0.39-1.17)	0.71 (0.42-1.22)
1, 4-dichlorobenzene (ng/mL) (N=3749)	**N=2747**	**N=1002**				
Log (Continuous)			0.96 (0.90-1.02)	0.97 (0.90-1.03)	0.95 (0.88-1.03)	0.95 (0.88-1.03)
T0 (BLD)	1301	496	1.00	1.00	1.00	1.00
T1 (0.040-0.095)	486	165	0.80 (0.60-1.06)	0.80 (0.60-1.07)	0.79 (0.59-1.05)	0.79 (0.59-1.06)
T2 (0.095-0.325)	485	166	0.91 (0.67-1.23)	0.95 (0.71-1.29)	0.94 (0.68-1.32)	0.94 (0.67-1.31)
T3 (0.325-82.6)	475	175	1.00 (0.75-1.33)	1.03 (0.75-1.40)	0.96 (0.67-1.37)	0.96 (0.67-1.37)

[a]Adjusted for NHANES year cycle

[b]Adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, and NHANES year cycle

[c]Adjusted for the same covariates as model 2 and alcohol, BMI (kg/m^2), and smoking

[d]Adjusted for the same covariates as model 2 and alcohol, BMI (kg/m^2), and cotinine (ng/mL)

*BLD = Below the limit of detection

** Statistically significant

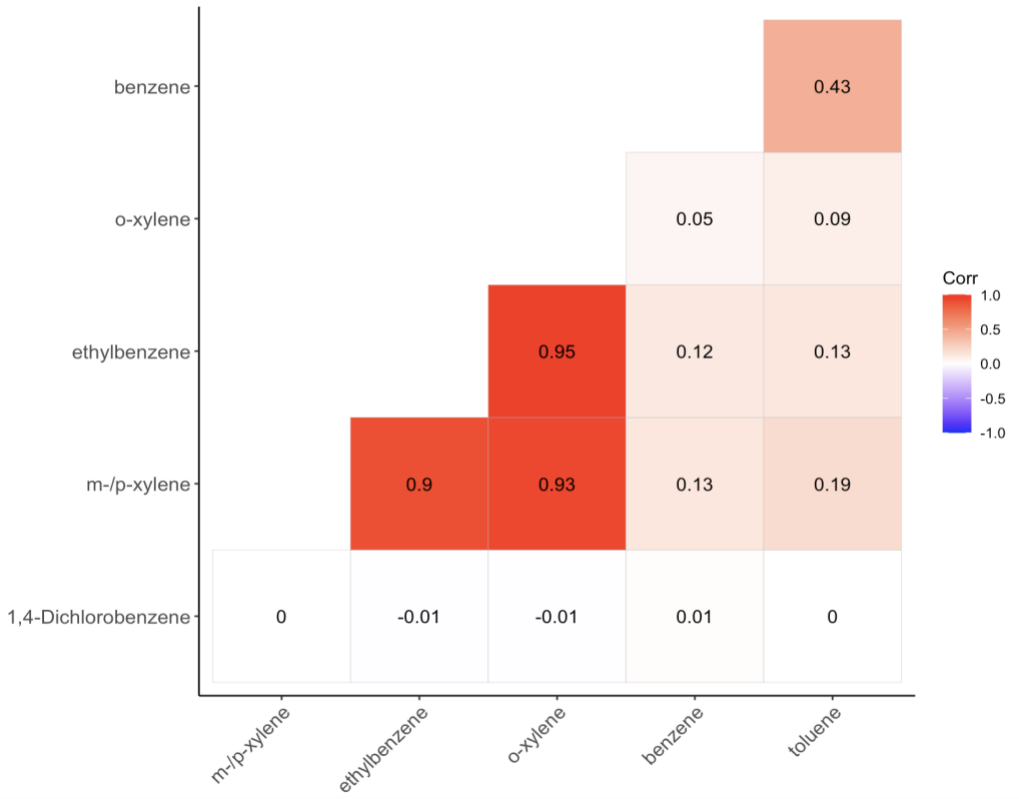

Figure 8. Pearson correlation analysis among specific VOCs

Table 16: Posterior probabilities of inclusion (PIPs) of VOCs in the probit-BKMR model

VOCs	group	group PIP	conditional PIP[a]
Benzene	1	0.42	
Toluene	2	0.22	
1,4-dichlorobenzene	3	0.84	
Ethylbenzene	4	0.74	0.12
m-/p-Xylene	4	0.74	0.00
o-xylene	4	0.74	0.88

[a]Conditional PIPs are only estimated for multi-exposure groups.

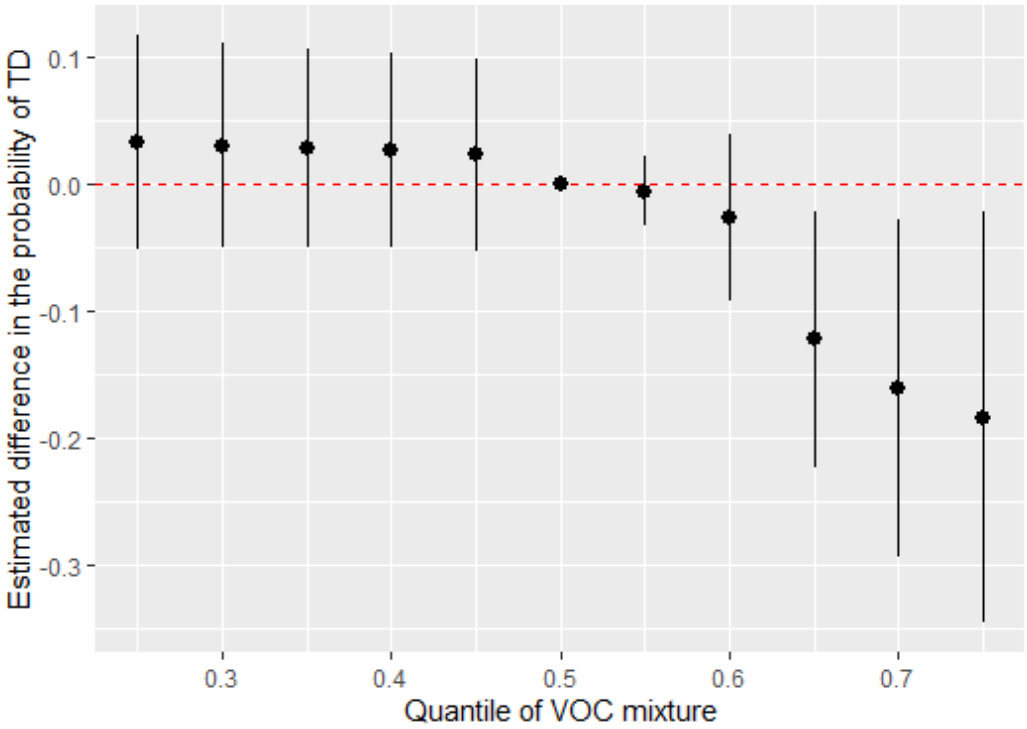

Figure 9. Overall effect of the VOCs mixture on TD

Overall effect (95% CI) of the VOC mixture on TD when all the VOCs at particular percentiles were compared to all the VOCs at their 50th percentile (median).

The results were assessed by the probit-BKMR model, adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, alcohol, BMI (kg/m^2), and smoking, and NHANES year cycle.

*Dots indicate the estimates and black vertical lines represent 95% CI

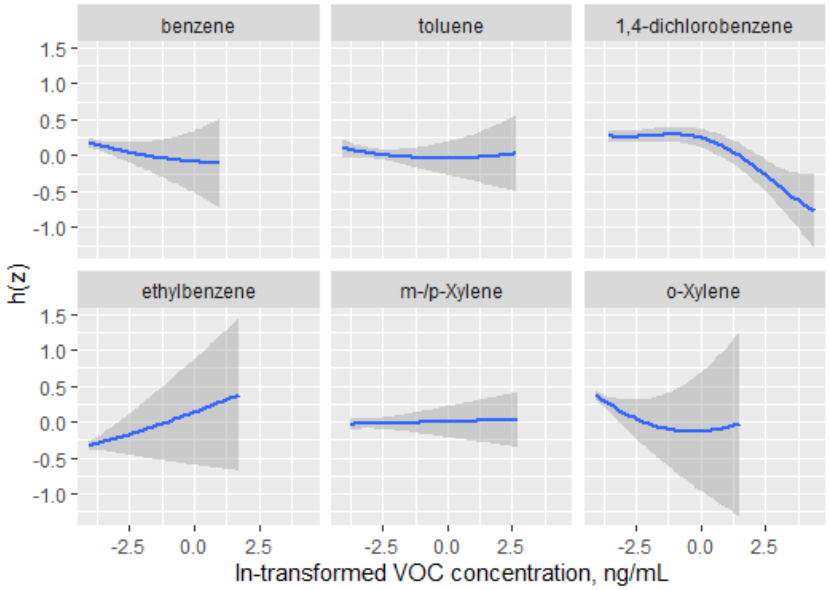

Figure 10. Univariate exposure–response function

Univariate exposure–response function with 95% CI (shaded areas) for each VOCs with the other VOCs fixed at the median values (50th percentile).

h(z) can be interpreted as the relationship between VOCs and the probability of developing TD.

BKMR model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, alcohol, BMI (kg/m^2), and smoking, and NHANES year cycle.

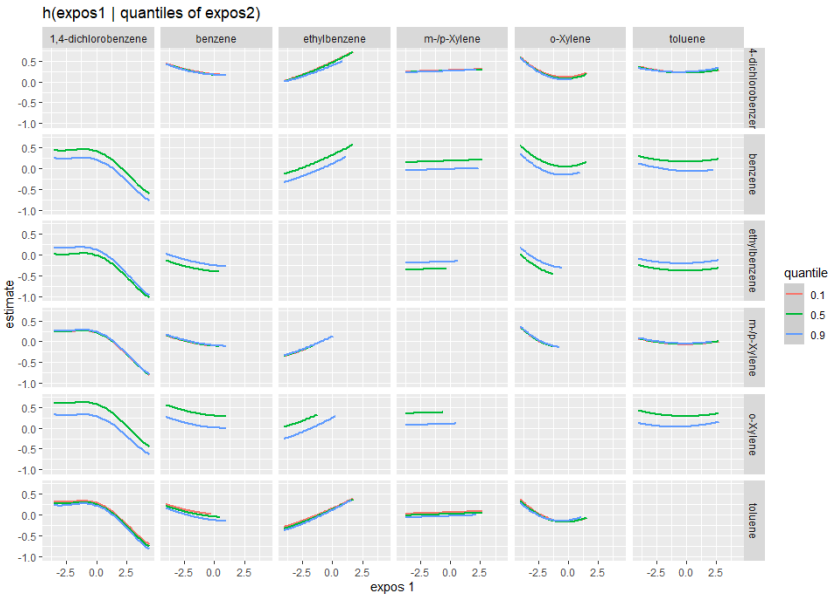

Figure 11. Bivariate exposure response functions

Bivariate exposure response functions for each VOCs presented on x-axis when VOCs on y-axis was fixed at 10% (red line), 50% (green line), and 90% (blue line) percentile respectively, and other VOCs were fixed at their median levels. Model was adjusted for age, race/ethnicity, education level, poverty income ratio, and marital status, alcohol, BMI (kg/m^2), and smoking, and NHANES year cycle.

Table 17: Odds ratios (95% confidence intervals) for each VOCs and TD among non-smokers

VOCs	No TD	TD	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 3[c] OR (95% CI)
Benzene (ng/mL) (N=2513)	**N=1786**	**N=727**			
Log (Continuous)			1.01 (0.84-1.22)	1.00 (0.82-1.22)	1.04 (0.85-1.29)
T0 (BLD*)	1458	613	1.00	1.00	1.00
T1 (0.024-0.033)	103	45	1.11 (0.74-1.68)	1.10 (0.69-1.76)	1.06 (0.63-1.79)
T2 (0.034-0.052)	111	36	0.99 (0.54-1.83)	0.97 (0.52-1.81)	0.95 (0.49-1.85)
T3 (0.052-0.650)	114	33	1.14 (0.76-1.71)	1.11 (0.74-1.69)	1.21 (0.77-1.90)
Toluene (ng/mL) (N=1781)	**N=1301**	**N=480**			
Log (Continuous)			1.06 (0.88-1.29)	1.03 (0.85-1.26)	1.00 (0.82-1.22)
T0 (BLD)	313	133	1.00	1.00	1.00
T1 (0.043-0.061)	320	125	1.05 (0.68-1.61)	1.03 (0.67-1.59)	1.05 (0.65-1.69)
T2 (0.061-0.098)	325	120	0.97 (0.64-1.48)	0.97 (0.63-1.49)	0.99 (0.61-1.61)
T3 (0.098-14.70)	343	102	1.03 (0.67-1.59)	0.97 (0.62-1.51)	0.93 (0.58-1.48)
m-/p-Xylene (ng/mL) (N=2530)	**N=1799**	**N=731**			
Log (Continuous)			1.09 (0.98-1.22)	1.05 (0.93-1.18)	1.03 (0.90-1.18)
T0 (BLD)	590	236	1.00	1.00	1.00
T1 (0.034-0.051)	383	185	1.31 (0.95-1.82)	1.29 (0.93-1.79)	1.23 (0.89-1.70)
T2 (0.051-0.083)	396	172	1.34 (0.94-1.91)	1.27 (0.89-1.82)	1.19 (0.82-1.75)
T3 (0.083-22.00)	430	138	1.12 (0.88-1.41)	1.00 (0.77-1.29)	0.91 (0.69-1.21)
o-xylene (ng/mL) (N=2581)	**N=1840**	**N=741**			
Log (Continuous)			1.07 (0.89-1.29)	1.03 (0.84-1.25)	1.01 (0.82-1.25)
T0 (BLD)	1441	588	1.00	1.00	1.00
T1 (0.024-0.031)	129	55	0.97 (0.55-1.70)	0.91 (0.51-1.61)	0.78 (0.42-1.48)
T2 (0.031-0.051)	133	51	1.08 (0.72-1.61)	0.96 (0.63-1.45)	0.90 (0.60-1.36)

T3 (0.051-4.600)	137	47	1.02 (0.67-1.54)	0.95 (0.62-1.45)	0.91 (0.58-1.43)
Ethylbenzene (ng/mL) (N=2514)	**N=1793**	**N=721**			
Log (Continuous)			1.01 (0.82-1.25)	0.97 (0.77-1.22)	1.01 (0.78-1.30)
T0 (BLD)	1459	609	1.00	1.00	1.00
T1 (0.0185-0.03)	111	38	1.20 (0.73-1.95)	1.10 (0.66-1.83)	1.04 (0.61-1.79)
T2 (0.03-0.0470)	114	35	0.69 (0.41-1.16)	0.61 (0.35-1.04)	0.63 (0.36-1.12)
T3 (0.047-8.180)	109	39	1.24 (0.75-2.06)	1.18 (0.70-1.98)	1.23 (0.68-2.23)
1, 4-dichlorobenzene (ng/mL) (N=2556)	**N=1820**	**N=736**			
Log (Continuous)			1.02 (0.95-1.09)	1.02 (0.94-1.11)	1.00 (0.91-1.09)
T0 (BLD)	883	355	1.00	1.00	1.00
T1 (0.040-0.094)	323	117	0.85 (0.57-1.26)	0.84 (0.57-1.25)	0.86 (0.58-1.26)
T2 (0.094-0.321)	325	124	0.98 (0.67-1.43)	1.01 (0.69-1.49)	0.99 (0.64-1.54)
T3 (0.322-82.60)	299	140	1.26 (0.92-1.73)	1.28 (0.89-1.83)	1.20 (0.80-1.78)

[a]Adjusted for NHANES year cycle

[b]Adjusted for age, race/ethnicity, education level, family income to poverty ratio, marital status, and NHANES year cycle

[c]Adjusted for the same covariates as model 2 and alcohol, and BMI (kg/m^2)

*BLD = Below the limit of detection

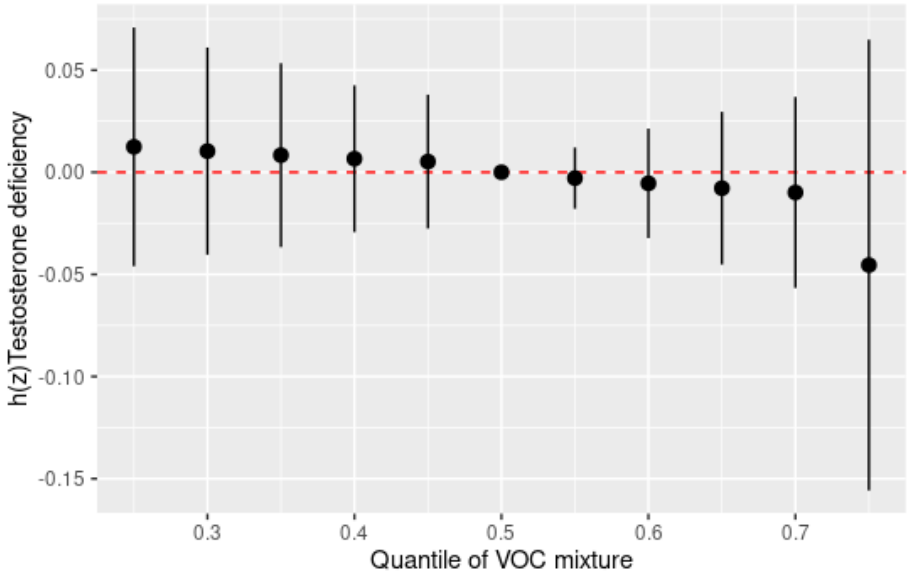

Figure 12: The overall effect of the VOC mixture on TD among non-smokers

Table 18: Table comparing median (IQR) of VOC's: current smokers vs other.

	Never N= 1,748	Current N= 1,289	Former N= 976	P value
Benzene (median [IQR])	0.02 (0.00)	0.11 (0.19)	0.02 (0.00)	<0.001
Toluene (median [IQR])	0.06 (0.06)	0.32 (0.47)	0.06 (0.06)	<0.001
Xylene (median [IQR])	0.05 (0.06)	0.15 (0.18)	0.05 (0.06)	<0.001
O-xylene (median [IQR])	0.02 (0.00)	0.03 (0.03)	0.02 (0.00)	<0.001
1,4, -dichlorobenzene (median [IQR])	0.05 (0.14)	0.05 (0.16)	0.04 (0.14)	0.104
ethylbenzene (median [IQR])	0.02 (0.00)	0.05 (0.07)	0.02 (0.00)	<0.001

CHAPTER FIVE

DISCUSSION

This dissertation investigated the effects of common (VOCs) and rare but toxic (tear gas) environmental exposures on common male reproductive issues. The result from the VOC analysis, which utilized NHANES data, showed that benzene, toluene, and ethylbenzene were inversely associated with TD; in other words, those with higher levels of these VOCs were less likely to have TD. However, when the data was restricted to non-smokers alone, these associations were no longer observed, and the effect estimates became positive in some cases. Higher levels of tear gas exposure, on the other hand, was strongly associated with higher risk of erectile dysfunction.

There are several studies available that have examined the effect of various environmental exposures on male sexual function (206, 212). These environmental factors have different pathways in which they may result in male reproductive health outcomes, such as erectile dysfunction (ED). Some environmental exposures like pesticides and radiation have been linked to low testosterone levels through hormonal disruption (303-305). These effects may potentially result in ED. Other air pollutants, such as $PM_{2.5}$, may have direct damage to the penile arteries, affecting penile blood flow, subsequently leading to ED (206, 306). In addition, some of these environmental contaminants, such as BPA and VOCs, are also regarded as endocrine disruptors, which through their interference in testosterone production and HPG axis may result in male

reproductive conditions, such as ED and ejaculatory dysfunction (59, 187, 227). Other environmental exposures that have been linked to these male sexual dysfunctions are lead (307, 308), 4,4′-Diaminostilbene-2,2′-disulfonic acid (DAS) (61), and arsenic (63).

Despite the body of work on the environmental exposure impact on male reproductive outcomes, there are other less common pollutants, such as tear gas, that have been understudied. In the past few years, there has been heightened use of tear gas by law enforcement officers to disperse crowds during protests and demonstrations (122), which has been a significant concern, primarily because of its effect on the population's health, potentially including reproductive health. These events and health concerns have been highlighted in various news reports and previous bills passed by US lawmakers. On October 27, 2023, there was a news report on a protest in Maputo, Mozambique, where the police officers detonated tear gas and other ammunition, resulting in at least three deaths, including a 10-year-old boy (309). Also, as reported by the US Embassy in Panama City, Panama, there were recent demonstrations in the country where law enforcement officers had to disperse the crowd using tear gas (310). In August 2020, a bill was passed in Oregon to curb the use of tear gas by police officers (311). The above evidence shows how important this tear gas research is in protecting the population's health; although acute effects of tear gas exposure are well-documented, chronic health effects are not well known. This dissertation further adds to the body of literature by investigating the understudied area of the association between tear gas and male reproductive outcomes.

Although the sample size used for the tear gas study analysis was small, the finding may serve as a basis for future larger studies on the effects of tear gas exposure

on male sexual function. This future research may include a prospective study that allows for the ascertainment of temporarily or causality. Also, more research is needed to thoroughly understand the mechanisms by which tear gas may lead to sexual dysfunction. Qualitative methods, including the use of interviews and focus groups, may also be utilized to get more context on the effects tear gas exposure had on the study participants and better assess the male reproductive outcomes. For example, ED may be measured using a validated questionnaire, such as the International Index of Erectile Function (IIEF) (312). The association between tear gas exposure and other male reproductive conditions, such as male infertility, may also be explored.

Aside from the tear gas section of this dissertation, the study on the association between VOCs and male reproductive outcomes is also a major contribution to the literature because of this chemical compound's ubiquitous nature (266). VOCs can be found in indoor and outdoor settings, with a higher level of human exposure in indoor spaces since people spend more time in enclosed spaces (217, 218). In addition, VOCs are found in products people use in their daily lives (215, 216). It is, therefore, important to understand its harmful effects on health, including reproductive health and the mechanisms in which they affect human health.

In this study, some of the VOCs were associated with decreased odds of testosterone deficiency (TD), and one of the VOCs, 1,4-dichlorobenzene, was associated with increased odds of erectile dysfunction (ED). This results may seem conflicting because some studies have identified low testosterone (or TD) as a risk factor of ED (313). Low testosterone and ED are common among older men. It is, therefore, not surprising that there may be a link between these conditions (16, 314, 315). In addition,

testosterone helps regulate the enzyme nitric oxide synthase, which is responsible for secreting NO that aids in dilating penile blood vessels for erection (316). However, low testosterone is just one of the risk factors of ED, among other risk factors, including type 2 diabetes (16, 46), age (317, 318), CVD (306, 319), and psychological factors (22). Therefore, an individual with a normal testosterone level (absence of TD) may develop ED due to exposure to other risk factors. Another possible reason for the varying results is that the testosterone in the NHANES study was measured using blood samples. In contrast, ED was assessed using a questionnaire. This implies that those who reported having ED may not necessarily have ED, and vice versa.

In addition to exploring the effect of VOCs on total testosterone levels, the different forms of testosterone may also be considered. Testosterone can be grouped into two categories--bound and unbound. A larger percentage of testosterone in the body is bound to proteins such as albumin and sex hormone-binding globulin (SHBG) (320). The unbound testosterones are regarded as free testosterone. Unlike the bound testosterone, free testosterone is more active and androgenic (320). In the current analysis, total testosterone comprising both protein-bound and unbound testosterone was utilized. In addition, total testosterone was categorized following the American Urological Association definition of TD (Total Testosterone<300ng/dL) (280). Additional studies may be required to explore the effects of VOCs on free testosterone, considering that it is the biologically available part of the total testosterone (unbound testosterone).

In this dissertation, the VOCs were measured using the participants' blood samples, which has its advantages and disadvantages. One of the advantages of using blood VOCs for analysis is that it allows for early detection of VOCs in the body. VOCs

are released into the blood before dispersing to other organs in the body. Therefore, the blood VOCs may be more reliable than other sources of VOCs. However, the measurement of VOCs in the blood is invasive, coupled with the issue of short half-lives (321). Some VOCs, such as benzene, easily evaporate in the air at room temperature, especially during sample collection (302, 322). Another reliable source of VOCs is urine-based VOCs. Upon exposure, VOCs usually metabolize in the liver into metabolites that are excreted via the urine (323). These urine-containing VOC metabolites may then be utilized to assess the level of VOCs in the individual's body. Compared to the blood VOCs, the urine VOC metabolites may be a better approach to assessing VOC levels in humans since they have longer half-life (324-326). Therefore, urine VOCs may be considered for future studies on the effect of VOCs on adverse male reproductive outcomes.

In the current study, all source VOCs were examined. Airborne sources of VOCs include cigarette smoke, automobile exhaust, paints, solvents, and other industrial-related exposure (215, 216). Aside from the inhalation of VOC, humans may also be exposed to VOCs through ingestion. Some sources of VOCs via ingestion are food, water, and other liquids (327). Prior studies have shown that VOC-containing water may be linked to the development of ED (221). As mentioned previously in aim 3, a veteran study reported a 12% increased odds of ED among those exposed to contaminated water containing VOCs, like trichloroethylene and tetrachloroethylene (221). Possible future research may be to investigate the relationship between specific source-VOC and male reproductive outcomes. As seen in aim 3, varying effect measures were observed when the entire dataset (inverse associations) was used compared to the nonsmoker dataset (mainly

positive associations). This difference suggests that VOCs from other sources may have a different effect on male reproductive health compared to VOCs from cigarette smoke; alternatively, there could be residual confounding due to characteristics associated with smoking or by components of smoke other than VOC's (namely, nicotine). Thus, the null findings among nonsmokers further highlight the possibility of other factors being responsible for the associations observed in prior research.

Another source of VOCs, automobile exhaust, is known to contain VOCs such as benzene, toluene, ethylbenzene, and xylene (328). Some studies have shown that the benzene content of vehicle exhaust may be associated with male infertility due to its resulting DNA damage to the human sperm (329). Additional studies may be required to show whether the VOCs in motor vehicle exhaust may affect erectile function or testosterone levels.

Multi-omics data may be employed to understand the molecular basis and complexities of these reproductive outcomes. There are several types of omics data, including genomics, epigenomics, transcriptomics, proteomics, and metabolomics (330). The Jorgenson et al. (36) paper performed a genome-wide association study (GWAS) of erectile dysfunction and identified a variation close to the SIM1 gene responsible for the association. Other areas to explore in this research may be to examine if there are interactions between genes and environment in relation to sexual function (combination of EWAS and GWAS).

In conclusion, this study adds two novel contributions to the literature; namely, it examines the effects of VOCs on male reproductive outcomes among nonsmokers; and is the first study to examine possible effects of tear gas exposure on male reproductive

outcomes. This dissertation provides further evidence of the need to pay attention to what we are exposed to in our surroundings. Some of the individual protective measures include the use of masks in outdoor spaces, especially when tear gas is detonated; taking a shower after exposure to tear gas; examining the labels of what we consume to determine the presence or level of VOC content; getting more insights or health education on environmental pollutants in general; and seeking medical help after prolonged exposure to these environmental compounds. Population-level health interventions may also be employed. Law enforcement officers and agencies should minimize the use of this tear gas in public places. Health departments, CDC, EPA, or other health-related government organizations may collaborate with law enforcement agencies and FDAs to minimize population exposure to these chemicals, thereby preventing known and unknown adverse health effects.

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APPENDIX

TEAR GAS HEALTH QUESTIONNAIRE

1. Are you at least 18 years old?

   _____ Yes _____No

2. Do you believe you were exposed to tear gas in 2020 or 2021?

   _____ Yes _____No

Demographic information

3. State of residence

   1, AK | **2**, AL | **3**, AR | **4**, AZ | **5**, CA | 6, CO | **7**, CT | **8**, DC | **9**, DE | **10**, FL | **11**, GA | **12**, HI | **13**, IA | **14**, ID | **15**, IL | **16**, IN | **17**, KS | **18**, KY | **19**, LA | **20**, MA | **21**, MD | **22**, ME | **23**, MI | **24**, MN | **25**, MO | **26**, MS | **27**, MT | **28**, NC | **29**, ND | **30**, NE | **31**, NH | **32**, NJ | **33**, NM | **34**, NV | **35**, NY | **36**, OH | **37**, OK | **38**, OR | **39**, PA | **40**, RI | **41**, SC | **42**, SD | **43**, TN | **44**, TX | **45**, UT | **46**, VA | **47**, VT | **48**, WA | **49**, WI | **50**, WV | **51**, WY | **52**, Don't wish to answer

4. What is your sex?

   ____Female ____Male ____non-binary or transgender ____Other

5. What is your race?

   ____White ____Black or African American ____Asian ____American Indian or

   Alaska Native ____Native Hawaiian or Other Pacific Islander ____Other

   ____Don't wish to answer

6. What is your ethnicity?

   ____Non-Hispanic ____Hispanic ____Don't wish to answer

7. What is your age?
8. What is your highest level of education?

   ____Less than high school ____High School Diploma ____Some college ____Bachelor's Degree ____Graduate degree ____Don't know/Don't wish to answer
9. What is your household annual income?

   ___Less than $10,000 ___$10,000-$19,999 ___$20,000-$29,999 ___$30,000-$39,999 ___$40,000-$49,999 ___$50,000-$59,999 ___$60,000-$69,999 ___More than $70,000 ___Don't know/Don't wish to answer
10. Do you smoke cigarettes?

    ___Yes ___No ___Don't wish to answer
11. How many cigarettes/packs do you smoke per day?

    ___1 to 9 (less than half a pack) ___11 to 19 (less than 1 pack) ___20 to 39 (less than 2 packs) ___40 or more (2 packs or more) ___Don't know/Don't wish to answer

The following questions will focus on your health after tear gas exposure (any information that you provide will remain anonymous).

12. If any, what effects do you think the tear gas exposure had on your eyes? Check all that apply:

    ___Watery eyes ___Burning, stinging eye ___Other ___Don't know/Don't wish to answer
13. Please describe "other" effects on the eye.

14. If any, what effects do you think the tear gas exposure had on your lungs? Check all that apply:

    ___Coughing ___Burning lungs ___Shortness of breath ___Other ___Don't know/Don't wish to answer

15. Please describe "other" effects on lungs.
16. If any, what effects do you think the exposure to tear gas had on your skin? Check all that apply:

    ___ Burning sensation ___Blistering ___Other ___Don't know/Don't wish to answer

17. Please describe the "other" effects on your skin.
18. If any, what effects do you think the tear gas exposure had on your heart? Check all that apply:

    ___Increased heart rate ___Irregular heartbeat ___Chest pain ___Other ___Don't know/Don't wish to answer

19. Please describe the "other" heart effects.
20. After your exposure, did you seek medical care from a healthcare provider?

    ___Yes ___No ___Don't wish to answer

Male Reproductive Outcomes

The following questions will focus on your health status (any information that you provide remain anonymous).

21. What reproductive or hormonal problems have you had since you started attending protests? Check all that apply:

___Erectile dysfunction ___Ejaculatory dysfunction ___Trouble conceiving

___Don't know/Don't wish to answer

Medical care

22. After your exposure, did you seek medical care from a healthcare provider?

___Yes ___No ___Trouble conceiving ___Don't know/Don't wish to answer

CURRICULUM VITA

Damilola Rukayat Owoade

EDUCATION

2020 – Present	PhD in Public Health Science, Epidemiology University of Louisville, Louisville, KY Dissertation: *Environmental exposures and male reproductive health*
2019 – 2020	M.Sc., Data Science Texas Tech University, Lubbock, TX
2017 – 2019	Master of Public Health (MPH) Texas Tech University Health Sciences Center, Lubbock, TX
2010 – 2014	B.S., Microbiology Babcock University, Ilishan-Remo, Nigeria

AWARDS, SCHOLARSHIPS, AND HONORS

2018	Rural Health Competitive Scholarship, Texas Tech University Health Sciences Center
2019	Phi Kappa Phi Honor Society, Texas Tech University Health Sciences Center
2020	University Fellowship, University of Louisville
2022	1st place in 2022 Research! Louisville poster presentation, PhD division
2023	Dissertation Completion Award, University of Louisville

RESEARCH EXPERIENCE

2021 – Present Graduate Research Assistant
University of Louisville, Louisville, KY
Project: "Adverse effects of tear gas on human health" – Partnership between the University of Louisville and Until Justice Data Partners

- Researched the effect of acute tear gas exposure on male reproductive outcomes.
- Analyze 200+ responses to an online survey created and maintained on REDCap using R and communicate results in conferences.

2018 – 2019 Graduate Research Assistant
Texas Tech University Health Sciences Center, Lubbock, TX

- Conducted literature searches.
- Analyzed data using SPSS.

TEACHING EXPERIENCE

Fall 2022 & Fall 2023 Graduate Teaching Assistant
University of Louisville, Department of Epidemiology and Population health
Courses:

- Statistical Foundations for Epidemiology
- Epidemiologic Methods and Concepts for Public Health

SPRING 2019 Graduate Teaching Assistant
Texas Tech University Health Sciences Center, Department of Public Health
Course:

- Introduction to Epidemiology

OTHER WORK EXPERIENCE

2018 Department of State Health Services, Public Health Region 1, Lubbock, TX

- Assessed a department-specific project on human/sex trafficking by developing an evaluation and tracking tool for stakeholders attending human trafficking outreaches.
- Maintained communication lines among project stakeholders in 5 Texas counties.

PUBLICATIONS

- Lovelace J, Shabaneh O, De La Cruz N, **Owoade DR**, Nwabuo CC, Nair N, Appiah D. The Joint Association of Septicemia and Cerebrovascular Diseases with In-Hospital Mortality Among Patients with Left Ventricular Assist Device in the United States. J Stroke Cerebrovasc Dis. 2021 30(4):105610.
- Appiah L, John D, **Owoade DR**, Mendenhall J, Appiah D. Factors Influencing Racial and Ethnic Differences in Prescription Opioid Misuse Among Young Adolescents in the USA, 2009-2019. J Racial Ethn Health Disparities. 2021.
- Appiah D, Nwabuo CC, **Owoade DR**, Samad J, Ebong I, Winters SJ. Family History of Premature Myocardial Infarction Modifies the Associations of Bilateral Oophorectomy with Cardiovascular Disease Mortality in a US National Cohort of Postmenopausal Women. Menopause. 2020 (In press].

PRESENTATIONS

- **Damilola R. Owoade**, Monica Unseld, Emily K. Reece, Madeline M. Tomlinson, Anne Wallis, Cynthia Corbitt, Ted Smith, Aruni Bhatnagar, and Kira C. Taylor. *Acute tear gas exposure symptoms and adverse male reproductive outcomes*. Poster presented at: Research Louisville; September 22, 2022; Louisville, KY.
- **Damilola R. Owoade**, Monica Unseld, Emily K. Reece, Madeline M. Tomlinson, Anne Wallis, Cynthia Corbitt, Ted Smith, Aruni Bhatnagar, and Kira C. Taylor. *Acute tear gas exposure symptoms and adverse male reproductive outcomes*. Oral presentation at; The Society for Pediatric and Perinatal Epidemiologic Research (SPER); June 14, 2022; Chicago, IL.
- **Damilola R. Owoade**, Drew Rasmussen, Summre Blakely, Nathan Villalpando, Jaffer Samad, Hridoy Haq, Susan Mengel, LisaAnn Gittner, Hafiz Khan. *Investigating Breast Cancer Incidence and Mortality in a Rural West Texas Parmer County*. Poster presented at: Spark Conference; April 23, 2018; Lubbock, TX.

TECHNICAL SKILLS

- R
- SAS
- Python
- MySQL