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Protest-related tear gas exposure and menstrual function

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By

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A Dissertation Approved on

April 14, 2022

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DEDICATION

This dissertation is dedicated to my family:

To those who went before, you are my inspiration.

To those with me now, you are my heart.

To those who come after, you are my hope.

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ABSTRACT

PROTEST-RELATED TEAR GAS EXPOSURE AND MENSTRUAL FUNCTION

Emily K. Reece

April 14, 2022

During the racial justice protests of 2020 and 2021, crowd control chemical irritants (referred to as “tear gas”) were deployed against protesters, after which there were anecdotal reports of altered menstrual cycles among exposed individuals. There is only one peer reviewed published study on tear gas exposure and menstrual health. This study examined whether tear gas exposure was associated with menstrual cycle outcomes among women attending the 2020-2021 protests.

Data from 103 women who attended racial justice protests in 2020 and 2021 were collected through an online questionnaire. Data included protest attendance, acute symptoms of tear gas exposure, whether medical care was sought for acute effects of tear gas exposure, and menstrual cycle outcomes. The associations between proxy measurements of tear gas exposure and menstrual cycle symptoms were determined through linear regression, adjusted for covariates. The proxies for tear gas exposure were number of protests attended; total number of acute symptoms of exposure; acute symptoms experienced in specific organ systems (eye, lung, skin, heart); and seeking medical care after exposure (yes/no). The outcome variables were total number of menstrual cycle outcomes, and two factors identified through exploratory factory analysis: factor 1 – intense outcomes (heavy bleed, long bleed, short bleed, long cycle,

irregular cycles, and period pain) and factor 2 – milder outcomes (light bleed, short bleed, and short cycle). All models were adjusted for age, race, ethnicity, education, income, and trying to conceive.

Higher protest attendance (> 9) had significant positive associations with total number of menstrual cycle symptoms (β: 2.12, 95% CI: 1.12, 3.11) and factor 1 (β: 1.22, 95% CI: 0.79, 1.65). Seeking medical care for tear gas exposure had a significant inverse association with factor 1 (β: -0.95, 95% CI: -1.56, -0.34), but was not associated with total number of menstrual cycle symptoms or factor 2. The total number of acute symptoms and acute symptoms in specific organ systems were not significantly associated with menstrual cycle outcomes. Results may be confounded by stress experienced during protests. Additional research is needed to determine whether there are long-term menstrual cycle and reproductive health outcomes after exposure to tear gas.

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INTRODUCTION

Overview

The years of 2020 and 2021 saw a marked increase in racial justice protests and demonstrations as part of the Black Lives Matter movement. The protestors involved in racial justice protests have frequently been met with the use of crowd control chemical irritants, such as tear gas and pepper spray, by law enforcement agencies (1). The widespread and ongoing nature of these protests creates the potential for a large number of individuals to be exposed to these irritants (2). Tear gas and pepper spray are intended as transient incapacitants, which work by inducing uncontrollable tearing (lacrimation), coughing and sneezing. While tear gas and pepper spray are considered safer than more forceful measures of crowd control and their effects are considered temporary when tested in healthy volunteers, there are still questions about the overall safety and longer-term effects when used on the general population (3, 4).

The main lacrimator agent in tear gas can be one of several compounds. These include 2-chlorobenzylidene malononitrile (CS), 1-chloroacetophenone (CN), and dibenz [*b,f*]-1,4-oxazepine (CR) (3). Tear gas agents can be deployed as aerosolized solids (projectile pellets and pyrotechnic canisters) or as liquid sprays (3). The active ingredient in pepper spray is oleoresin capsicum (OC), which is an oil isolated from hot peppers, or a synthetic analogue (pelargonic acid vanillylamide or capsaicin II) (4). Deployment methods for pepper spray include the use of liquid sprays and aerosolized solids

(projectile pellets) (3). Of these lacrimating agents, CS and OC are the most common as they are considered the safest options (4).

While the immediate effects of tear gas and pepper spray on eyes, lungs, and skin are well documented, effects on other organ systems are not. One potential health effect that has not been well studied is altered menstrual function. Menstrual function includes menstrual cycle length (the first day of menstrual bleeding until the day before the start of the next menstrual bleeding), bleed length, and bleed intensity. These characteristics can be indicative of reproductive health and have been associated with fertility and reproductive cancers (5, 6). The following review of literature outlines the biology behind the menstrual cycle and ovulation, known effects of tear gas and pepper spray, and studies of tear gas in relation to the reproductive system. The literature suggest that tear gas may affect the reproductive system by disrupting the endocrine system that orchestrates it.

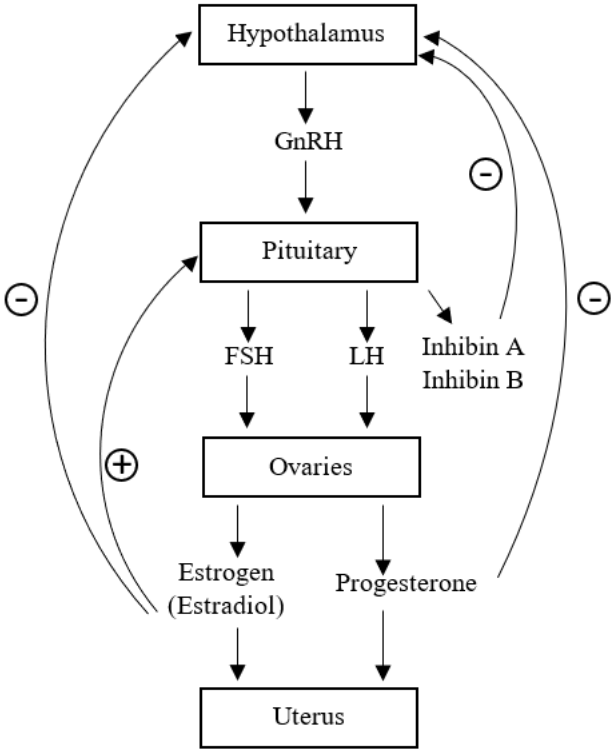
Menstrual Function and Ovulation

To understand how tear gas and pepper spray might affect menstrual health, the characteristics of normal functioning of the menstrual cycle and ovulation must first be established. The following section outlines the phases of the menstrual and ovarian cycles and the associated hormone actions and fluctuations.

The menstrual cycle and ovulation are coordinated through the interplay of several hormones. These include gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and the

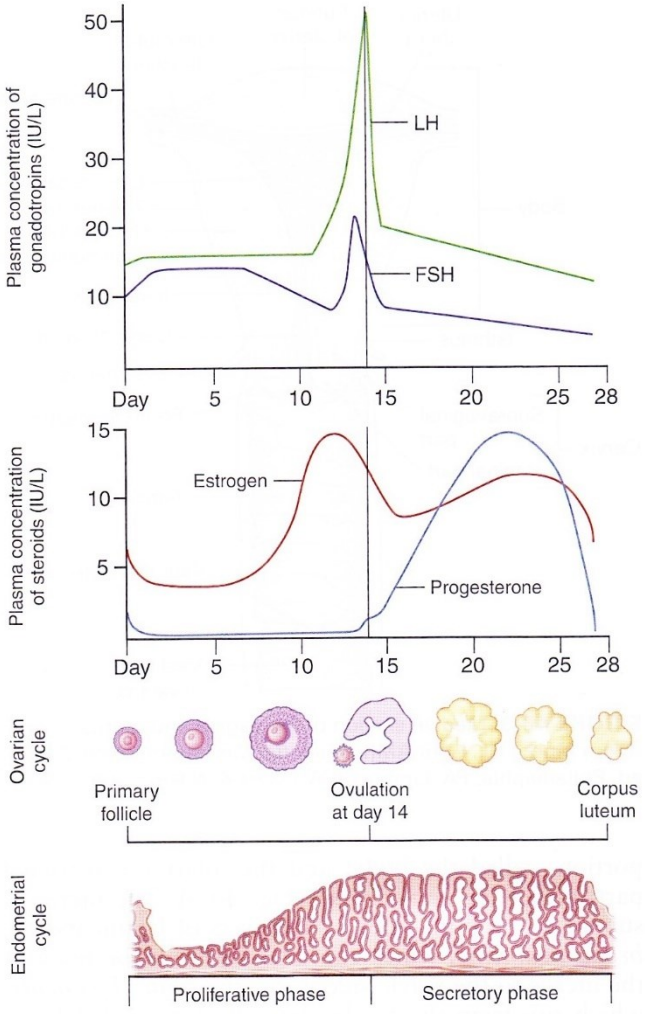
proteins inhibin A and inhibin B (7). GnRH is produced in pulses by the hypothalamus and stimulates the pituitary to release FSH and LH (7). FSH and LH both work on the ovaries. FSH stimulates ovarian follicles to release estradiol and LH stimulates the follicles to release estrogen. Estradiol and estrogen both act on the uterus. Estradiol also has a negative feedback function on the production of GnRH by the hypothalamus and a positive feedback function on the production of FSH and LH by the pituitary (7). Inhibin A and Inhibin B are protein dimers secreted by ovarian follicles and the corpus luteum (7). Inhibin A release is stimulated by both FSH and LH (7). FSH also causes inhibin B levels to rise by stimulating proliferation of the cells in the follicle and corpus luteum that secrete it (7). Inhibins A and B suppress FSH secretion by the pituitary (7). The hypothalamus, pituitary, and reproductive organs are known as the hypothalamus-pituitary-gonadal axis (HPG axis) [Figure 1].

Figure 1: The Hypothalamus-Pituitary-Gonadal Axis



The menstrual cycle is divided into two phases: the follicular (or proliferative) phase and the luteal (or secretory) phase [Figure 2]. The follicular phase begins with the onset of menstrual bleeding and ends the day before ovulation (7). The first day of the follicular phase is also the first day of the menstrual cycle. The average day of ovulation is day 14 of the cycle. The menstrual bleeding that defines the beginning of the follicular phase generally lasts seven to nine days (8). In these initial days, GnRH, LH, estrogen, and estradiol release are relatively low, while FSH is at its highest (9). The elevated FSH stimulates the recruitment of several ovarian follicles, which began maturing in the preceding late luteal phase (9). As a dominant follicle emerges around the 5^{th} to 7^{th} day and begins to release inhibin B, FSH levels drop, further favoring the dominant follicle (9). During this same period, the uterine lining proliferates to build the endometrium back up in preparation for a fertilized ovum (7).

Figure 2: Changes in Hormones, Ovaries, and Endometrium Over the Menstrual Cycle



Reed et al. 2000 (9)

In the middle of the follicular phase, GnRH release increases, but LH production is still depressed by the estradiol made by the dominant follicle (7). As the follicular phase progresses, the level of estradiol released by the dominant follicle rapidly increases, which engages the positive feedback loop to the pituitary, leading to a surge in FSH and LH (7). The LH surge triggers ovulation approximately 36 hours later (7).

The luteal phase follows ovulation and lasts approximately 14 days (9). The post-ovulatory remains of the dominant follicle in the ovary form the corpus luteum and begin

to secrete progesterone and estradiol and continue to secrete inhibin A (9). The increase in progesterone and estradiol causes the release of GnRH from the hypothalamus (7). In the middle of the luteal phase and in the absence of fertilization and implantation, the corpus luteum begins to functionally decline (7). As it secretes less progesterone and estradiol, suppression of FSH production in the pituitary eases (7). The decline in progesterone, which was supporting the blood supply to the endometrium that had formed during the follicular phase, causes the endometrium to begin to degrade (9). The luteal phase ends the day before the onset of menstrual bleeding (7).

The ovaries contain a population of resting primary follicles (10). These are composed of an oocyte enclosed in a single layer of granulosa cells which provide metabolic support (11). Follicles are recruited in multiple waves during the menstrual cycle (10). Each wave can include 4 to 14 follicles (10). During recruitment, FSH and LH stimulate the primary follicles to become secondary follicles, which become more metabolically active; and the granulosa cells form multiple layers and develop an external layer of theca cells (10, 11). FSH then further stimulates the secondary follicles to form fluid-filled cavities surrounded by granulosa cells (10). At this stage, the follicles are referred to as atrial follicles (10). The dominant atrial follicle will go on to develop into a large Graafian follicle, with an expanded fluid pocket and a larger number of granulosa cells (10). This is the mature follicle that will be ovulated (10).

While follicles can be recruited during several points in the menstrual cycle, follicle maturation and selection of the dominant follicle occur during the follicular phase (10). The elevated levels of FSH in the follicular phase promote follicle maturation, and as follicles mature, less viable follicles die off (10). As one follicle becomes dominant, it

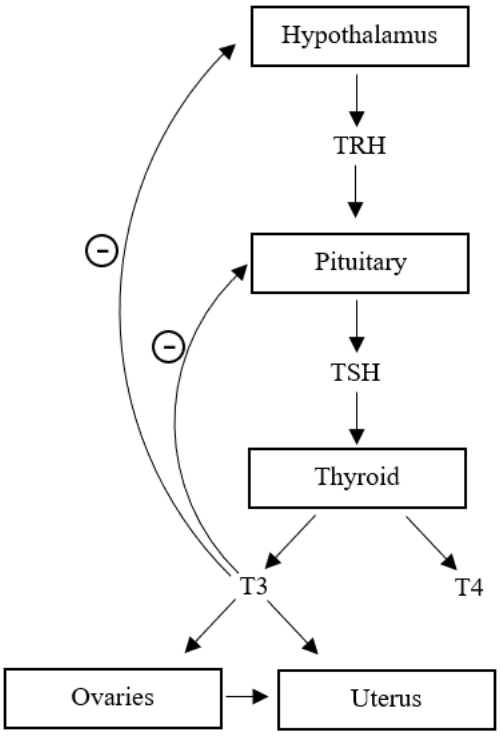
begins secreting estrogen, which suppresses the FSH that has been supporting the less developed recruited follicles (12). The estrogen suppresses FSH production until a threshold is met, and then it stimulated the release of FSH followed closely by LH (the LH surge) (10). The FSH surge prepares the dominant follicle for ovulation, and the LH surge causes the rupture of the follicle that releases the oocyte in ovulation and its immediately surrounding granulosa cells (11).

In the luteal phase, the granulosa cells that remain in the ovary after ovulation take on a yellow pigmentation and become more vascularized to form the corpus luteum (10, 11). The corpus luteum produces progesterone and estradiol to maintain the endometrium for implantation. If implantation does not occur, the corpus luteum regresses and forms a white scar called the corpus albicans (11). The cycle of follicle recruitment, maturation, and selection then begins again as the next follicular phase begins.

The thyroid also plays an important role in menstrual function and ovulation, though the mechanisms are not as well defined as for the HPG axis. The coordinated functioning of the hypothalamus, pituitary, and thyroid is known as the hypothalamus-pituitary-thyroid axis (HPT axis) [Figure 3]. In the HPT axis, the hypothalamus secretes thyroid releasing hormone (TRH) which stimulates the pituitary to release thyroid stimulating hormone (TSH). In response to TSH, the thyroid secretes thyroid hormones (THs) T3 and T4 (13). T3 has a negative feedback effect on the secretion of TRH and TSH (13). T3 stimulates granulosa cells and is believed to also play a role in ovulation, endometrium proliferation, and placental development (13, 14). The importance of the thyroid to reproductive functions is demonstrated by the association that abnormally low

thyroid activity (hypothyroidism) and abnormally high thyroid activity (hyperthyroidism) have with fertility issues as well as a number of menstrual disorders (14, 15).

Figure 3: The Hypothalamus-Pituitary-Thyroid Axis and the Female Reproductive System



Menstrual Disorders

Menstrual disorders occur when the interplay of hormones detailed above is disrupted. These disorders include the absence of bleeding (amenorrhea), short or light bleeding (hypomenorrhea), heavy or long bleeding (menorrhagia), period pain (dysmenorrhea), cycles of less than 21 days (polymenorrhea), cycles that are irregular or more than 35 days (oligomenorrhea), and bleeding between periods (metrorrhagia) (16). Amenorrhea, hypomenorrhea, menorrhagia, polymenorrhea, oligomenorrhea, and

metrorrhagia are also referred to as abnormal uterine bleeding (AUB) (17). AUB is estimated to affect 14% to 25% of women during their reproductive years (17). Understanding the risk factors associated with developing menstrual disorders such as AUB is important as they can impact both the quality of life and professional performance of affected women (18).

Excessive menstrual bleeding (heavy, long, or frequent bleeding) has many known underlying causes and risk factors. Known causes include coagulation disorders; uterine fibroids; endometrial polyps; adenomyosis, in which the uterine lining grows into the uterine wall; cancer; polycystic ovarian syndrome (PCOS), hypothyroidism, and anovulatory cycles (15, 17). Risk factors for excessive bleeding include hypoxia; extremes of the reproductive age range; and some medications, such as blood thinners, corticosteroids, antipsychotics, and tricyclene antidepressants (17, 19).

Long or irregular cycles affect approximately 14% of women of childbearing age (20). This irregularity can be caused by PCOS, hyperthyroidism, hypothyroidism, androgen secreting tumors, diabetes, adrenal hyperplasia, Cushing syndrome, and pelvic inflammatory disease (15, 20). Risk factors include recent menarche or nearing menopause, stress, and antipsychotic medications (15, 20).

Metrorrhagia, which is also known as spotting, breakthrough bleeding, or intermenstrual bleeding, is a common side effect of many forms of hormonal birth control. Smoking and sexually transmitted infections increase the risk of spotting. Breakthrough bleeding can also be caused by uterine fibroids (21).

Amenorrhea, or lack of menstrual bleeding for at least three cycles, is experienced by approximately 1% of women in the United States (22). Amenorrhea is often associated with pregnancy, but there are other known causes and risk factors (15). Nutritional deficiencies, such as those caused by anorexia and bulimia; extreme exercise; antipsychotic medications; and psychological stress are all risk factors for amenorrhea (15). Additional causes include PCOS, hypothyroidism, hyperthyroidism, ovarian failure, and hyperprolactemia (23, 24). Some types of birth control can also cause menstrual bleeding to stop (22). Light bleeding shares many of the same causes and risk factor as amenorrhea, including PCOS, extreme exercise, nutritional deficiencies, stress, and hormonal birth control (25).

The reported prevalence of period pains among women of reproductive age varies widely, ranging from 16% to 91% (26). Pain associated with menstruating is often due to uterine muscle spasms and restricted blood flow (15). Risk factors for this type of pain include age, high body mass index, menorrhagia, no history of pregnancy, depression, and a family history of period pain (26). Painful periods can also be caused by endometriosis, polyps, and fibroids (26).

History of Tear Gas and Pepper Spray

The tear gas agent CN was first developed during World War I (27). CS was developed in 1928, CR in 1962, and OC in the 1970's (27, 28). The use of chemical agents, including tear gas, were banned for use in military conflicts by the Geneva Protocol (1925) and the Chemical Weapons Convention (1993). The United States used

tear gas the Vietnam War despite the Geneva Protocol (29). While banned in wartime, tear gas and pepper spray were not banned for domestic use and were adopted by law enforcement agencies (3, 27). CS and OC are currently the most common crowd control chemical irritants used by law enforcement (27).

The federal government of the United States does not regulate crowd control irritants, although a bill to do so was introduced by U.S. Senator Tim Kaine in May 2021 (30). Laws to regulate the use of crowd control irritants have passed in several states. Specifically, the states of California, Oregon, and Washington have passed laws restricting the use of tear gas, though the Washington law does not regulate the use of pepper spray (31-33).

Concerns over unexpected effects of tear gas and pepper spray exposure among protesters have been raised in recent decades, especially after large scale civilian exposures in Israel in the 1980s and Bahrain in 2011 (29, 34). In both instances, there were reports of increased miscarriages among exposed populations (29, 34). Concern was great enough that in 2011, Chile temporarily banned the use tear gas during protests (29). There is still a great deal of uncertainty as to whether tear gas and pepper spray have any reproductive effects.

More recently, tear gas and pepper spray have been deployed against racial justice demonstrators in cities such Louisville, Kentucky. The protests in Louisville began May 28, 2020 and were met with crowd control irritant chemical deployed by police on the first day (35). The following June, the Louisville Metro Police updated their tear gas policy so that the chief of police (or designee) would have to approve of the use of tear gas before it was deployed (36). Protests continued for at least 365 consecutive days (34).

In August of 2020, American Civil Liberties Union of Kentucky filed a motion for a restraining order to keep police officers from using tear gas against protesters. The motion was denied (37). Restrictions on tear gas were proposed in September 2020 in an ordinance to limit use of force by the police department, but were removed from the final version citing a reduction in tear gas use by officers (38). It is unknown how many people were exposed to tear gas during the Louisville protests.

Health Effects of Tear Gas and Pepper Spray

While the potential reproductive effects of tear gas and pepper spray are poorly understood, their effects on eyes, the respiratory system, and skin are well-documented (3). Heart effects have also been documented (3). The damaging effects of CS and CN are believed to be a result of their ability to deactivate enzymes (39). CS and CN are both SN2-alkylating agents and react with enzymes in areas such as sulfhydryl and thiol groups (39). The deactivation of essential enzymes can cause damage in affected tissues that persists after the exposure has ended (39). Short term effects of CS and CN, such as pain and cough, result from the interaction with a type of transient receptor potential (TRP) channel called TRPA1 (3). OC interacts with similar TRP channels known as TRPV1 (3). TRP channels are present in nerves that are capable of sensing pain (3). Pain and injury from the deployment of tear gas and pepper spray can also occur as a result of carrier solvents in sprays, abrasion from powders, burns from incendiary devices, and blunt force trauma from launched cannisters and projectile pellets (40).

The eyes are one of the primary targets of tear gas and pepper spray. CS, CN, and OC all cause tearing (lacrimation), pain, and involuntary blinking or closing of the eyes (blepharospasm) (40, 41). CS exposure can result in conjunctivitis and in reduced vision that resolves within a couple of days (10). CS is generally not associated with long term eye effects (40). Aerosolized CN can cause corneal opacity for hours to months if it is not quickly flushed out (40). CN powder has more potential to cause permanent injury and has been associated with long-term corneal clouding, necrotizing keratitis, and optic nerve damage (40). The powder form appears to be able to penetrate deeper into tissues and can also cause injury due to its abrasive properties (40). OC exposure can cause conjunctivitis, increase intraocular pressure, and transient vision loss (40, 41). Longer-term effects include corneal ulcers and necrosis (40). OC and CS are less likely than CN to have long-term effects, but repeated exposure make long-term injury more likely (40).

The respiratory system is the other primary target of tear gas and pepper spray. Acute effects of CS, CN, and OC include cough, shortness of breath, and runny nose, and sore throat (2). While these effects are usually temporary and not life threatening, serious reactions can occur in individuals with asthma and those who have become sensitized through repeat exposures to tear gas and pepper spray (3). Potential serious reactions include spasming and swelling of the airways, which can impair breathing, and in some cases, lead to death (39). OC, in particular, is associated with pulmonary edema (3). Long-term effects of tear gas and pepper spray include persistent cough, asthma, and chronic bronchitis (3). CS exposure is associated with the development of respiratory infections (3). Severe effects, including death, are more likely when tear gas or pepper spray is deployed in an enclosed space (39).

The most common effect of tear gas and pepper spray on skin is a burning sensation (2, 39). They can also cause blistering, rashes, and chemical burns (39). CN is more likely to cause severe burns than CS or OC, though OC is more difficult to wash off as it is an oil (39, 42). All three agents can cause long-term sensitization resulting in contact dermatitis with repeat exposure, but CN is the most potent sensitizer (39). Sensitization to OC can cause contact dermatitis after preparing or ingesting culinary chili peppers (43).

Tear gas and pepper spray can also affect the cardiovascular system. CS, CN, and OC all cause a transient rise in blood pressure (42). There is disagreement in the published literature as to whether CS, CN, and OC trigger bradycardia, tachycardia, or both (39, 42). In at least two cases, tear gas or pepper spray has been suspected in the precipitation of myocardial infarction (40, 41). One involved CS exposure and the other OC exposure (44, 45).

Previous Research

An area that has received little attention is the potential effects of tear gas and pepper spray on the female reproductive system. This includes fertility, pregnancy outcomes, and menstrual cycle characteristics. Only three original research articles and an abstract were identified through PubMed and searches of the references in peer-reviewed papers on tear gas and pepper spray (2, 46, 47). A non-peer-reviewed article in preprint was also identified through a general internet search (48).

The earliest paper was published by Upshall in 1973 (46). In this study, pregnant rats and rabbits were exposed to CS (as an aerosol or as an injection). The initial phase of the aerosol study, pregnant rats were exposed to either 20 mg/m^3 or silica dust for 5 minutes a day for 15 days. The dose-response phase included both rats and rabbits. Rats were exposed on days 6 through 15 of pregnancy and rabbits were exposed on days 6 through 18 of pregnancy. Test animals were initially divided into two exposure categories: 20 mg/m^3 aerosolized CS and silica dust controls. In a later arm of the study, animals were divided into four exposure categories to test for a dose response: 6 mg/m^3, 20 mg/m^3, and 60 mg/m^3 aerosolized CS, and silica dust exposure controls. Twenty-two rats and 12 rabbits were assigned to each exposure group. Rats were euthanized at day 21 of pregnancy and rabbits at day 30 of pregnancy. These dates were selected as they are one day before the expected dates of delivery. There was also an intraperitoneal injection arm of the study in rats (46). The number of rat fetuses in each inhalation exposure group ranged from 222 to 242 and the number of rabbit fetuses ranged from 45 to 79 fetuses. In the initial CS versus control phase of the study, visible gross malformations, total number of pregnancies, mean litter size, total number of fetuses, fetal loss, and fetal weight were not significantly different. The pregnancy weight gain in the treated pregnant rats was significantly decreased (by 22.9%) when compared to controls. In both rats and rabbits, the following outcomes were not significantly different between exposures: visible gross malformations, total number of pregnancies, mean litter size, total number of fetuses, fetal loss, and pregnancy weight gain. There was a dose-response decrease in rat fetal weight as CS dose increased (6 mg/m^3: 7.6%, 20 mg/m^3: 8.5%, and 60 mg/m^3: 11.0% decrease) (46).

The injection study in the Upshall study was restricted to rats. Pregnant rats were injected with either CS (20 mg/kg in polyethylene glycol 300) or with polyethylene glycol (control) intraperitoneally on one day of pregnancy. The day of injection was day 6, 8, 9, 10, 12, or 14 of pregnancy. The rats were euthanized the day before expected delivery. There were 8 to 18 pregnant rats per injection group, and 100 to 174 fetuses. There were no significant differences in visible gross malformations, total number of pregnancies, mean litter size, total number of fetuses, fetal loss, and pregnancy weight gain between the groups. Mean fetal weight in the group injected with CS at day 12 was 8% lower than in controls. The fetal weights of the remaining exposure groups were not significantly different (46).

In 1978, Chowdhury et al. published a study investigating whether CS exposure was associated with histological thyroid changes in rats (49). The study included three exposure groups of female rats with 10 rats per group. Rats in each group were given intraperitoneal injections of either CS dissolved in olive oil at 10 mg/kg or 20 mg/kg, or just olive oil (control). Injections were administered each day for ten days and the rats were euthanized on day 11. The thyroid from each rat was fixed in Bouin's fluid for histological examination. Degeneration of the thyroid follicle was observed in both of the CS groups. In the 10 mg/kg group the degeneration was mild and in the 20 mg/kg group the degeneration was severe. The degeneration was believed to either be from a reduction of thyroid stimulating hormone (THS) release from the pituitary or from direct damage to the thyroid by the CS (49).

An abstract published in 2004 by McElhatton et al. examined CS exposure in pregnant women and pregnancy outcomes (47). This study was a prospective case series

that included 30 pregnant women. All of the women were exposed to CS; however, the circumstances around the exposures were not reported. Approximately 63% of the pregnant women experienced temporary acute symptoms after exposure to CS gas. One neonate with malformation, one spontaneous abortion, and one elective abortion were reported. The prevalence of these outcomes was not significantly different from the general population (47).

Torgrimson-Ojerio et al. published a study in 2021 that looked at exposure to general crowd-control irritants in Portland, Oregon and the associated short-term and long-term symptoms (2). The data were collected through an online survey and that was available from July 30, 2020 to August 20, 2020. One thousand six hundred fifty participants provided menstrual health data. The survey included both quantitative and qualitative questions. The main quantitative exposure variable was the number of days that respondents were exposed to tear gas. Exposure was split into three categories for analysis: 1 day, 2 to 4 days, and greater than or equal to 5 days. Outcomes were divided into immediate physical health issues, delayed physical health issues, and psychological health issues. The quantitative delayed health issues included menstrual cycle issues, such as increased cramping, increased bleeding, increased clots, increased days of bleeding, unusual bleeding, change in bleed color, decreased days of bleeding, breast tenderness, and other. Associations between the exposure groups and menstrual outcomes were analyzed using the Cochran-Armitage trend test. All of the menstrual outcomes, except “other”, had significant positive associations with tear gas exposure. Answers to the open-ended qualitative questions demonstrated that many respondents experienced

early and unusually long menstrual bleeding. The authors suggest that tear gases may be endocrine disruptors (2).

The article in preprint that was not peer-reviewed was published online in 2020 by Mahfud et al. (48). Yellow Vest protesters in France were invited to participate in an online survey in February 2020, until the start of the SARS-CoV-2 pandemic in March 2020. Exposures fell into four categories: never, fewer than 5 exposures, 5 to 10 exposures, and greater than 10 exposures. General symptoms of CS tear gas exposure and the presence of menstrual cycle symptoms were collected from 145 participants. Specific menstrual cycle outcomes were not included as part of the questionnaire. Rather, participants were asked to indicate whether menstrual cycle anomalies. Participants were instructed to indicate whether symptoms were short-term or long-term. Correlation and logistic regression models were also used to determine whether tear gas exposure was associated with menstrual cycle abnormalities. Exposure number and general symptoms of tear gas were correlated with abnormal menstrual cycles. In the logistic models, exposures were associated with significantly higher odds of reporting menstrual abnormalities of any duration, as well as long-term menstrual abnormalities. However, the associations were no longer significant after adjusting for unspecified demographic and health covariates (48).

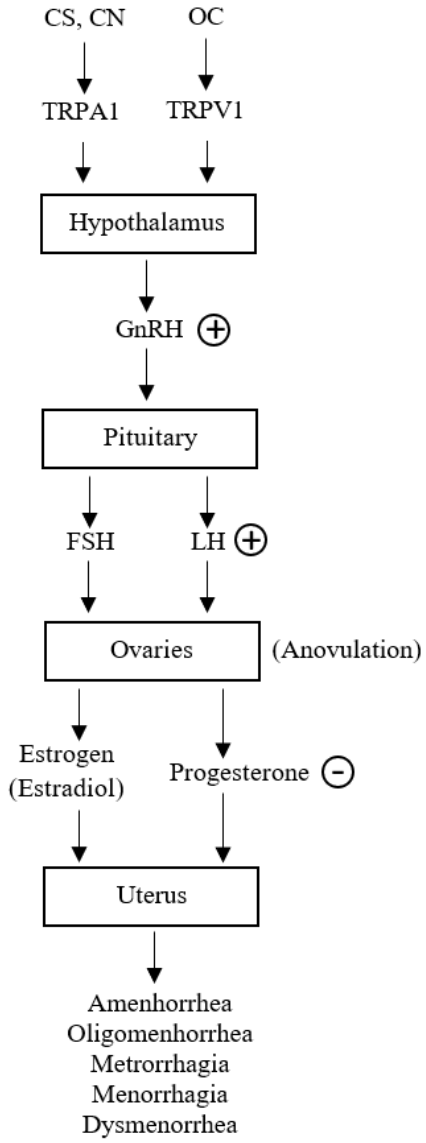
Biological Mechanism

Tear gas and pepper spray primarily interact with the body through TRPA1 and TRPV1 channels, respectively. While not extensively studied in humans, these channels

have been found in the hypothalamus in rat and mouse models (50, 51). As study published in 2020 by Surkin et al. provided evidence that stimulation of TRPV1 channels may activate the hypothalamus-pituitary-gonadal axis in male rats, resulting in GnRH and LH (52). In the human body, elevated levels of GnRH and LH are believed to be the cause of many of the symptoms of PCOS, such as amenorrhea, oligomenorrhea, menorrhagia, and metrorrhagia (15, 53, 54).

The first proposed mechanism by which tear gas (CS and CN) and pepper spray (OC) influence menstrual cycle outcomes is illustrated in Figure 4. OC activates TRPV1 and CS and CN activate TRPA1. TRPV1 stimulates the hypothalamus to produce a greater frequency of GnRH. TRPA1 activation is hypothesized to have similar effects as TRPV1 in the hypothalamus. The increase in GnRH pulsatility (frequency of pulses) signals the pituitary to release LH. Elevated levels of LH act on the ovaries by inhibiting ovulation. In the absence of ovulation, menstrual cycle may be absent (amenorrhea) or irregular (oligomenorrhea). The release of progesterone is depressed due to the lack of ovulation, and the endometrium is allowed to continue to grow. The buildup of the endometrium can lead to breakthrough bleeding (metrorrhagia), or in heavy and prolonged bleeding (menorrhagia) and period pain (dysmenorrhea) should the menstrual cycle resume.

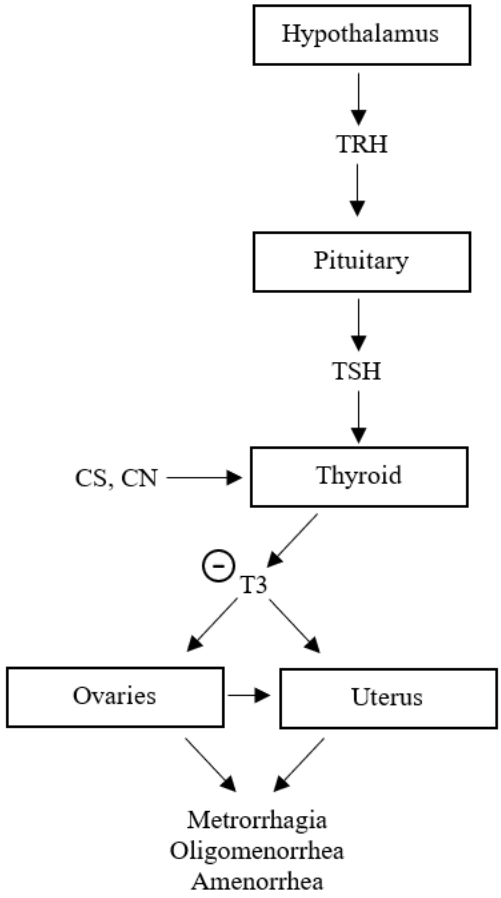
Figure 4: Tear Gas, Pepper Spray, and the Hypothalamus-Pituitary-Gonadal Axis



Tear gas, specifically CS, also been shown to result in damage the thyroid in rats, as demonstrated by Chowdhury et al., though it was not clear whether the CS damaged the thyroid directly or indirectly (through inhibition of TSH) (49). Based on this prior research, the second proposed mechanism is illustrated in Figure 5. CS and CN cause damage to the thyroid which reduces the production of T3. Low T3 levels inhibit

ovulation and the support of the endometrium, which can lead to metrorrhagia, oligomenorrhea, and amenorrhea.

Figure 5: Tear Gas and the Hypothalamus-Pituitary-Thyroid Axis



Conclusion

Menstrual disorders can have a significant negative impact on women's overall quality of life. Women with severe symptoms can experience fatigue, pain, psychological distress, and missed days of school and work (55). It's estimated that absence from work and lower productivity due to menstrual disorders results in an annual loss of $12 to $36

billion in the United States (55). That loss does not include the medical bills that result from women seeking treatment (55). Understanding the causes of menstrual orders, for both prevention and treatment, is important for the quality of women's lives and the economic advancement of women and society. The study by Torgrimson-Ojerio et al. provided evidence that exposure to tear gas may be associated with menstrual disorders (2). Considering the exposure of large numbers of protesters in 2020 and 2021, further understanding this association has become increasingly urgent.

SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1

Determine whether there is an association between the number of protests attended and female reproductive health outcomes.

a. Determine whether there is an association between the number of reported protests and the number of reported menstrual health outcomes.

   Hypothesis: There will be a significant positive association between the number of reported protests and the number of reported menstrual health outcomes.

b. Determine whether there is an association between the number of reported protests and any menstrual health outcome factors that were identified through exploratory factor analysis.

   Hypothesis: There will be a significant positive association between the number of reported protests and the reported menstrual health outcome factors.

Specific Aim 2

Determine whether there is an association between the number acute teargas effects and female reproductive health outcomes.

a. Determine whether there is an association between the composite score of reported acute tear gas effects (eye, lung, skin, heart) and the number of reported menstrual health outcomes.

   Hypothesis: There will be a significant positive association between the number of reported composite score of reported acute tear gas effects and the number of reported menstrual health outcomes.

b. Determine whether there is an association between the composite score of reported acute tear gas effects (eye, lung, skin, heart) and any identified menstrual health outcome factors.

   Hypothesis: There will be a significant positive association between the number of reported composite score of reported acute tear gas effects and menstrual health outcome factors.

Specific Aim 3

Determine whether there is an association between acute effects in any specific organ system (eye, lung, heart, skin) and female reproductive health outcomes.

a. Determine whether there is an association between acute effects in any specific organ system (eye, lung, skin, heart) and the number of reported menstrual health outcomes.

   Hypothesis: There will be a significant positive association between reported acute tear gas effects in the eye, lung, skin, and heart and the number of reported menstrual health outcomes.

b. Determine whether there is an association between acute effects in any specific organ system (eye, lung, skin, heart) and any identified menstrual health outcome factors.

   Hypothesis: There will be a significant positive association between reported acute tear gas effects in the eye, lung, skin, and heart and menstrual health outcome factors.

Specific Aim 4

Determine whether there is an association between seeking medical care for acute effects and female reproductive health outcomes.

a. Determine whether there is an association between seeking medical care for acute effects and the number of reported menstrual health outcomes.

   Hypothesis: There will be a significant positive association between seeking medical care for acute effects and the number of reported menstrual health outcomes.

b. Determine whether there is an association between seeking medical care for acute effects and any identified menstrual health outcome factors.

   Hypothesis: There will be a significant positive association between seeking medical care for acute effects and menstrual health outcome factors.

METHODS

Population

The target population of the study was individuals aged 18 year and older who believed that they were exposed to tear gas in the years 2020 and 2021. To be included for analyses for this dissertation, respondents had to identify as female, report their age as less than 46 years, and have complete data for all covariates that were included in the models.

Study Design

The data were drawn from the University of Louisville Investigation of Possible Health Effects of Tear Gas Among Protestors in the U.S. Study, also known as the Tear Gas Study. This was a cross-sectional study designed to determine whether self-reported exposures to tear gas resulting from protests in 2020 and 2021 were associated with short-term and long-term health outcomes, as well as whether pre-existing conditions were associated with tear gas related health outcomes. The study was also intended to report findings directly back to the community that contributed data through updates to participants who indicated that they would like to be recontacted for this purpose. The Tear Gas Study was approved by the Institutional Review Board of University of Louisville, KY (IRB 20.0802).

The initial questionnaire was retrospective, but the study was designed to also include prospective follow-up questionnaires for participants who consented to be recontacted. The questions included on the questionnaires were based on the literature on tear gas exposure and health outcomes (2). Initial questions were drafted by researchers and community members directly involved with the Tear Gas Study. A committee of epidemiological and environmental researchers was also convened to review the drafted questions and propose additional questions. The final version of the main questionnaire (version 4) is included in Appendix A.

The Tear Gas Study originally targeted residents of Louisville, Kentucky who had been exposed to tear gas during the 2020 racial justice demonstrations. The target population was then expanded to residents of the United States who had been exposed to tear gas during 2020 and 2021. Participants were recruited through social media (Facebook and Twitter) posts and advertisements, as well as flyers distributed to community partners. The study was also covered in several news outlets (56-58). Individuals interested in participating were initially asked to send a message to the study email address for a participant identification number and the link to the online questionnaire. After the 236th participant, the requirements of emailing and acquiring a participant identification number were eliminated. Direct links (hyperlinks and QR codes) to the questionnaire were available on social media posts, the study website, and flyers. Recruiting materials initially offered twenty-dollar gift cards to participants who submitted a questionnaire. This was discontinued due to the high volume of blank and identical questionnaires that were submitted. Criteria for eliminating potential duplicates are discussed in further detail below.

The electronic questionnaire was created and maintained on Research Electronic Data Capture (REDCap), a secure, online platform for entering and storing data (59, 60). The platform is compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).Data could be downloaded in multiple formats, including as a comma-separated values (CSV) file or as a datafile for several statistical software packages. Each questionnaire that was initiated was automatically assigned a record identification number by REDCap. The questionnaire was preceded by a preamble that explained the purpose of the Tear Gas Study and the type of questionnaire that would follow. The preamble included the phone number of the primary investigator (PI) with instructions to contact the PI if the participant had any questions about the study. Contact information for the Human Subjects Protection Office and complaint hotline was also provided. Participants could decide whether to continue with the questionnaire that followed the preamble. The questionnaire included two screener questions: whether the participant was 18 years of age or older and whether the participant was exposed to tear gas. A "no" answer to either question ended the questionnaire. Participants were not able to view any of the participant-level or aggregate data while on the REDCap site.

Exposure Assessment

The exposure of interest for this project was intensity of crowd control chemical irritants, under the umbrella term "tear gas". All participants reported being exposed to tear gas. Participants were unlikely to know what kind of tear gas (CS or CN) that they were exposed to and may not have been able to differentiate between tear gas and pepper spray. The questionnaire used the term "tear gas" with the understanding that participants

may refer to either true tear gas or pepper spray products and is used as such throughout the study. The exposure was assessed three ways; each serves as an estimate of the extent of tear gas exposure. The first was the number of protests attended, regardless of the presence tear gas. Protests number options on the questionnaire included the following: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and >20. The second method was a composite score of acute health effects of tear gas. These health effects include eye (watering, burning/stinging, other), lung (coughing, burning, shortness of breath, other), skin (burning, blistering, other), and heart (increased heart rate, irregular heartbeat, chest pain, other). Participants were instructed to select all that applied. Composite scores were computed by summing the number of "yes" responses for the 13 health effects (from: 0 - 14) and were treated as continuous variables. The third method treated acute effects in each target organ system separately (eye, lung, skin, heart). These were also composite scores (lung range and heart ranges: 0 - 4; eye and skin ranges: 0 - 3) that were handled as continuous variables. The final analysis used seeking medical treatment (yes/no) as an exposure, and determined whether seeking medical treatment for acute symptoms of tear gas was associated with menstrual cycle outcomes.

Outcome Assessment

The outcome of interest was menstrual health. The outcome was assessed using a composite score of all menstrual cycle outcomes on the questionnaire (light bleeding, heavy bleeding, period pains, irregular cycles, long bleeding, short bleeding, short cycle, and long cycle) after exposure to tear gas. Participants were instructed to select all that applied. The composite score could range from 0 to 8 and was treated as a continuous

variable. Exploratory factor analysis was performed to determine whether specific outcomes were interrelated and should be split into factors with separate models. The estimated factor scores provided an additional outcome measure of menstrual symptoms.

Statistical Analyses

To be included in the analyses, questionnaire records must have met the set inclusion criteria. The survey collected data from male, female, and transgender/non-binary participants, but only female-identified records were included this analysis (n=103). Participants also had to self-identify as being 18 to 45 years of age. In the event that records were submitted with timestamps within one minute of one another with identical or nearly identical data, only one of the records was included in the analyses. Duplicates were excluded. This was a quality control measure intended to eliminate serial submissions by the same participants. One hundred three records were eligible for analysis based on these criteria.

Descriptive statistics for covariates were calculated for each exposure proxy and each outcome. Protest number (1 – 9, > 9) and seeking medical care (yes, no) were binary variables and chi-square *p*-values were calculated for each characteristic. Nine was chosen as the cutoff as the resulting groups were close in size (n = 34 and 36, respectively). T-test *p*-values were calculated for characteristics by acute symptoms score, acute symptoms in specific organ systems, menstrual cycle outcome score, factor 1 and factor 2 as these predictors and outcomes were continuous variables. The *p*-values

presented for all the analyses in this dissertation are two-sided and were considered statistically significant when less than 0.05.

Correlation calculations and t-tests were performed to examine the unadjusted associations between exposures and outcomes. Multiple linear regression was used to determine the relationships between the exposures and the outcomes, after adjusting for covariates. Statistical analyses were conducted using Statistical Analysis Software (SAS) Version 9.4 (61). Potential covariates were identified through published literature and directed acyclic graphs (DAGs) [Figures 6-8].

Figure 6: Directed Acyclic Graph for Protest Number and Menstrual Cycle Outcomes

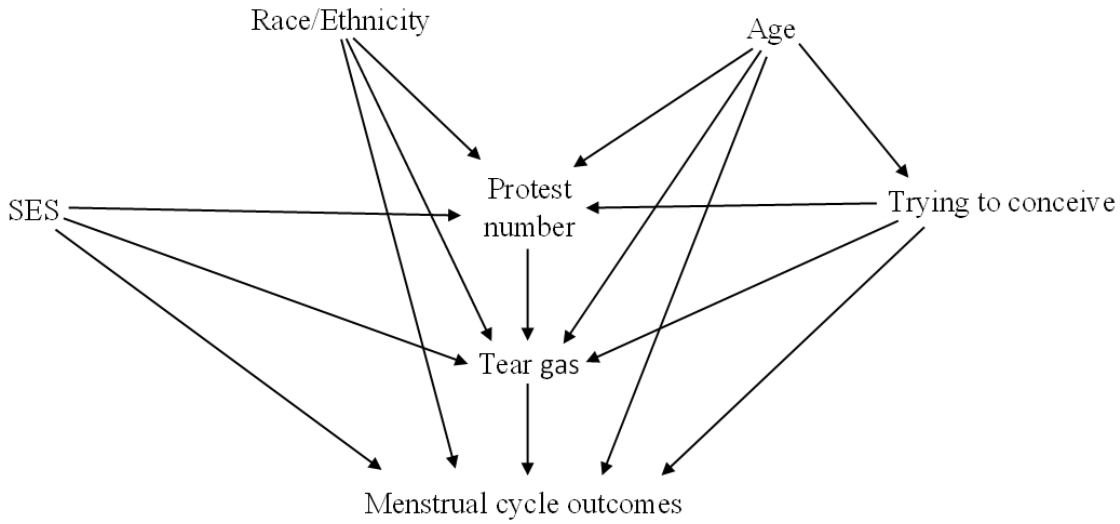


Figure 7: Directed Acyclic Graph for Acute Effects and Menstrual Cycle Outcomes

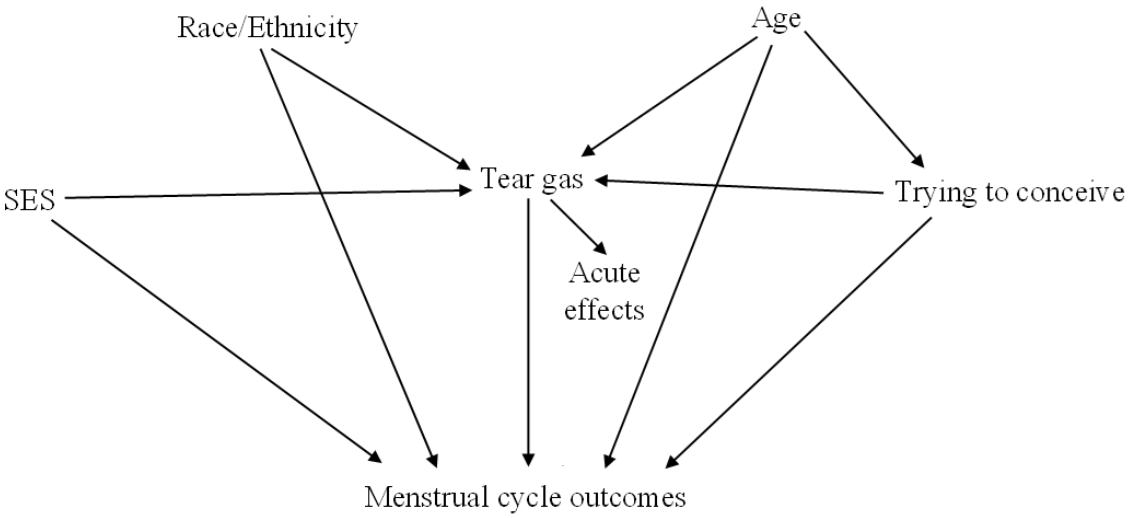
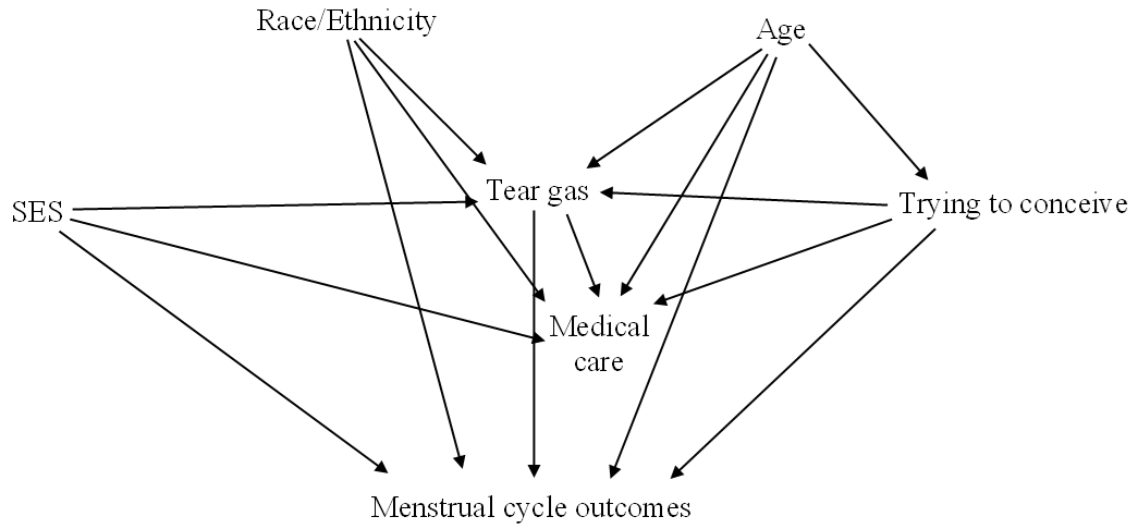


Figure 8: Directed Acyclic Graph for Medical Care for Acute Effects and Menstrual Cycle Outcomes



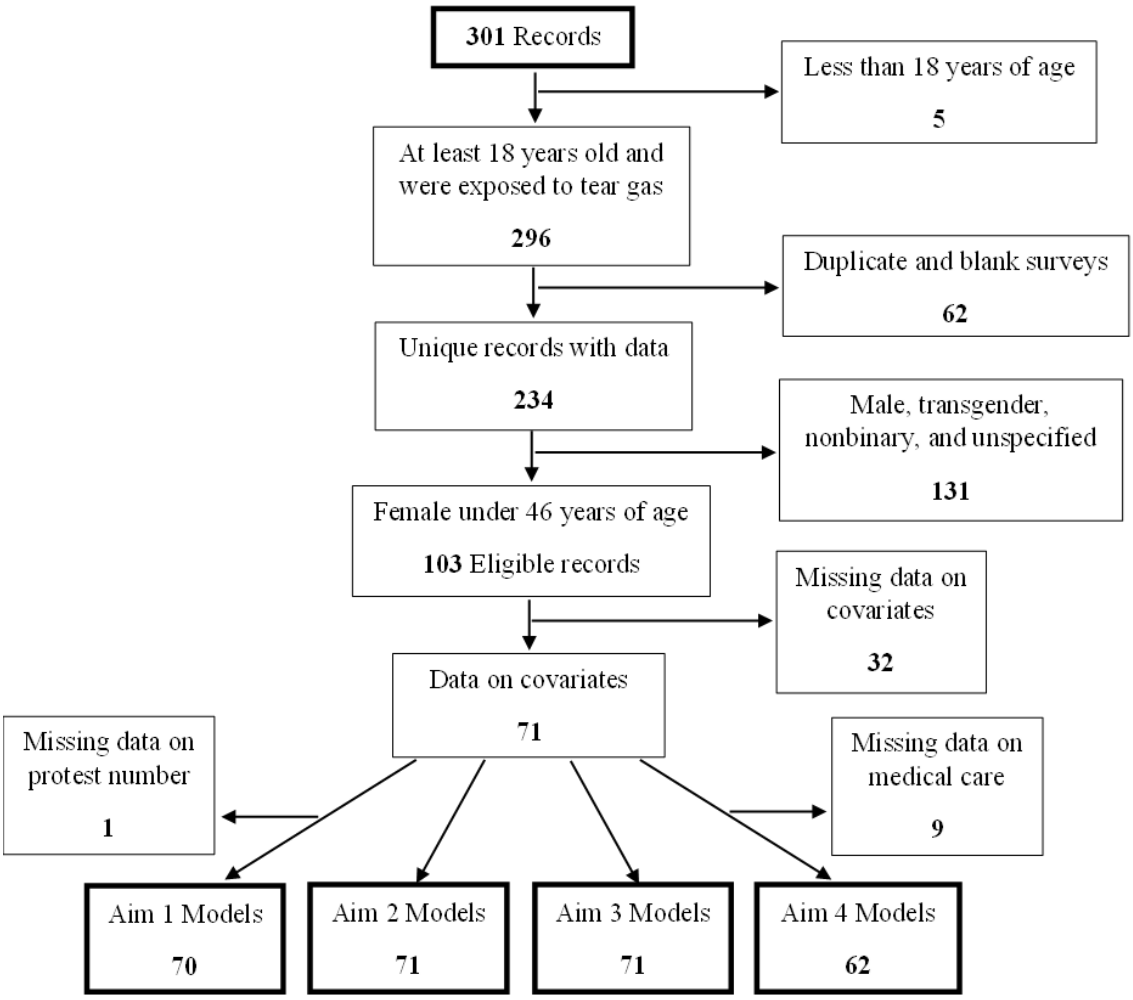
Menstrual cycle outcomes were potentially correlated. Exploratory factor analyses (EFA) were conducted to identify the factor structure of the outcomes in the questionnaire. The number of factors was determined by reading a scree plot of Eigenvalues for values greater than 1 [Figure 9]. Item factor loadings were examined

using promax nonorthogonal rotation. Items that loaded well on a single factor, with a rotated factor loading of greater than 0.6 in absolute value were assigned to that factor [Table 5, Figure 10]. Factor scores were then generated using the regression method (62). The resulting factor scores are normalized as standard deviations around a mean of zero. Normalizing the scores to the same scale allows for comparison between the factors. The identified factors were included as outcome variables in linear regression models for each research aim.

Confounders were determined through the use of directed acyclic graphs [Figures 6-8]. Identified confounders were age, race, ethnicity, socio-economic status (SES) indicators (education and income), and trying to conceive. Additional model-building steps to evaluate the confounding, multicollinearity, precision, and interactions of the confounders identified through DAGs were not conducted. Due to the small sample size, the validity of the evaluations would have been questionable.

The final models included the main exposure (proxy for tear gas exposure) and outcome variables; as well as the confounders identified through the DAGs. Different proxies were not included together in models because of the interrelatedness detected through Chi-square tests, t-tests, and correlation calculations (Appendix B). The flow diagram for participant record inclusion is illustrated in Figure 9.

Figure 9: Flow Diagram for Participant Records



Linear regression models with imputed data were also conducted as a sensitivity analysis. Missing data on predictors and covariates were imputed for the 103 eligible records. Twenty imputations were performed for each missing datapoint. Results from the analyses with imputed data are in Appendix C.

RESULTS

Descriptive Statistics

Between March 15 and September 23, 2021, 301 records (questionnaires) were logged on REDCap. Of these, 103 were eligible to be included in the analysis based on the previously outlined criteria (18 - 45 years of age, reported tear gas exposure, no duplication of records, and female). The final analyses for each aim excluded records that were missing data on the predictors and covariates specified for each model. The models for Aim 1 included 70 records, Aims 2 and 3 included 71 records, and Aim 4 included 62 records [Figure 9].

Descriptive statistics were performed for the 103 eligible participants [Tables 1-4]. Forty percent reported Kentucky as their state of residence. All other reported states of residence made up 6% or less of the sample. Fifty percent were aged 18 to 32, 54% were white, 49% reported an income of $50,000 or higher, and 42% had graduated from college. 27% sought medical care for acute symptoms of tear gas exposure and 21% were trying to conceive. The majority of respondent either attended 1 to 2 protests (30%) or greater than 20 protests (30%). The median acute symptom score was 5 (IQR = 0 - 8). The median menstrual cycle outcome score (total number of 8 menstrual cycle symptoms) was 1 (IQR = 0 - 2). Factor 1 had a median score of -0.59 (IQR = -0.75 - 0.75) and factor 2 had a median score of -0.57 (IQR = -0.57 - 0.50). Factor 1 (representing high severity symptoms) and factor 2 (representing milder symptoms) were

normalized as standard deviations from the mean of 0. Descriptive statistics for eligible records were stratified by protest number [Table 1]; acute symptom composite score, as well as eye, lung, skin, and heart symptom scores [Table 2]; seeking medical care [Table 3]; and menstrual cycle, factor 1, and factor 2 scores [Table 4].

Table 1: Characteristics of Participants by Protest Number, n=101

Characteristic	1 - 9 Protests N=54 N (%)	> 9 Protests N=47 N (%)	Chi-square *p*-value
Age (years)			0.0036
18 - 32	14 (35)	27 (68)	
33 - 45	26 (65)	13 (33)	
Missing	14	7	
Race			0.90
White	24 (55)	25 (53)	
Other	20 (45)	22 (47)	
Missing	10	0	
Ethnicity			0.13
Hispanic	12 (24)	4 (11)	
Non-Hispanic	39 (76)	33 (89)	
Missing	3	10	
Highest education			0.068
No undergraduate degree	35 (66)	22 (48)	
Undergraduate degree	18 (34)	24 (52)	
Missing	1	1	
Income			0.014
≤ $49,999	20 (38)	28 (64)	
≥ $50,000	32 (62)	16 (36)	
Missing	2	3	
Trying to conceive			0.74
No	41 (82)	33 (85)	
Yes	9 (18)	6 (15)	
Missing	4	8	

Table 2: Characteristics of Participants by Acute Symptoms, n=103

Characteristic	n (%)	Total Acute Symptoms Mean±SD	Total Acute Symptoms t-test *p*-value	Eye Mean±SD	Eye t-test *p*-value	Lung Mean±SD	Lung t-test *p*-value	Skin Mean±SD	Skin t-test *p*-value	Heart Mean±SD	Heart t-test *p*-value
Age (years)			0.0044		0.0011		0.0052		0.041		0.14
18 - 32	41 (50)	7.20±2.59		1.98±0.69		2.46±0.78		1.29±0.75		1.46±1.25	
33 - 45	41 (50)	5.27±3.32		1.41±0.81		1.83±1.18		0.95±0.74		1.07±1.10	
Missing	21										
Race			0.93		0.40		0.27		0.30		0.44
White	50 (54)	5.58±2.79		1.58±0.76		2.04±1.05		0.92±0.63		1.04±1.07	
Other	43 (46)	5.51±4.23		1.42±1.07		1.77±1.32		1.09±0.95		1.23±1.31	
Missing	10										
Ethnicity			0.50		0.38		0.14		> 0.9999		0.87
Hispanic	16 (18)	5.00±3.83		1.31±0.95		1.50±1.21		1.00±0.89		1.19±1.17	
Non-Hispanic	74 (82)	5.66±3.52		1.54±0.92		1.99±1.19		1.00±0.79		1.14±1.21	
Missing	13										
Highest education			0.016		0.0008		0.0060		0.023		0.73
No undergraduate degree	59 (58)	4.22±4.23		1.08±1.07		1.42±1.38		0.75±0.90		0.97±1.26	
Undergraduate degree	42 (42)	6.02±2.59		1.74±0.70		2.12±0.97		1.12±0.63		1.05±1.03	
Missing	2										
Income			< 0.0001		< 0.0001		< 0.0001		0.0035		0.017
≤ $49,999	49 (51)	6.31±3.22		1.71±0.84		2.22±1.07		1.12±0.78		1.24±1.25	
≥ $50,000	48 (49)	3.40±3.71		0.94±1.00		1.13±1.25		0.65±0.79		0.69±0.99	
Missing	6										
Positive COVID-19 Test			0.12		0.19		0.15		0.049		0.48
No	66 (83)	6.35±3.00		1.68±0.79		2.18±1.01		1.17±0.76		1.32±1.14	
Yes	14 (18)	4.86±4.20		1.36±1.08		1.71±1.44		0.71±0.83		1.07±1.33	
Missing	23										
Trying to conceive			0.62		0.68		0.43		0.68		0.53
No	75 (83)	5.56±3.70		1.50±0.96		1.93±1.23		0.97±0.84		1.15±1.24	
Yes	15 (17)	5.07±2.55		1.40±0.63		1.67±0.98		1.07±0.80		0.93±0.96	
Missing	13										

Table 3: Characteristics of Participants by Seeking Medical Care for Acute Effects, n=73

Characteristic	Medical Care - No n=52 n (%)	Medical Care - Yes n=21 n (%)	Chi-square *p*-value
Age (years)			0.33
18 - 32	30 (58)	9 (45)	
33 - 45	22 (42)	11 (55)	
Missing	0	1	
Race			0.23
White	35 (67)	11 (52)	
Other	17 (33)	10 (48)	
Ethnicity			0.0060
Hispanic	5 (10)	8 (38)	
Non-Hispanic	44 (90)	13 (62)	
Missing	3	0	
Highest education			0.082
No undergraduate degree	19 (37)	12 (60)	
Undergraduate degree	32 (63)	8 (40)	
Missing	1	1	
Income			0.90
≤ $49,999	29 (62)	12 (60)	
≥ $50,000	18 (38)	8 (40)	
Missing	5	1	
Trying to conceive			0.025
No	44 (88)	13 (65)	
Yes	6 (12)	7 (35)	
Missing	2	1	

Table 4: Characteristics of Participants by Menstrual Cycle Symptoms, n=103

		Total Menstrual Cycle Symptoms		Factor 1		Factor 2	
Characteristic	**n (%)**	**Mean±SD**	**t-test *p*-value**	**Mean±SD**	**t-test *p*-value**	**Mean±SD**	**t-test *p*-value**
Age (years)			0.12		0.20		0.80
18 - 32	41 (50)	2.49±2.69		0.33±1.04		0.10±1.12	
33 - 45	41 (50)	1.63±2.13		0.04±1.00		0.16±1.02	
Missing	21						
Race			0.073		0.24		0.12
White	50 (54)	2.24±2.50		0.19±1.06		0.22±1.16	
Other	43 (46)	1.35±2.19		-0.05±0.93		-0.12±0.82	
Missing	10						
Ethnicity			0.36		0.77		0.017
Hispanic	16 (18)	2.25±2.29		0.14±0.95		0.57±1.08	
Non-Hispanic	74 (82)	1.66±2.35		0.06±1.02		-0.09±0.95	
Missing	13						
Highest education			0.0099		0.0068		0.14
No undergraduate degree	59 (58)	1.12±2.11		-0.23±0.86		-0.14±0.89	
Undergraduate degree	42 (42)	2.29±2.32		0.30±1.05		0.15±1.05	
Missing	2						
Income			0.0071		0.0050		0.75
≤ $49,999	49 (51)	2.12±2.52		0.23±1.04		-0.01±1.12	
≥ $50,000	48 (49)	0.92±1.71		-0.31±0.81		0.08±0.73	
Missing	6						
Positive COVID-19 Test			0.0028		0.0064		0.11
No	66 (83)	2.42±2.54		0.29±1.04		0.21±1.14	
Yes	14 (18)	0.29±0.61		-0.52±0.54		-0.30±0.55	
Missing	23						
Trying to conceive			0.32		0.56		0.27
No	75 (83)	1.88±2.47		0.07±1.00		-0.03±1.04	
Yes	15 (17)	1.20±2.34		-0.09±0.86		0.28±0.75	
Missing	13						

Participants who reported an income of at least $50,000 attended significantly fewer protests and had significantly fewer total acute symptoms of tear gas, acute symptoms in specific organ systems, total menstrual cycle symptoms, and factor 1 scores. Having at least an undergraduate degree was positively associated with total acute symptoms of tear gas, acute symptoms in specific organ systems (excluding heart), total menstrual cycle symptoms, and factor 1 scores. Participants over the age of 32 reported a significantly lower protest attendance; as well as fewer total acute symptoms and acute symptoms of the eyes, lungs, and skin. Hispanic ethnicity was significantly associated with higher factor 1 score and with seeking medical care for acute exposure. Participants who were trying to conceive also reported more medical care seeking behavior.

Two factors were identified for inclusion in the linear regression models [Figure 10]. Long bleed, short bleed, heavy bleed, long cycle, irregular cycles, and period pain loaded on factor 1. Short bleed, light bleed, and short cycle loaded on factor 2 [Table 5, Figure 11]. Generally, factor 1 represented more severe menstrual symptoms, while factor 2 represented milder symptoms, though the two factors were correlated ($\rho = 0.504$, $p < 0.0001$). Together, factor 1 and factor two explain 89.9% of the variance in the menstrual symptoms.

Figure 10: Scree Plot of Eigenvalues

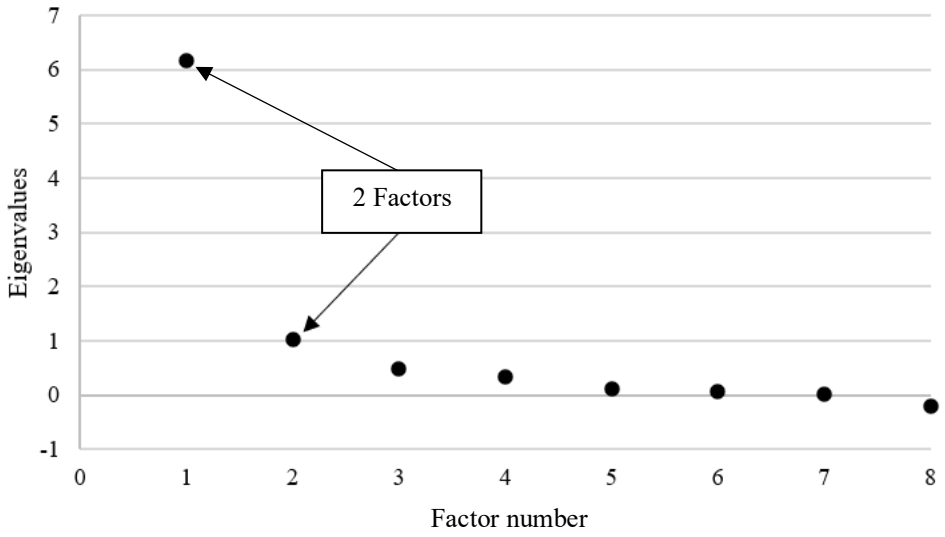
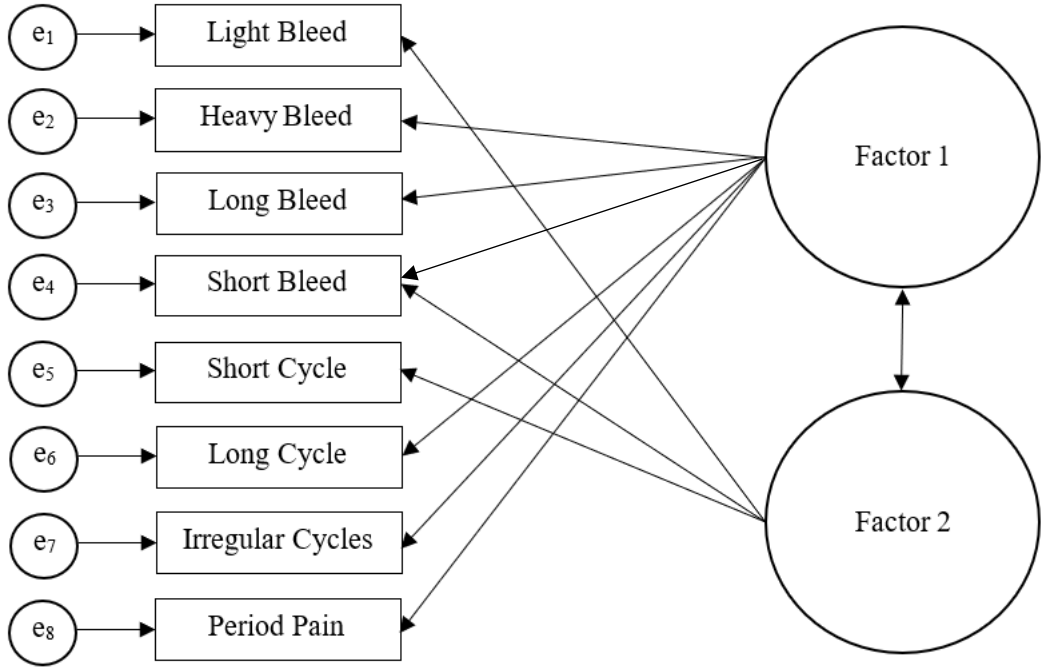


Table 5: **Rotated Factor Loadings**

	Factor Loadings	
Cycle Outcome	**Factor 1**	**Factor 2**
Light bleed	0.14207	**0.93922**
Heavy bleed	**0.93389**	0.17817
Long bleed	**0.93183**	0.25749
Short bleed	**0.62030**	**0.80062**
Short cycle	0.40165	**0.77963**
Long cycle	**0.78103**	0.49616
Irregular cycles	**0.87585**	0.37628
Period pain	**0.82730**	0.45100

Figure 11: Menstrual Cycle Outcomes Loaded on Two Factors



e = error

Unadjusted associations between the tear gas exposure proxies and the outcomes were examined through the use of correlation coefficients and t-test *p*-values, depending on whether the proxy was a continuous or binary variable [Tables 6 & 7]. Total acute symptom score, and acute symptoms of the eye, lung, skin, and heart were correlated with total menstrual cycle symptom score and factor 1, but only heart symptoms were correlated with factor 2. Protest attendance had significant positive associations with menstrual cycle symptom score, factor 1, and factor 2. Seeking medical care for tear gas exposure had significant inverse associations with menstrual cycle symptoms score, and factor 1.

Table 6: Correlation Coefficients (ρ) for Acute Symptoms of Tear Gas and Menstrual Cycle Outcomes, n=103

	Total Cycle Symptoms	Factor 1	Factor 2
Total Acute Symptoms	0.3404**	0.3563**	0.1914
Eye	0.3045*	0.4047***	0.1451
Lung	0.3703**	0.4088***	0.1714
Skin	0.2462*	0.2197*	0.1310
Heart	0.2572*	0.2007*	0.2114*

* p-value < 0.05
** p-value < 0.001
*** p-value < 0.0001

Table 7: Unadjusted Associations of Menstrual Cycle Outcomes with Protest Attendance (n=101) and Seeking Medical Care (n=73) for Tear Gas Exposure

		Total Menstrual Cycle Symptoms		Factor 1		Factor 2	
Tear Gas Proxy	**n (%)**	**Mean±SD**	**t-test *p*-value**	**Mean±SD**	**t-test *p*-value**	**Mean±SD**	**t-test *p*-value**
Protests			< 0.0001		< 0.0001		0.046
1 - 9	54 (53)	0.67±1.20		-0.47±0.62		-0.20±0.72	
> 9	47 (47)	2.68±2.70		0.52±1.05		0.19±1.17	
Medical Care			0.014		0.0003		0.75
No	52 (71)	2.60±2.61		0.53±1.03		0.15±1.13	
Yes	21 (29)	1.00±1.95		-0.52±0.58		0.24±1.06	

Models and Variable Coding

Twenty-one linear regression models were implemented in the investigation of the specific aims [Table 8]. Beta coefficients and 95% confidence intervals were calculated for protest number, acute symptom score, acute eye score, acute lung score, acute skin score, acute heart score, and seeking medical care according to the model for the particular aim. Outcome variables were menstrual cycle outcome score, normalized factor 1 score, and normalized factor 2 score. The definitions and methods of handling for each variable included in the models are included in Table 9.

Table 8: Final Linear Models

Aim 1

Model 1: $Factor\ 1 = \beta_0 + \beta_1 * Protest\ Number + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 2: $Factor\ 2 = \beta_0 + \beta_1 * Protest\ Number + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 3: $Cycle\ Score = \beta_0 + \beta_1 * Protest\ Number + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 2

Model 4: $Factor\ 1 = \beta_0 + \beta_1 * Acute\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 5: $Factor\ 2 = \beta_0 + \beta_1 * Acute\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 6: $Cycle\ Score = \beta_0 + \beta_1 * Acute\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 3 (Eye)

Model 7: $Factor\ 1 = \beta_0 + \beta_1 * Eye\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 8: $Factor\ 2 = \beta_0 + \beta_1 * Eye\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 9: $Cycle\ Score = \beta_0 + \beta_1 * Eye\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 3 (Lung)

Model 10: $Factor\ 1 = \beta_0 + \beta_1 * Lung\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 11: $Factor\ 2 = \beta_0 + \beta_1 * Lung\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 12: $Cycle\ Score = \beta_0 + \beta_1 * Lung\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 3 (Skin)

Model 13: $Factor\ 1 = \beta_0 + \beta_1 * Skin\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 14: $Factor\ 2 = \beta_0 + \beta_1 * Skin\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 15: $Cycle\ Score = \beta_0 + \beta_1 * Skin\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 3 (Heart)

Model 16: $Factor\ 1 = \beta_0 + \beta_1 * Heart\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 17: $Factor\ 2 = \beta_0 + \beta_1 * Heart\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 18: $Cycle\ Score = \beta_0 + \beta_1 * Heart\ Score + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Aim 4

Model 19: $Factor\ 1 = \beta_0 + \beta_1 * Medical\ Care + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 20: $Factor\ 2 = \beta_0 + \beta_1 * Medical\ Care + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Model 21: $Cycle\ Score = \beta_0 + \beta_1 * Medical\ Care + \beta_2 * Age + \beta_3 * Race + \beta_4 * Ethnicity + \beta_5 * Education + \beta_6 * Income + \beta_7 * Trying\ to\ Conceive$

Table 9: Definitions for Model Variables

Variable	Definition
Age	
	18 - 32 years (reference)
	33 - 45 years
Race	
	White (reference)
	Other
Ethnicity	
	Hispanic
	Non-Hispanic (reference)
Education	

	No undergraduate degree (reference)
	Undergraduate degree
Income	
	≤ $49,999 (reference)
	≥ $50,000
Trying to Conceive	
	Yes
	No (reference)
Protest Number	
	1 - 9 (reference)
	> 9
Acute Symptom Score	
	Continuous (0 - 14)
Eye Score	
	Continuous (0 - 3)
Lung Score	
	Continuous (0 - 4)
Skin Score	
	Continuous (0 - 3)
Heart Score	
	Continuous (0 - 4)
Sought Medical Care	
	Yes
	No (reference)
Menstrual Cycle Score	
	Continuous (0 - 8)
Factor 1 Score	
	Continuous (-1.32 - 2.57)
Factor 2 Score	
	Continuous (-1.71 - 3.59)

Aim 1: Protest Number

Three models were run for Aim 1. Protest number was the predictor of interest. Seventy records were included in the models. Compared to the reference of 1 to 9 protests, attending greater than 9 protests was significantly associated with higher

menstrual cycle outcome scores and factor 1 [Table 10]. The only covariate that had a significant effect was ethnicity. In model 3, Hispanic ethnicity was associated with higher menstrual cycle outcome scores than non-Hispanic ethnicity.

Table 10: Multivariable Linear Models for Protest Number and Menstrual Cycle Outcomes

Number of protests attended n = 70				
Protests	**n (%)**	**β[a]**	**(95% CI)[a]**	***p*-value**
		Factor 1 (Model 1)		
1 - 9	38 (54)	0.000	Reference	
> 9	32 (46)	1.219	(0.788, 1.650)	<0.0001
		Factor 2 (Model 2)		
1 - 9	38 (54)	0.000	Reference	
> 9	32 (46)	0.462	(-0.050, 0.974)	0.082
		Cycle Score (Model 3)		
1 - 9	38 (54)	0.000	Reference	
> 9	32 (46)	2.116	(1.120, 3.112)	<0.0001

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Aim 2: Acute Symptoms of Tear Gas

The analyses for Aim 2 included three models in which acute tear gas exposure symptom score was the predictor. Seventy-one records contributed data to the models. Acute symptom score had non-significant positive associations with menstrual cycle outcome score, factor 1 score, and factor 2 score [Table 11]. Education was significantly positively associated with cycle score.

Table 11: Multivariable Linear Models for Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes

Acute symptoms score n = 71		
β[a]	**(95% CI)[a]**	***p*-value**
Factor 1 (Model 4)		
0.004	(-0.078, 0.085)	0.93
Factor 2 (Model 5)		
0.018	(-0.062, 0.099)	0.66
Cycle Score (Model 6)		
0.027	(-0.147, 0.200)	0.76

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Aim 3: Acute Symptoms in Specific Organ Systems

Aim 3 examined acute scores for eye, lung, skin, and heart symptoms as separate predictors. Twelve models were run with data from 71 records. All associations with the outcomes of interest were non-significant. Eye, lung, skin, and heart scores had positive associations with menstrual cycle score and factor 2 score. Skin and heart scores had inverse associations with factor 1 score, while the associations were positive for eye and lung scores [Table 12]. Education had a significant positive associated with cycle score for each organ system.

Table 12: Multivariable Linear Models for Specific Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes

Specific acute symptoms n = 71			
Symptom	**β[a]**	**(95% CI)[a]**	***p*-value**
Eye			
		Factor 1 (Model 7)	
	0.156	(-0.157, 0.469)	0.33
		Factor 2 (Model 8)	
	0.057	(-0.256, 0.370)	0.72
		Cycle Score (Model 9)	
	0.048	(-0.626, 0.722)	0.89
Lung			
		Factor 1 (Model 10)	
	0.081	(-0.158, 0.319)	0.51
		Factor 2 (Model 11)	
	0.021	(-0.216, 0.259)	0.86
		Cycle Score (Model 12)	
	0.088	(-0.423, 0.599)	0.74
Skin			
		Factor 1 (Model 13)	
	-0.072	(-0.401, 0.256)	0.67
		Factor 2 (Model 14)	
	0.033	(-0.293, 0.360)	0.84
		Cycle Score (Model 15)	
	0.084	(-0.619, 0.787)	0.82
Heart		**Factor 1 (Model 16)**	
	-0.077	(-0.285, 0.131)	0.47
		Factor 2 (Model 17)	

	0.066	(-0.141, 0.273)	0.53
Cycle Score (Model 18)			
	0.056	(-0.391, 0.502)	0.81

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Aim 4: Medical Care for Acute Symptoms

The main predictor for Aim 4 was seeking medical treatment for acute tear gas symptoms. Three models were run with the records from 62 participants. Medical treatment had a significant inverse association with factor 1. The associations were non-significantly positive for factor 2 score, and non-significantly inverse for menstrual cycle outcome score [Table 13]. None of the covariates were significantly associated with menstrual cycle outcomes.

Table 13: Multivariable Linear Models for Seeking Medical Attention for Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes

	Sought medical care for acute effects n = 62			
Medical care	**n (%)**	**β[a]**	**(95% CI)**	***p*-value**
		Factor 1 (Model 19)		
No	42 (68)	0.000	Reference	
Yes	20 (32)	-0.952	(-1.563, -0.340)	0.0035
		Factor 2 (Model 20)		
No	42 (68)	0.000	Reference	
Yes	20 (32)	0.190	(-0.489, 0.870)	0.59
		Cycle Score (Model 21)		
No	42 (68)	0.000	Reference	
Yes	20 (32)	-0.987	(-2.420, 0.447)	0.18

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Imputed Models

Each model was run again with data from the multiple imputation. These models each included the full eligible sample of 103. The significant associations between protest number and factor 1 (model 1), protest number and total menstrual cycle symptoms (model 3), and seeking medical care and factor 1 (model 19) held. Significant positive associations with imputed data that had not been seen in the unimputed models were between protest number and factor 2 (model 2), total acute symptoms and total menstrual cycle symptoms (model 6), eye symptoms and factor 1 (model 7), and lung symptoms with both factor 1 and total menstrual cycle symptoms (models 10 & 12). The only significant inverse association in the imputed models that was not in the unimputed models was between seeking medical for acute symptoms of tear gas and total menstrual cycle outcomes (model 21). Tables for the imputed data analyses are in Appendix C.

DISCUSSION

The purpose of this study was to investigate whether exposure to tear gas during the racial justice protests was associated with menstrual cycle characteristics or abnormalities. The hypothesis was that tear gas exposure, as estimated by the number of protests attended, number of acute tear gas symptoms, tear gas symptoms of specific organ systems (eye, lung, skin, and heart), and seeking medical care for acute tear gas symptoms would be significantly associated with menstrual cycle. The number of protests attended, but not the other measures of tear gas exposure, was significantly and positively associated with two measures of menstrual cycle characteristics (the number of menstrual cycle symptoms, and factor 1 score). Seeking medical treatment for tear gas exposure had significant inverse relationship with factor 1 score.

Protest Number

Protest number was a main tear gas exposure proxy of interest. Participants who reported attending more protests (> 9) reported statistically higher menstrual cycle outcomes scores and factor 1 scores. Factor 1 generally included more intense menstrual cycle outcomes (heavy bleed, long bleed, short bleed, long cycle, irregular cycles, and period pain), while the menstrual outcomes included in factor 2 were generally milder (light bleed, short bleed, and short cycle). However, there was a significant positive association between protest attendance and factor 2 in the larger imputed dataset. These

results generally support the hypothesis that protest number would have a significant positive association with menstrual cycle outcomes. The association may be reflecting protest number as an appropriate proxy for tear gas, as expected. If this is the case, the pathway from protest number to menstrual cycle outcomes should go through tear gas exposure [Figure 6]. The association may also be due to unidentified confounders or alternative pathways. These possibilities will be explored in more detail below.

Acute Symptoms

Acute symptoms, both as a composite score and as symptoms of specific organ systems, were not significantly associated with menstrual cycle outcomes. This may indicate that acute symptoms are poor proxies for estimating tear gas exposure, and that the frequency of tear gas exposure might be a better proxy for overall tear gas exposure, as demonstrated by the significant association between number of tear gas exposures and menstrual outcomes shown in the supplementary analyses. It is also possible that tear gas is able to impact the menstrual cycle in doses that are too low to produce the acute symptoms included in the questionnaire. Analyses of acute symptoms would, then, be unable to differentiate between exposures that were above and below the threshold for menstrual cycle symptoms to manifest. Based on available published literature, there does not appear to be any research on the minimum dose required for menstrual cycle effects.

Medical Care

Seeking medical care for tear gas acute symptoms of tear gas exposure had a significant inverse association with factor 1, but non-significant associations with factor 2 and menstrual cycle outcomes. Per the stated hypotheses, medical care seeking behavior was expected to be positively associated with menstrual cycle symptoms, factor 1, and factor 2. Seeking medical care was intended to be a proxy for tear gas exposure, in which higher exposure would lead more individuals to seek treatment. A possible explanation for the inverse association seen with factor 1 is that seeking (and receiving) medical treatment for tear gas exposure was not a proxy for exposure, but an effect modifier. Medical treatment may have interrupted the processes that lead to factor 1 outcomes, but not factor 2 outcomes. Seeking medical care is also correlated with overall self-care; individuals who are more thoughtful and active about their health may have also taken other measures to mitigate the effect of tear gas exposure (e.g., changing clothes or showering after exposure). Total menstrual cycle outcomes include those of both factor 1 and factor 2, so the effect of medical treatment may have been diluted. In the imputed models, the associations between seeking medical treatment and factor 1 was stronger (smaller 95% confidence intervals and *p*-values), which may have contributed to the significant inverse association between medical care and total menstrual cycle outcomes.

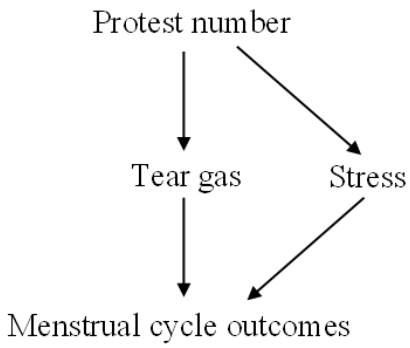
Biological Mechanisms

Two potential biological mechanisms to explain how tear gas may affect menstrual cycle outcomes were proposed in Figures 4 and 5 and were incorporated in the proposed hypotheses. In the first pathway, TRPV1 and TRPA1 receptors in the hypothalamus are activated by OR, CS, and CN (50, 51). The overstimulation of the

GnRH release leads to anovulation and abnormal menstrual cycle outcomes (53). In the second pathway, CS and CN cause damage to the thyroid, reducing the production of T3, which leads to altered menstrual cycles (13-15, 49).

There is the possibility that the association between protest number and menstrual cycle scores was not a result of the tear gas exposure, but of the stress of the protests themselves [Figure 12]. Previous research has provided evidence for an association between stress and abnormal menstrual cycle outcomes. For example, Gordley et al. published a study in 2000 that demonstrated a link between acute stress from life events and menstrual cycle outcomes, such as period pain, long or heavy bleeding, and altered cycle length (18). Anovulation has also been linked to stress and can present as a long menstrual cycle (39). It has been hypothesized that the glucocorticoids released as part of the stress response may disrupt hormone release by the pituitary and ovaries, thus altering the menstrual cycle (5). The addition of a variable measuring stress within the models, as well as a main predictor, may shed some light on whether stress affects the association of tear gas exposure and menstrual cycle outcomes. However, data on stress was only collected for 32 of the 103 eligible participants and was not included in these analyses.

Figure 12: Alternate Pathway Through Stress from Protest Number to Menstrual Cycle Outcomes



Published Literature

The results from these analyses in which protest number was used as a proxy for tear gas exposure are in agreement with currently available literature on tear gas exposure and menstrual cycle outcomes (2, 48). Torgrimson-Ojerio et al. found the number of tear gas exposures to be significantly associated with cycle outcomes, such as clots, cramping, changes in bleed length and color, and a lack of bleeding (2). Mahfud et al. reported tear gas exposure number to have a significant association with increased reporting of total abnormal menstrual cycle outcomes and long-term abnormal cycle outcomes before adjusting for covariates. General symptoms of tear gas exposure were correlated with menstrual cycle outcomes in the Mahfud et al. study; however, they were not significant predictors in the current analyses. Differences may have been due to the Mahfud et al. study analyzing a larger sample size and focusing specifically on CS gas, whereas the current analyses likely included a mixture of crowd-control agents (48).

Implications for Public Health

In the dataset for this study, attending more than 9 protests was positively associated with severe menstrual outcomes, while medical care for tear gas exposure had an inverse association. These results could be used to inform members of the public who are interested in attending protests on measures to reduce severe menstrual outcomes after exposure to crowd control irritants. These measures include attending fewer protests and seeking medical care in the event of exposure to tear gas or pepper spray. Medical

treatment for tear gas and pepper spray includes decontamination, and information on effective decontamination techniques need to be made widely available (3, 39).

On the policy side of public health, this study adds to the body of research that indicates that crowd control irritants are not benign and can have short-term and long-term health effects that range from relatively mild to very serious (39). Tear gas and pepper spray are known to cause chemical burns, blindness, and respiratory failure (39, 40). There is mounting evidence they can also have reproductive effects (2, 46-48). Regulations at the national, state, and local levels are needed to limit the use of crowd control irritants to extreme cases, such as violent riots. Steps have already been taken in this direction, such as the previously mentioned laws passed in California, Oregon, and Washington (31-33). Some of these laws do not include limitations on the use of pepper spray (OC) with the limits on tear gas (33). While this study was not able to distinguish between tear gas and pepper spray, previous research has shown that pepper spray can also have severe health outcomes (including fatal lung edema) (3). The existing data do not appear to support regulating pepper spray differently from tear gas (3, 39).

Any steps to reduce the use of crowd control irritants by law enforcement agencies should include educating law enforcement agents on the proper use of tear gas and pepper spray, the potential health outcomes for civilians and law enforcement agents who are exposed, situations in which severe health outcomes are more likely (such as deploying crowd control irritants in enclosed spaces), and decontamination methods to avoid contaminating the homes and families of law enforcement agents (3). It should be stressed that tear gas and pepper spray are not benign and should not be used unless absolutely necessary.

Limitations

There are several potential limitations to this study. There may be residual confounding from variables that were not identified through literature and DAGs, or that were not collected in the questionnaire. For example, there may be unaccounted for variables that make participants both more likely to be exposed to tear gas and more likely to experience the menstrual outcomes of interest. An individual's occupation could influence both their protest attendance and menstrual health. Some individuals may have underlying conditions that predispose them to both acute symptoms after tear gas exposure and menstrual disorders. A person who is willing to seek medical care for acute tear gas exposure may have other health habits that are associated with fewer menstrual cycle disturbances. These could result in spurious associations between the predictors and the outcomes. Residual confounding could also result from the dichotomous coding of the covariates included in the models.

This study may also be subject to selection bias inherent to studies in which participants are self-selected. Individuals who experienced more severe tear gas exposure symptoms may be more motivated to participate in the study. This could result selection bias if these same individuals were also more likely to experience menstrual cycle disturbances, apart from those being caused by tear gas exposure. Possible sources of information bias include the reliance on self-reported data, which may not be as accurate as more objective data collection methods. The data were also collected retrospectively, which introduces the possibility of recall bias. Participants who experienced more of the menstrual cycle outcomes may be more likely to recall more severe tear gas exposure than if they had not experienced those outcomes.

Another limitation is that the study is unable to determine exactly what type(s) of chemical irritant each participant was exposed to and at what dose. Participants may not have been able to distinguish between tear gas and pepper spray, and there are different formulations of these crowd control agents. Collecting data on individual exposures through personal monitors was not feasible; thus only proxy measures of exposure were available (protest number, acute exposure symptoms, and exposure number). Therefore, the study was unable to determine which chemical agents at which concentration are responsible for any associations between exposure and menstrual cycle issues that may be identified. However, the data do reflect real world exposures experienced by protesters.

Number of protests attended was used as a proxy for tear gas exposure (as were acute symptoms and seeking medical care) because exposure could not be measured directly through personal air monitoring units in this study. Wrist bands that pick up environmental chemicals have been developed, by they are not currently designed to capture tear gas and pepper spray (63). Laboratory tests to identify crowd control irritants post exposure are also generally unavailable (27). The question of how many times participants believed that they were exposed to tear gas was added later to questionnaire. Analyses of the 58 eligible participants who provided data on exposure number showed similar results to protest number [Appendix D]. The binary protest number and exposure number were also directly associated with one another (Chi-square $p < 0.0001$). These results indicate that protest number is a reasonable proxy for tear gas exposure number.

The models also included small sample sizes, which may have impacted whether they were sufficiently powered to detect significant outcomes. A post hoc power exploration is included in Appendix E.

A limitation specific to the exploratory factor analyses was the cross-loading of short bleed on factor 1 and factor 2. Ideally, no item (symptom) would load strongly on more than one item. Doing so indicates that the two factors poorly present short bleed. A different non-orthogonal rotation could potentially resolve the cross-loading.

Strengths

One strength of this study is that it collected data on a variety of menstrual cycle outcomes. This allowed for the examination of different ways that menstrual cycles can be altered and to determine which were potentially related (in that they had common underlying processes). Relatedness of the outcomes was determined through the use of EFA, which is another strength of the study. Identifying factors and including them in separate models reduced the model dimensionality.

Multiple proxies for tear gas exposure were modeled and compared to exposure number. This demonstrated that protest number may be a good proxy for tear gas exposure, but that acute symptoms and seeking medical care may not. The inclusion of medical care also indicated that treatment for acute symptoms may be important for preventing menstrual cycle outcomes, which could be of interest from a public health standpoint.

This study contributes data to an area of public health (tear gas and menstrual health outcomes) that has been the subject of little research. This work provides further evidence that exposure to tear gas is associated with menstrual cycle outcomes. Data on tear gas and health outcomes is of importance to policy makers, medical providers, and

individuals who have been exposed to tear gas or who may be exposed in future demonstrations.

Suggestions for Future Research

While the current study demonstrated an association between tear gas and menstrual cycle outcomes, it is uncertain which specific tear gas agents are implicated. Future research is needed on specific agents, such as OC, CS, and CN. It is also important to understand which agents are and were being used in different areas of the United States. This information would give protesters some idea of what specifically they were exposed to. The development of inexpensive and easy to use personal monitors that could be employed to analyze exposures of individuals would be an important tool when trying to answer this question.

The results of the study indicate that seeking medical treatment for tear gas exposure is associated with lower factor 1 (severe menstrual cycle symptoms) score. Research on the effects of medical treatment is needed, including whether specific interventions are more protective. Data on different interventions would help inform medical professionals who may be treating exposed individuals. For example, are immediate eye flushing and skin decontamination both important to preventing menstrual disturbances? Do the formulations of cleaning solutions impact menstrual cycle outcomes? Is there an optimal time frame for treatment for acute tear gas symptoms? Research on these questions could inform best practices for reducing harm to patients who have been exposed to crowd control irritants.

Research is also needed on the duration of menstrual cycle disturbances after exposure to tear gas. Long-term adverse menstrual changes could negatively impact quality of life and financial stability of affected individuals (55). There is also the question of whether there are long-term consequences as far as the ability of affected individuals to achieve pregnancy and carry to term. McElhatton et al. conducted a study with 30 women who were exposed to CS during pregnancy, in which adverse outcomes (malformations and pregnancy loss) were not significantly different from the general population (47). However, no studies were identified that examined fertility and pregnancy in individuals who had been exposed to tear gas prior to conception. Data on long-term reproductive outcomes could be important to individuals who have been exposed (or who plan to participate in activities that may include exposure) and wish to become pregnant in the future.

CONCLUSION

The purpose of this study was to determine whether protest-related tear gas exposure was associated with menstrual cycle disturbances. The results indicate that there is a positive relationship between tear gas exposure and menstrual cycle symptoms: participants who reported higher tear gas exposures also tended to report more menstrual cycle symptoms. The study also found that seeking medical care for acute symptoms of tear gas exposure may be protective. These results add to a small, growing body of literature investigating health outcomes of tear gas exposure that have previously gone undocumented. Tear gas exposure affects not only the nervous, cardiovascular, and integumentary systems, but may cause hormonal disturbances which then alter reproductive function. Future research is needed to better define the role of other confounding variables (such as stress); examine the duration of menstrual cycle outcomes post-exposure; determine the importance of seeking medical treatment for acute tear gas exposure and whether it can mitigate adverse reproductive health outcomes; and evaluate the potential for long-term fertility and pregnancy complications.

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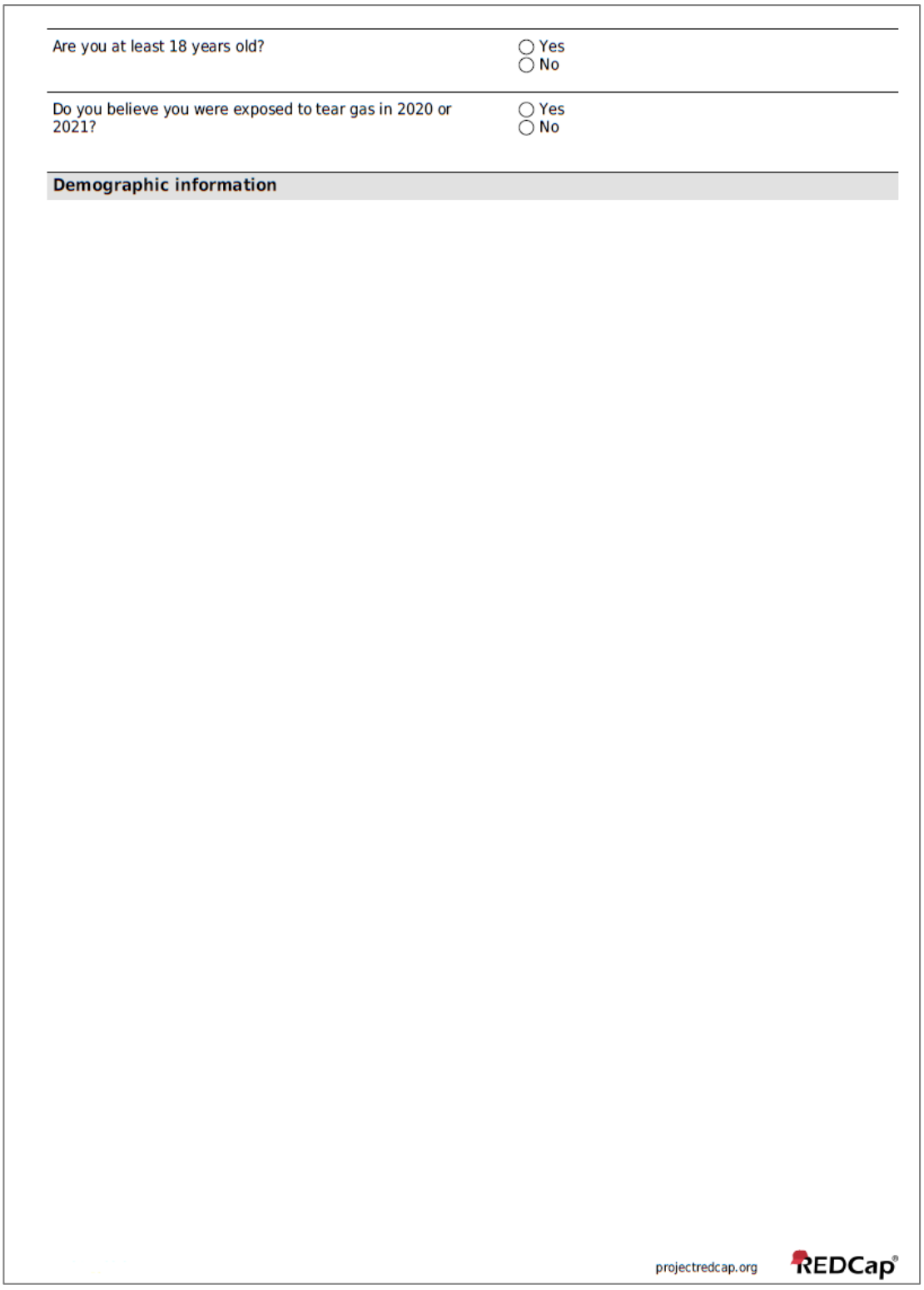
APPENDIX A: TEAR GAS HEALTH QUESTIONNAIRE

Figure 13: Tear Gas Health Questionnaire, Version 4



Are you at least 18 years old?	◯ Yes ◯ No
Do you believe you were exposed to tear gas in 2020 or 2021?	◯ Yes ◯ No

Demographic information

projectredcap.org REDCap

State of residence	◯ AK ◯ AL ◯ AR ◯ AZ ◯ CA ◯ CO ◯ CT ◯ DC ◯ DE ◯ FL ◯ GA ◯ HI ◯ IA ◯ ID ◯ IL ◯ IN ◯ KS ◯ KY ◯ LA ◯ MA ◯ MD ◯ ME ◯ MI ◯ MN ◯ MO ◯ MS ◯ MT ◯ NC ◯ ND ◯ NE ◯ NH ◯ NJ ◯ NM ◯ NV ◯ NY ◯ OH ◯ OK ◯ OR ◯ PA ◯ RI ◯ SC ◯ SD ◯ TN ◯ TX ◯ UT ◯ VA ◯ VT ◯ WA ◯ WI ◯ WV ◯ WY ◯ Don't wish to answer
What is your sex?	◯ Female ◯ Male ◯ Non-binary or transgender ◯ Other

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
What is your ethnicity?	◯ Non Hispanic ◯ Hispanic ◯ Don't wish to answer
What is your age?	_____
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
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
What is your occupation?	◯ Health provider ◯ Law enforcement or police officer ◯ Media ◯ Other ◯ Don't wish to answer
If you selected 'other', please describe your occupation.	_____
Do you smoke cigarettes?	◯ Yes ◯ No ◯ Don't wish to answer
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 ask about protest that you attended (any information that you provide will remain anonymous).

How many protests did you attend in 2020-2021?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ 11 ◯ 12 ◯ 13 ◯ 14 ◯ 15 ◯ 16 ◯ 17 ◯ 18 ◯ 19 ◯ 20 ◯ More than 20 ◯ Don't know/Don't wish to answer
Please estimate the date that you attended your first protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your second protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your third protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your fourth protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your fifth protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your sixth protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your seventh protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your eighth protest in 2020-2021. Leave blank if you do not remember.	_____

Please estimate the date that you attended your ninth protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended your tenth protest in 2020-2021. Leave blank if you do not remember.	_____
Please estimate the date that you attended any other protests not listed above in 2020-2021. Leave blank if you do not remember.	_____ ((MM/DD/YYYY))
Where were the protests? (City, State) Leave blank if you don't wish to answer.	_____
What was your role at the protest? Please check all that apply:	☐ Protestor ☐ Law enforcement ☐ Media ☐ Street medic ☐ Passerby ☐ Lived or worked nearby ☐ None of the above ☐ Don't know/Don't wish to answer
Do you believe you were exposed to tear gas during any of the protests?	◯ Yes ◯ No
How many times do you think you were exposed?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ 11 ◯ 12 ◯ 13 ◯ 14 ◯ 15 ◯ 16 ◯ 17 ◯ 18 ◯ 19 ◯ 20 ◯ More than 20 ◯ Don't know/Don't wish to answer
On what date(s) do you think you were exposed? Give your best estimate or leave blank if you do not remember.	_____ ((MM/DD/YYYY))
Did you handle any tear gas canisters?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer

Have you ever deployed (released) tear gas?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Was your home exposed to tear gas from a nearby protest? (For example, did tear gas enter your home through windows or under doors?)	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Was your car within 500 feet of tear gas at any time?	◯ Yes ◯ No ◯ Not applicable ◯ Don't know/Don't wish to answer
After being exposed to tear gas, what steps did you take to protect yourself? Please check all that apply:	☐ Removed and disposed of clothes and shoes before returning to vehicle or home ☐ Showered before returning to vehicle or home ☐ Nothing ☐ Other ☐ Don't know/Don't wish to answer
Please describe the 'other' steps you took to protect yourself after exposure.	_____
The following questions will focus on your health status (any information that you provide remain anonymous).	
What chronic health conditions did you have prior to attending protests or being exposed to tear gas? Please check all that apply:	☐ Asthma ☐ Chronic Obstructive Pulmonary Disorder (COPD) ☐ Other lung-related condition ☐ Cancer ☐ Diabetes ☐ Obesity ☐ Heart condition ☐ Thyroid condition ☐ Reproductive health problems ☐ Depression ☐ Anxiety ☐ Other ☐ Don't know/Don't wish to answer
Please describe the heart condition you had before attending protests or being exposed to tear gas.	_____
Please describe the reproductive health problems you had before attending protests or being exposed to tear gas.	_____
Please describe the 'other' chronic health condition you had before attending protests or being exposed to tear gas.	_____
Have you ever tested negative for COVID-19?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please estimate the date of your most recent negative test?	_____

Have you ever tested positive for COVID-19?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please estimate the date of your most recent positive test?	_____
The following questions will focus on what you know about tear gas.	
Are you aware of the potential short-term and long-term health effects of exposure to tear gas? (A fact sheet will be provided to you after you complete this questionnaire.)	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Do you feel the city provided adequate information on what to do after exposure?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
What are your thoughts on the safety of using tear gas?	◯ It is safe; it does not harm health ◯ It causes short-term adverse health effects ◯ It causes both short-term and long-term adverse health effects ◯ 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).	
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
Please describe "other" effects on the eye.	_____
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
Please describe "other" effects on lungs.	_____
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
Please describe the "other" effects on your skin.	_____
If any, what effects do you think the tear gas exposure had on your heart? Check all that apply:	☐ Increased heart rate ☐ Irregular heart beat ☐ Chest pain ☐ Other ☐ Don't know/Don't wish to answer

Please describe the "other" heart effects.	_____
Did you have any other symptoms after the tear gas exposure that were not listed above?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe.	_____
Have you noticed any differences or changes in your health since being exposed to tear gas?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe.	_____
The following questions will focus on medical care for tear gas exposure (any information that you provide will remain anonymous).	
After your exposure, did you seek medical care from a healthcare provider?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, how soon did you seek care after the exposure?	◯ Less than 4 hours ◯ 4 to 8 hours ◯ 8 to 24 hours ◯ 24 to 48 hours ◯ 3 to 7 days ◯ 8 or more days ◯ Don't know/Don't wish to answer
Do you feel your healthcare provider gave you adequate information on what to do after exposure?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Do you feel your medical provider was knowledgeable about exposure to tear gas?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
The following questions will focus on your mental health (any information you provide will remain anonymous). Please provide us with the first answer that comes to mind. **If you are experiencing suicidal thoughts or need help, please seek professional help.**	
On a scale of 1 to 5, how often did you feel nervous and/or stressed in the time leading up to the protests?	◯ 1 Never ◯ 2 Rarely ◯ 3 Sometimes ◯ 4 Often ◯ 5 All the time ◯ Don't know/Don't wish to answer

On a scale of 1 to 5, how often have you felt nervous and/or stressed in the time since you attended protests?	◯ 1 Never ◯ 2 Rarely ◯ 3 Sometimes ◯ 4 Often ◯ 5 All the time ◯ Don't know/Don't wish to answer

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

Are you pregnant now?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Were you pregnant when you were attending protests?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
Did you have any pregnancy-related health conditions or complications that existed before you started attending protests?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe?	_____
Have you had any pregnancy-related health conditions or complications since you started attending protests?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe?	_____
Are you using birth control?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, what kind?	_____
How many other children do you have?	_____
Are you trying to conceive?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer

What reproductive or hormonal problems have you had since you started attending protests? Check all that apply:	☐ Trouble conceiving ☐ Spontaneous abortion (miscarriage) ☐ Light bleeding ☐ Heavy bleeding ☐ Period pains ☐ Irregular cycles ☐ Long bleeding ☐ Short bleeding ☐ Short cycle ☐ Long cycle ☐ Don't know/Don't wish to answer
Of the reproductive or hormonal problems that you checked above, which, if any, are you still experiencing? Check all that apply:	☐ Trouble conceiving ☐ Spontaneous abortion (miscarriage) ☐ Light bleeding ☐ Heavy bleeding ☐ Period pains ☐ Irregular cycles ☐ Long bleeding ☐ Short bleeding ☐ Short cycle ☐ Long cycle ☐ Don't know/Don't wish to answer
The following questions will focus on your reproductive health (any information that you provide will remain anonymous).	
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
Of the reproductive or hormonal problems that you checked above, which, if any, are you still experiencing? Check all that apply:	☐ Erectile dysfunction ☐ Ejaculatory dysfunction ☐ Trouble conceiving ☐ Don't know/Don't wish to answer
Have you experienced any other reproductive or hormonal problems since you since you started attending protests?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe.	_____
The following questions will focus on your reproductive health (any information that you provide will remain anonymous).	
Have you had unexpected bleeding in spite of hormone treatment since you started attending protests?	◯ Yes ◯ No ◯ Not applicable ◯ Don't know/Don't wish to answer
Are you still experiencing unexpected bleeding in spite of hormone treatment?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer

Have you experienced any other reproductive or hormonal problems since you started attending protests?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
If yes, please describe.	_____

Invitation to future participation

Would you like a periodic newsletter explaining the results of this study and its implications?	◯ Yes ◯ No
May we contact you for follow-up research on longer-term effects of tear gas exposure?	◯ Yes ◯ No
Please enter the email address that you would like us to contact you through. If you prefer to create an unidentifiable email address please do so.	_____

The following questions will invite you to describe concerns you may have.

What concerns do you have about exposure to tear gas?	_____
Do you feel like you are well informed about exposure to tear gas?	◯ Yes ◯ No ◯ Don't know/Don't wish to answer
How would information about the potential short-term health effects be useful to you and the community?	_____
Is there anything else you would like us to know? Please explain.	_____

APPENDIX B: TEAR GAS PROXY INTERRELATEDNESS

Table 14: Seeking Medical Care for Tear Gas Exposure by Protest Number, n=71

Medical Care	Protests		Chi-square *p*-value
	1 - 9 n (%)	> 9 n (%)	
			0.017
No	13 (28)	17 (94)	
Yes	33 (72)	1 (6)	

Table 15: Correlation Coefficients (ρ) for Acute Symptoms of Tear Gas, n=103

	Total Acute Symptoms	Eye	Lung	Skin	Heart
Total Acute Symptoms	1				
Eye	0.874*	1			
Lung	0.934*	0.858*	1		
Skin	0.885*	0.736*	0.741*	1	
Heart	0.826*	0.507*	0.660*	0.704*	1

*p-value < 0.0001

Table 16: Unadjusted Associations of Acute Symptoms of Tear Gas Exposure with Protest Attendance (n=101) and Seeking Medical Care (n=73) for Tear Gas Exposure

		Total Acute Symptoms		Eye		Lung		Skin		Heart	
	n (%)	Mean±SD	t-test *p*-value	Mean±SD	t-test *p*-value	Mean±SD	t-test *p*-value	Mean±SD	t-test *p*-value	Mean±SD	t-test *p*-value
Protests			0.039		<0.0001		0.0007		0.38		0.40
1 - 9	54 (53)	4.22±3.88		1.00±0.89		1.31±1.26		0.81±0.80		1.09±1.23	
> 9	47 (47)	5.74±3.37		1.74±0.94		2.15±1.12		0.96±0.81		0.89±1.11	
Medical Care			0.48		0.028		0.037		0.016		0.0065
No	52 (71)	6.73±2.41		1.96±0.63		2.48±0.75		1.10±0.72		1.19±1.10	
Yes	21 (29)	7.19±2.69		1.62±0.50		2.05±0.86		1.52±0.51		2.00±1.14	

APPENDIX C: MULTIPLE IMPUTATION

Table 17: Pattern of Missing Data, n=103

	Variable									
Group	Age	Race	Ethnicity	Education	Trying to Conceive	Income	Protests	Medical Care	Frequency	Percent
1	X	X	X	X	X	X	X	X	61	59.2
2	X	X	X	X	X	X	X	.	9	8.7
3	X	X	X	X	X	X	.	X	1	1.0
4	X	X	X	X	X	.	X	X	4	3.9
5	X	X	X	X	.	X	X	X	1	1.0
6	X	X	X	X	.	.	.	X	1	1.0
7	X	X	X	.	X	X	X	X	1	1.0
8	X	X	.	X	X	X	X	X	3	2.9
9	X	X	.	X	X	X	X	.	1	1.0
10	.	X	X	X	.	X	X	.	1	1.0
11	.	X	X	.	.	.	X	X	1	1.0
12	.	X	.	X	.	X	X	.	9	8.7
13	.	.	X	X	X	X	X	.	10	9.7

X = data present

. = data missing

Table 18: Multivariable Linear Models for Protest Number and Menstrual Cycle Outcomes with Imputed Data

Number of protests attended

n = 103

Protests	β[a]	(95% CI)[a]	*p*-value
	Factor 1 (Model 1)		
1 - 9	0.000	Reference	
> 9	0.924	(0.516, 1.332)	<0.0001
	Factor 2 (Model 2)		
1 - 9	0.000	Reference	
> 9	0.516	(0.078, 0.954)	0.021
	Cycle Score (Model 3)		
1 - 9	0.000	Reference	
> 9	1.892	(0.945, 2.840)	<0.0001

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Table 19: Multivariable Linear Models for Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes with Imputed Data

Acute symptoms score n = 103		
β[a]	**(95% CI)[a]**	***p*-value**
	Factor 1 (Model 4)	
0.063	(0.000, 0.126)	0.052
	Factor 2 (Model 5)	
0.054	(-0.008, 0.116)	0.090
	Cycle Score (Model 6)	
0.147	(0.003, 0.292)	0.046

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Table 20: Multivariable Linear Models for Specific Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes with Imputed Data

Specific acute symptoms n = 103			
Symptom	**β[a]**	**(95% CI)[a]**	***p*-value**
Eye			
		Factor 1 (Model 7)	
	0.295	(0.061, 0.528)	0.013
		Factor 2 (Model 8)	
	0.149	(-0.099, 0.394)	0.23
		Cycle Score (Model 9)	
	0.401	(-0.164, 0.966)	0.16
Lung			
		Factor 1 (Model 10)	
	0.235	(0.049, 0.422)	0.014
		Factor 2 (Model 11)	
	0.152	(-0.042, 0.346)	0.12

	Cycle Score (Model 12)		
	0.488	(0.039, 0.938)	0.033
Skin			
	Factor 1 (Model 13)		
	0.134	(-0.137, 0.406)	0.33
	Factor 2 (Model 14)		
	0.151	(-0.118, 0.421)	0.27
	Cycle Score (Model 15)		
	0.482	(-0.133, 1.110)	0.12
Heart	**Factor 1 (Model 16)**		
	0.110	(-0.078, 0.297)	0.25
	Factor 2 (Model 17)		
	0.172	(-0.005, 0.348)	0.057
	Cycle Score (Model 18)		
	0.388	(-0.022, 0.798)	0.064

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

Table 21: Multivariable Linear Models for Seeking Medical Attention for Acute Symptoms of Tear Gas Exposure and Menstrual Cycle Outcomes with Imputed Data

Sought medical care for acute effects n = 103			
Medical care	**β[a]**	**(95% CI)**	***p*-value**
	Factor 1 (Model 19)		
No	0.000	Reference	
Yes	-0.796	(-1.286, -0.306)	0.0015
	Factor 2 (Model 20)		
No	0.000	Reference	
Yes	-0.037	(-0.593, 0.519)	0.90
	Cycle Score (Model 21)		
No	0.000	Reference	
Yes	-1.187	(-2.365, -0.010)	0.048

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

APPENDIX D: TEAR GAS EXPOSURE NUMBER ANALYSES

Table 22: Multivariable Linear Models for Tear Gas Exposure Number and Menstrual Cycle Outcomes

Number of exposures n = 51				
Exposures	**n (%)**	**β[a]**	**(95% CI)[a]**	***p*-value**
		Factor 1		
1 - 2	26 (51)	0.000	Reference	
> 2	25 (49)	0.938	(0.420, 1.457)	0.0010
		Factor 2		
1 - 2	26 (51)	0.000	Reference	
> 2	25 (49)	0.361	(-0.234, 0.956)	0.24
		Cycle Score		
1 - 2	26 (51)	0.000	Reference	
> 2	25 (49)	2.182	(1.045, 3.320)	0.0005

[a]Adjusted for age, race, ethnicity, education, income, and trying to conceive.

APPENDIX E: POST HOC POWER EXPLORATION

A post hoc power investigation was conducted using G*Power Version 3.1.9.7 (64). F-tests with an alpha of 0.05 were performed for each main model [Table 23], as well as the models with exposure number as the predictor [Table 24]. A calculated power of 0.80 (80%) or above was considered to be sufficiently powered to detect an effect. Three models were sufficiently powered: the Aim 1 models in which factor 1 and cycle score were the outcomes, and the Aim 4 model in which factor 1 was the outcome. Post hoc power calculations should be interpreted with caution as there are questions as to how useful they are for indicating the true power of models (65, 66).

Table 23: Post Hoc Power Calculations for Main Models (α = 0.05)

Model	Main Predictor	Main Outcome	Sample (n)	Partial R^2	Power
			Aim 1		
1	Protests	Factor 1	70	0.4959	> 0.999
2		Factor 2	70	0.0504	0.475
3		Cycle score	70	0.2795	> 0.999
			Aim 2		
4	Acute score	Factor 1	71	0.0002	0.052
5		Factor 2	71	0.0030	0.074
6		Cycle score	71	0.0015	0.062
			Aim 3		
7	Eye score	Factor 1	71	0.0153	0.178
8		Factor 2	71	0.0019	0.065
9		Cycle score	71	0.0002	0.052
10	Lung score	Factor 1	71	0.0071	0.108
11		Factor 2	71	0.0004	0.053
12		Cycle score	71	0.0017	0.064
13	Skin score	Factor 1	71	0.0030	0.074
14		Factor 2	71	0.0005	0.054
15		Cycle score	71	0.0009	0.057
16	Heart score	Factor 1	71	0.0084	0.119

17		Factor 2	71	0.0062	0.100
18		Cycle score	71	0.0010	0.058
			Aim 4		
19	Medical care	Factor 1	62	0.1724	0.942
20		Factor 2	62	0.0055	0.089
21		Cycle score	62	0.0338	0.304

Table 24: Post Hoc Power Calculations for Tear Gas Exposure Models (α = 0.05)

Main Outcome	Sample (n)	Partial R^2	Power
Factor 1	51	0.2928	0.994
Factor 2	51	0.0329	0.251
Cycle score	51	0.3286	0.998

CURRICULUM VITA

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EDUCATION

2020 - Present	**PhD in Public Health Science, Epidemiology** University of Louisville, Louisville, KY Dissertation: *Protest-Related Tear Gas Exposure and Menstrual Function*
2015 - 2017	**MS in Public Health Science, Epidemiology** University of Louisville, Louisville, KY Thesis: *The Effect of Caffeine on Fecundability: Differences in Coffee, Tea, and Cola Consumption*
2001 - 2005	**BA in Biology** Bellarmine University, Louisville, KY Senior Thesis: *The Pesticide Malathion and Rate of Movement in the Phiddipus audax Jumping Spider*

AWARDS, FELLOWSHIPS, AND GRANTS

2022	**Dissertation Completion Award**, University of Louisville
2005	**Graduated Cum Laude**, Bellarmine University
2003	**KBRIN (Kentucky Biomedical Research Network) Summer Research Program Recipient**, University of Kentucky
2001 - 2005	**Monsignor Horrigan Scholarship**, Bellarmine University

RESEARCH EXPERIENCE

2020 - Present	**Doctoral Student**

University of Louisville Investigation of Possible Health Effects of Tear Gas Among Protestors in the U.S. Study
University of Louisville, Louisville, KY

- Developing, editing, and maintaining online questionnaires
- Contacting participants for follow-up
- Downloading and analyzing data
- Utilized exploratory factor analysis to reduce data dimensionality

2017 **Research Assistant**
Coal Ash and Children's Health Study
University of Louisville, Louisville, KY

- Recruited participants
- Set up air monitoring equipment
- Collected and entered data
- Conducted literature searches

2016 - 2017 **Graduate Research Assistant**
Louisville Tobacco Smoke Exposure, Genetic Susceptibility and Infertility (LOUSSI) Study
University of Louisville, Louisville, KY

- Recruited participants
- Collected and entered data
- Analyzed urine samples using ELIZA and real time PCR
- Assisted with obtaining approval from the Institutional Review Board

2016 - 2017 **Masters Student**
Thesis: *The Effect of Caffeine on Fecundability: Differences in Coffee, Tea, and Cola Consumption*
University of Louisville, Louisville, KY

- Analyzed data from the *Mount Sinai Study of Women Office Workers* (Emory University)

2015 – 2016 **Graduate Research Assistant**
University of Louisville, Louisville, KY

- Analyzed human blood samples through CBC and flow cytometry
- Managed stored samples in the FreezerPro database

2005 - 2006 **Student**
Miami University, Oxford, OH

- Conducted preliminary research on behavior of harvestmen

2001 - 2005 **Student**

Senior Thesis: *The Pesticide Malathion and Rate of Movement in the Phiddipus audax Jumping Spider*
Bellarmine University, Louisville, KY

- Exposed jumping spiders and ladybugs to experimental treatments
- Collected and analyzed data

2003 **Research Intern**
Kentucky Biomedical Research Infrastructure Network (KBRIN)
University of Kentucky, Lexington, KY

- Entered a competition and won a ten-week internship through KBRIN
- Conducted genetic research on *Drosophila*
- Presented the results at the Kentucky Academy of Science at Western Kentucky University in the fall of 2003

OTHER WORK EXPERIENCE

2017 - 2020 **Quality Improvement Coordinator II**
University of Louisville Hospital, Blood and Marrow Transplant Program, Louisville, KY

- Ensured that policies and procedures complied with standards set forth by various accrediting and regulatory bodies (such as FACT and FDA)
- Successfully guided the program through the Foundation for the Accreditation of Cellular Therapy (FACT) inspection and renewal process, that took place every three years
- Performed scheduled and ad hoc quality audits to determine efficacy of, and adherence to, policies
- Coordinated quality management activities between UofL Hospital and Norton Children's Hospital
- Developed and tracked various quality indicators
- Held monthly and quarterly quality meetings to discuss quality indicator trends, deviations from procedures, and to review and implementation of documents
- Responded to requests from insurance companies for program level data
- Instructed collaborators in basic statistics and quality tracking

2010 - 2015 **Research Technologist II**
University of Louisville, Diabetes and Obesity Center, Louisville, KY

- Maintained and managed distinct mouse colony lines
- Performed phenotype tests on mice using the TSE Phenomaster Physiological Cage System, the LunarPIXImus densitometer
- Maintained genotyping and phenotyping equipment
- Trained personnel and students in genotyping and phenotyping methodology

2007 - 2010 **Associate Chemist**
CreoSalus
Peptide Department, Louisville, KY
- Manually synthesized peptides and amino acids
- Operated automated synthesizers
- Purified products through HPLC
- Assisted with the preparation of the company safety manual

Quality Control Department, Louisville, KY
- Analyzed product purity by HPLC, mass spectrometry, IR, and TLC

POSTER PRESENTATIONS

Emily Reece, Madeline Tomlinson, Aastha Kakar, Anne Wallis, Cynthia Corbitt, Ted Smith, Aruni Bhatnagar, Kira Taylor. Tear Gas Exposure During the 2020 and 2021 Protests and Female Reproductive Health. Research! Louisville. Louisville, KY. October 2021.

Lindsay Poling, **Emily Steinmetz**, Alice Wright, John Rawlings. Mutations of Pyrimidine Degradation in *Drosophila*. 89th Annual Meeting of the Kentucky Academy of Science. Bowling Green, KY. November 2003.

PUBLICATIONS

Haberzettl P, Conklin D, **Steinmetz E**, and Bhatnagar A. *Age-Dependent Insulin Resistance in Aldose Reductase-Null Mice*. Circulation 2011;124:A13129.