CANNABIS USE AND REPRODUCTIVE HEALTH: AN INVESTIGATION OF TIME
TRENDS AND ADVERSE BIRTH OUTCOMES

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ABSTRACT

Devika Chawla: Cannabis Use and Reproductive Health: An Analysis of Time Trends and
Adverse Birth Outcomes
(Under the direction of Julie L. Daniels)

Cannabis is the most commonly used illicit drug among populations of reproductive age
in the United States (U.S.) [2-4]. Despite changing policies and attitudes, little is known about
how patterns of cannabis use are changing among populations of reproductive age, or about how
cannabis might affect fetal development.

Understanding trends requires careful consideration of underlying demographic factors,
such as age, generation, and period effects due to significant events such as legalization.
Therefore, we first aim to estimate age, period, and cohort effects of past-month cannabis use
among U.S. populations of reproductive age from 2002-2014 using the National Survey of Drug
Use and Health (NSDUH, n=534,679). As policies and patterns of cannabis use continue to
change, health effects are increasingly important to understand. Animal studies suggest cannabis
affects sperm quality and may alter DNA packaging, but effects on fetal development remain
unknown. Therefore, we secondly aim to estimate effects of paternal preconception cannabis use
on structural birth defects in the National Birth Defects and Prevention Study (NBDPS,
n=34,320).

Past-month cannabis use among U.S. populations of reproductive age increased from
9.2% in 2002 to 12.3% in 2014. Distinct age, period, and cohort effects were observed, though
age effects were largest in magnitude. In NBDPS, cannabis use during the 3-month
preconception period was reported for 8.8% of control-fathers. After adjustment for confounders,

paternal cannabis use was associated with gastroschisis (aOR: 1.23, 95% CI: 1.00, 1.52), and meaningfully associated with diaphragmatic hernia (aOR: 1.33, 95% CI: 0.99, 1.80), cleft lip alone (aOR: 1.23, 95% CI: 0.95, 1.60), and hypoplastic left heart syndrome (aOR: 1.38, 95% CI: 0.99, 1.92).

Past-month cannabis use is prevalent and increasing among men and women of reproductive age. While distinct age, period, and cohort effects are at play, age remains the strongest correlate of past-month use. Moreover, our results may suggest increased risk of some birth defects following paternal preconception cannabis use, though studies with better exposure measurement are needed. Future research is needed to understand how paternal cannabis use affects fetal development, especially in light of changing cannabis policies and documented increases in prevalence of use.

I dedicate this thesis to my maternal grandfather Dr. Benarsi Lal Talwar, who taught me by example to never shy away from hard work, to embrace curiosity, and to pursue knowledge for myself; and to my parents Anu and Sandeep Chawla, who were my first teachers.

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LIST OF ABBREVIATIONS

APC   age-period-cohort

aOR   adjusted odds ratio

aPR   adjusted prevalence ratio

CB1   cannabinoid receptor type 1

CB2   cannabinoid receptor type 2

CHD   congenital heart defect

cOR   crude odds ratio

cPR   crude prevalence ratio

CDC   Centers for Disease Control and Prevention

CI   confident interval

HLHS   hypoplastic left heart syndrome

MMWR   Morbidity and Mortality Weekly Report

MTF   Monitoring the Future study

NBDPS   National Birth Defects Prevention Study

NPV   negative predictive value

NSDUH   National Study of Drug Use and Health

PPV   positive predictive value

SAMHSA   Substance Abuse and Mental Health Services Administration

THC   tetrahydrocannabinol

US   United States

CHAPTER 1: SPECIFIC AIMS

Cannabis is the most commonly used illicit drug among men and women of reproductive age in the United States [2, 3]. As of 2015, an estimated 1 in 5 young adults (aged 18-25) were current users of cannabis [8]. Prevalence of current cannabis use has steadily increased from 16% in 2004 to 20% in 2015 in this age group [8]. Moreover, cannabis attitudes among young adults continue to move towards greater acceptance; the percentage of teenagers who perceive "great risk from regular use" has significantly decreased in the past decade [9, 10]. Policies on cannabis legality are also rapidly changing. To date, 24 states have legalized medical use, and four states and the District of Columbia have legalized recreational use, and six states have legislation pending [11]. **Despite changing policies and attitudes, little is known about what drives these changes in prevalence of cannabis use over time.** Identifying drivers of time trends can shed light on social-cultural changes in cannabis use, providing useful information for policy-makers.

Understanding how trends change over time requires careful consideration of underlying factors that contribute to observed trends, such as shifts in the population distribution of age, period effects due to significant events (such as legalization), and generational effects. Age-period-cohort (APC) analysis is a method specifically designed to examine time trends and the underlying factors that contribute to them. Four APC analyses on cannabis use in adult populations have been published, but all but one examined data only through 2009. Cannabis laws and social norms have changed drastically since then, so it is essential to understand drivers of time trends in the past seven years. Additionally, no APC analyses to date have focused on

individuals of reproductive age [12, 13]. The three published studies evaluated ever/never use in the past 12 months, but not more recent use, which is more biologically relevant for cannabis' effect on reproductive health. **Therefore, I aim to conduct an APC analysis of past-month cannabis use among men and women of reproductive age from 2002 to 2014 using the National Survey of Drug Use and Health (NSDUH), a nationally representative annual survey of drug use conducted by the Department of Health and Human Services.**

To parse out the independent age, period, and cohort effects that contribute to the observed trends in cannabis use, we plan to conduct sub-analyses to better characterize these patterns. First, to better understand the user population, we plan to estimate socio-demographic risk factors for current cannabis use in populations of reproductive age. Second, we will evaluate how the APC effects differ by gender. We know that men are more likely to use cannabis than women [14]. However, recent trends show that cannabis use is increasing faster among women than among men, and little research has shown why. Therefore, we hypothesize that the period effects (changing policies and social norms) are stronger for women than for men. Third, because social norms surrounding cannabis use are rapidly changing, we plan to evaluate time trends in *risk perception* of cannabis use in populations of reproductive age.

As policies and attitudes towards cannabis use change rapidly, it is essential to understand how cannabis use patterns may impact health. Because legalization may increase use among men and women of reproductive age, the reproductive health effects are especially important to understand. Animal studies have shown that THC – the main psychoactive component of cannabis – crosses the placenta. Epidemiologic studies suggest that prenatal exposure is associated with fetal growth restriction, specific birth defects, increased likelihood of neonatal intensive care, and changes in brain morphology [6, 15-19]. While more

research is needed to understand the effects of maternal exposure, we know *even less* about the potential reproductive effects of paternal exposure. THC has been shown to reduce sperm concentration and alter sperm motility in human semen samples [20]. A recent epidemiologic study of Danish men aged 18-28 showed significant differences in sperm quality measures between regular marijuana smokers (more than once a week) versus non-smokers (n=1215) [21]. In addition to altering sperm concentration and morphology, THC has been shown to damage DNA packaging during spermatogenesis [22]. **While the effect of cannabis on sperm quality has been demonstrated, very little research has focused on the potential effects of paternal preconception cannabis use on embryonic development.** Since cannabis affects sperm quality, it warrants investigation as a potential risk factor for birth defects [5, 23]

Major birth defects, defined as a structural malformation with a significant impact on the health and development of a child, are the leading cause of infant mortality and lifelong disability [24, 25]. While some genetic and environmental risk factors have been identified, little is known about the etiology of most birth defects. Though most research has focused on maternal risk factors, epidemiologic investigations have shown that *paternal* exposures during the preconception period can increase risk of birth defects [26-29].

Only three studies to date have investigated the association of paternal cannabis use and birth defects. Two have suggested an increased risk for cardiac-related birth defects among infants of fathers with preconception paternal cannabis use [30, 31]. One study reported a null association between preconception cannabis use and neural tube defects [32]. However, all three studies had insufficient control of confounding and lacked clinical verification of the reported birth defects among offspring. Moreover, no studies have evaluated an association of paternal cannabis use and other types of birth defects. **Therefore, I aim to estimate the association**

between paternal preconception cannabis use and risk of major structural birth defects in the National Birth Defects and Prevention Study (NBDPS) – the largest case-control study of birth defects in the U.S. with rigorous clinical outcome verification and classification.

Cannabis is an increasingly prevalent exposure among men and women of reproductive age, yet little is known about patterns of use or effects on reproductive health in this population. Twenty-five U.S. states have already legalized cannabis either medically or recreationally, and other states are currently considering similar laws. As policies and social norms surrounding cannabis continue to rapidly evolve, we must gain a better understanding of how cannabis affects reproductive health. Therefore, our specific aims are as follows:

Aim 1: Conduct an age-period-cohort analysis of recent (past 30 days) cannabis use among men and women of reproductive age from 2002 to 2014 using a nationally-representative data source.

- *Sub Aim 1a:* Estimate risk factors for recent cannabis use in this population.

- *Sub Aim 1b:* Quantify age, period, and cohort effects of past-month cannabis use.

- *Sub Aim 1c:* Evaluate if age, period, cohort effects differ by gender.

Aim 2: Estimate the association between paternal 3-month preconception cannabis use and prevalence of major birth defects.

- *Sub Aim 2a***:** Estimate prevalence of cannabis use among men in preconception period.

- *Sub Aim 2b:* Quantify crude and adjusted effect estimates for paternal cannabis use and specific birth defect phenotypes.

- *Sub Aim 2c:* Evaluate potential bias due to exposure misclassification using a probabilistic bias analysis approach.

CHAPTER 2: BACKGROUND & SIGNIFICANCE

2.1 Aim 1

2.1.1 Patterns of cannabis use in the U.S.

Cannabis, also known as marijuana, marihuana, or weed, is any preparation of the *cannabis sativa* plant, which is generally used by smoking, vaping, or eaten as a food or extract [33]. Cannabis is the most commonly used illicit drug among men and women of reproductive age in the United States [2, 3]. As of 2015, an estimated 1 in 5 young adults (aged 18-25) were current users of cannabis [8]. Approximately 44% of U.S. adults reported ever using cannabis [34]. Since legal, social, and cultural norms surrounding cannabis use are rapidly changing, patterns of use are likely to change as well. Here, we provide a brief overview of how cannabis is consumed, demographic patterns of cannabis use in the United States today, and how patterns of use are changing over time.

Cannabis can be consumed in a few different ways. The most common method of consumption is smoking the cannabis plant in the form of a joint, bowl, or pipe. Less frequently, bongs, water pipes, or hookah devices are used [35]. Cannabis can also be vaporized, or "vaped", where active ingredients are released into a smokeless vapor, then inhaled using a vaporizer or e-cigarette device. Some studies suggest that vaping is on the rise, due to increased prevalence of e-cigarettes in the U.S. and perceptions that vaping is healthier than smoking cannabis [36, 37]. Tetrahydrocannabinol (THC), the main psychoactive component of cannabis, can be extracted in oil form, called "hash". Extracts of the cannabis plant can also be prepared in various types of food or drinks, called "edibles". While no consistent estimate is apparent in the literature, a

recent study using social media data found that approximately 16% of cannabis users consume edibles [35]. A relatively new method of consuming cannabis is using butane hash oil, colloquially known as "dabs", which involves butane extraction of the THC from flowering cannabis [38]. The resulting substance is much more THC-concentrated than tradition forms of cannabis. Though "dabbing" is not common and understudied, it appears to be on the rise in the United States [38].

Cannabis use is an ancient practice, and socio-cultural norms surrounding cannabis use vary greatly across cultures. Archeologic sources date cannabis use back to 2000 B.C. in India and China, where it was used for medicinal or spiritual purposes [39]. In fact, the THC-induced cannabinoid activation is mediated by a compound called *anandamide*, which comes from the Sanskrit word 'anande', which means 'joy, bliss, delight'. The practice of using cannabis for medicinal, spiritual, and recreational purposes has since spread to the rest of the world – cannabis is by far the most widely cultivated, trafficked, and abused illicit drug in the world [40].

Individuals generally use cannabis for one of two reasons: (1) **medical**, including but not limited to pain management, appetite management during cancer treatment, post-traumatic stress disorder (PTSD), irritable bowel syndrome (IBS), or self-diagnosed conditions, or (2) **recreational**, i.e. to experience a high [41]. A majority of cannabis users report recreational reasons, though approximately 10% report medical reasons only, and approximately 36% of users report both medical and recreational reasons [35]. Characteristics of cannabis use differ for medical and recreational users. Individuals using cannabis for medical purposes are more likely to vape or eat, while recreational cannabis users are more likely to smoke [42]. Medical users tend to use cannabis more frequently than recreational users, though frequency of use varies greatly [42, 43]. Medical users are more likely to concurrently use opioids, both prescribed and

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non-prescribed, as compared to recreational users [43]. Women are more likely to use cannabis medically as compared to recreationally [43]. In states where medical cannabis is legal, medical users are more likely to obtain cannabis from dispensaries, where cannabis is regulated and doses are labeled. Potency and strain of cannabis vary greatly, and recreational users are often unaware of the potency of the cannabis they consume [44].

Cannabis use is considerably more prevalent among young adults (age 18-25), as compared to all other age groups in the United States [2]. Men are more likely than women to use cannabis [2, 45, 46]. Frequency of cannabis use varies by state, likely due to the different legal status in various states. According to the 2016 SAMSHA report, the states with the highest proportion of past-month cannabis users among ages 12 and older are Vermont (17%), Oregon (16%), Alaska (16%), and Colorado (16%), and Rhode Island (15%) [47]. Racial and socio-economic status (SES) patterns are less clear. Most studies suggest that Whites have the highest past-year cannabis use prevalence, followed by African-Americans, then Hispanics [43, 45]. However, most nationally-representative studies on drug use exclude incarcerated populations [48, 49]. Since African-Americans are more likely than Whites to be incarcerated for cannabis use, available demographic data on racial patterns might be under-representing African-Americans. In some study populations, cannabis users tend to have higher educational attainment as compared to non-users, while the opposite trend is observed in other study populations [43, 46, 50] . Racial and SES characteristics of cannabis users are perhaps more nuanced than these studies reflect, or perhaps they depend on geographic location and study population.

2.1.2 Time trends of cannabis use in the U.S.

How have cannabis use patterns among U.S. men and women changed over time? There are a few ways to answer this question, which we will briefly review here. The most

comprehensive study of *youth* drug trends in the U.S. in the Monitoring the Future (MTF) study, which surveys high school students across the nation annually. From 1976 to 2013, the MTF study found that current cannabis use slowly declined from the 1970s to the 1990s, but has steadily increased from the 1990's until now (see Figure 2.1) [51]. By 2010, the rate of cannabis use exceeded that of cigarette use among U.S. youth [1]. More recent trends from MTF (past 10 years) suggest that cannabis use is steadily increasing among high school students (Figure 2.2) [1].
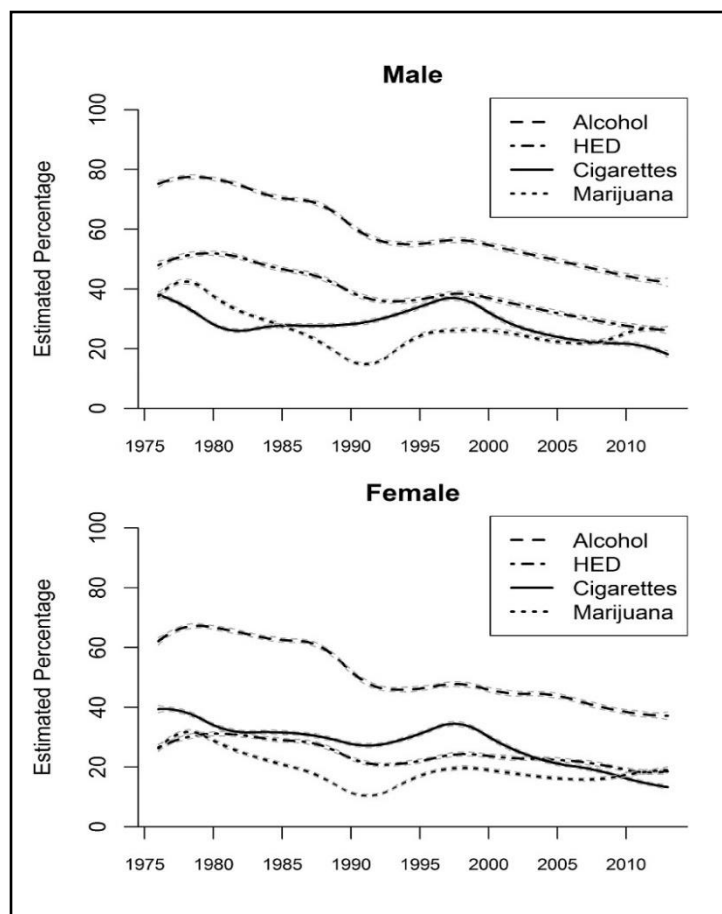
Figure 2.1. Trends of recent cannabis use among male and female high school students in Monitoring the Future Study, 1975-2013 [1]

The most comprehensive study of drug use in the *general* population is the National Drug Use and Health Survey (NSDUH), a nationally-representative annual survey among non-institutionalized adults age 12 and older [2]. The most recent NSDUH report shows a steady increase in current cannabis use from 2002 to 2015. Since cannabis use rates differ by age, NSDUH presents their time trends stratified by age group (see Figure 2.2). Cannabis use is highest among the 18-25 age group, and rates in all groups have been steadily increasing since 2005. Though qualitative, NSUDH trends suggest a recent spike in reported cannabis use from 2012 to 2015, driven by the '26 or older' age group. Broadly, both

MTF and NSDUH show a steady increase in current cannabis use in the past 15 years among men and women of reproductive age.
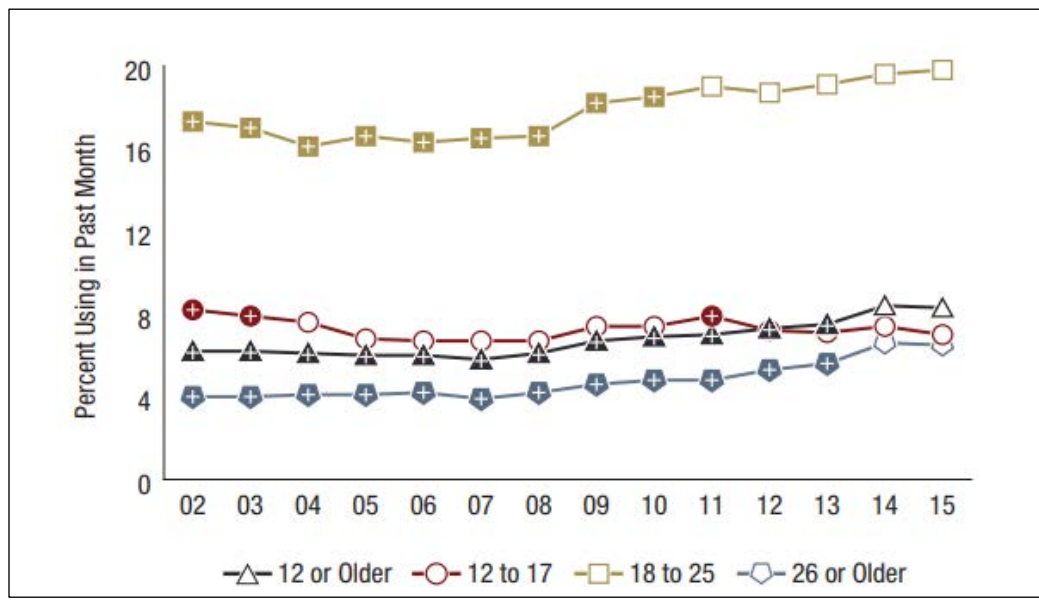
Figure 2.2. Trends of recent cannabis use among U.S. individuals (age 12 and older) from the National Survey of Drug Use and Health, 2002-2015

2.1.3. Age, period, and cohort effects

When measuring time trends of cannabis use, a big analytic challenge is the parsing out changes over time into the differential contributions of three time-related effects: age effects, period effects, and cohort effects [52]. These three effects represent distinct phenomena of interest, which we will contextualize with our outcome – cannabis use. Age effects represent the effect of an individual's age at the time of observation on the outcome [52]. In other words, how does one's age influence likelihood to use cannabis? Period effects represent the influence of the time period of measurement of the outcome [52]. For example, how does the socio-cultural context of the 1994 influence likelihood of cannabis use, as compared to the socio-cultural context of the 2014 Period effects especially of interest, due to the recent changes in recreational and medical cannabis legality, and shifting social attitudes towards cannabis [53]. Cohort effects represent the influence in the year of birth or some other shared life events for a set of

individuals. These are also known as "generational effects." For example, how does being born in the 1960's influence likelihood of cannabis use, compared to being born in the 1990's? Age, period, and cohort are distinct important effects of interest for cannabis use, but time trends reported in repeated cross-sectional designs – like the MTF or NSDUH graphs above (Figures 2.1 and 2.2) – fail to show these distinct effects. To parse out time trends in these three effects, a specific type of analysis is needed: age-period-cohort (APC) analysis [52].

2.1.3.1 Current literature

To date, five APC analyses on various aspects of cannabis use have been published. These four studies are summarized below in Table 2.1.

Johnson et al. analyzed age, period, and cohort effects of "marijuana incidence", which they define as they age at first marijuana use. Authors found that period effects became weaker over the course of the study period (1961-1990), which they suggest is due to the anti-drug policies of the 1980s. They also found that cohort effects became stronger over the course of the study period, especially for women. While this study focused on the *age at which individuals first try marijuana*, it did not report APC effects related to *recent marijuana use* or among individuals older than 24 years of age. Cohort effects, may differ for age at first use compared to recent use. Further investigation is needed regarding trends in recent use among individuals of reproductive age, which would be most important for policy and public health messaging around the prevention of adverse birth outcomes.

Kerr et al. conducted an APC analysis on cannabis use prevalence, which is defined as ever/never use in the past 12 months. To our knowledge, this was the first published APC analysis of prevalence cannabis use. Using "ever/never use in the past 12 months" is the most common way to operationalize prevalent cannabis use in the literature, and is used by three out

of four of the APC analyses. Authors found that overall time trends differed by sex; cannabis use decreased for men, but remained stable for women, during the study period (1984-2000). Authors also found strong age and cohort effects. However, this analysis relied on only four study years to extrapolate trends for a 16-year period, thus limiting the validity of the trends. An APC analysis is strengthened by having more consistent time points of measurement, like annual or semi-annual measurement [52]. Another limitation of this study is the data source. The National Alcohol Study (NAS), as its name suggest, was primarily designed to ascertain accurate estimates of alcohol use, and was not specifically designed to ask about illicit drug use. The NAS has face-to-face interviews, and unlike other drug use surveys, lacks specific techniques for making respondents feel safe and comfortable reporting illegal activities. This data ascertainment method likely increased measurement error, with more people underreporting their cannabis use as compared to anonymous drug use surveys.

Table 2.1. Overview of four age-period-cohort analyses on marijuana-related outcomes

Author	Year	Outcome	Data source	Study population	Main findings
Johnson	2000	age at first marijuana use	National Household Surveys on Drug Abuse	US men and women aged 10-24, interviewed between 1961-1990	- Period effects decreased, cohort effects increased over course of study period. - Cohort effects were stronger for women. - Authors suggest that anti-drug policies of 1980s explain decreasing period effects.
Kerr	2007	ever/never use in past 12 months	National Alcohol Surveys	US men and women aged 18-80, interviewed 1984, 1990, 1995, and 2000	- Marijuana use trends differed by sex; trends were decreasing for men; stable for women. - Birth cohorts born after 1945 have higher rates of marijuana use. - Age effects strongly predicted use, especially for women. - Period effects were stronger for men.
Piontek	2011	ever/never use in past 12 months	German Epidemiological Survey of Substance Abuse	German men and women aged 18-65, interviewed some years between 1990-2009	- Age was strongest predictor of cannabis use. Prevalence peaks in young adulthood and decreasing with age. - Counter to hypothesis, cohort effects were not significant. - Authors suggest "cannabis boom" of early 2000s Europe was mostly experimental use, thus not reflected in past-12-month use variable.
Miech	2012	ever/never use in past 12 months	National Survey on Drug Use and Health	US men and women aged 15-64, interviewed some years between 1985-2009	- Age effects are strong for both men and women. - Cohort effects were strong for older individuals, weaker for younger individuals. - Recent increase in marijuana use due to period effects. - Period effects becoming stronger for all ages/cohorts.
Kerr	2017	ever/never use in past 12 months	National Alcohol Surveys	US men and women aged 18-80, interviewed 1984-2015	- Age effects were strong, especially at younger ages and especially among men - Cohort and period effects were moderate but increasing - Cannabis policy changes did not significantly increase use

Piontek et al. conducted an APC analysis on prevalent cannabis use, also defined as ever/never use in the past 12 months. However, this study was conducted in a German study population. Authors found strong age effects, which is consistent with results found by Kerr et al. and Meich et al. Authors were specifically interested in the "cannabis boom" of the early 2000s in Europe and expected to see cohort or period effects that reflect that boom. Authors did not find significant age and period effects, and suggest that their outcome measure (ever/never use in the past 12 months) was not sensitive enough to capture these effects. A major limitation of this study is changing age eligibility over the study period (1990-2009). The upper age limit was 39 in 1990, 59 in 1995, and 64 in 2006 onwards. Since the age distribution is artificially different across the study period, the ability of the APC analysis to parse out separate age, period, and cohort effects is limited. Additionally, the socio-cultural norms surrounding cannabis use likely differ between German and U.S. populations, making it difficult to compare APC analyses from these two countries.

Meich et al. is the most recent APC analysis on prevalent cannabis use, both in terms of publishing date (2012) and study years (1985-2009). It is the only APC analysis that includes U.S. cannabis use data from the 21st century. Similar to previous APC analyses, authors also define their outcome as ever/never use in the past 12 months. This analysis uses data from the National Study on Drug Use and Health (NSDUH), a nationally-representative study of drug use in the U.S. (which we plan to use for Aim 1). Like previous studies, authors found a strong age effect. But, authors also found an increase in marijuana use prevalence for all birth cohorts, and suggest that period effects were growing stronger. This finding was unique from previous APC analysis, and perhaps reflects the more recent trends of reduced stigma, lower risk perception, and loosening of cannabis policies in the U.S.

Most recently, Kerr et al. conducted an age-period-cohort analysis of past-year cannabis use among US adults age 18 and older using the National Alcohol Surveys from 1984-2015 [54]. Authors found strong age effects at younger (18-30) ages, and age effects were slightly stronger in magnitude for women as compared to men. This study was able to utilize a longer study period than previous study years and identified that cohort effects were small but increased with later birth cohorts, especially for women. Period effects were moderately strong and increased in recent years (2005-2015). However, when authors examine specific cannabis policy changes (legalization of: recreational use, medical marijuana grown at home, medical marijuana sold at dispensaries), no significant effects on past-year use were observed. Authors suggest the strong period effects but non-significant policy change effects are due to changing social norms and risk perceptions that drive both trends in use and policy-change, so the policies themselves do not causally result in increased use.

In summary, there is a small literature on age, period, and cohort effects of cannabis use in the US, though these trends are of major public health significance. A major limitation of the literature is the limited number of analyses with data from 2000 onwards, despite the rapidly changing socio-cultural norms, laws, and use patterns of cannabis in this century. The one analysis with data since 2012 – Kerr et al. 2017 – found that period effects grew stronger in the past decade, though they did not use a nationally-representative data source. Therefore, this literature needs updated, rigorous APC analyses from nationally-representative data to accurately reflect drivers of more recent time trends in cannabis use.

2.2 Aim 2

2.2.1 Cannabis use and reproductive health

The primary psychoactive element of cannabis – delta-9-tetrahydrocannabinol (THC) – interacts with the body's endocannabinoid system by stimulating cannabinoid receptors, specifically CB1 and CB2 receptors [55]. Cannabinoid receptors are highly expressed in the nervous system, which explains the short-term neurophysiological effects of cannabis use, like altered senses (i.e. feeling "high"), changes in mood, pain modulation, and impaired memory [33]. However, cannabinoid receptor are also located in other parts of the human body, including the testis, vas deferens, uterus, and ovaries [55-59]. Because of THC's strong role in activating cannabinoid receptors in reproductive organs, animal studies and a small epidemiologic literature have investigated the effect of cannabis use on various aspect of reproductive health. Results differ by sex and are summarized below.

2.2.1.1 Women

The effect of cannabis use on female reproductive health can be viewed in two contexts: preconception health and pregnancy health. Little is known about the effects of cannabis use on preconception health among women, but animal studies have found that chronic use can disrupt the menstrual cycle, suppressing oogenesis, and impair embryo implantation [60, 61]. In human studies, cannabis use has been shown to disrupt menstrual cycles, specifically by suppressing luteinizing hormone levels during the luteinizing phase, increasing risk of anovulatory cycles, affecting embryo implantation [61-64]. Findings from both animal and human studies suggest that acute THC suppresses the release of gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) from the hypothalamus, thus preventing the release of menstruation-regulating hormones [62]. Additionally, a recent study in mice found that prenatal cannabis exposure at low doses reduced birthweight in offspring, higher proportion of male pups,

and reduced maternal weight gain [65]. In summary, the literature on the effects of cannabis use on preconception reproductive health in women is small, but animal models show consistent effects on various aspects of menstruation and suggest biologic pathways for adverse effects on fertility.

While our study aims do not directly address cannabis use during pregnancy, understanding effects during pregnancy are of interest because (1) women who use cannabis prior to getting pregnancy are more likely to use cannabis during pregnancy as compared to non-users, and (2) pregnancy is an extremely sensitive window of exposure, both for the mother and the infant. Endocannabinoid signaling is involved in fertilization, implantation, embryo development, and early pregnancy maintenance [66]. Cannabinoid receptors have recently been identified in human uterus tissue, and THC exerts a relaxant effect on oxytocin-induced human myometrial contractility – an important factor in labor induction [67]. Animal studies have shown that THC crosses the placenta, achieving concentrations in the fetus that are consistent with that of the mother [68]. Additionally, a recent 2018 showed that THC inhibited the migration of human amniotic epithelial cells, which may affect amniotic fluid development and spontaneous preterm birth risk [69]. A handful epidemiologic studies have investigated the effects of cannabis use during pregnancy and birth or infant outcomes, with mixed results. A recent meta-analysis suggests that prenatal cannabis exposure is associated with fetal growth restriction and increased likelihood of neonatal intensive care, but not with preterm birth [6, 15, 16]. A 2017 study from the NICHD Stillbirth Collaborative Research network showed that biomarker-validated prenatal cannabis use was not associated with small-for-gestational-age or spontaneous preterm birth, but was associated with neonatal mortality [70]. Recent epidemiologic studies suggest that prenatal cannabis exposure may alter brain morphology and

neurodevelopment in the infant [17, 71, 72]. After adjustment, periconceptional cannabis use was associated with increased odds of specific birth defects phenotypes (namely anencephaly and gastroschisis) in the National Birth Defects Prevention Study [16]. In summary, the animal and epidemiologic literature suggests *some* adverse effects of cannabis use during pregnancy, but more research is needed to fully understand the range and severity of these effects.

2.2.1.2 Men

A growing epidemiologic literature has shown that current cannabis use has adverse effects on various aspects of semen quality. A 2014 case-control study on modifiable risk factors for poor sperm morphology found that cannabis use in the 3 months prior to sample collection significantly increased risk for poor sperm morphology (OR: 1.94; 95% CI: 1.05, 3.60) [73]. A 2015 review article summarizes the consistent effects of cannabis on reducing sperm count, motility, viability, and morphology [74]. Most notably, a recent epidemiology study of 1215 young Danish men found that regular marijuana use significantly lowered sperm concentration and sperm count [21]. In response to this study, Eisenberg et al. published a commentary in the American Journal of Epidemiology stressing the need for more research on the effect of cannabis on male reproductive health [75]. Though cannabis has negative effects on sperm quantity, a 2017 study showed it was positive associated sexual frequency among U.S. men and women [76], so it presumably does not affect sexual function.

Both CB1 and CB2 receptors are present in human sperm [74]. Human studies have shown that CB1 activation increases the proportion of immobile sperm, and CB2 activation increases the proportion of "sluggish" sperm, providing biologic evidence that cannabis affects sperm motility [77]. Figure 2.3 depicts the location of CB1 and CB2 receptors in human sperm cells. Additionally, mouse studies have shown that CB1 receptors – which are significantly

activated by THC – influence chromatin remodeling in sperm, suggesting that cannabis has potential epigenetic effects on sperm cells [22].
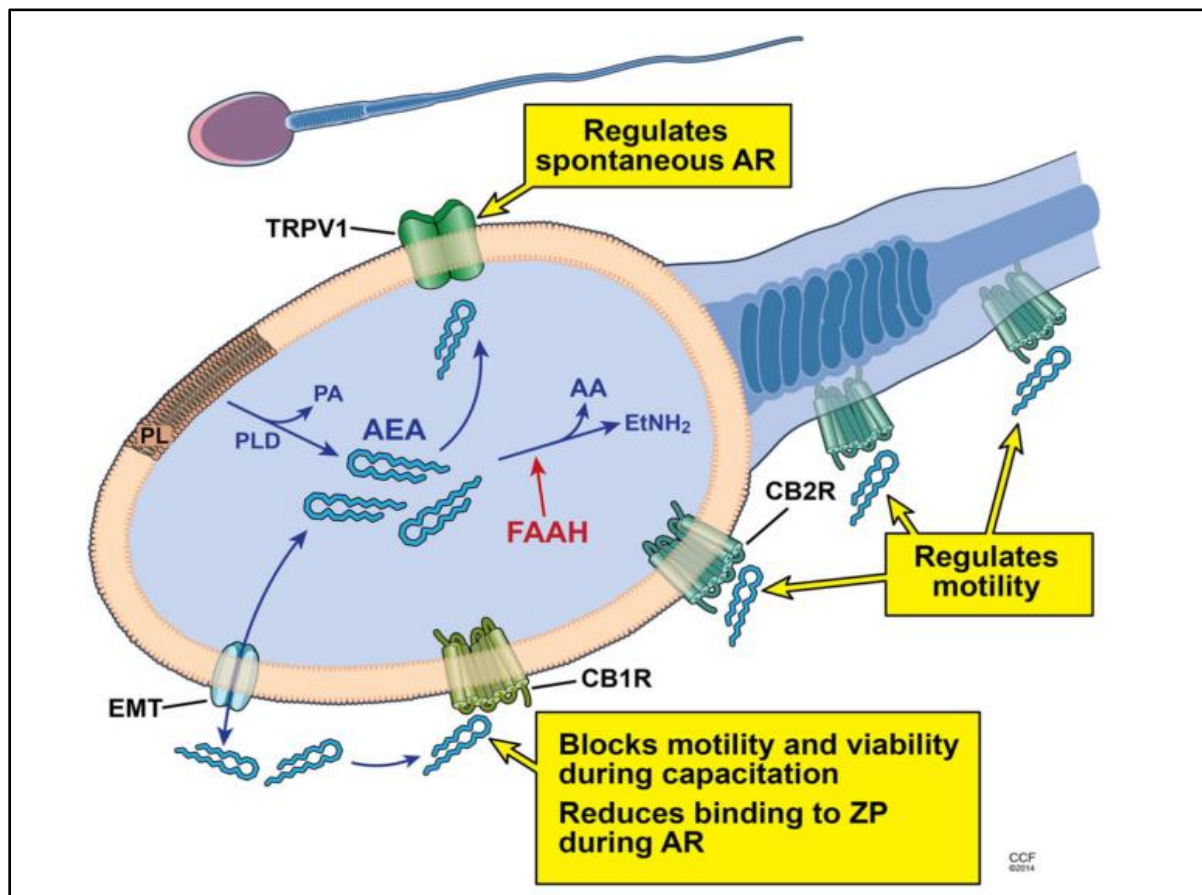
Figure 2.3. Location and influence of CB1 (cannabinoid receptor 1) and CB2 (cannabinoid receptor 2) on human sperm function [35]

While the effects of chronic cannabis use on the endocannabinoid system and sperm quality measures have been demonstrated, the effects on reproductive *outcomes* are less established. While clinic-based studies suggest that men with fertility issues are more likely to use cannabis, no population-based time-to-pregnancy studies have published on paternal cannabis use [78, 79]. Two case-control study of cardiac heart defects (CHDs) suggest increased risk among men who use cannabis 3 months prior to conception; these studies are discussed in more detail in section 2.2. [30, 31]. Some epidemiologic studies suggest a potential *mutagenic* effect of cannabis on male reproductive organs. Specifically, a recent meta-analysis suggests that

weekly cannabis use increases risk of testicular cancer (summary OR: 2.59, 95% CI: 1.60, 4.19) [55]. Despite the strong evidence and biologic mechanism for the effect of cannabis on sperm quality, very few epidemiologic studies to date have investigated preconception cannabis use and adverse reproductive outcomes.

2.2.1.3 Summary

There is a strong literature on the biologic mechanisms for cannabis's effect on both female and male reproductive health. Less is known about the effects on female preconception health, though animal studies suggest cannabis interferes with regular menstrual function. More is known about the adverse effects on male reproductive health, namely on sperm count, motility, and morphology. Recent epidemiologic population-based studies have confirmed these effects. Animal studies suggest that cannabis has epigenetic effects on sperm, but very few studies have investigated the effects of preconception cannabis use on fertility or infant outcomes. In summary, cannabis use has some proven and some hypothesized effects of reproductive health, but little is known about the effect of preconception cannabis use on offspring development.

2.2.2 Brief epidemiology of birth defects

Birth defects are the leading cause of infant mortality and lifelong disability [25, 80]. The Centers for Disease Control and Prevention (CDC) estimate that birth defects affect every 1 in 33 babies born in the United States [25, 80]. Babies born with a birth defect are five times more likely to die in the first year of life, as compared to healthy babies [80]. Additionally, caring for children with birth defects can place significant emotional and financial strains on families and caretakers [81]. A recent US study showed birth defects led to more than 130,000 hospital stays during a single year, result in $2.6 billion in hospital costs alone [82]. Although some genetic and environmental risk factors have been identified, little is known about the etiology of most

birth defects [83]. Identifying modifiable risk factors for birth defects is essential for reducing the incidence of these common, costly, and critical conditions.

Animal studies have shown that most structural birth defects develop early in embryogenesis, typically during the first 10 weeks of pregnancy [84, 85]. Most defects occur in isolation, affecting only one organ system. Multiple defects – when an infant has a defect in more than one organ system – account for approximately a quarter of all birth defects, though this varies by phenotype [86]. While some birth defects have a genetic cause, most defects cannot be explained by a single gene or chromosomal abnormality – these are called 'non-chromosomal defects'. The etiology of non-chromosomal defects are likely a complex interplay between genetic, epigenetic, and environmental factors [84].

There are hundreds of birth defect phenotypes that are clinically and etiologically heterogeneous. For reasons described elsewhere, Aim 2 will focus on 21 specific subtypes of structural birth defects. Here we provide a brief background on some (but not all) of the birth defect phenotypes included in the Aim 2 analysis: (1) hypospadias, (2) congenital heart defects (CHDs), (3) oral clefts, and (4) gastroschisis. These phenotypes are common, non-chromosomal defects with unknown etiologies.

2.2.2.1 Hypospadias

Hypospadias is a birth defect in male infants where the urethra is not located at the tip of the penis. During fetal development, this abnormal urethral development occurs during weeks 8 to 14 post-conception [87]. Severity of this defect can vary; minor defects are considered "first degree", while more sever defects are considered "second degree" or "third degree" [88]. There are three subtypes of hypospadias; classification depends on the location of the opening of the urethra. Boys with hypospadias often have problems with urinating, and sometimes have difficulty performing sexual intercourse later in life [87]. Hypospadias is one of the most

common birth defects in the U.S., affecting approximately 1 in every 150 male live births (64 cases per 10,000 male live births) [89]. Prevalence of hypospadias seems to be increasing in certain populations, though this is debated in the literature [90-92].

Etiology of hypospadias is largely unknown, though some genetic and environmental risk factors have been identified. Some studies suggest genetic variation, specifically on androgen receptor and estrogen receptor genes, may contribute to risk of hypospadias [91]. Exposure to endocrine-disrupting chemicals appears to be a risk factor [93, 94]. A recent meta-analysis showed that maternal occupational exposure to pesticides was associated with increased odds of hypospadias (pooled RR=1.4; 95% CI: 1.0, 1.8) [95]. Paternal occupational exposure was also associated with increased odds of hypospadias (pooled RR=1.2; 95% CI: 1.0, 1.4), suggested a paternally-mediated pathway for hypospadias is plausible.

2.2.2.2 Congenital heart defects

Congenital heart defects (CHDs) are conditions present at birth that can affects the structure or functioning of an infant's heart. CHDs are the most common type of birth defects [96, 97]. CHDs are the most common type of birth defect at time of live birth, with an estimated prevalence of 1 case per 100 live births, though severity of these defects range widely [98]. Note that true incidence in utero is unknown due to missing data on pregnancy loss potentially due to CHDs [99]. While infant mortality rates remain high (approximately 25% of infants born with a severe CHD die in the first year of life), advancing medical treatment has allowed infant with CHDs to live longer and healthier lives [96]. Most infants born with severe CHDs need surgery or other procedures in their first year of life [96]. Among those who survive to adulthood, considerable morbidity persists for patients with CHDs as compared to patients with healthy hearts [100]. Prevalence of some subtypes, specifically atrial and ventricular septal defects, has

steadily increased in the past 30 years, while prevalence of other subtypes have remained stable [101].

Etiology remains largely unknown, though specific genetic causes have been identified for specific CHD phenotypes. Less is known about non-inherited causes, but established risk factors include maternal rubella, pre-gestational diabetes, exposure to thalidomide, vitamin A cogeners, and phenylketonuria [97]. A recent review paper on CHDs identified these exposures as areas for future investigation: peri-conceptional multivitamin intake, maternal drug exposures, environmental exposures, and paternal exposures [97].

CHDs are a broad category with clinically and etiologically heterogeneous sub-types. Here we briefly outline the levels of CHD classification relevant for this project. The selected CHDs for this analysis fall in two broad categories: (1) septal defects and (2) obstructive defects. *Atrial septal defects (ASDs*) and *ventricular septal defects (VSDs)* fall under the category of septal CHDs. *Left ventricular outflow tract obstruction (LVOTO)* and *right ventricular outflow tract obstruction (RVOTO)* fall under the category of obstructive defects [98]. Septal defects generally indicate a "hole" exists between two chambers of the heart, so oxygen-rich blood can leak into oxygen-poor regions, and vice versa. ASDs indicate a hole in the atrial chambers, and VSDs indicate a hole in the ventricular chambers (Sadler book) [102]. Obstructive defects generally include defects of blood flow. LVOTO refers to defects that obstruct blood flow out of the left ventricular chamber, and RVOTO refers to defects that obstruct blood flow out of the right ventricular chamber [102]. Figure 2.4 below outlines this categorical hierarchy for the selected CHDs for this analysis. Note that many more sub-types exist within the CHD category; we focus on these selected phenotypes for reasons described in the Aim 2 Methods section.
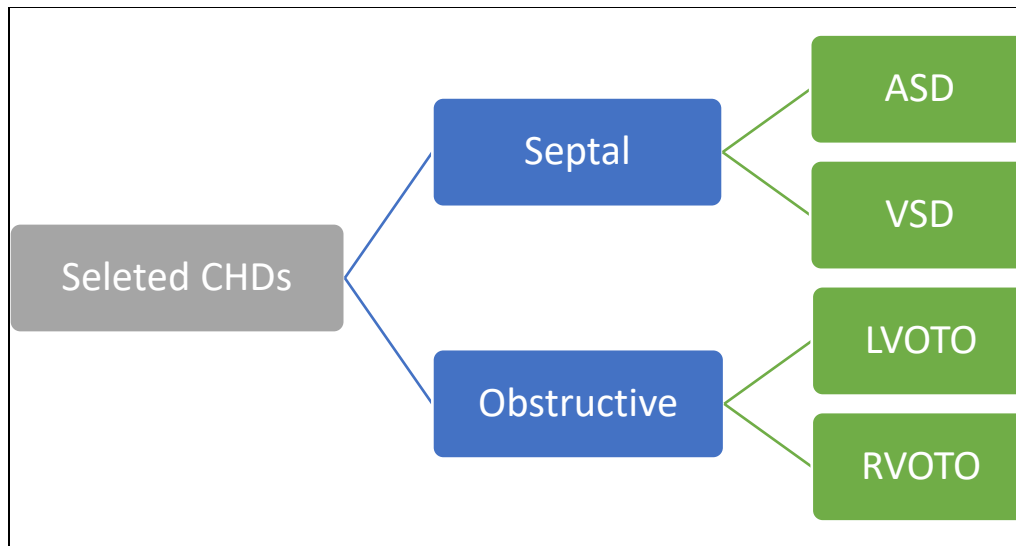
Figure 2.4. Hierarchy of selected congenital heart defect (CHD) categories

2.2.2.3 Oral clefts

Oral clefts are defects of the infant's lip or mouth. Oral clefts include two types of defects: (1) cleft lip and (2) cleft palate. During fetal development, the lip forms between the 4th and 7th week of pregnancy and the palate forms between the 6th and 9th week of pregnancy [103]. Oral clefts have an estimated prevalence of 16 cases per 10,000 live births, or 1 in 600 live births [89] . Infants born with cleft lip and/or cleft palate often have issues with feeding, speaking, and hearing; they are at higher risk for dental problems and ear infections. Children might need continued services, like special dental care or speech therapy [104]. Surgery to repair cleft lip and/or palate is usually recommended in the first 18 months of life. With treatment, most children with oral cleft are able to lead a healthy life, though some dental and speech issues may persist [103, 104].

Causes remain unknown, though a complex etiology involving gene-by-environment interactions is suggested [105]. Identified risk factors include: cigarette smoking during pregnancy, maternal diabetes prior to pregnancy, and specific medication use during first trimester [103, 106]. A recent meta-analysis found that older paternal age (>40 years) was

associated with increased odds of having a newborn with cleft palate (OR:1.58, 95% CI: 1.15, 2.17), suggesting that paternal factors may play a role in oral cleft etiology [107].

2.2.2.4 Gastroschisis

Gastroschisis is an abdominal wall defect that results in the infant's intestines exiting the body through the belly button. This defects results from aberrations in early fetal development where the infant's abdominal wall does not form correctly [108, 109]. Gastroschisis is an extremely adverse birth defect that results in increased risk of medical complications and infant mortality [110]. Severity of the defect varies, since the size of the hole and organs impact differ in each case. Generally, babies born with gastroschisis need surgery to place abdominal organs back inside the body. They also need specific treatments, such as antibiotics to prevent infection and an IV to provide essential nutrients, during the neonatal period [109].

Reports from multiple surveillance systems show that prevalence has been increasing since the 1980s [109, 110]. While increase in prevalence are documented among all ages groups, the increase is particularly striking among young (<20) mothers. Young maternal age was first documented as a risk factor for gastroschisis in the 1970s. A study in Norway reported an independent association with paternal age, after accounting for confounding by maternal age [111]. While etiology of gastroschisis remains unknown, the most recent MMWR suggests that "epidemiologic patterns indicate that lifestyle behaviors, environmental exposures, or other risk factors disproportionately affecting young women might play a role" [110]. More research is needed to understand etiology of gastroschisis and explain the recent spikes in prevalence.

2.2.3 Paternal exposures and birth defects

While most research on birth defects etiology focuses on the mother, recent epidemiologic investigations have shown that paternal exposures during the preconception period can increase risk of birth defects [29, 112]. Certain paternal occupations – notably

painters, chemical workers, agricultural workers, and janitors – are associated with increased

odds of birth defects [113, 114]. A small literature has examined the association between

paternal exposure to organic solvents and neural tube defects. A meta-analysis of these studies

found that paternal exposure to organic solvents is associated with an increased risk of any

neural tube defect (summary OR: 2.18, 95% CI: 1.52-3.11) [115]. While the exact mechanisms

behind these paternal effects is unknown, these studies suggest a morphologic and/or mutagenic

effect on the sperm, which then increases risk of a birth defect. This growing literature shows the

plausibility of paternal exposures during the preconception causing particular birth defects,

potentially mediated through sperm quality.

Additional evidence of male-mediated teratogenicity comes from the childhood cancer

literature. Animal studies have shown that male rodents exposed to chemical carcinogens in the

weeks prior to mating result in significant increases in tumor incidence in progeny [29].

Epidemiologic studies have consistently found that paternal occupational exposures to metals are

associated with increased risk of specific childhood cancers in offspring [29]. While childhood

cancer is a distinct outcomes from birth defects, they may share a causal mechanism of male-

mediated epigenetic effects and are therefore of interest.

Two studies have examined preconception paternal cigarette smoking and risk of birth

defects. A 1992 study from China found that paternal cigarette smoking increased risk of

anencephaly and spina bifida. A 2013 study from China found that preconception paternal

cigarette smoking increased certain subtypes of cardiac heart defects (CHDs) among offspring

[116]. A recent molecular review paper suggests this effect could be mediated by the adverse

effects of *polycyclic aromatic hydrocarbons* (PAHs) on spermatocytes. Briefly, the PAHs in

cigarettes can impact *aryl hydrocarbon receptor* (AHR) activation, which leads to oxidative

stress-mediated DNA damage in sperm [117]. While cigarette smoking and cannabis smoking are different exposures with different biologic components, the fact that paternal cigarette smoking increases risk of specific birth defects warrants investigation of cannabis smoking as a potential teratogen.

2.2.4 Association between paternal cannabis use and major structural birth defects

Since cannabis affects the way genetic material is transferred from father to developing fetus, it warrants investigation as a potential risk factor for birth defects. Figure 2.5 depicts the conceptual diagram for Aim 2, which is focused on estimating the effect of 3-month paternal preconception cannabis use and risk of major structural birth defects. Here, we summarize the previous literature on this association and explain how our proposal addresses gaps in this literature.
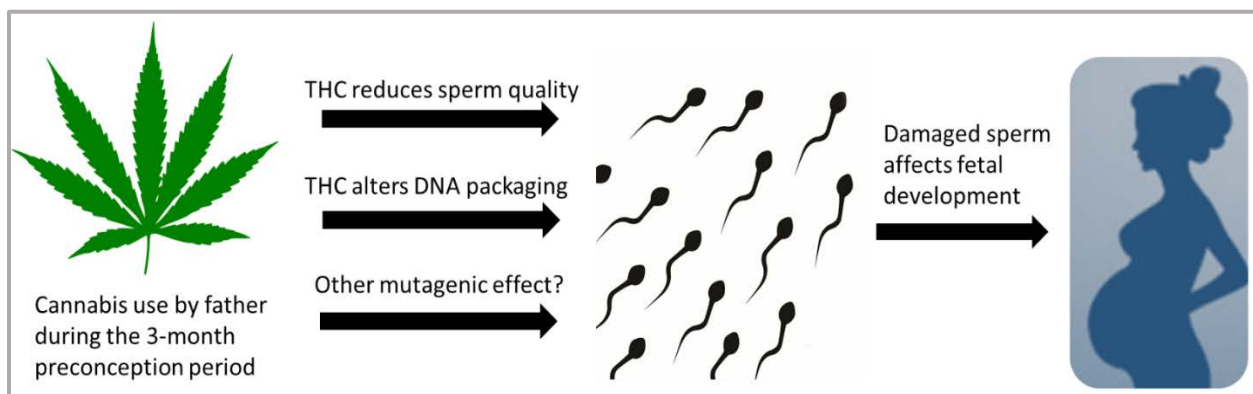
Figure 2.5. Conceptual diagram for potential effects of paternal cannabis on fetal development

2.2.4.1 Current literature

Three studies have addressed some aspect of the paternal cannabis use – birth defects association, summarized below in Table 2.2.

Table 2.2. Overview of three studies of preconception paternal cannabis use and risk of specific birth defect

Author	Year	Outcome	Exposure	Data source	Study population	Main findings
Shaw	1996	Neural tube defects (NTDs)	Face-to-face maternal interview; mothers asked about paternal drug use 3 months prior to pregnancy	California Birth Defects Monitoring Program	**Cases**: Singleton fetuses and live-born infants diagnosed with NTD (includes fetal deaths) between June 1989 and May 1991 **Controls**: randomly selected from local hospitals, singleton infants born without a reportable congenital anomaly	- Paternal use of marijuana/hash in the 3-month period prior to conception was not associated with NTDs (crude OR: 0.86, 95% CI: 0.63, 1.2). - Approximately 17% of fathers reported regular marijuana/hash use during preconception period. - Confounder-adjusted estimates not reported; authors state adjustment did not meaningfully change estimates [32].
Ewing	1997	Isolated membranous ventricular septal defects (IMVSD)	Face-to-face parental interview; primary respondent was generally mother, father involved in approx. 20% of interviews [118]	Baltimore-Washington Infant Study (BWIS)	**Cases**: Live-born infants with confirmed diagnosis of IMVSD before 1 year of age (does not include fetal deaths). Excluded chromosomal defects. **Controls**: Randomly selected live-born infants unaffected by congenital heart defects.	- Paternal use of marijuana in the 6-month period prior to conception was associated with increased risk of IMVSD (adjusted OR: 1.36, 95% CI: 1.05, 1.76). - Approximately 16% of fathers reported marijuana use during preconception period [30]

Wilson	1998	Congenital heart defects (CHDs)	Face-to-face parental interview; primary respondent was generally mother, but father participated in approx. 20% of interviews [31].	Baltimore-Washington Infant Study (BWIS)	**Cases**: Live-born infants with confirmed diagnosis of CHDs before 1 year of age (does not include fetal deaths). Excluded chromosomal defects. **Controls**: Randomly selected live-born infants unaffected by congenital heart defects.	- Authors investigate paternal risk factors for 8 subtypes of CHDs, but only report associations for paternal marijuana use and two specific CHDs:
						    - *Transposition of great arteries with intact ventricular septum*: RR =1.7; AF=12.1 (95% CI: 8.5, 15.8)
						    - *IMVSID*: RR =1.4; AF=6.0 (95% CI: 2.2, 9.7)
						 - Approximately 25% and 22% case fathers reported marijuana use, respectively.

*OR = odds ratio; RR = risk ratio; CI = confidence interval.

*Wilson et al. did not report confidence intervals for their risk ratios.

Shaw et al. was the first study published on paternal cannabis use in relation to any birth defect. Authors focused specifically on neural tube defects (NTDs), due to the unknown etiology of this defect. They used a case-control study design with data from the California Birth Defects Monitoring Program, a state-level active surveillance program. Medical records were reviewed for infants delivered at hospitals in elected California counties between June 1989 and May 1991. Fetuses diagnosed prenatally with an NTD and electively terminated were eligible to be considered a case. This analysis focused on 473 eligible cases and 474 eligible controls. Participation rates were comparable for cases and controls (87.8% and 88.2%, respectively). Women in this study underwent a two-hour face-to-face interview regarding various exposures before and during their pregnancy. During this interview, mothers were also asked about illicit drug use by the father of the baby during the 3-month period prior to conception. Notably, paternal heroin use appeared to be associated with NTDs (OR: 4.6, 95% CI: 0.92, 30.6), though this estimate is imprecise likely due to very few fathers with reported heroin use. For paternal marijuana/hash use, authors report a crude OR of 0.85 (95% CI: 0.63, 1.2). The effect estimate is slightly below the null, but the estimate is somewhat imprecise and includes the null. Authors acknowledged that maternal report of this paternal exposure is subject to potential measurement error, but did not conduct a bias analysis to see how that measurement error would affect effect estimates [32].

Ewing et al. 1997 and Wilson et al. 1998 both use data from the Baltimore-Washington Infant Study (BWIS). However, they focus on different subtypes of cardiac heart defects (CHDs), and use different methodological approaches for estimating the association. Case data from BWIS come from five pediatric cardiology centers in the Maryland/Washington DC area that permit enrollment of infants as soon as they are diagnosed with a CHD.). A strength of the

BWIS study is that a cardiologist reviewed participants' medical records to determine specific CHD diagnoses. Exposure was ascertained from a face-to-face parental interview, which may increase social desirability bias and result in under-ascertainment of exposure. Wilson et al. note that the mother was usually the primary responder, and the father self-reported exposures on approximately 20% of the interviews [31]. Ewing et al. do not report how often the father self-reported exposure (versus the mother reporting about the father), but we assume that the rates are comparable to the Wilson paper, since they use the same parent study. While father self-reported exposure is presumably more accurate that maternal report, both are imperfect measures that could result in under-ascertainment of exposure.

Ewing et al. 1997 focus on isolated membranous ventricular septal defects (IMVSD), which are the most common type of CHDs in the US [30]. Authors suggest that prior studies on paternal age and CHDs influenced their decision to study paternal risk factors for IMVSD. Paternal cannabis use was defined as any use of marijuana in the 6-month period prior to conception. The adjusted association was OR = 1.36 (95% CI: 1.05, 1.76), and was adjusted for paternal cocaine, maternal age, maternal cocaine use, sex of infant, and race/ethnicity; however, not adjusted for other potentially important confounders, such as other paternal drug use, maternal nutritional factors, and SES. Authors found that paternal age did not meaningfully modify the effect (though it was a strong modifier for the paternal cocaine-IMVSD association).

Unlike the other two papers, Wilson et al. was not a typical exposure-outcome analysis. Instead, authors aimed to estimate attributable fractions for various risk factors for cardiac malformations (another term for CHDs). The authors' rationale was that attributable fractions can directly inform how effective various interventions would be in reducing absolute numbers of CHD cases. Because attributable fractions assume causality, authors limited their analysis to

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exposures they with "potentially causal effects" on CHDs. The analysis focused on eight

subtypes of CHDs that were clinical confirmed by a cardiologist in the first year of life.

However, they only report estimates for paternal cannabis use for two of the subtypes,

assumingly because paternal cannabis did not have a sufficiently large attributable fraction for

the other subtypes. The relative risk for paternal marijuana use and risk of *Transposition of great*

arteries with intact ventricular septum was 1.7 (no confidence interval provided). The relative

risk of paternal marijuana use and risk of *Isolated/simplex membranous ventricular septal defects*

was 1.4 (no confidence interval provided). Among the associations considered, the largest

attribute fraction was for paternal use of marijuana and transposition of great arteries with intact

ventricular septum (AF=30.2, 95% CI: 24.2, 36.1). Authors suggest the potential for exposure

misclassification to impact results may be minimal given the case control differences persisted

when using "affects controls". Authors noted their results were consistent with animal models of

male-mediated teratogenicity, concluding that paternal marijuana use is a plausible risk factor for

these specific subtypes of CHDs [31].

In summary, the epidemiologic literature on paternal cannabis use and risk of birth

defects is extremely sparse. The three studies described above (see Table 2.2) were from the

1990s. All three studies used case-control designs, as is common in birth defects research. Shaw

et al. used surveillance data from California, and the other two studies used data from the

Baltimore-Washington Infant Study, so the current literature is derived from only two study

population. Shaw et al. found a null association between paternal cannabis use and neural tube

defects (NTDs), while Ewing et al. and Wilson et al. found that paternal cannabis use was

associated with increased risk of specific subtypes of CHDs.

2.2.5 Exposure misclassification

A major challenge of this literature is ensuring accurate exposure measurement of cannabis use. Ideally, the most accurate exposure measurement would be a biomarker for cannabis use (hair or urine) taken multiple times during the critical window of exposure – in this case, during the 3-month window prior to conception. Unfortunately, this approach is impossible with the retrospective case-control design that is inherent to most studies of rare outcomes. The next best option would be real-time paternal self-report of cannabis use during the critical window of exposure, which are not always available. Because studying rare events like birth defects is most efficient with a case-control design, previous studies used data from maternal report of paternal cannabis.

Exposure measurement error is possible due to (1) mother's lack of awareness of father's true cannabis use, (2) mother's inability to recall father's cannabis use, or (3) mother's discomfort or hesitancy in reporting father's true cannabis use. Of particular interest is whether this exposure misclassification is differential by case status, since differential misclassification is more likely to bias the effect estimates. Our proposal will use a computer-assisted maternal report of paternal cannabis, acknowledging it is an imperfect but acceptable method of exposure ascertainment if we take care to examine and quantify the possible impact of exposure misclassification (see Aim 2 Methods). We aim to conduct a probabilistic bias analysis to quantify how potential exposure misclassification could change our effect estimates. Observed data and information from the cannabis literature will inform semi-Bayesian priors and distributions of sensitivity and specificity. Details of our analytic approach can be found in Section 3.2.4. Given the issues with potential exposure misclassification in the literature, we hope this rigorous bias analysis will put the results of Aim 2 in appropriate context.

CHAPTER 3: RESEARCH METHODS

3.1 Aim 1 Methods

The goal of Aim 1 is to conduct an age-period-cohort analysis of current (past 30 days) cannabis use among men and women of reproductive age from 2002 to 2014 using a nationally-representative data source. Sub-aims include (1a) estimating risk factors for recent cannabis use, (1b) evaluating if period effects differ by gender, and (1c) evaluating time trends in risk perception towards regular cannabis use.

3.1.1 Study design and population

The National Survey on Drug Use and Health (NSDUH) is the largest nationally-representative survey of drug use in the United States. NSDUH uses an annual cross-sectional survey design. The survey provides national data on the use of tobacco, alcohol, and illicit drugs. In addition to detailed questions on drug use patterns, the survey also covers mental health issues, behavioral issues, and attitudes towards drug use. The survey began in 1971 and was originally conducted every other year, but demand for current, accurate information on drug use in the 1990s prompted the survey to be conducted annually [119]. NSDUH is authorized by Section 505 of the Public Health Service Act, which requires regular data collection on the level and patterns of substance use [120]. The survey is run by the Substance Abuse and Mental Health Services Administration (SAMSHA), an operating division within the US Department of Health and Human Services [2]. Since 1988, the operational duties of the survey are conducted by Research Triangle Institute (RTI) International [119].

Population: The source population is the civilian, noninstitutionalized US population aged 12 years or older from 2002 to 2014. Individuals excluded from the sample are active military personnel, residents of institutional group quarters (e.g., prisons, nursing homes, mental institutions, long-term hospitals), and homeless persons not living in a shelter at the time of the survey (Methods guide). Data collection takes places in all 50 states and Washington DC.

While the survey has been conducted regularly since 1971, it underwent major changes in 2002. Specifically, some drug use questions were modified and all participants were offered a $30 compensation for participation from 2002 onwards. Since this modification changed the quantity and demographics of survey respondents, NSDUH administrators advise against making inferences on temporal trends before and after 2002. Therefore, we focus our analysis on the temporal trends from 2002 to 2014, the most recent study year with available data. Moreover, this time period captures significant changes in cannabis legality and social norms that are of interest in this analysis.

Sampling method: A random sample of US households is selected, and a professional interviewer from RTI makes a personal visit to each selected household. Specifically, a multistage clustered sampling design is used. Briefly, the source population is grouped by geographic "clusters", then simple random samples are taken within these clusters. Each state was geographically partitioned into roughly equal-sized regions according to population, called *state sampling regions* (SSRs). The cluster sampling had three stages: (1) census tract, (2) census block, then (3) compact clusters within census block. The goal of this sampling division was that each area yielded, in expectation, roughly the same number of interviews within each state during each quarterly data collection period [119]. All SSRs had samples sizes sufficient to support reliable direct estimates, while maintaining efficiency for national estimates [119].

Note that the inclusion criteria of "households" also includes non-institutional group quarters, e.g. shelters, boarding houses, college dormitories, migratory workers' camps, and halfway houses [2]. Once a household is chosen, no other household can be substituted. After a brief screener interview, one or two residents of the household (over 12 years of age) may be asked to participate in the survey. Participation is voluntary; participants that complete the survey receive $30 in cash. All selected persons are encouraged to participate, whether or not they use or know anything about tobacco, alcohol, or drugs [120]. The NSDUH study has a strict quarterly schedule to ensure that interviews are conducted equally across the calendar year. The final sample size of completed surveys varies by year; the most recent (2016) NSDUH is designed to yield 67,500 completed interviews (field guide).

Data collection: Participants complete the survey in the privacy of their own home. The trained RTI interviewer sets up the computer-assisted interview on a touch-screen Samsung Galaxy tablet, and the participant completes the entire survey on the tablet. The interview is trained to step away and not view the computer, so the participants' responses remain confidential. No prior computer skills are necessary to complete the survey, and the trained interview sets up the tablet in such a way that the participant need not worry about the technical aspects of the computer-assisted interview. Small portions of the interview are conducted via computer-assisted personal interviewing (CAPI), where the interviewer asks the questions and records the answers on the tablet. Sensitive questions, including questions on cannabis use, are completed using audio computer-assisted self-interviewing (ACASI), where the respondent listens to the questions and enters his/her own response [119]. The ACASI approach ensures that the interviewer is unaware of the respondent's answers. On average, the survey takes about an

hour to complete [120]. Interviewers undergo rigorous training and have a detailed "Interviewer

Field Guide" to guide them through ambiguous situations during data collection. Figure 2.6 is a

flowchart that details the data collections steps for NSDUH.
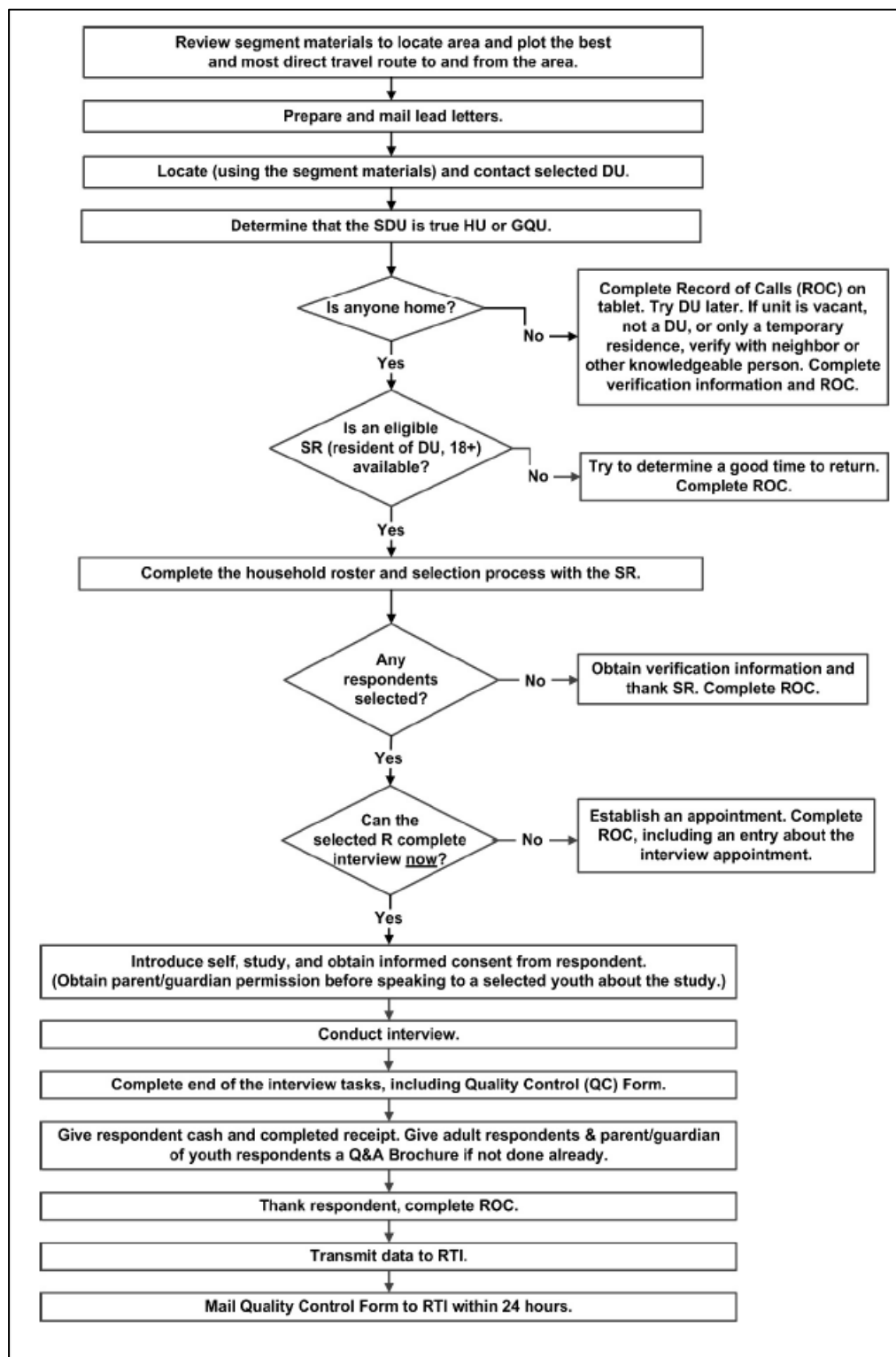
Figure 3.1. Flowchart of NSDUH screener and interview process

Confidentiality of data: Full names are never recorded or associated with a participant's answers, and participants are informed about this anonymity prior to completing the survey. The confidentiality of the answers provided are actually protected under federal law by the Confidential Information Protection and Statistical Efficiency Act of 2002 (CIPSEA), so by law, all responses can only be used for research purposes. Each survey data file is identified only by a code number, and is electronically submitted to RTI the same day the survey is completed [120].

3.1.2 Exposure Assessment

The exposure – any cannabis use in past 30 days – is ascertained from the NSDUH survey. Specifically, the cannabis interview questions are asked using audio computer-assisted self-interviewing (ACASI), where the respondent listens to the questions and enters his/her own response (described in Section 3.1.1). This method is advantageous, as compared to exposure assessment methods from previous APC analyses, since the question is *not* asked face-to-face by an interviewer, therefore reducing stigma and potential misreporting.

The NSDUH survey contains multiple questions regarding cannabis use. The cannabis section of the survey opens up with a brief definition of cannabis, and re-emphasized that this information is for research purposes only and will be kept confidential. See Figure 3.2 for the exact wording of the survey's introduction to cannabis.

Figure 3.2. NSDUH 2014 survey introduction to cannabis questions

Then, the survey asks a handful of questions about ever/never use, age at first use, and frequency of use. For this analysis, we focus on any cannabis use in the past 30 days, which is derived directly from the question labeled 'MJLAST3'. See Figure 3.2 for the exact wording of this specific cannabis use question. Note that the question asks about 'time since last use', and the survey repeats this question up to three times. For example, if a participant responded 'don't know or refuse' to the first question, the question pops up again on the screen asking for their '*best guess* of time since last use'. If the participant again responded 'don't know or refuse', then the question pops up a third time, acknowledging that this information is personal and reminding the participant that the information is kept confidential (see Figure 3.3). This repeated-question method ensures that respondents who initially respond 'don't know or refuse' are given ample opportunities to report their true cannabis use.

Figure 3.3. NSDUH 2014 survey questions about time since last cannabis use

The exposure will be coded as a binary variable (yes/no use in last 30 days). If participants click response '1' for MJLAST3, then they are coded as 'yes'. If participants click '2', '3', or reported never using cannabis in question MJO1, then they are coded as 'no'. If participants changed their response on questions 'MJRECDK' or 'MJRECRE', then the *most recent* response is used (see Figure 3.3). For example, if participant Bob clicks 'don't know or refuse' for MJLAST3, but then clicks '1' for 'MJRECDK', then he is classified as a 'yes'. These follow-up questions hopefully improve measurement of true exposure by repeating the question and remind participants of the confidentiality of their responses. If a participant reports 'don't know or refuse' to all three questions, he/she will be excluded from the main analysis. However, we will conduct a sensitivity analysis – including all the 'don't know or refuse' as 'yes', then including them all as 'no' – to estimate the bounds of the effect estimates.

3.1.3 Age, period, and cohort definitions

An age-period-cohort analysis requires specific definitions for age, period, and cohort that remain consist across the study period. Here, we briefly specify the exact coding of these three variables. In the Core Demographics section of the survey, the survey asks date of birth, and automatically calculates current age (see Figure 3.4). This variable will be used to specify **age** and **cohort** (represented by year of birth). Individuals less than 12 years of age are ineligible for the study, so the age variable will be truncated, by design, at age 12. There is no upper age limit for participation in the study. The age variable was categorized as follows by NSDUH study administrators: integers from age 12-21, two-year categories from 22-25, five-year categories from 26-34, and 14-year categories from 35 onwards. These categories best represented change in drug use patterns, since habits often fluctuate greatly among teenagers and became stable later in life. Therefore, we will categorize age in accordance with the NSDUH cut points. Since this aim is focused on populations of reproductive age, age eligibility will be 15-49 for men and women (based on World Health Organization definitions) [121]. Indicator variables in the regression model will indicate each age category; the age group with the lowest cannabis use prevalence will serve as the reference group.

```
Core Demographics

age1      What is your date of birth?

          ENTER MM-DD-YYYY

          DOB: _____
          DK/REF

          DEFINE CALCAGE:
          CALCAGE = AGE CALCULATED BY "SUBTRACTING" DATE OF BIRTH FROM DATE OF INTERVIEW.

confdob   [IF AGE1 NE DK OR REF] I have entered your date of birth as [AGE1]. Is this correct?

          1       YES
          2       NO
          DK/REF
```

Figure 3.4. NSDUH 2014 survey questions for age and cohort variables

The exact birthdate is not available in the public-access data, so 'cohort' will be calculated by subtracting 'age' from 'study year'. For example, if a participant is 14 years old when completing the 2014 survey, then cohort will be calculated as: 2014 (study year) – 14 (age) = 2000 (cohort). Cohort will then be categorized by decade (e.g. born in the 1970s, born in the 1980s, etc.). This categorization best represents generational effects while simplifying interpretation, and is consistent with previous APC analyses in this literature. Indicator variables will be used to represent each cohort; the cohort with the lowest cannabis use prevalence will serve as the reference group.

The **period** will be represented by the year the survey was administered, and will be dichotomize to *before* and *after* 2012 (see Section 3.1.4.1 for explanation). Briefly, dichotomizing the period effect *constrains* one of the three parameters, thereby addressing the model identification issue. Moreover, we hypothesize that period effects will likely occur in 2012, since the first states legalized recreational use of marijuana in November 2012. Table 3.3 depicts the categorization of the age, period, and cohort variables.

Table 3.1. List of categories for age, cohort, and period variables for Aim 1 analysis

Age	Cohort	Period
15-17	1930s	2002
18-21	1940s	2003
22-23	1950s	2004
24-25	1960s	2005
26-29	1970s	2006
30-34	1980s	2007
35-49	1990s	2008
	2000s	2009
		2010
		2011
		2012
		2013
		2014

3.1.4 Analysis

3.1.4.1 Specific Aim 1 – Main analysis

The goal of this APC analysis is to quantitatively estimate the independent effects of age, period, and cohort on prevalence of past-month cannabis use in the US. We will use nationally-representative NSDUH data from 2002 to 2014. The measurement and definition of the outcome of interest – prevalence of past-month cannabis use – is detailed in sections 3.1.1-3.1.3. All analyses for this aim will be conducted in R, specifically 'base R' for data management, 'ggplot' package for graphics, and 'apc' package for the age-period-cohort modeling [122].

Introduction to APC models: Conceptually, the APC model is a regression model that is incorporating age, period, and cohort as predictors of the outcome of interest. The basic linear form of this regression model can be written as:

$$R_{ij} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ij}$$

where *R* represent the rate of the outcome of interest for the *i*th age group at the *j*th time period, μ denotes the intercept, alpha denotes the *i*th row age effect, β denotes the *j*th row period effect, γ

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denotes the *k*th row cohort effect, ε denotes random error. Subscript *i* refers to the age group,

subscript *j* refers to the time period, and subscript *k* refers to the cohort category. [52].

In the context of Aim 1, the outcome (R_{ij}) is a binary variable for *any cannabis use in the*

past month. This model uses individual-level data for all parameters. The age, period, and cohort

parameters will be categorical indicator variables. Categories of these three parameters are laid

out in Table 3.3. Since no covariates predict age, period, or cohort (e.g. no arrow on the DAG

from covariate to exposure), no confounders need to be included in the model. We will assess

potential effect-measure modification by gender in Sub-aim 1b (see 3.1.4.2).

This analysis will be conducted using the 'apc' package in R, an open-source tool created

by Bent Nielsen at Oxford University [122]. The analysis will follow statistical guidelines laid

out in the 'Age-Period-Cohort Models: New Models, Methods, and Empirical Applications'

book by Yang Claire Yang and Kenneth C. Land [52].

Model identification problem: A major issue in APC analysis is the model identification

problem. Briefly, a key assumption in the APC data structure is that this linear relationship holds

true: "Period – Age = Cohort." Because of this perfect linear relationship, a unique solution to

the ordinary least squares (OLS) estimator does not exist in the linear APC regression model. In

other words, there are an infinite number of possible solutions of the matric equation, so it is not

possible to estimate the effect of age, period, and cohort separately without imposing at least one

constraint on the coefficients [52]. In the current literature, there are a few different solutions to

the model identification problem. We plan to use unequal categorization of age, period, and

cohort indicators to 'break' the algebraic linearity between the three variables and therefore solve

the model identification problem [52]. Specifically, age will be categorized in unequal categories

available in NSDUH public-access data, cohort will be categorized in 10-year periods to capture

generational effects by decade, and period effects will be categorized in 1-year categories since period effects are more susceptible to short-term changes in social norms and policies. See Table 3.1 for details on parameter categorization.

3.1.4.2 Specific Aim 1a – Correlates of current cannabis use

The demographic make-up of the cannabis user population has changed over time, and varies by study population (see Section 2.1.2). Moreover, previous descriptive epidemiology on the cannabis user population mostly focused on past-year use. To characterize the current (past month) cannabis user population, we will report the distribution of key covariates among users and non-users in the NSDUH population. Distribution of covariates will be reported for the overall study population, then stratified by period (before and after 2012), to qualitatively assess demographic changes in the user population. Additionally, we will use a logistic regression model to assess how covariates are associated with odds of past-month cannabis use, both crude and adjusted. Covariates of interest include: age, gender, race/ethnicity, educational attainment, urbanity, age at first cannabis use, cigarette smoker status, alcohol use, use of hard drugs (i.e. heroin, cocaine, methamphetamines, etc.), and risk perception towards regular cannabis use. Covariate data will come from the NSDUH survey; results will be presented in a Table 3.1 format.

3.1.4.3 Specific Aim 1b – Age, period, cohort effects

We plan to estimate age, period, and cohort effects from a fully-specified model as detailed in section 3.1.4.1 (main analysis of Aim 2).

3.1.4.4 Specific Aim 1c – Stratified by gender

Cannabis use frequency differs by gender; US men are more likely than US women to report past-year cannabis use [2]. National trends of cannabis use also appear to differ by gender, with trends for women increasing while trends for men remain stable in the past decade [2].

Moreover, women's risk perception of cannabis use is declining faster than men's in the past decade [10]. Due to these gender difference in cannabis use and trends of use of time, we aim to qualitatively compare if age, period, and cohort effects differ by gender – with a particular interest in the period effect. To achieve this aim, we will stratify the APC analysis by self-reported gender. In our results section, we plan to present model results for the overall study population, then results stratified by gender. No statistical tests will be conducted to test differences by gender.

3.1.5 Power calculations

Since the APC analysis is not a hypothesis test, traditional power calculations do not apply. However, we do want to ensure that we have sufficient data to validly parse out the age, period, and cohort effects of cannabis use.

Figure 3.5 depicts the distribution of cannabis use across the study period (2002-2014). The number of past-month users for each study year ranges from 5272 to 6336; no study year has less than 5000 past-month users. Therefore, we should have sufficient exposed samples for each study year. Table 3.4 depicts the age distribution by study year in the NSDUH population, showing sufficient sample size for the age by period comparisons. Note that the cohort variable is still being created; once this is complete, we will assess age by cohort by period cross-tabulations to ensure sufficient sample size in all cells.
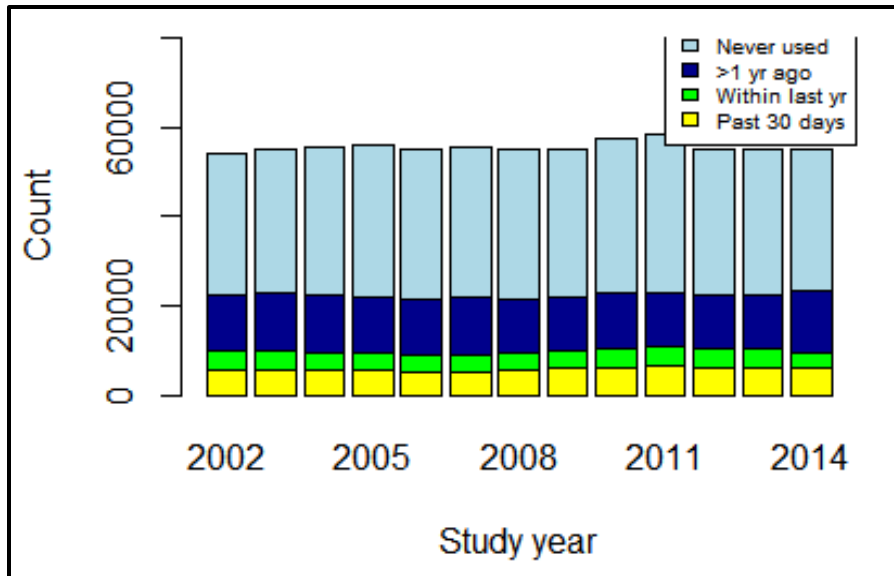
Figure 3.5. Bar plot of cannabis use distribution by study year

Table 3.2. Age distribution of NSDUH population by study year

AGE	15	16	17	18	19	20	21	22-23	24-25	26-29	30-34	35-49	50-64
STUDY YEAR													
2002	2844	2776	2774	2397	2329	2177	2253	4378	4194	2396	3134	8306	2787
2003	3060	3011	3015	2630	2280	2302	2292	4511	4368	2434	3128	8096	3052
2004	3116	3010	2969	2686	2370	2255	2277	4545	4342	2609	3101	8052	3072
2005	3232	3209	3047	2563	2387	2296	2257	4512	4461	2608	2887	8133	3111
2006	3193	3159	3030	2524	2283	2143	2263	4396	4323	2646	2860	7697	3606
2007	3079	3124	3032	2603	2383	2256	2260	4426	4389	2810	3017	8213	3298
2008	3062	3180	3084	2811	2468	2280	2342	4520	4468	2732	2806	7788	3290
2009	3106	3102	3152	2673	2516	2299	2307	4542	4424	2690	2913	7802	3439
2010	3117	3188	3208	2638	2460	2388	2380	4629	4580	2783	3106	8313	3506
2011	3239	3409	3323	2639	2347	2423	2448	4711	4615	2670	2956	7619	4219
2012	2956	3058	3038	2469	2223	2271	2354	4707	4591	2628	2864	7391	3923
2013	3006	3058	2983	2343	2122	2244	2220	4643	4570	2557	2889	7511	3936
2014	2344	2356	2217	1664	1543	1639	1611	3526	3356	3789	4601	11235	5361

3.1.6 Addressing gaps in literature

Broadly, there are three main limitations of this literature. First, there is a lack of APC analyses with data from 2000 onwards, which is a major gap in the literature in light of the rapidly changing socio-cultural norms, laws, and use patterns of cannabis. Meich et al. did

include data up until 2009, and the overall time trends, cohort effects, and period effects were substantially different as compared to previous APC analyses on the same outcome variable. This suggests that the landscape of APC effects and overall time trends in cannabis use are changing in recent years. We plan to address this gap by including data up until 2014. This would be the most up-to-date APC analysis, and would include five additional years of data as compared to the Meich et al. analysis. Moreover, these five additional years (2010-2014) reflect major changes in cannabis policy 28 states have medically or recreationally legalized cannabis in this time period, so it is crucial to understand how patterns of use are changing in response to these policy shifts [11].

Second, two out of the four studies do not have comparable study populations. Piontek et al. is focused in German adults. While results of this analysis are interesting, the socio-cultural norms surround cannabis use are likely very different between German and U.S. populations. Moreover, period effects are very different in the two settings, since the U.S. has rapidly changing laws about medical and recreational cannabis, whereas Germany's cannabis laws have remained fairly steady over the past few decades. Therefore, the Piontek et al. analysis does not translate to our population of interest. Johnson et al. conducted their APC analysis on age at first marijuana use, which is a very different variable than marijuana use prevalence. Given that many individuals try cannabis once or twice in their youth but do not continue to use, age at first use is probably not a strong indicator of current cannabis use. Therefore, the Johnson et al. analysis does not translate to our study question.

Third, most APC analyses on cannabis use prevalence focus on ever/never use in the past 12 months. This measure, while useful in some contexts, does not represent "recent" or "current" cannabis use. As mentioned by Piontek et al, ever/never use in past 12 months lumps together

occasional users and regular users, and therefore might not be a sensitive outcome definition for changing use trends. Additionally, we are interested specifically in cannabis use among men and women of reproductive age, due to the potential adverse effects on reproductive health. Though evidence is limited, the adverse effects on reproductive health appear to be due to recent (i.e. past three months or sooner) use. As detailed in Section 2.2.1, cannabis use in the past three months can adversely affect sperm quality. The three-month window is biologically relevant, since spermatogenesis takes approximately three months. The window of exposure for women is less clear, though a few preliminary studies (detailed in Section 2.2.1) suggest that past month cannabis use can affect menstruation. Due to our focus on reproductive health, and the need for a more specific measure of recent cannabis use, we plan to conduct our APC analysis on past-month (defined as past 30 days) cannabis use. To our knowledge, this would be the first study to examine APC effects of *current* cannabis use.

3.2 Aim 2 methods

The goal of Aim 2 is to estimate the association between paternal 3-month preconception cannabis use and prevalence of major birth defects. Sub-aims include (2a) estimating prevalence of cannabis use among men in preconception period, (2b) evaluating effect-measure modification by paternal age, and (2c) evaluating potential bias due to exposure misclassification.

3.2.1 Study design and population

The National Birth Defects and Prevention Study (NBDPS) is the largest and most comprehensive population-based case-control study of birth defects in the United States [123]. NBDPS has ten study centers located in geographically diverse parts of country (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah). Some study centers capture cases and controls from the entire state, while others focus on select geographic districts that are representative of the state's population (see Figure 3.6).

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During the study period of 1997-2011, approximately 6 million births occurred in the NBDPS catchment area. All birth defects cases are identified through state birth defects surveillance registries; controls are randomly selected from the source population using birth certificates. NBDPS has a total of approximately 44,000 participants. Specifically, a total of 32,187 case-mothers and 11,814 control-mothers participated in the study. The study design used a 3:1 case-to-control ratio to ensure sufficient data of a wide variety of birth defects [123]. Because the etiology of birth defects is likely heterogeneous, and specific birth defects are rare, it is essential to have an extremely large study to have sufficient power to study etiology of specific birth defects. Moreover, the case-control design is the most cost-effective approach for rare outcomes like specific birth defects [123].

The source population of this case-control study is all babies born during the study period (1997-2011) in selected counties of Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah. Specifically, study eligibility started with pregnancies ending on or after October 1, 1997, and concluded with pregnancies with estimated dates of delivery (EDD) on or before December 31, 2011. A woman was ineligible to participate in the NBDPS if she already participated in the study with a previous pregnancy, could not complete the interview in English or Spanish, was incarcerated, or did not have legal custody of the infant. For three out of the ten centers, women under 18 years at the end of her pregnancy were ineligible [123]. Figure 3.6 below shows the catchment area for select NBDPS study sites, along with the approximate annual birth population of the catchment areas.

NBDPS Study Site	Catchment area	Annual birth population of catchment area
Arkansas	Entire state	37,000
California	San Joaquin Valley counties (Fresno, Kern, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare)	65,000
Georgia	Metropolitan Atlanta counties (DeKalb, Fulton, and Gwinnett)	35,000
Iowa	Entire state	40,000
Massachusetts	Central and Eastern counties (Barnstable, Essex, Middlesex, Norfolk, Plymouth, Suffolk, and Worcester)	58,000
North Carolina	Perinatal Care Regions II and IV counties (Alamance, Alexander, Allegheny, Ashe, Avery, Burke, Caldwell, Caswell, Catawba, Chatham, Davidson, Davie, Durham, Forsyth, Franklin, Granville, Guilford, Iredell, Johnston, Lee, Orange, Person, Randolph, Rockingham, Rowan, Stokes, Surry, Vance, Wake, Warren, Watauga, Wilkes, and Yadkin)	51,000
New York	Selected Western and Downstate counties (Allegany, Bronx, Cattaraugus, Chautauqua, Erie, Genesee, Monroe, Niagara, Orange, Orleans, Putnam, Rockland, Westchester, and Wyoming)	66,000

Figure 3.6. Overview of NBDPS catchment area for select study sites [101]

3.2.2 Outcome ascertainment

The case and control selection process was consistent across study site. A broad overview of the case and control selection processes are laid out in Figure 3.7.
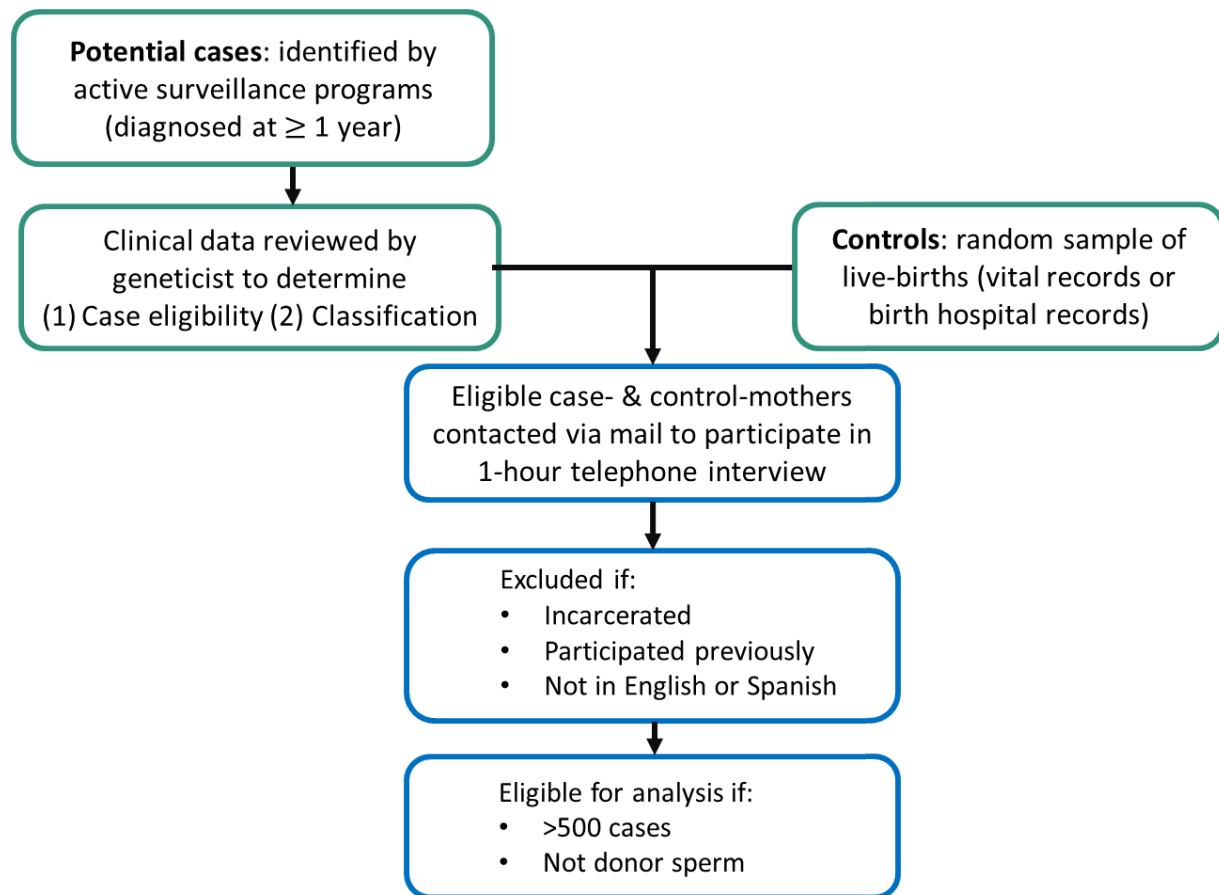
Figure 3.7. Flowchart of NBDPS case and control selection process

Case ascertainment: All potential cases underwent rigorous evaluation by a clinical geneticist experienced with birth defects to ensure accurate classification of all birth defect cases. NBDPS specifically focused on twenty-four types of structural birth defect that currently have unknown etiologies 21 (see Figure 3.8). A unique feature of NBDPS is that many centers included stillbirths and induced abortions in the case group, in addition to live births. This detailed outcome data addresses issues related to conditioning on live birth, which is problematic in other birth defects studies (see Section 3.2.6). Moreover, since all cases are ascertained

through active state-level surveillance, potential for missing cases or systematic error in case selection are minimized.

Clinical verification of all cases:

After cases are identified from each state's Birth Defects Surveillance program, cases undergo a rigorous verification process by clinical geneticists with expertise in birth defects phenotyping. Some birth defect phenotypes were classified by the same clinical geneticist for all cases for the entire study period. For other defect types that were more complex to diagnose, classification was completed by more than one geneticists. The NBDPS clinical geneticists developed guidelines that detailed the (1) inclusion and exclusion criteria for each defect eligible for the NBDPS; (2) rationale for including certain

Birth defect
Anencephaly, craniorachischisis
Spina bifida
Encephalocele, cranial meningocele, encephalomyelocele
Holoprosencephaly
Hydrocephalus
Dandy-Walker malformation
Anophthalmia, microphthalmia
Cataracts, glaucoma and related eye defects[a]
Anotia, microtia
Conotruncal heart defects
Single ventricle
Septal heart defects (atrial septal defects, ventricular septal defects)
Atrioventricular septal heart defects
Ebstein malformation
Obstructive heart defects (right and left ventricular outflow tract defects)
Anomalous pulmonary venous return
Heterotaxia
Choanal atresia
Cleft lip +/− palate
Cleft palate
Esophageal atresia +/− tracheoesophageal fistula
Intestinal atresia/stenosis
Biliary atresia
Hypospadias, second or third degree
Renal agenesis/hypoplasia
Exstrophy, bladder
Exstrophy, cloacal
Limb deficiency, intercalary
Limb deficiency, longitudinal
Limb deficiency, transverse
Limb deficiency, not elsewhere classified
Craniosynostosis
Diaphragmatic hernia
Sacral agenesis
Omphalocele
Gastroschisis
Amnion rupture sequence

Figure 3.8. List of eligible NBDPS birth defects

diagnostic codes for defects and related defects; (3) instructions and rationale for designating the final case classification (isolated, multiple, complex); and (4) instructions and recommendations to analysts on how the defect type could be analyzed in epidemiologic studies. All these guidelines were consistent across study site to ensure that the classification process did not differ

geographically or by clinical geneticist. Figure 3.8 depicts a broad overview of the NBDPS case

identification process and where clinical verification of cases fits in this process.

Specific phenotypes for analysis: Birth defect phenotypes are clinically and etiologically

heterogeneous. Therefore, specific phenotypes should be analyzed separately when estimating

causal effects. Various to approaches exist for deciding which phenotypes to focus on to best

address Aim 2. Traditionally, we would rely in current literature to inform specific hypotheses

for which birth defects might be associated with paternal cannabis use. However, this literature is

too limited for meaningful inference. Another approach would rely on the proposal biological

mechanism of preconception cannabis use on fetal development. As described in section 2.2.1,

cannabis affects sperm quality by binding cannabinoid receptors, but how this mechanism affects

specific birth defects differently is unknown.

Therefore, we focus broadly on the *most prevalent* birth defect phenotypes NBDPS,

which reflect conditions arguably significant to public health. Specifically, we focus on NBDPS

defects with at least 500 isolated cases during the 1997-2011 study period. Since no strong

rationale exists for focusing on specific birth defect phenotypes, we take a more exploratory

approach: we are interested if paternal cannabis has an effect any of the most common birth

defect phenotypes. This approach best suits the nature of the current literature, research question,

and public interest. Moreover, this approach ensures sufficient power to investigate this

relationship between a relatively rare exposure and quite rare outcome. Defects included in our

analysis are outlined in Table 3.3, alongside the number of cases available in NBDPS (1997-

2011). While we focus on the most prevalent defects in NBPDS, we include Table 3.4 as a

reference to show that the seven most prevalent birth defect phenotypes in the US and consistent

with the defects included in our analysis. Data in Table 3.4 are aggregated from state-specific

active and passive surveillance systems from 2008-2012 and are weighted to be nationally

representative [89].

Table 3.3. Selected birth defect phenotypes for Aim 2 (N)

Birth defect phenotype	Total cases	Isolated cases	Multiple cases
Anencephaly and craniorachischisis	662	592	70
Spina bifida	1297	1140	154
Cleft palate	1631	1310	321
Cleft lip with cleft palate	2052	1748	304
Cleft lip without cleft palate	1109	1031	78
Hypospadias second/third degree	2607	2328	278
Transverse limb deficiency	732	613	118
Craniosynostosis	1627	1472	154
Diaphragmatic hernia	883	673	200
Gastroschisis	1450	1315	135
DTransposition of the great arteries (Level 2 Code)	781	579	43
Hypoplastic left heart syndrome (Level 2 Code)	669	594	59
RVOT defects - restricted (Level 3 and Level 2 Codes)	2149	1422	97
RVOT defects - excluding Ebstein cases (Level 3 and Level 2 and Level 1 Codes)	1998	1310	87
Pulmonary valve stenosis (Level 2 Code)	1591	1071	62
VSD perimembranous (Level 2 Code)	1700	916	128
VSD muscular (Level 2 Code)	685	.	.
ASD secundum (Level 2 Code)	2508	1260	285

*Congenital heart defect sample sizes reflect 'simple' and not 'associated' defects

Table 3.4. Most prevalent major structural birth defect phenotypes in the US, 2008-2012 [67]

Birth defect phenotype	Estimated # of cases per 10,000 live births
(1) Hypospadias	64.7 (23.0, 106.3) (among *male* live births)
(2) Atrial septal defects (ASD)	64.7 (0, 171.7)
(3) Ventricular septal defect (VSD)	43.4 (10.1, 76.6)
(4) Pulmonary valve atresia and stenosis	8.3 (0.5, 16.2)
(5) Cleft palate alone	6.1 (2.2, 10.0)
(6) Cleft lip w/ cleft palate	5.9 (2.1, 9.6)
(7) Coarction of the aorta	5.6 (0, 14.2)

*Excludes chromosomol defects

*Estimated among live births only

Due to potential etiologic heterogeneity, we will estimate the effect of paternal cannabis use on each *specific phenotype.* For example, we will separately estimate the effect of paternal cannabis on gastroschisis and hypospadias. The analysis will be conduct on 'isolated' defects for non-heart defects and 'simple isolated for heart defects. Power calculations for these specific phenotypes are presented in section 3.2.5. Table 3.3 summarizes the broader birth defect groupings and specific phenotypes within each grouping.

Control ascertainment: NBDPS has population-based control selection. Controls were randomly selected from the source population that represents the geographic region and time period of the cases. Specifically, they are simple random sample of the birth certificates of the source population, and are contacted for participation in the same way as cases (e.g. by mail and phone). All controls are reviewed by the clinical geneticist; infants that have a major birth defects are excluded from the control group. Note that potential controls that are later found to have a birth defect do not move to the case group; they are removed entirely from the study population. Address information for all cases and controls were reviewed by study staff to ensure residence in the catchment area during the pregnancy. The monthly number of controls selected was proportional to the number of births in the same month in the previous year to minimize seasonal effects on control sampling. This rigorous population-based control sampling reduces potential selection bias by ensuring that control accurately represent the source population.

3.2.3 Exposure assessment

Interview: Trained interviewers conducted a one-hour computer-assisted telephone interview (CATI) with each mother in the study. Interviewers guided participants through the questions, while participants entered information into the online questionnaire. These interviews were scheduled at the mother's convenience, offered in both English and Spanish, and sometimes completed over the course of multiple telephone calls. The CATI asked detailed

questions regarding diet, drug use, demographics, lifestyle factors, occupation, environmental

exposures, pregnancy history, fertility treatments, medical conditions, and psychological

conditions during both the preconception and pregnancy periods. The CATI also asks the mother

about the father of the baby, notably about his drug use during the periconceptional period.

Exposure definition: The exposure of interest - preconception paternal cannabis use -

will be defined as **ever/never use of marijuana during the 3-month preconception period.**

This will be derived from maternal report of paternal cannabis use from the CATI, explained

above. At one point during the study period, the CATI underwent some slight changes. The

CATI version used during the first part of the study period will be referred to as the *classic*

CATI, and the version used during the latter part of the study period will be referred to as the *new*

CATI. The classic and new CATI ask about paternal cannabis use slightly differently.

The classic CATI was administered approximately to 1997-2005 EDDs, and it asks about

marijuana and hash use from 3-months preconception to *date of infant birth*. Specifically,

question G1 asks "Between (-3) and (DOIB), did (NOIB)'s father use any of the following

recreational or street drugs?" and asks separately about 'marijuana' and 'hash' (see Figure 3.9).

If the response is yes to any of the drugs, the respondent is then asked to specify the exact

month(s) of use in context of conception, e.g. B3 represent 3-months prior to conception (see

Figure 3.10).

The new CATI was administered to 2006-2011 EDDs, and it asks about marijuana and

hash use from 3-months preconception to *time of conception*. Specifically, question F17 asks "In

the 3 months before pregnancy, which would be (B3) through (B1), did ([NOIB]'s/the) father use

any of the following recreational or street drugs?" then asks about 'marijuana' (see Figure 3.11).

No follow-up questions are asked regarding exact timing of drug use.

Figure 3.9. Classic CATI question regarding paternal marijuana use, used for expected delivery dates 1997-2005

See Figures 3.9 and 3.10 for the exact wording of the *classic* CATI questions, and Figure 3.11

for the exact wording of the *new* CATI questions.

Figure 3.10. Classic CATI question regarding timing of paternal marijuana use, used for expected delivery dates 1997-2005

Figure 3.11. New CATI question regarding paternal marijuana use, used for expected delivery dates 2006-2011

In summary, there are two main differences in the classic and new CATI questions about cannabis use. First, the classic CATI asks about three months prior to conception up until date of infant birth, so it includes exposure during the pregnancy time frame, whereas the new CATI only asks about the timeframe of interest (three months prior to conception up until time of conception). However, the classic CATI includes a follow-up question, specifying when in the periconceptional period marijuana was used. To appropriately ascertain exposure during the preconception period, we will recode fathers as 'exposed' only if the cannabis use occurred during the preconception period. In other words, fathers that used cannabis during the pregnancy, but not during the 3-month period prior to conception, will be categorized as 'unexposed'. Second, the classic CATI asks about both marijuana use and hash use, whereas the new CATI asks about only about marijuana use. For the classic CATI study years (1997-2005), we will include 'hash use' in the exposed category, since hash has the same psychoactive components as marijuana, and the term is often used interchangeability with marijuana. However, we will

conduct a sensitivity analysis where we exclude 'hash' from the exposed group to see if this

change in exposure definition meaningfully changes our effect estimates.

Handling 'DK' responses: Note that respondents can respond 'yes', 'no', or 'DK' (don't

know) for the questions regarding paternal cannabis use (see Figures 3.10-3.11). The 'yes' and

'no' answers clearly will be categorized as 'exposed' and 'unexposed'; how to handle the 'DK'

responses are less clear. The main analysis will be a complete case analysis, where participants

with missing exposure data are excluded. We will then conduct a few different bias analysis to

assess how exposure misclassification may impact our results. These bias analyses are laid out in

detail in Section 3.2.4.3 (Sub-aim 2b – Probabilistic Bias Analysis).

Covariate ascertainment: The CATI, described above, asked detailed questions

regarding diet, drug use, demographics, lifestyle factors, occupation, environmental exposures,

pregnancy history, fertility treatments, medical conditions, and psychological conditions during

both the preconception and pregnancy periods. The CATI also asks the mother about the father

of the baby, specifically about his demographics, occupation, and drug use during both the

preconception and pregnancy period. This extremely detailed questionnaire contains data on all

known confounders of the paternal cannabis-birth defects association, therefore allowing us to

control for potential confounding in our analysis (Aim 2). Moreover, the paternal age variable

from the CATI is essential for investigating potential effect-measure modification by paternal

age (Aim 2b).

3.2.4 Analysis

3.2.4.1 Specific aim 2

The goal of Aim 2 is to investigate the relationship between paternal self-reported

preconception cannabis use and major birth defects. Due to the case-control nature of the study

design, logistic regression will be used to analyze the association between paternal cannabis use

(yes/no) and specific birth defect phenotypes (yes/no). The exposure data will come from the

CATI questionnaire described above (see Section 2.3.3). The outcome data will come from the

clinically-verified case classifications of birth defects included in the NBDPS data. Since birth

defects are etiologically heterogeneous, separate logistic models will be run for each birth type

phenotype group.
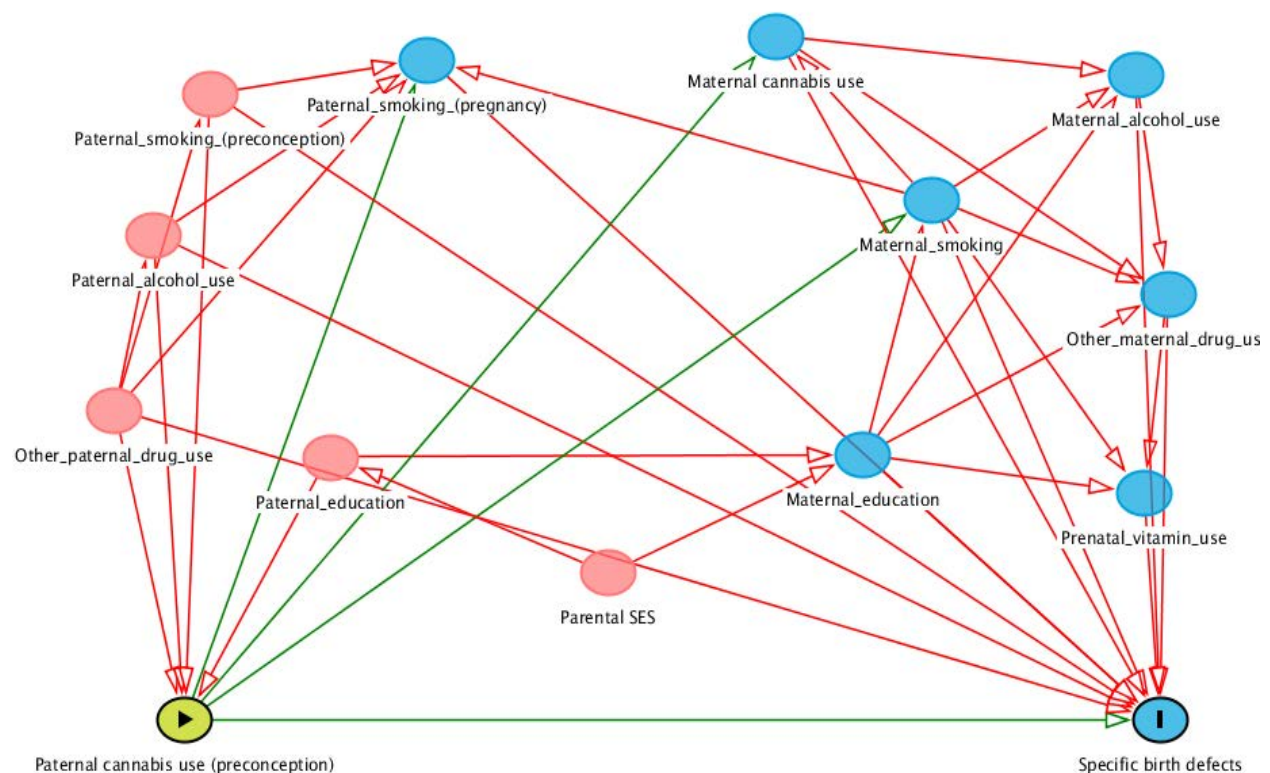
Figure 3.12. Directed acyclic graph (DAG) for hypothesized relationship between paternal cannabis use and birth defects in offspring

Confounding: All potential confounders will be established a priori using a directed

acyclic graph (DAG) that will be informed by current literature and NBDPS birth defects

experts. Figure 3.12 depicts the DAG for Aim 2. A minimally-sufficient adjustment set will be

identified from this DAG and used in all etiologic analyses. Gestational age at delivery and birth

weight will not be included in the adjustment set since they are thought to be on the causal

pathway of the paternal cannabis-birth defects association [124, 125]. Potential confounders

include other paternal drug use, paternal cigarette smoking, paternal age, maternal cannabis use, other maternal drug use, maternal education, maternal nutritional status, and pregnancy intention.

Multiple comparisons: We plan to estimate the effect of preconception cannabis use on eight birth defect phenotypes (see Table 3.5). Since birth defects are clinically and etiologically heterogeneous, these eight phenotypes will be analyzed separately. We will **not** perform any corrections for multiple testing. Corrections for multiple comparisons take a frequentist approach to hypothesis testing, assume that the null hypothesis is true, and aims to reduce Type I error. However, interpretation of results should not be based solely on hypothesis tests and p-values. Instead, inferences should be made on the patterns, magnitude, and precision of results in the context of clinical interventions or public health policy [126-128].

Additionally: while correcting for multiple comparisons reduces likelihood of false positives (reduces Type I error), it also increases likelihood of false negatives (increases Type II error) [129, 130]. The relative value of false positives and false negatives is context-specific. In our context of understanding if a prevalent easily-modifiable exposure affects risk of an adverse fetal outcomes, false negatives are – arguably – more harmful for public health than false positives. Therefore, we will not perform any corrections for multiple comparisons. Resulting manuscript(s) will be clear about the number of tests conducted, so the reader has this knowledge when interpreting our results.

3.2.4.2 Sub-aim 2a – Estimating prevalence of preconception cannabis use

While the NSDUH study population in Aim 1 can provide nationally-representative estimates of cannabis use prevalence among persons of reproductive age, the NBDPS study population in Aim 2 is uniquely suited to provide prevalence estimates *during the preconception period*. Therefore, we will report the prevalence of any reported cannabis use during the preconception period among control-fathers in the NBDPS study population. We will report the

(1) overall prevalence in study population, (2) prevalence stratified by study year (1997-2011), and (3) prevalence stratified by state to qualitatively assess geographic and time trends. To our knowledge, this will be the first estimate of preconception paternal cannabis use prevalence in the 21st century.

3.2.4.3 Sub-aim 2b – Crude and adjusted results

We aim to estimate crude and adjusted odds ratios and 95% confidence intervals for the association between paternal cannabis use during the preconception period and odds of specific birth defect phenotypes, as described in section 3.2.4.1 (Aim 2 main analysis).

3.2.4.4 Sub-aim 2c – Probabilistic bias analysis

The exposure in this study is susceptible to misclassification for two main reasons. First, cannabis is a sensitive exposure to ask about, and participants may have various reasons for misreporting their cannabis use. For example, they may not want to admit to using an illegal substance to a government-funded research study, or they think that cannabis use is "socially undesirable" and therefore feel uncomfortable reporting it. Second, the exposure data is derived from maternal report, and mothers may not know the true level of paternal exposure. Of particular interest is whether this exposure misclassification is differential by case status, since differential misclassification is more likely to bias the effect estimates. It is possible that the first mechanism of misclassification is differential by case status (i.e. variations of recall bias), but it is less likely that the second mechanism of misclassification is differential by case status. We plan to address this exposure misclassification in by conducting probabilistic bias analyses with various assumption.

Specifically, we will use a semi-Bayesian probabilistic sensitivity analysis to minimize misclassification of the paternal self-reported cannabis use variable. Briefly, this method uses different assumptions about the sensitivity and specificity of the observed exposure to get closer

to "true" exposure values [131]. For example, we assume *perfect specificity* (those who report

'yes' to cannabis use are truly exposed) but *imperfect sensitivity* (20% of participants who report

'no' are actually exposed) – based on priors from the cannabis literature. Moreover, probabilistic

bias analyses draws from a sample distribution of sensitivity and specificity values, rather than

assigning fixed values for sensitivity and specificity. We will define an *a priori* trapezoidal

distribution of a reasonable range of values for sensitivity and specificity based on priors from

previous literature. This approach more adequately incorporates uncertainty of exposure

misclassification as compared to deterministic approaches.

We will follow the approach established by Tim Lash and colleagues for conducting a

probabilistic bias analysis [132-134]. We create our own macro based publicly-available SAS

macro for probabilistic sensitivity analysis called 'sensmac', developed by Lash and colleagues

[134]. This macro simulates the data that would have been observed had the misclassified

variable been correctly classified given the sensitivity and specific of classification [134]. Using

this macro, we will simulate two scenarios: (1) non-differential misclassification, where the

sensitivity and specificity of exposure misclassification is the same cases and controls, and (2)

non-differential misclassification, where the sensitivity and specificity of exposure

misclassification differs by case status.

While this approach makes assumptions of specificity and sensitivity that cannot be

validated within our data, it at least allows us to understand how measurement error with

informative priors might, or might not, change the results (>10% change-in-estimate). A similar

approach was used to minimize exposure misclassification in a 2014 study of maternal cannabis

use and birth defects in the NBDPS population [135]. Results of this bias analysis will indicate

the magnitude and direction of expected bias, given a range of input assumptions regarding the

distribution of exposure misclassification. This bias analysis will ultimately provide meaningful interpretation of effect estimates in this analysis in the context of unknown misclassification of exposure.

3.2.5 Addressing gaps in literature

The existing epidemiologic literature on paternal cannabis use and birth defects has four major limitations. First, the current literature is extremely sparse and outdated. The most recent study was published in 1998, so the data is over 20 years old. Cannabis use has changed drastically in the past two decades in terms of frequency of use, demographic patterns of use, and changing legal and social norms surrounding cannabis use. Moreover, the potency of marijuana has become stronger in the past few decades, so cannabis exposure today is at a higher dose as compared to when these studies were published [136, 137]. Our study will be using data from 1997-2011, so the study population and exposure data is much more up-to-date as compared to previous studies.

Second, the existing studies have only focused on two types of birth defects – NTDs and CHDs. No studies have examined the effect of paternal cannabis on any other type of birth defect. Birth defects are phenotypically and etiologically heterogeneous, so it is unlikely that results about NTDs and CHDs can be generalized to other types of defects. Therefore, we urgently need epidemiologic investigation of paternal cannabis and other subtypes of defects. Our study plans to investigate the effect of paternal cannabis on the most prevalent birth defect phenotypes, as noted in Table 3.2. Specifically, our study includes *hypospadias* and *oral clefts* – adverse and prevalent birth defects which no previous studies of cannabis use have investigated. Therefore, our study would greatly expand our knowledge on the association of paternal cannabis and a wider range of major structural defects.

Third, the existing studies have imperfect outcome ascertainment. Shaw et al. used an active surveillance system, but they lacked clinical verification of cases. This means that the healthcare providers at birth determine the birth defects diagnosis, and these diagnoses were not reviewed by a clinical geneticist (or other expert), thus opening up this study to potential missing data or potential misclassification of outcomes. Ewing et al. and Wilson et al. – both using BWIS data – did have clinical verification of their cases by a cardiologist, which is appropriate since they focused on CHDs. These two studies also ascertained cases up until age 1, which is important especially for CHDs, which are sometimes not detected at time of birth. However, both analyses only included live-born infants; in other words, they both condition on live birth. This is problematic, since the exposure (cannabis use) could result in pregnancy loss or miscarriage that would be missed by only ascertain live-born cases. In other words, the effect estimates from these studies have potential to be biased if (1) authors missed fetal deaths resulting from the birth defect under study, and (2) some proportion of those fetal deaths was caused by the exposure. Since congenital anomalies often result in fetal death, and since paternal cannabis use could plausibly affect risk of fetal death, conditioning on live birth introduces potential for this live-birth bias [138]. Our study addresses these two limitations by (1) using active surveillance and rigorous clinical verification of all cases by a clinical geneticists with expertise in birth defects, and (2) including fetal deaths as eligible for the case group. Though the fetal death data is limited to deaths after 20 weeks, so there is potential missing data from early pregnancy loss, our proposal still improves upon previous studies and increases our ability to control any bias due to conditioning on live birth. Our proposal's approach improves upon the measurement error present in previous studies, and aims to provide more accurate outcome data necessary to estimate causal effects.

The fourth limitation relates to exposure assessment. All three existing studies conducted face-to-face interviews with the mother (and some cases, the father) regarding paternal cannabis use. Cannabis use is a sensitive topic, especially since it is federally illegal and carries social stigma [139]. When asking participants about cannabis use, it is essential to use methods that make the respondent feel safe, comfortable, and anonymous to ensure accurate reporting. Studies comparing accuracy of drug reporting across various questionnaire methods found that lack of response to sensitive drug questions was higher in face-to-face studies as compared to other survey methods [140]. A recent study specifically compared face-to-face interviewing computer-assisted self-interviewing, and found that the latter method resulting in higher reporting of stigmatized behavior [141]. The mechanism behind this difference is the increased feeling of anonymity and decreased personal judgment of using a computer-assistant self-interview as compared to a face-to-face interview. Our study will address this limitation by using a computer-assisted self-interview to ascertain the exposure. However, our exposure measurement – maternal report of paternal cannabis use – remains imperfect. The *ideal* measurement would be a biomarker measured during the preconception window. Since birth defects are a rare outcome usually studied in a case-control design, measuring a biomarker during preconception period would be nearly impossible for any study. A *superior* method would have been paternal self-report, which is not available in the NBDPS data. Maternal report of paternal cannabis use is a sufficient – but not ideal – exposure measurement. To assess if measurement error introduces potential bias in our effect estimate, we will conduct a probabilistic bias analysis, as described in the Aim 2 analysis plan.

In summary, our proposal contributes meaningfully to our understanding of the paternal cannabis-birth defects association by (1) providing much more recent data, (2) investigating birth

defects phenotypes apart from NTDs and CHDs for the first time, (3) improving validity of

outcome ascertainment and reducing live-birth bias, and (4) assessing potential bias from

measurement error.

CHAPTER 4: TRENDS IN PAST-MONTH CANNABIS USE AMONG MEN AND WOMEN OF REPRODUCTIVE AGE FROM 2002-2014: AN AGE-PERIOD-COHORT ANALYSIS[1]

4.1. Introduction

Cannabis is the most commonly used illicit drug among men and women of reproductive age in the United States (U.S.) [2-4]. The adverse and beneficial health effects of cannabis use are widely debated, but recent evidence suggested that cannabis use may have negative effects on reproductive and perinatal health [5-7].

Animal studies have shown that tetrahydrocannabinol (THC) – the main psychoactive component of cannabis – can disrupt regular menstrual cycles, suppress oogenesis, and reduce female fertility [60-62]. THC crosses the placenta [68, 142] and can transfer to breast milk, though the infant's level of exposure via breast milk is uncertain [143, 144]. Epidemiologic studies have found maternal cannabis use during pregnancy to be associated with fetal growth restriction, certain birth defects, and neonatal intensive care admission [6, 15, 16]. Due to this growing evidence on the potential effects of cannabis use on reproductive and perinatal health, the American College of Obstetricians and Gynecologists recommends counseling pregnant women and women considering pregnancy to discontinue cannabis use. [145]

Less is known about the potential reproductive effects of paternal exposure. In a recent study of Danish men, sperm quality was significantly lower for regular marijuana smokers (more than once a week) compared to non-smokers [21]. THC has been shown to reduce sperm

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[1] This chapter was submitted to Drug Alcohol Dependence on 9 August 2016.

concentration and alter sperm motility in humans [5, 20] and to damage DNA packaging during spermatogenesis in both human and animal studies [5, 22].

Understanding trends in cannabis use among women and men of reproductive age is an essential step toward developing health policy, health education, and targeted interventions to mitigate potential adverse reproductive and perinatal health effects from cannabis use [7]. The prevalence of recent cannabis use (defined as at least one occurrence in the past month) has increased among adults aged 18 to 25 from 16% in 2004 to 20% in 2014 [2]. Moreover, attitudes among younger cohorts continue to move towards greater acceptance of cannabis, with the percentage of teenagers who perceive "great risk from regular use" significantly declining in the past decade [9, 10]. Policies on cannabis legality are also rapidly changing. To date, 24 states have legalized medical use, and eight states and the District of Columbia have legalized recreational use [11]. Disentangling the effects of age, policy period, and cohort is critical to understanding changes in cannabis use over time, as these three parameters are inextricably correlated over time but may have independent, potentially modifiable effects. Additionally, few contemporary studies have investigated gender differences in cannabis trends, despite substantial gender differences and social norms regarding use [146, 147].

We conducted an age-period-cohort (APC) analysis to estimate the independent effects of age, period, and cohort on past-month cannabis use among men and women of reproductive age in the U.S. from 2002 to 2014 using nationally-representative surveillance data. We also investigated potential differences in cannabis use trends by gender.

4.2. Material and methods

4.2.1 Study population

We analyzed data from the National Survey on Drug Use and Health (NSDUH). Conducted by the Department of Health and Human Services since 1972, the NSDUH uses a

repeated annual cross-sectional survey design to measure tobacco, alcohol, and illicit drugs use. In addition to drug use patterns, the sur vey also covers mental health conditions, behavioral disorders, and attitudes towards drug use.

The survey uses multistage clustered sampling to ascertain a nationally-representative sample of U.S. households [119]. Professional interviewers visit selected household to conduct the computer-assisted interviews [119]. Monetary compensation for study participation began in 2002 and resulted in increased participation; thus, we restricted our sample to 2002 onwards. We merged publicly-available NSDUH data across survey years 2002 to 2014. We restricted the sample to those ages 15-49 years at time of interview (n=534,679) to represent populations of reproductive age [121, 148].

4.2.2 Measures

The cannabis survey questions were asked using audio computer-assisted self-interviewing (ACASI), during which the respondent listens to pre-recorded questions on headphones and enters his/her own response. This survey method has been shown to improve response accuracy, since the question is not asked face-to-face by an interviewer, thereby reducing perceived stigma and potential misreporting [140, 141]. During this section, the respondent were first briefed on different formats and names for cannabis (e.g. marijuana, hash, pot, grass, joints) that are considered exchangeable in the survey. Respondents were then asked: "How long has it been since you last used marijuana or hash?" with the following possible responses: (1) Within past 30 days, (2) More than 30 days ago but within past 12 months, (3) More than 12 months ago, or (4) Never used. Our primary outcome of interest for this analysis was any cannabis use in the past 30 days; thus, we combined response categories to create a dichotomous variable indicating *use in the past 30 days* vs. *use more than 30 days ago or never use* (referent group for all analyses).

Socio-demographic characteristics, tobacco, alcohol, and other illicit drug use were also ascertained from the NSDUH survey. Socio-demographic questions were asked by the trained interviewer, while more sensitive questions pertaining to substance use were ascertained using the ACASI method described above.

4.2.3 Statistical analysis

We examined the distribution of socio-demographic characteristics, tobacco, alcohol, and other drug use stratified by individual-level past-month cannabis use in our study sample (see Table 4.1).

First we created age-period contingency tables, overall and stratified by gender, for the proportion of past-month cannabis use for each age category and study year. Publicly-available NSDUH data provided most ages categorically for confidentiality (e.g. 30-34). Therefore, we calculated a range of birth years for a given age/study year intersection and used the median birth year for a 10-year period to represent each birth cohort.

We used log-linear multivariable models to estimate adjusted prevalence ratios (aPR) and 95% confidence intervals (CI) for associations between age, period, and cohort and proportion of individuals reporting past-month cannabis use. The conventional age-by-period array of aggregate outcomes categorize age and period in equal interval lengths, thereby creating the exact linear dependency between the independent variables (period = age + cohort) that leads to the model identification problem [52]. However, in our data, cohort was categorized in 10-year intervals - conventional in demographic studies of population based data - to represent potential generational effects. Age was categorized in unequal intervals based on NSDUH statisticians' recommendations to best represent age-related changes in drug use. Period was categorized in 1-year intervals to assess potential year-by-year changes in cannabis use, in the context of changing legislation during the study period. We therefore used the differential time interval

groupings to break the exact linear dependency between the three variables.[52] To ensure

results were robust to effect estimate contrast, we additionally used linear models to estimated

adjusted prevalence differences (aPD) and 95% CIs for age, period, and cohort effects.

Descriptive models included confidential survey weight and variance estimation stratum

from the NSDUH to account for sampling, non-response, and variance adjustment. Weighted

results are meant to be generalizable to the target population of U.S. adults age 15-49. No

additional covariates were included in the age-period-cohort models, since no potential

confounders were identified using a directed acyclic graph [149, 150]. This approach is

consistent with previous age-period-cohort analysis of substance use [12, 13, 151]. No

interaction terms were included in the model, since cohort effects can be conceptualized as an

interaction between age and period. In other words, cohort effects capture how age effects vary

by time period. Sensitivity analyses were conducted to assess whether results were sensitive to

different age, period, or cohort parameterization and referent groups.

Analyses were conducted using R 2.14.0 and SAS 7.3.

4.3 Results

4.3.1 Sociodemographic characteristics

Overall, 9.9% of the study population reported past-month cannabis use. Past-month use

varied greatly by age, with prevalence as high as 19.7% for 18-21 year olds and as low as 5.4%

for 35-49 year olds (see Table 4.1). Compared to those reported no cannabis use in the past

month, past-month users were more likely to be male, unemployed, and unmarried. Past-month

users did not differ substantially in terms of family income, educational attainment, and overall

health status. Past-month cannabis use was associated with current cigarette smoking, past-

month binge drinking, and ever-use of cocaine, heroin, LSD, methamphetamines, and non-

medical use of painkillers (see Table 4.1).

4.3.2 Trends from 2002-2014

The overall prevalence of past-month cannabis use increased from 9.2% in 2002 to 12.3% in 2014. While the absolute prevalence remained higher for males through the study period, the overall trend appeared similar for males and females (see Figure 4.1).

4.3.3 Age, period, cohort effects

By simultaneously modeling age, period, and cohort effects on proportion of individuals reporting past-month cannabis use, we estimated each independent effect while holding the other two factors constant. Note that Figures 4.2-4.4 have varying y-axis limits to best depict results. Age had considerably stronger effects on proportion of past-month cannabis use than period or cohort. The strongest age effects were observed for 18-21 year olds (aPR: 2.91, 95% CI: 2.57, 3.30) and 22-25 year olds (aPR: 2.28, 95% CI: 2.03, 2.57), compared to 35-49 year olds (see Figure 4.2). Cohorts born in the 1970s, 1980s, and 1990s had slightly higher proportions of past-month use compared to the 1960s birth cohort, with the strongest cohort effect for 1980 (PR: 1.21, 95% CI: 1.05, 1.39) (see Figure 4.3). We observed a small but consistently increasing period effect for study years 2009 to 2014 compared to 2002 (see Figure 4.4). Most confidence intervals included the null; however, period effects for 2013 (aPR: 1.08, 95% CI: 1.01, 1.15) and 2014 (aPR: 1.16, 95% CI: 1.09, 1.24) were more pronounced.

4.3.4 Gender differences

Prevalence of past-month cannabis use was higher among males than females for all age groups. The difference was most pronounced for 22-25 and 26-29 year olds: prevalence among males was nearly two-fold that among females. The overall increasing trend in past-month use from 2002 to 2014 was similar across gender.

Age effects were stronger for females, most notably for the 18-21 age group (aPR for females: 3.32, 95%CI: 2.79, 3.96; aPR for males: 2.62, 95% CI: 2.26, 3.03) (see Figure 4.2).

Cohort and period effects were overall similar across gender (see Figures 4.3 and 4.4). However, significant period effects were observed for study years 2011 through 2014 among males, but only for study year 2014 among females.

When estimating prevalence differences, the same patterns emerged, though gender differences in age effects were mitigated (see Supplemental Table 4.1). Sensitivity analyses for alternative referent groups did change the magnitude and direction of effect estimates in some cases, but the overall trends remained the same (see Supplemental Table 4.2). Sensitivity analyses for alternative categorizations for age, period, and cohort parameters did not substantively change our results (see Supplemental Table 4.3).

4.4 Discussion

4.4.1 Overall

This was the first study to examine age, period, and cohort effects on cannabis use in the United States since the first states legalized recreational use of cannabis in 2012 and the first study to explore differences among men and women of reproductive age. This was also the first APC study to consider past-month cannabis use, a more sensitive marker for regular cannabis use and potentially related health effects [152-154].

Cannabis use was prevalent in this study population, with approximately 10% of participants reporting past-month use. We observed a meaningful increase in past-month cannabis use prevalence from 9.2% in 2002 to 12.3% in 2014, though trends varied by age group. The age-specific trends may reflect cannabis use among young adults that continues into adulthood, more now than in prior generations, suggesting a shift in the life-course patterns of cannabis use. [49, 155].   This potential explanation is consistent with the subtle cohort effect observed, since they reflect that trends (e.g., period effects) differ by age (e.g., age effects) [156].

While we found distinct age, period, and cohort effects on past-month cannabis use, age was the strongest independent source of variation, especially for the 18-21 and 22-25 age groups, which is consistent with current literature on risk factors for cannabis use. Age was a stronger correlate of cannabis use prevalence among women when estimating prevalence ratios. However these gender differences were mitigated when estimating prevalence differences, likely because the baseline prevalence was lower in females, thus resulting in stronger prevalence ratios. We therefore conclude that age effects are strong for both men and women, and the magnitude of effects are similar by gender.

A consistent increase in use across periods suggests that time-dependent socio-cultural factors may influence past-month cannabis use. Notably, the overall period effect was strongest for study years 2013 and 2014, which reflect the time period immediately after Colorado and Washington legalized recreational cannabis use in 2012.

State-level legalization in 2012 could influence national period effects in past-month cannabis use through multiple pathways [157-159]. First, individuals may have increased access to cannabis; this would primarily affect individuals living in states with legalization. Second, legalization may reduce risk perception and change social norms surrounding acceptance and use of cannabis. Significant decreases in the risk perception surrounding cannabis use in US populations from 2002 to 2014 have been documented [10]. Drug use patterns are known to be strongly influenced by social norms and other group-level processes [158, 160, 161]. Future research could assess these potential mediators, and how effects differ for medical versus recreational cannabis use [43]. We cannot necessarily infer that state-level legalization caused this period effect because we were unable to account for other time-varying factors during this

period that may influence cannabis use. Additionally, more time may need to elapse before we see the true period effects from state-level legalization [162].

Three earlier studies found strong age effects with a peak in young adulthood, consistent with our findings [12, 13, 163]. Kerr et. al. reported a decline in past-year cannabis use among men, but stable use among women from 1984 to 2000 [163]. More recently, we observed distinct patterns by gender, but increased use among both men and women from 2002-2014. Consistent with our results, Kerr et al. also found that age effects were stronger for women than for men [163]. Miech et al found a distinct period effect in study years 2000-2009 across all ages and cohorts [12], speculating trends may have reflected changing social norms and attitudes towards cannabis use [12]. Similarly, we observed a distinct increasing period effect, but of more consistent magnitude and precision than was observed by Miech et al. This may suggest that period effects were already emerging in the US from 2000-2009 but have since become stronger in 2002-2014, perhaps due to even stronger social norms or legislation changes.

Laws and social norms have changed drastically since the last APC study reported on trends through 2009 [10, 157, 162]. The most notable change was in 2012 with the first state legalization of recreational cannabis use, so it is timely to examine time trends and drivers those trends over the past seven years [158]. Our efforts to understand trends among individuals of reproductive age are crucial to public health guidelines and planning, especially in light of the growing evidence of adverse reproductive health effects of cannabis use. Moreover, we have investigated past-month use, which provides a more temporally relevant measure of use with regard to reproductive health effects than would be available from previous studies that evaluated past-year cannabis use [164-166].

4.4.2 Limitations

Self-report of cannabis may be under-reported, and the extent of measurement error may differ by age and gender. Moreover, our results could be vulnerable to time-varying changes in reporting accuracy either by all participants or by participants of specific age groups: if study participants in later years are more comfortable reporting true cannabis use, we may mistake increased reporting for increases in actual prevalence. These concerns may be mitigated by measures taken within the NSDUH data collection protocol to improve the accuracy of self-report, including: anonymous reporting without face-to-face interactions with the interviewer, reminders of anonymity, and a repeated follow-up approach to minimize non-response.

By restricting to a 13-year period (2002 to 2014), we are 'capturing' each birth cohort at specific ages. For example, individuals born in 1965 would be captured in our study at ages 37 to 49, whereas individuals born in the 1990 would be captured at ages 17 to 29. Since cannabis use is most prevalent among young adults, we may be missing the heaviest cannabis use for the 1960 and 1970 birth cohorts. We adjusted for period and age in the cohort effect estimates, which may mitigate this issue by providing cohort trends averaged over all period and age groups, but generalization of our results beyond this sample is limited. Longer study periods could help corroborate estimates of cohort effects in past-month cannabis use.

Finally, we were unable to investigate effect-measure modification by state because the public-access NSDUH files do not include data on participants' residence. Many states decriminalized possession and legalized medical and/or recreational use of cannabis over the study period. Future studies could look at state-specific data to elucidate how period effects differ geographically and by type of legislation change.

4.5. Conclusions

Cannabis use is prevalent and increasing among both men and women of reproductive age in the United States, with approximately 12% reporting past-month use in 2014. Prevalence almost doubled among women aged 26-34 in particular, which reflects women with the highest birth rate. Age, period, and cohort each have independent effects on past-month cannabis use, with age having the strongest influences. When holding age and cohort constant, small but consistent period effects were observed. Most notably, period effects – which arose from time-dependent socio-cultural factors – were strongest for 2013 and 2014. State-level cannabis legalization in 2012 may partly explain these recent national period effects, though recent period effects also fit a larger trend of steady increases in cannabis use in the past decade.

Cannabis use is common among men and women of reproductive age, and is rapidly increasing in the age group of women with the highest birth rates. Understanding trends in cannabis use among women and men of reproductive age is an essential step toward developing health policy, health education, and targeted interventions to mitigate potential adverse reproductive health effects from cannabis use. More epidemiologic research is needed to understand the risk factors and reproductive health effects of cannabis use.

Table 4.1. Study population characteristics by past-month cannabis use

	Past-month cannabis use (%)	
	No	Yes
Overall	90.1	9.9
Age		
15-17	87.8	12.2
18-21	80.3	19.7
22-25	84.1	15.9
26-29	88.8	11.2
30-34	91.5	8.5
35-49	94.6	5.4
Gender		
Female	92.8	7.2
Male	87.3	12.7
Race/ethnicity		
Non-Hispanic black	88.6	11.4
Non-Hispanic white	89.1	10.9
Hispanic	93.1	6.9
Asian	96.8	3.2
More than one race	83.5	16.5
Native American/AK native	87.1	12.9
Native HI/Pacific Islander	90.6	9.4
Education		
17 or younger	87.8	12.2
Less than high school	88.0	12.0
High school graduate	89.1	10.9
Some college	89.2	10.8
College graduate	93.8	6.2
Marital status		
Married	95.7	4.3
Not married	85.6	14.4
Employed		
Full-time	91.6	8.4
Part-time	87.4	12.6
18 or younger	87.8	12.2
Unemployed	83.3	16.7
Other	90.4	9.6
Family income		
< $20,000	86.0	14.0
$20,000-$49,999	89.4	10.6
$50,000-$74,999	91.5	8.5
≥ $75,000	92.5	7.5

	Past-month cannabis use (%)	
	No	Yes
Cigarette smoking status		
Current	78.2	21.8
Former	93.2	6.9
Never	97.5	2.5
Binge drink in past month[b]		
Yes	79.1	20.9
No	94.8	5.2
Chewing tobacco		
Never	91.4	8.6
Ever	82.4	17.6
Cocaine		
Never	93.9	6.1
Ever	72.5	27.5
Crack		
Never	91.2	8.8
Ever	65.8	34.2
Heroin		
Never	90.6	9.4
Ever	63.6	36.4
Ecstasy		
Never	92.9	7.1
Ever	60.3	39.7
LSD		
Never	92.9	7.1
Ever	68.7	31.3
Methamphetamine		
Never	91.1	8.9
Ever	71.2	28.8
Painkiller (non-medical use)		
Never	93.9	6.1
Ever	73.0	27.0
Overall health		
Excellent	92.6	7.4
Very good	89.8	10.2
Good	88.5	11.5
Fair	87.6	12.4
Poor	87.6	12.4

[a] Frequencies are weighted for NSDUH sampling structure and reflect combined NSDUH 2002-2014 data

[b] Binge-drinking is defined as 5 or more drinks in one setting
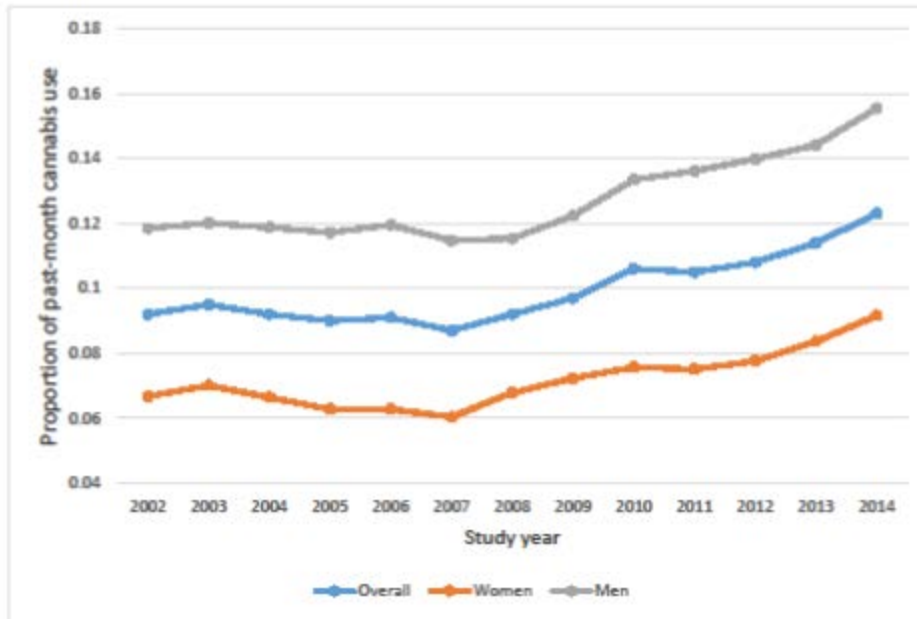
Figure 4.6. Trends in past-month cannabis use among men and of reproductive age from 2002-2014
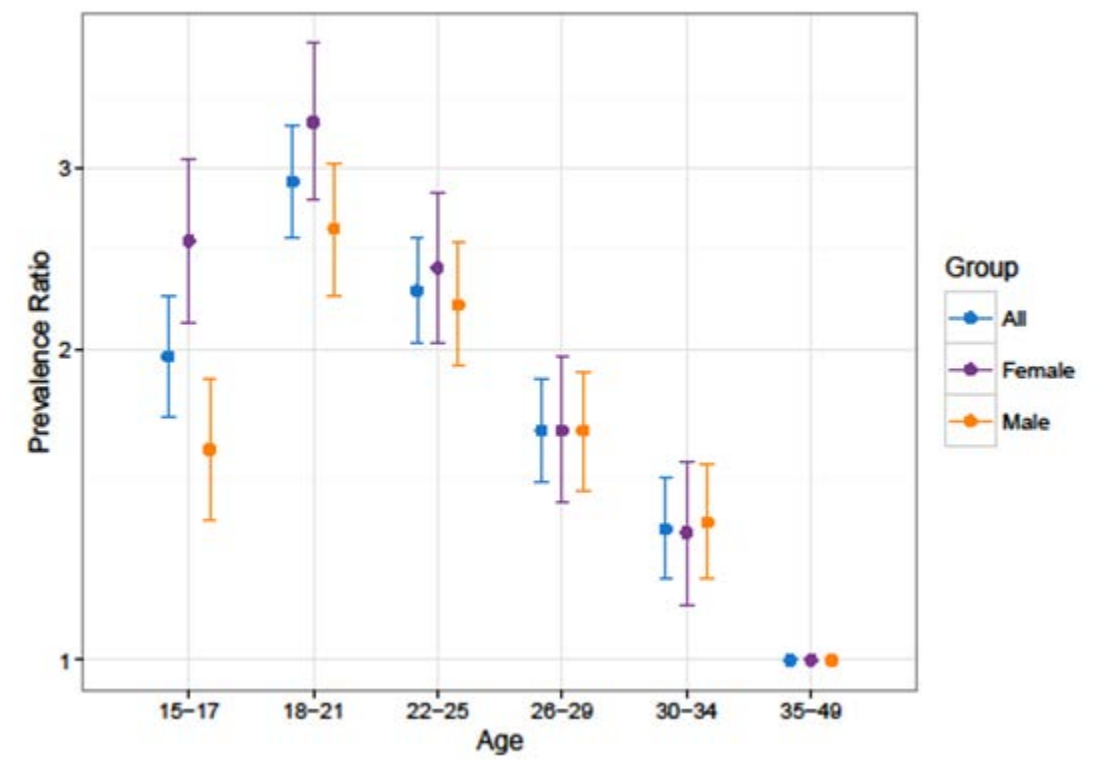
Figure 4.7. Age effects on prevalence of past-month cannabis use
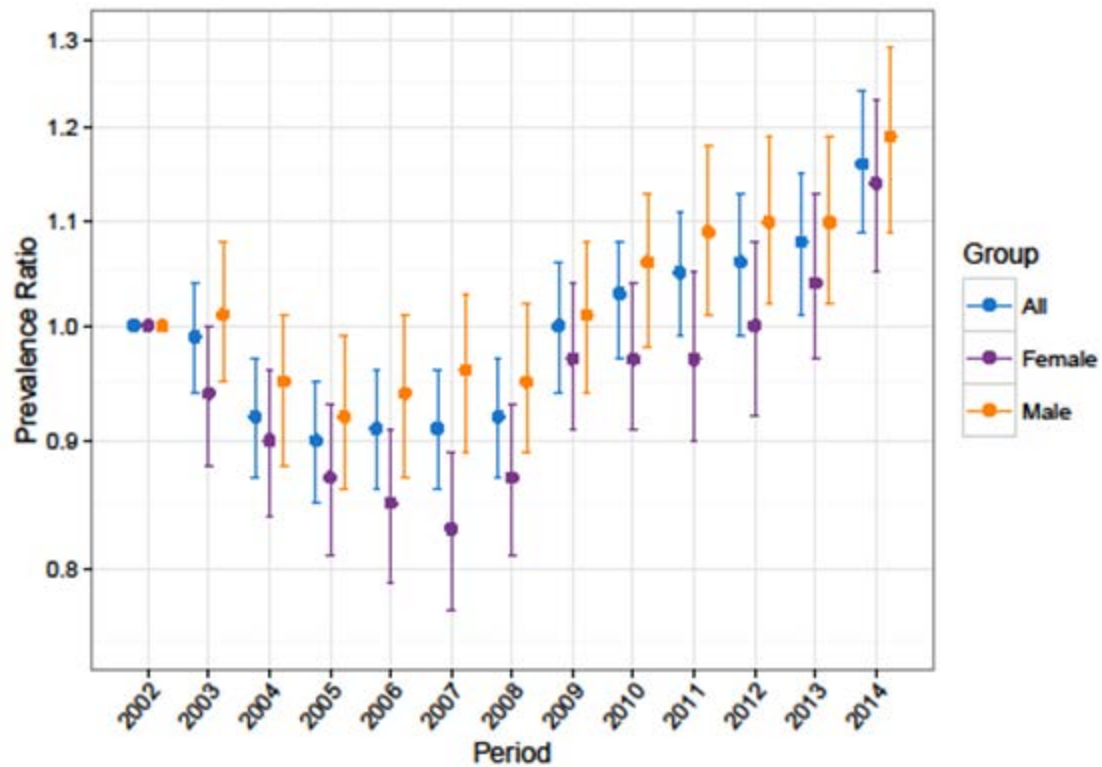
Figure 4.8. period effects on prevalence of past-month cannabis use
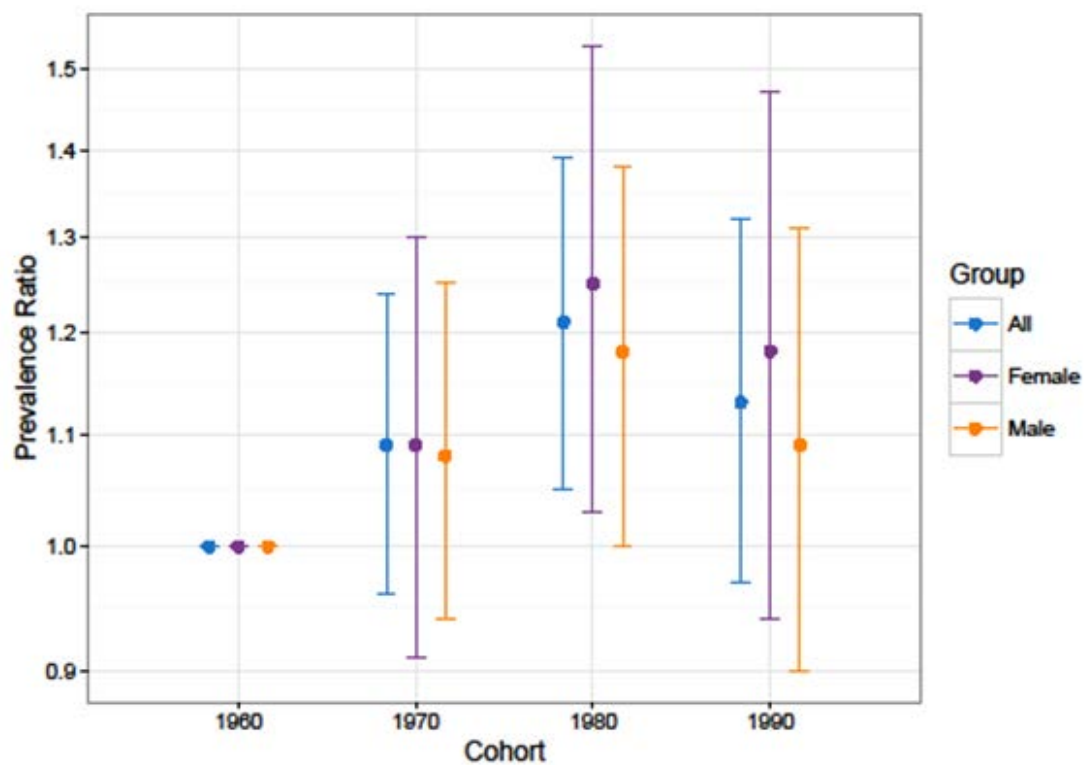
Figure 4.9. Cohort effects on prevalence of past-month cannabis use

Supplemental Table 4.1. Age, period, and cohort effects on prevalence of past-month cannabis, aPD (95% CI)[a]

Parameter	Category	Overall	Women	Men
Age	15-17	0.074 (0.061, 0.088)	0.083 (0.070, 0.097)	0.063 (0.042, 0.083)
	18-21	0.138 (0.126, 0.150)	0.120 (0.108, 0.132)	0.155 (0.136, 0.174)
	22-25	0.095 (0.084, 0.106)	0.075 (0.064, 0.086)	0.118 (0.101, 0.135)
	26-29	0.053 (0.043, 0.062)	0.040 (0.030, 0.049)	0.068 (0.053, 0.083)
	30-34	0.030 (0.021, 0.039)	0.023 (0.014, 0.032)	0.039 (0.026, 0.053)
	35-49	REF	REF	REF
Period	2002	REF	REF	REF
	2003	0.000 (-0.007, 0.007)	-0.005 (-0.012, 0.002)	0.004 (-0.007, 0.014)
	2004	-0.007 (-0.013, 0.000)	-0.007 (-0.014, 0.000)	-0.005 (-0.016, 0.005)
	2005	-0.009 (-0.016, -0.002)	-0.008 (-0.015, -0.001)	-0.009 (-0.020, 0.001)
	2006	-0.009 (-0.015, -0.002)	-0.012 (-0.019, -0.005)	-0.006 (-0.017, 0.005)
	2007	-0.009 (-0.016, -0.002)	-0.015 (-0.022, -0.007)	-0.004 (-0.015, 0.007)
	2008	-0.007 (-0.014, 0.000)	-0.009 (-0.017, -0.002)	-0.005 (-0.016, 0.006)
	2009	0.003 (-0.004, 0.011)	0.002 (-0.006, 0.009)	0.005 (-0.006, 0.016)
	2010	0.007 (0.000, 0.014)	0.002 (-0.005, 0.009)	0.012 (0.001, 0.023)
	2011	0.008 (0.000, 0.015)	0.000 (-0.008, 0.008)	0.016 (0.004, 0.027)
	2012	0.012 (0.003, 0.020)	0.005 (-0.004, 0.013)	0.020 (0.007, 0.032)
	2013	0.015 (0.007, 0.023)	0.012 (0.003, 0.020)	0.020 (0.007, 0.033)
	2014	0.026 (0.017, 0.034)	0.020 (0.011, 0.029)	0.032 (0.019, 0.046)
Cohort	1960	REF	REF	REF
	1970	-0.006 (-0.015, 0.003)	-0.008 (-0.017, 0.001)	-0.005 (-0.019, 0.009)
	1980	0.002 (-0.010, 0.014)	-0.002 (-0.014, 0.011)	0.005 (-0.014, 0.024)
	1990	-0.007 (-0.022, 0.009)	-0.008 (-0.024, 0.008)	-0.007 (-0.031, 0.017)

[a] aPD: adjusted prevalence difference; CI: confidence interval; REF: referent group

Supplemental Table 4.2. Sensitivity analyses for referent groups: age, period, and cohort effects on prevalence of past-month cannabis use, aPR (95% CI)[a]

Parameter	Category	Original analysis	Sensitivity 1[b]	Sensitivity 2[c]
Age	15-17	1.97 (1.72, 2.25)	REF	1.97 (1.72, 2.25)
	18-21	2.91 (2.57, 3.30)	1.48 (1.43, 1.53)	2.91 (2.57, 3.30)
	22-25	2.28 (2.03, 2.57)	1.16 (1.11, 1.21)	2.28 (2.03, 2.57)
	26-29	1.67 (1.49, 1.87)	0.85 (0.80, 0.90)	1.67 (1.49, 1.87)
	30-34	1.34 (1.20, 1.50)	0.68 (0.64, 0.73)	1.34 (1.20, 1.50)
	35-49	REF	0.51 (0.44, 0.58)	REF
Period	2002	REF	0.86 (0.80, 0.92)	1.11 (1.05, 1.17)
	2003	0.99 (0.94, 1.04)	0.85 (0.79, 0.91)	1.10 (1.04, 1.16)
	2004	0.92 (0.87, 0.97)	0.79 (0.74, 0.84)	1.02 (0.97, 1.08)
	2005	0.90 (0.85, 0.95)	0.78 (0.73, 0.83)	REF
	2006	0.91 (0.86, 0.96)	0.78 (0.73, 0.83)	1.00 (0.95, 1.06)
	2007	0.91 (0.86, 0.96)	0.78 (0.73, 0.83)	1.00 (0.95, 1.06)
	2008	0.92 (0.87, 0.97)	0.79 (0.74, 0.84)	1.02 (0.96, 1.08)
	2009	1.00 (0.94, 1.06)	0.86 (0.81, 0.91)	1.11 (1.05, 1.17)
	2010	1.03 (0.97, 1.08)	0.88 (0.83, 0.93)	1.14 (1.08, 1.20)
	2011	1.05 (0.99, 1.11)	0.90 (0.86, 0.95)	1.16 (1.10, 1.23)
	2012	1.06 (0.99, 1.13)	0.91 (0.87, 0.95)	1.17 (1.10, 1.25)
	2013	1.08 (1.01, 1.15)	0.93 (0.88, 0.97)	1.19 (1.12, 1.27)
	2014	1.16 (1.09, 1.24)	REF	1.29 (1.21, 1.37)
Cohort	1960	REF	0.88 (0.76, 1.04)	REF
	1970	1.09 (0.96, 1.24)	0.97 (0.89, 1.04)	1.09 (0.96, 1.24)
	1980	1.21 (1.05, 1.39)	1.07 (1.03, 1.12)	1.21 (1.05, 1.39)
	1990	1.13 (0.97, 1.32)	REF	1.13 (0.97, 1.32)

[a] aPR: adjusted prevalence ratio CI: confidence interval; REF: referent group
[b] Sensitivity analysis 1: referent groups are opposite of original analysis
[c] Sensitivity analysis 2: referent groups are groups with lowest past-month marijuana use

Supplemental Table 4.3. Sensitivity analyses for categorization: age, period, and cohort effects on prevalence of past-month cannabis use, aPR (95% CI)[a]

Parameter	Category	Sensitivity 3[b]	Sensitivity 4[c]
Age	15	1.04 (0.53, 2.04)	REF
	16	1.56 (0.81, 3.01)	1.50 (1.25, 1.81)
	17	1.94 (1.01, 3.70)	1.86 (1.56, 2.22)
	18	2.20 (1.17, 4.16)	2.12 (1.78, 2.52)
	19	2.35 (1.26, 4.37)	2.26 (1.88, 2.70)
	20	2.33 (1.26, 4.30)	2.24 (1.85, 2.71)
	21	2.22 (1.21, 4.07)	2.14 (1.75, 2.6)
	22-23	1.97 (1.10, 3.55)	1.90 (1.52, 2.35)
	24-25	1.70 (0.96, 3.00)	1.63 (1.27, 2.1)
	26-29	1.39 (0.80, 2.40)	1.22 (100, 1.78)
	30-34	1.17 (0.71, 1.94)	1.13 (0.78, 1.62)
	35-49	REF	0.96 (0.49, 1.89)
Period	2002-03	REF	0.92 (0.74, 1.14)
	2004-05	0.91 (0.82, 1.01)	0.83 (0.69, 1.01)
	2006-07	0.88 (0.78, 0.99)	0.80 (0.68, 0.95)
	2008-09	0.93 (0.81, 1.07)	0.85 (0.74, 0.98)
	2010-11	1.00 (0.84, 1.18)	0.91 (0.81, 1.03)
	2012-13	1.01 (0.84, 1.23)	0.93 (0.83, 1.04)
	2014	1.09 (0.88, 1.36)	REF (REF, REF)
Cohort	1960-64	REF	0.71 (0.3, 1.68)
	1965-69	1.01 (0.58, 1.76)	0.72 (0.33, 1.59)
	1970-74	1.13 (0.63, 2.03)	0.81 (0.49, 1.34)
	1975-79	1.35 (0.68, 2.67)	0.97 (0.67, 1.38)
	1980-84	1.46 (0.72, 2.98)	1.04 (0.80, 1.36)
	1985-89	1.53 (0.72, 3.26)	1.09 (0.90, 1.32)
	1990-94	1.50 (0.67, 3.36)	1.07 (0.94, 1.22)
	1995-99	1.40 (0.60, 3.29)	REF (REF, REF)

[a] aPR: adjusted prevalence ratio; CI: confidence interval; REF: referent group
[b] Sensitivity analysis 3: finer age categories, two-year period categories, 5-year cohort categories
[c] Sensitivity analysis 4: finer age categories, two-year period categories, 5-year cohort categories, opposite referent groups as original analysis

CHAPTER 5: PATERNAL CANNABIS USE AND RISK OF BIRTH DEFECTS IN THE NATIONAL BIRTH DEFECTS PREVENTION STUDY[2]

5.1 Introduction

Cannabis is the most commonly used illicit drug among men of reproductive age in the United States [3, 167]. As of 2015, an estimated 1 in 5 young men (aged 18-25) had used cannabis in the past month [167, 168]. Moreover, cannabis attitudes among young adults continue to move towards greater acceptance. The percentage of young adults who perceive "great risk from regular use" has drastically decreased in the past decade [9, 10]. Policies on cannabis legality are also rapidly changing. To date [as of December 2017], over half of US states have legalized medical use, eight states have legalized recreational use, and other states have legislation pending [169, 170]. Considering the increasing prevalence of use, changing social norms, and evolving policies of cannabis use, we must better understand how cannabis potentially impacts male reproductive health.

A growing literature has shown that cannabis use has adverse effects on various aspects of semen quality [21, 55, 73, 74, 79, 171]. Most notably, a recent epidemiologic study of 1215 young Danish men found that regular cannabis use significantly lowered sperm concentration and sperm count [21]. In response to this study, Eisenberg et al. published a commentary in the American Journal of Epidemiology stressing the need for more research on the effect of cannabis on male reproductive health [75]. The main psychoactive component of cannabis – tetrahydrocannabinol (THC) – interacts with the human endocannabinoid system, specifically

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[2] This chapter will be submitted to the American Journal of Epidemiology.

CB1 and CB2 receptors. Human studies have shown that CB1 activation increases the proportion of immobile sperm, and CB2 activation increases the proportion of lower motility sperm [77]. Mouse studies have shown that CB1 receptors – which are significantly activated by THC – influence chromatin remodeling in sperm, suggesting that cannabis has potential epigenetic effects on sperm cells [22].

Although the effect of cannabis on sperm quality has been demonstrated, very little research has focused on the potential effects of paternal preconception cannabis use on embryonic development. Since cannabis affects semen quality, it warrants investigation as a potential risk factor for birth defects [5, 23]. Major birth defects, defined as a structural malformation with a significant impact on the health and development of a child, are the leading cause of infant mortality and lifelong disability [24, 25] in the U.S. While some genetic and environmental risk factors have been identified, little is known about the etiology of most birth defects. Though most research has focused on maternal risk factors, epidemiologic investigations have shown that paternal exposures (e.g. cigarette smoking, occupational exposures) during the preconception period may increase risk of birth defects [26-29, 172, 173].

Three previous studies investigating the association between paternal cannabis use and subsequent risk of specific birth defects produced mixed results [30-32]. Shaw et al. (1996) found that paternal cannabis use was not associated with neural tube defects (NTDs) (cOR: 0.86, 95% CI: 0.63, 1.2; no adjusted estimates reported) in the California Birth Defects Monitoring program [32]. Ewing et al. (1997) and Wilson et al. (1998) both investigated the association between paternal cannabis use and specific types of congenital heart defects (CHDs) in the Baltimore-Washington Infant Study and found increased risk for isolated membranous ventricular septal defects and transposition of great arteries with intact ventricular septum [30,

31]. All three studies used maternal report of paternal cannabis use, and none investigated potential impacts of exposure misclassification. All studies were conducted in the late 1980s, but cannabis potency has considerably increased in the past two decades [137, 174, 175]. In summary, this small literature suggests potential increased risk for CHDs but is outdated and faces challenges with exposure measurement, appropriate confounder adjustment, and clinical case verification. Moreover, other defects besides CHDs and NTDs have not been investigated.

Therefore, we aimed to investigate the association between paternal cannabis use during the preconception period and risk of 21 structural birth defect phenotypes in the National Birth Defects Prevention Study (NBDPS), a large national case-control study of risk factors for birth defects.

5.2 Materials and methods

5.2.1 Study design and population

The National Birth Defects Prevention Study (NBDPS) is the largest and most comprehensive population-based case-control study of birth defects in the United States [123]. NBDPS has ten study centers located in geographically diverse parts of country (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah). During the study period of 1997-2011, approximately 6 million births occurred in the NBDPS catchment area.

Study eligibility started with pregnancies ending on or after October 1, 1997, and concluded with pregnancies with estimated dates of delivery (EDD) on or before December 31, 2011 eligible study sites. A woman was ineligible to participate in the NBDPS if she already participated in the study with a previous pregnancy, could not complete the interview in English or Spanish, was incarcerated, or did not have legal custody of the infant. For three out of the ten centers, women under 18 years at the end of her pregnancy were ineligible [123]. Women were

excluded if they had participated with a previous pregnancy, were unable to complete the interview in English or Spanish, were incarcerated, or did not have legal custody of the infant. For this analysis, we additionally excluded pregnancies conceived from donor sperm (n=170). Details on the NBDPS study design and population have been previously published [123].

To focus on more prevalent defects and to ensure sufficient statistical power, analyses included only phenotypes represented by over 500 cases available in NBDPS.

5.2.2 Case and control ascertainment

The goal of NBDPS was to understand risk factors for birth defects with unknown etiologies. Therefore, cases were not eligible for NBDPS if they had a chromosomal anomaly, recognized single-gene disorder, or known teratogenic syndrome [123]. Cases are identified from state-level Birth Defects Surveillance programs, then undergo a rigorous verification process by clinical geneticists with expertise in birth defects phenotyping. Identified cases are contacted by mail or phone to request participation in the study. The NBDPS clinical geneticists developed guidelines that detailed the (1) inclusion and exclusion criteria for each defect eligible for the NBDPS; (2) rationale for including certain diagnostic codes for defects and related defects; (3) instructions and rationale for designating the final case classification (isolated, multiple, complex); and (4) instructions and recommendations to analysts on how the defect type could be analyzed in epidemiologic studies. Separate guidelines were developed for classifying congenital heart defects, since they are unique in etiology and clinical presentation [98]. All guidelines were consistent across study site to ensure that the classification process did not differ geographically or by clinical geneticist. Most centers included stillbirths and induced abortions in the case group, in addition to live births [98, 176].

Controls were randomly sampled live births selected from the source population that represents the geographic region and time period of cases. Mothers were contacted for

participation in the same way as cases (e.g. by mail and phone). If a potential control was later found to have an eligible birth defect, they were converted to a case. The monthly number of controls selected was proportional to the number of births in the same month in the previous year to minimize seasonal effects on control sampling. The study design used a 3:1 case-to-control ratio to ensure sufficient statistical power to investigate a wide variety of birth defect phenotypes [123].

5.2.3 Exposure and covariate measurement

Trained interviewers conducted a one-hour computer-assisted telephone interview (CATI) with each mother in the study, which asked detailed questions regarding diet, drug use, demographics, lifestyle factors, pregnancy history, fertility treatments, medical conditions, and paternal characteristics during the preconception and pregnancy periods. Interviews were scheduled at the mother's convenience, offered in both English and Spanish, and sometimes completed over the course of multiple telephone calls. One section of the CATI asks women to report about the baby's father, including information about demographics, family history, occupation, smoking, and substance use.

For this analysis, the exposure of interest – preconception paternal cannabis use – is defined as ever/never use of marijuana during the 3-month period prior to conception. Data on potential confounders were ascertained from the CATI.

5.2.4 Statistical analyses

Regression

Multivariable logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between paternal preconception cannabis use (yes/no) and specific birth defect phenotypes (yes/no) while accounting for possible confounding. Since birth defects are etiologically heterogeneous, separate logistic models were

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fit for each birth phenotype group. Congenital heart defects (CHDs) are clinically and

etiologically distinct from non-heart defects and were therefore handled slightly differently in the

analysis [98]. Non-heart defects were analyzed at the 'isolated' level to represent cases where

only a single major defect was diagnosed, while CHDs were analyzed at the 'simple isolated'

level to represent non-association and non-complex cases where only a single major defect was

diagnosed. A directed-acyclic-graph (DAG) was used to identify potential confounders

including: other paternal drug use, paternal age, paternal education, maternal cannabis use, other

maternal drug use, maternal education, maternal cigarette smoking, maternal alcohol use,

household income, and study site [177, 178]. All potential confounders were included in the final

adjustment set, which was consistent across birth defect models. Gestational age at delivery and

birth weight were not included in the adjustment set since they are potential mediators on the

paternal cannabis-birth defects causal pathway [124, 125].

Probabilistic bias analysis

Since some degree of misclassification is expected in maternal report of paternal cannabis

use, we conducted probabilistic bias analyses to assess how various types of exposure

misclassification would impact our results. Whereas simple bias analyses simulate results under

specified parameters (e.g. exact sensitivity and specificity of observed exposure), probabilistic

bias analyses take into account uncertainty of exposure correction by drawing from a distribution

of parameters (e.g. distribution of probable sensitivity and specificity values). This latter

approach was well-suited for our analysis since no validation data to deterministically specify

accuracy of exposure were available. More details about this approach have been previously

published [132-134]. We created a SAS macro to estimate results under positive predictive

values (PPVs) and negative predictive values (NPVs) drawn from a specified trapezoidal

distribution, using bootstrap methods to appropriately estimate confidence intervals. We conducted the bias analyses for both differential and non-differential misclassification for adjusted results prior to multiple imputation. We conducted bias analyses for a six non-heart defects and two heart defects to get a sense of the magnitude and direction of the bias. Details of the probabilistic bias analysis are provided in Supplemental Table 5.1.

5.3 Results

Descriptive results

A total of 22,522 cases and 11,798 controls were eligible for analysis. Paternal cannabis use during the 3-month period prior to conception was reported for 8.8% of control fathers and 10.4% of eligible case fathers. While paternal and maternal cannabis use were correlated (r=0.49; r2=0.24), paternal use was higher than maternal use during pregnancy (5.0%) among controls. Cases and controls were similar in terms of maternal and paternal age, education, and household income (see Table 5.1). Among controls, exposure prevalence differed across study site, with the highest prevalence in Arkansas (13.0%) and the lowest in Utah (4.4%). Exposure prevalence also shifted over the course of the study period from 4.0 % in 1997 to 9.6% in 2011.

Main effects

Any paternal cannabis use during the 3-month preconception period was crudely associated with anencephaly (OR: 1.43, 95% CI: 1.11, 1.84), cleft lip and palate (cOR: 1.21, 95% CI: 1.04, 1.43), cleft lip alone (cOR: 1.34, 95% CI: 1.09, 1.63), transverse limb deficiency (cOR: 1.41, 95% CI: 1.11, 1.79), diaphragmatic hernia (cOR: 1.28, 95% CI: 1.02, 1.61), and gastroschisis (cOR: 3.07, 95% CI: 2.66, 3.54) (see Table 5.2). After adjustment, four results had suggestive associations including: diaphragmatic hernia (aOR: 1.33, 95% CI: 0.99, 1.80), cleft

lip alone (aOR: 1.23, 95% CI: 0.95, 1.60), gastroschisis (aOR: 1.26, 95% CI: 1.00, 1.52), and

hypoplastic left heart syndrome (aOR: 1.38, 95% CI: 0.99, 1.24).

Probabilistic bias analysis

Table 5.3 shows predicted effect estimates under pre-specified trapezoidal distributions

for PPV and NPV for non-differential and differential exposure misclassification frameworks,

alongside adjusted results from complete-case analyses. Generally, bias analyses shifted odds

ratios towards the null or remained consistent. The largest shift in magnitude was observed for

diaphragmatic hernia, where the original effect estimate aOR: 1.33 (95% CI: 0.99, 1.80) shifted

to aOR: 1.08 (95% CI: 0.84, 1.40) and aOR: 1.03 (95% CI: 0.83, 1.27) under assumptions of

non-differential and differential misclassification, respectively. A similar shift in magnitude was

seen for cleft lip alone, gastroschisis, and hypoplastic left heart syndrome.

5.4 Discussion

In a comprehensive case-control study of birth defects, we found that reported paternal

cannabis use during the 3-month period prior to conception was associated with slightly

increased risk of diaphragmatic hernia, gastroschisis, cleft lip alone, and hypoplastic left heart

syndrome. This is the first study to investigate the association between paternal cannabis use and

birth defects since the 1980s, since then cannabis potency has changed considerably [136, 137,

174]. These associations persisted after adjustment for important confounders, and remain

unchanged after probabilistic bias analyses under a range of plausible assumptions about the

structure of any anticipated exposure misclassification. However, these positive findings must be

balanced with the null effects observed for 19 other birth defects in this analysis.

Our findings are consistent with some of the previous investigations of paternal cannabis

use and birth defects. Shaw et al. (1996) found that paternal preconception cannabis use was not

associated with neural tube defects (NTD) in California from 1989-1991 (cOR: 0.86, 95% CI:

0.63, 1.2) [32]. We had the ability to investigate more specific NTD phenotypes and found similar null results (anencephaly and craniorachischisis: aOR: 1.14, 95% CI: 0.80, 1.64; spina bifida: aOR: 1.02, 95% CI: 0.78, 1.33). Ewing et al. (1997) and Wilson et al. (1998) found increased risk for two specific CHDs in the Baltimore-Washington Infant Study (BWIS) from 1981-1989: Ventricular septal defects (VSDs) and Transposition of great arteries with intact ventricular septum (TGA) [30, 32]. These results are somewhat inconsistent with our findings: we found slightly increased risk of hypoplastic left heart syndrome, but not of the 8 remaining CHD phenotypes we investigated. However, all three previous studies had very limited sample size (e.g. the BWIS study had only 26 exposed cases for TGAs) and lacked sufficient adjustment for important confounders. No previous studies investigated other defects besides NTDs and CHDs, so our positive findings for gastroschisis, diaphragmatic hernia, and cleft lip alone are novel contributions to the literature.

Epidemiologic and animal studies have consistently shown that cannabis use can adversely affect semen quality, specifically sperm count and motility, but the epigenetic effects are less clear [21, 75]. A recent study in mice showed that the CB1 receptor, an important part of the endocannabinoid system that is activated by THC, influences chromatin remodeling in sperm [22, 79, 179]. This suggests that cannabis has the potential to induce epigenetic changes on sperm. However, additional research is needed to confirm and clarify this effect. Although epigenetic pathways are the most likely mechanism for this association given the epigenetic etiologies for many birth defects, another potential mechanism is secondhand cannabis smoke exposure to the mother. This assumes that cannabis was consumed via smoking and occurred around the mother during early pregnancy. However, a study of maternal cannabis use in NBDPS did not find increased risk for diaphragmatic hernia (aOR: 1.3, 95% CI: 0.8–2.2) or

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gastroschisis (aOR: 1.3, 95% CI: 0.9, 1.8), suggesting that mechanisms for paternal versus maternal cannabis use may be distinct from one another [16], at least for some birth defects.

Our findings are also inconsistent with literature on other male-mediated substances than may cause birth defects. A study of paternal cigarette smoking during the peri-conception period in a Chinese population found increased risk of conotruncal heart defects, septal defects, and left ventricular outflow tract obstructions, but did not investigate non-heart defects [180]. Savitz et al. found that paternal cigarette smoking was associated with increased risk of cleft lip with or without cleft palate, hydrocephalus, ventricular septal defects, and urethral stenosis in a U.S. population [181]. Shaw et al. found that paternal heroin use was associated with increased risk of NTDs (OR: 4.6, 95% CI: 0.92, 30.6), but few other studies have investigated paternal drug use [32]. Defects identified from these paternal smoking and drug use studies do not overlap with the four defects we identified as associated with paternal cannabis use (diaphragmatic hernia, gastroschisis, cleft lip alone, hypoplastic left heart syndrome), suggesting these mechanisms may also be distinct.

Strengths of this study include its use of population-based controls, standardized clinical verification of cases, large sample size, and analytic methods to handle potential misclassification bias. The NBDPS identifies controls from birth records that represent the source population that gave rise to cases, thus providing a population-based control group that results in less potential for selection bias, compared to clinical or convenience-based control sampling [182, 183]. The NBDPS protocol includes a rigorous verification process for all potential cases, where clinical geneticists confirm exact birth defect diagnosis [123]. This detailed case classification increases phenotypic and etiologic homogeneity within birth defect group. Additionally, the sheer sample size of the NBDPS population allows for sufficiently-

powered analysis of rare birth defect phenotypes [123]. Since we only included phenotypes with at least 500 eligible cases, we had sufficient sample size for all analyses. Finally, we had the ability to control for important confounders that previous studies lacked, like maternal cannabis, other maternal/paternal drug use, and maternal/paternal education.

A major limitation of this study is our exposure measurement. While exposure was asked in reference to the etiologically-relevant timeframe, mothers reported retrospectively on paternal exposure and is subject to increased measurement error. Mothers may not have known fathers' true cannabis use patterns, or they may have misreported due to recall error or social desirability bias [184-186]. However, superior exposure measures like urine or hair biomarkers were not available given the retrospective nature of this case-control study. After conducting probabilistic bias analyses to assess how exposure misclassification may impact results, we found that reasonable amounts of exposure misclassification – as defined by trapezoidal distributions for PPV and NPV – resulted in negligible changes in effect estimates. This held true for both non-differential and differential (with respect to case-control status) structures of misclassification. Therefore, we feel that our results are robust to reasonable amounts of exposure misclassification. However, it is possible that the true misclassification was different than the distributions we assumed in our bias analysis. Future studies should consider conducting validation studies with biomarker data, or using both paternal self-report and maternal report together, to more accurately measure paternal cannabis use. Additionally, this study only assessed ever/never use of cannabis, but the true effect may differ by dosage, method of consumption (e.g. smoked, vaped, edibles), frequency of consumption (e.g. daily vs. monthly use) or strain (e.g. indica vs. sativa). Future studies should elucidate effect heterogeneity by these various types of exposure characteristics.

Our study may suffer from unmeasured confounding. Crude and adjusted results in our study differed meaningfully, suggesting that strong confounding was at play. While we were able to adjust for many important confounders -- such as maternal cannabis use, other maternal and paternal drug use, maternal and paternal education, and maternal income – results may still be biased by unmeasured or unknown confounders.

Finally, a challenging limitation of this study is potential selection bias resulting from restricting on live birth [138, 187, 188]. If paternal cannabis use increased risk of early pregnancy loss, then we are investigating a select population of infants who were healthy enough to survive until birth. Eight out of ten NBDPS study sites included stillbirths and induced abortions in their case group, which somewhat mitigates the conditioning on live birth issue [123]. Furthermore, we may have selection bias from conditioning on conception, that is, if paternal cannabis use decreases the probability of getting pregnant, then we are investigating a select group of fathers that were able to conceive in the first place. The latter scenario is more likely, given that cannabis has proven effects on reducing sperm count and motility. Unfortunately, we lack the ability to quantify this particular bias. Future studies could measure exact effects of cannabis use on time-to-pregnancy and conduct bias analysis for selection bias given bounds of these selection effects.

This is the first study to show that paternal cannabis use during the 3-month preconception period is associated with slightly increased risk of some structural birth defect phenotypes, including diaphragmatic hernia, gastroschisis, cleft lip alone, and hypoplastic left heart syndrome and not associated with other defects. These results warrant further investigation into cannabis as potential male-mediated teratogen, especially in light of changing cannabis policies and increases in prevalence of use among men of reproductive age.

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Table 5.1. Study population characteristics by case status, n (%)

	Cases*	Controls
Maternal characteristics		
Cannabis use†		
Yes	1240 (5.7%)	577 (5.0%)
No	20709 (94.4%)	10909 (95.0%)
Missing	573	312
Other drug use†§		
Ever	340 (1.6%)	129 (1.1%)
Never	21618 (98.5%)	11365 (98.9%)
Missing	564	304
Cigarette smoking†		
Ever	4545 (20.7%)	2074 (18.0%)
Never	17428 (79.3%)	9424 (82.0%)
Missing	549	300
Alcohol use†		
Ever	8075 (36.9%)	4263 (37.2%)
Never	13822 (63.1%)	7197 (62.8%)
Missing	625	338
Age at delivery		
<20	2345 (10.4%)	1177 (10.0%)
20-29	11408 (50.7%)	5935 (50.3%)
30-39	8139 (36.1%)	4420 (37.5%)
≥40	630 (2.8%)	266 (2.3%)
Missing	0	0
Education		
Less than high school	3713 (17.0%)	1905 (16.6%)
High school graduate	10776 (49.1%)	5346 (46.7%)
College graduate or higher	7407 (33.8%)	4202 (36.7%)
Missing	626	345
Household annual income		
<$10,000	4020 (19.5%)	2004 (18.9%)
$10,000-50,000	7061 (34.3%)	3862 (36.4%)
>$50,000	9509 (46.2%)	4759 (44.8%)
Missing	1932	1173
Paternal characteristics		
Cannabis use‡		
Yes	2203 (10.4%)	985 (8.8%)
No	19083 (89.7%)	10230 (91.2%)
Missing	1236	583
Other drug use‡§		
Yes	833 (3.8%)	301 (2.6%)

	Cases*	Controls
No	20860 (96.2%)	11101 (97.4%)
Missing	829	396
Cigarette smoking‡		
Yes	2434 (28.2%)	1250 (26.4%)
No	6200 (71.8%)	3478 (73.6%)
Missing	13888	7070
Age at delivery		
<20	992 (4.6%)	543 (4.8%)
20-29	9556 (43.9%)	4944 (43.3%)
30-39	9193 (42.2%)	4961 (43.5%)
40-49	1876 (8.6%)	890 (7.8%)
≥50	174 (0.8%)	75 (0.7%)
Missing	731	385
Education		
Less than high school	3769 (17.8%)	1813 (16.3%)
High school graduate	10648 (50.2%)	5335 (48.0%)
College graduate or higher	6797 (32.0%)	3974 (35.7%)
Missing	1308	676
Infant characteristics		
Sex		
Female	9043 (40.4%)	5781 (49.0%)
Male	13341 (59.6%)	6005 (51.0%)
Missing	138	12

*Includes all cases included in analysis

† Reflects use during pregnancy

‡ Reflect use during periconception period (3-months prior to conception to infant birth)

§ Any drug use includes any use of cocaine, crack, hallucinogens, heroin, or mushrooms

Table 5.2. Effect estimates of paternal preconception cannabis use and selected birth defect phenotypes

Birth defect phenotype	Cases N (% exposed)		Controls N (% exposed)		Crude OR (95% CI)	Adjusted OR (95% CI)‡
Non-heart defects†						
Anencephaly and craniorachischisis	656	(12.1%)	11799	(8.8%)	**1.43 (1.11, 1.84)**	1.14 (0.80, 1.64)
Spina bifida	1292	(8.2%)	11799	(8.8%)	0.93 (0.75, 1.15)	1.02 (0.78, 1.33)
Cleft palate alone	1625	(12.2%)	11799	(8.8%)	1.02 (0.84, 1.23)	0.88 (0.69, 1.12)
Cleft lip with cleft palate	2044	(10.6%)	11799	(8.8%)	**1.21 (1.04, 1.43)**	1.07 (0.87, 1.31)
Cleft lip alone	1104	(11.5%)	11799	(8.8%)	**1.34 (1.09, 1.63)**	1.23 (0.95, 1.60)
Esophageal atresia	762	(9.4%)	11799	(8.8%)	1.07 (0.83, 1.39)	1.17 (0.84, 1.63)
Anorectal atresia/stenosis	1090	(8.8%)	11799	(8.8%)	1.00 (0.80, 1.25)	1.18 (0.89, 1.56)
Hypospadias second/third degree	2582	(8.5%)	6005	(8.9%)	0.96 (0.81, 1.13)	1.03 (0.82, 1.29)
Transverse limb deficiency	731	(11.9%)	11799	(8.8%)	**1.41 (1.11, 1.79)**	1.20 (0.86, 1.66)
Craniosynostosis	1622	(6.9%)	11799	(8.8%)	0.77 (0.63, 0.95)	0.92 (0.71, 1.20)
Diaphragmatic hernia	882	(11.0%)	11799	(8.8%)	**1.28 (1.02, 1.61)**	1.33 (0.99, 1.80)
Gastroschisis	1449	(22.8%)	11799	(8.8%)	**3.07 (2.66, 3.54)**	**1.23 (1.00, 1.52)**
Heart defects†						
DTransposition of the great arteries	716	(8.4%)	11799	(8.8%)	0.95 (0.72, 1.26)	0.88 (0.62, 1.24)
Hypoplastic left heart syndrome	608	(10.7%)	11799	(8.8%)	1.24 (0.95, 1.63)	1.38 (0.99, 1.923)
RVOT defects	1967	(9.8%)	11799	(8.8%)	1.13 (0.96, 1.34)	1.13 (0.91, 1.39)
RVOT defects excluding Ebstein	1829	(9.8%)	11799	(8.8%)	1.13 (0.95, 1.34)	1.14 (0.92, 1.42)
Pulmonary valve stenosis	1464	(9.5%)	11799	(8.8%)	1.08 (0.89, 1.31)	1.11 (0.88, 1.42)
VSD perimembranous	1408	(9.3%)	11799	(8.8%)	1.07 (0.88, 1.30)	1.04 (0.81, 1.33)
VSD muscular	560	(8.0%)	11799	(8.8%)	0.90 (0.65, 1.24)	0.89 (0.60, 1.32)
ASD secundum	2029	(10.0%)	11799	(8.8%)	1.15 (0.98, 1.35)	1.08 (0.88, 1.32)
ASD NOS	516	(11.1%)	11799	(8.8%)	1.30 (0.97, 1.73)	0.70 (0.46, 1.06)

*OR: odds ratio; CI: confidence interval; VSD: ventricular septal defect; ASD: atrial septal defect; NOS: not otherwise

‡ Adjusted for paternal other drug use, age, education; maternal cannabis use, other drug use, cigarette smoking, age, education, alcohol use; household income; study site; study year

†Heart defects are simple isolated; non-heart defects are isolated

Table 5.3. Probabilistic bias analysis under non-differential and differential exposure misclassification for effects of paternal cannabis use on select birth defects, OR (95% CI)

Birth defect phenotype	Original analysis‡	Non-differential probabilistic bias analysis‡	Differential probabilistic bias analysis‡
Non-heart defects			
Anencephaly and craniorachischisis	1.14 (0.80, 1.64)	0.98 (0.80, 1.21)	1.00 (0.76, 1.33)
Spina bifida	1.02 (0.78, 1.33)	0.98 (0.78, 1.22)	0.98 (0.82, 1.16)
Cleft palate alone	0.88 (0.69, 1.12)	0.98 (0.81, 1.19)	0.99 (0.86, 1.13)
Cleft lip alone	1.23 (0.95, 1.60)	1.05 (0.90, 1.24)	1.02 (0.82, 1.26)
Transverse limb deficiency	1.20 (0.86, 1.66)	1.06 (0.88, 1.29)	1.06 (0.79, 1.41)
Diaphragmatic hernia	1.33 (0.99, 1.80)	1.08 (0.84, 1.40)	1.03 (0.83, 1.27)
Gastroschisis	1.23 (1.00, 1.52)	1.06 (0.83, 1.35)	1.09 (0.88, 1.35)
Heart defects			
DTransposition of the great arteries	0.88 (0.62, 1.24)	0.96 (0.74, 1.26)	0.99 (0.79, 1.24)
VSD perimembranous	1.04 (0.81, 1.33)	1.01 (0.85, 1.20)	1.03 (0.83, 1.28)

*OR: odds ratio; CI: confidence interval

‡ Adjusted for *paternal* other drug use, age, education; *maternal* cannabis use, other drug use, cigarette smoking, age, education, alcohol use, househould income; study site

*Supplemental Table 5.1. Specified trapezoidal distributions for positive predictive value (PPV)
and negative predictive value (NPV) for probabilistic bias analyses under non-differential and
differential exposure misclassfication*

		Non-differential	Differential
Case PPV	Leftmost end of trapezoid (bottom)	0.95	0.95
	Leftmost end of trapezoid (plateau)	0.98	0.98
	Rightmost end of trapezoid (plateau)	0.99	0.99
	Rightmost end of trapezoid (bottom)	1.00	1.00
Case NPV	Leftmost end of trapezoid (bottom)	0.50	0.50
	Leftmost end of trapezoid (plateau)	0.70	0.70
	Rightmost end of trapezoid (plateau)	0.90	0.90
	Rightmost end of trapezoid (bottom)	0.99	0.99
Control PPV	Leftmost end of trapezoid (bottom)	0.95	0.80
	Leftmost end of trapezoid (plateau)	0.98	0.90
	Rightmost end of trapezoid (plateau)	0.99	0.98
	Rightmost end of trapezoid (bottom)	1.00	1.00
Control NPV	Leftmost end of trapezoid (bottom)	0.50	0.50
	Leftmost end of trapezoid (plateau)	0.70	0.75
	Rightmost end of trapezoid (plateau)	0.90	0.85
	Rightmost end of trapezoid (bottom)	0.99	0.85

CHAPTER 6: DISCUSSION

6.1 Overview

State-level policies on medical and recreational cannabis legality have changed rapidly in the past decade, fueling a lively and important debate around the safety, merits, and health effects of cannabis use. In a climate where political ideologies and ignorance often direct policy decisions, sound science is needed to direct to the ongoing debate on cannabis use. While health effects of cannabis use remain unknown and vastly understudied, the reproductive health effects are *especially* understudied. This is of utmost public health concern, since cannabis use is prevalent and increasing among U.S. men and women of reproductive age and potential consequences on offspring may be severe.

This dissertation sought to address the broader knowledge gap of the reproductive health effects of cannabis use by tackling two unanswered questions. Specifically, our first aim addressed how trends in past-month cannabis use correlate with the inter-related time factors of age, period, and cohort among U.S. men and women of reproductive age. These results elucidate demographic shifts in the prevalence of past-month cannabis use in this particular population and are designed to be nationally-representative. Our second aim assessed how paternal cannabis use during the 3-month preconception window affected risk of subsequent birth defects in the offspring. This is the first rigorous and adequately-powered study to address this study questions, and results from this aim contribute to our understanding of male-mediated teratogenicity of cannabis use. Ultimately, results from this dissertation contribute rigorous evidence to our

understanding of the reproductive health effects of cannabis use that may eventually influence clinical guidance and policy decisions regarding cannabis use.

6.2. Aim 1

6.2.1 Summary of findings

Results from Aim 1 confirmed that cannabis use is prevalent and increasing among both men and women of reproductive age (15-49) in the United States, with approximately 12% reporting past-month use in 2014. Prevalence almost doubled among women aged 26-34 in particular, which reflects women with the highest birth rate. Age, period, and cohort each have independent effects on past-month cannabis use, with age having the strongest influences. When holding age and cohort constant, small but consistent period effects were observed. Most notably, period effects – which arose from time-dependent socio-cultural factors – were strongest for 2013 and 2014. State-level cannabis legalization in 2012 may partly explain these recent national period effects, though recent period effects also fit a larger trend of steady increases in cannabis use in the past decade. Understanding trends in cannabis use among women and men of reproductive age is an essential step toward developing health policy, health education, and targeted interventions to mitigate potential adverse reproductive health effects from cannabis use.

6.2.2 Strengths and limitations

Strengths

This was the first study to examine age, period, and cohort effects on cannabis use in the United States since the first states legalized recreational use of cannabis in 2012 and the first study to explore differences among men and women of reproductive age. Laws and social norms have changed drastically since the last APC study reported on trends through 2009 [10, 157, 162]. The most notable change was in 2012 with the first state legalization of recreational cannabis use, so it is timely to examine time trends and drivers those trends over the past seven

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years [158]. Our efforts to understand trends among individuals of reproductive age are crucial to public health guidelines and planning, especially in light of the growing evidence of adverse reproductive health effects of cannabis use. Moreover, we have investigated past-month use, which provides a more temporally-relevant measure of use with regard to reproductive health effects than would be available from previous studies that evaluated past-year cannabis use [164-166]. Since patterns for past-year and past-month use are distinct, our investigation of age-period-cohort effects for past-month cannabis use are novel and may portray age, period, and cohort effects for a more biologically-relevant exposure. Finally, we were able to address this study aim using data from the National Survey of Drug Use and Health, an extremely large and comprehensive survey designed to be nationally representative. This data source, along with the analytic techniques in our analysis to appropriately handle the study's complex survey weighting, provide us external validity, e.g. the ability to generalize our results to the broader target population of all U.S. men and women of reproductive age.

Limitations

Due to the data source, we had to rely on self-report of cannabis use. Self-report of cannabis may be under-estimate exposure, and the extent of measurement error may differ by age and gender. In fact, a study was just published during the writing of this dissertation thesis that shows how true cannabis exposure (measured by toxicology reports) is almost twice as high as self-reported cannabis use among pregnancy women in the Kaiser Permanente population in California, USA [189]. In addition, our results could be vulnerable to time-varying changes in reporting accuracy either by all participants or by participants of specific age groups: if study participants in later years are more comfortable reporting true cannabis use, we may mistake increased reporting for increases in actual prevalence. These concerns may be mitigated by

measures taken within the NSDUH data collection protocol to improve the accuracy of self-report, including: anonymous reporting without face-to-face interactions with the interviewer, reminders of anonymity, and a repeated follow-up approach to minimize non-response.

This study also has limitation in ascertain cohort effects. By restricting to a 13-year period (2002 to 2014), we are 'capturing' each birth cohort at specific ages. For example, individuals born in 1965 would be captured in our study at ages 37 to 49, whereas individuals born in the 1990 would be captured at ages 17 to 29. Since cannabis use is most prevalent among young adults, we may be missing the heaviest cannabis use for the 1960 and 1970 birth cohorts. We adjusted for period and age in the cohort effect estimates, which may mitigate this issue by providing cohort trends averaged over all period and age groups, but generalization of our results beyond this sample is limited. Longer study periods could help corroborate estimates of cohort effects in past-month cannabis use. Additionally, more recent data would allow for a longer assessment of period effects after state-level legalization in 2012.

Finally, we were unable to investigate effect-measure modification by state because the public-access NSDUH files do not include data on participants' residence. Many states decriminalized possession and legalized medical and/or recreational use of cannabis over the study period, which likely results in effect heterogeneity by state specifically for the period effect. Additionally, it would have been interesting to descriptively assess how trends in past-month cannabis use different across state, and how the gender difference in prevalence of use varied across state. Future studies could look at state-specific data to elucidate how period effects differ geographically and by type of legislation change.

6.2.3 Future directions

To better characterize age, period, and cohort effects of past-month cannabis use, future studies should consider conducting state-specific analyses. Since state-level policies on medical use, recreational use, and criminalization have changed dramatically in the past decade and differ by state, there is likely effect heterogeneity of trends and age, period, cohort effects by state that we missed in Aim 1. Moreover, state-specific analyses may be more useful to state-level policy development and decision-making.

Future studies could also examine trends using more accurate measures of cannabis use, like biomarker measure or toxicology data. There is an important tradeoff when deciding between self-report or biologic measures of cannabis use. Biologic measures are much more challenging and costly to obtain, and nearly impossible to obtain in a nationally-representative way. However, biologic measures have much higher validity and therefore more accurately capture true exposure. In our age-period-cohort analysis, we had no way to parsing out changes in trends into changes in actual use or changes in reporting bias (e.g. perhaps people felt more comfortable reporting in study years). Moreover, a paper just published in JAMA shows how toxicology measure of prenatal cannabis use are almost twice as high as self-report measures [189], thus highlighting the need to validate our results using biologic measure of cannabis use.

6.3 Aim 2

6.3.1 Summary of findings

Results from Aim 2 show that paternal cannabis use during the 3-month period prior to conception was associated with slightly increased risk of diaphragmatic hernia, gastroschisis, cleft lip alone, and hypoplastic left heart syndrome in a comprehensive case-control study of birth defects. This was the first study to investigate the association between paternal cannabis use and birth defects since the 1980s, since when cannabis potency has changed considerably [136,

137, 174]. These findings persisted after adjustment for important confounders, imputation for missing confounder data, and probabilistic bias analyses for exposure misclassification. However, these positive findings must be balanced with the null effects observed for 19 other birth defects in this analysis.

Our findings are somewhat inconsistent with previous investigations of paternal cannabis use and birth defects. While our null results for neural tube defects were consistent with findings from Shaw et al. (1996), our null results for subtypes of congenital heart defects (CHDs) were inconsistent with the increased risk for specific CHD subtypes found by Ewing et al. and Wilson et al. However, all three previous studies has very limited sample size (e.g. the BWIS study had only 26 exposed cases for TGAs) and lacked sufficient adjustment for important confounders. No previous studies investigated other defects besides NTDs and CHDs, so our positive findings for diaphragmatic hernia, gastroschisis, and cleft lip alone are novel contributions to the literature. Our findings are also inconsistent with literature on other male-mediated substance use teratogens. Previous studies found that paternal smoking was associated with increased risk of cleft lip with or without cleft palate and specific CHD subtypes [28, 181]. Another study found that paternal heroin use was associated with increased risk of NTDs, but few other studies have investigated paternal drug use [32]. Defects identified from these paternal smoking and drug use studies do not overlap with the two defects we identified as associated with paternal cannabis use (diaphragmatic hernia, gastroschisis), suggesting these mechanisms may also be distinct.

In conclusion, this is the first study to show that paternal cannabis use during the 3-month preconception period is associated with slightly increased risk of four structural birth defect phenotypes: diaphragmatic hernia, gastroschisis, cleft lip alone, and hypoplastic left heart syndrome. In contrast to previous studies, only one association with congenital heart defects

(CHDs) were observed. These results warrant further investigation into cannabis as potential male-mediated teratogen, especially in light of changing cannabis policies and increases in prevalence of use among men of reproductive age.

6.3.2 Strengths and limitations

Strengths

Strengths of this study include its use of population-based controls, clinical verification of cases, large sample size, and analytic methods to handle potential biases. The National Birth Defects Prevention Study (NBDPS) identifies controls from birth records that represent the source population that gave rise to cases, thus providing a population-based control group that results in less potential for selection bias, compared to clinical or convenience-based control sampling [182, 183]. The NBDPS protocol includes a rigorous verification process for all potential cases, where clinical geneticists confirm exact birth defect diagnosis [123]. This detailed case classification minimizes potential for outcome measurement error and allows for more specific case identification compared to other birth defect studies. Additionally, the sheer sample size of the NBDPS population allows for sufficiently-powered analysis of rare birth defect phenotypes [123]. Since we only includes phenotypes with at least 500 eligible cases, we had sufficient power for all analyses. Finally, we had the ability to control for important confounders that previous studies lacked, like maternal cannabis and other maternal/paternal drug use. Our crude and adjustment results were meaningfully different, suggesting these covariates were likely confounding the association.

Limitations

A major limitation of this study is our exposure measurement. While exposure was asked in reference to the etiologically-relevant timeframe, mothers reporting retrospectively on paternal exposure was subject to measurement error. Mothers may not have known fathers' true cannabis

use patterns, or they may have misreported due to recall error or social desirability bias [184-186]. However, superior exposure measures like urine or hair biomarkers were not possible given the retrospective nature of this case-control study. After conducting probabilistic bias analyses to assess how exposure misclassification may impact results, we found that reasonable amounts of exposure misclassification – as defined by trapezoidal distributions for PPV and NPV – resulted in negligible changes in effect estimates. This held true for both non-differential and differential structures of misclassification. Therefore we feel that our results are robust to reasonable amounts of exposure misclassification. However, it is possible that the true misclassification was different than the distributions we assumed in our bias analysis. Future studies should consider conducting validation studies with biomarker data, or using both paternal self-report and maternal report together, to more accurately measure paternal cannabis use.

Another limitation of this study is potential selection bias resulting from conditioning on live birth [138, 187, 188]. If paternal cannabis use increased risk of early pregnancy loss, then we are investigating a select population of infants who were healthy enough to survive until birth. Eight out of ten NBDPS study sites included stillbirths and induced abortions in their case group, which somewhat mitigates the conditioning on live birth issue [123]. Furthermore, we may have selection bias from conditioning on conception: if paternal cannabis use decreases the probability of getting pregnant, then we are investigating a select group of fathers that were able to conceive in the first place. The latter scenario is more likely, given that cannabis has proven effects on reducing sperm count and motility. Unfortunately we lack the ability to quantify this particular bias. Future studies could measure exact effects of cannabis use on time-to-pregnancy and conduct bias analysis for selection bias given bounds of these selection effects.

Finally, this study only assessed ever/never use of cannabis, but the true effect may differ by dosage, method of consumption (e.g. smoked, vaped, edibles), frequency of consumption (e.g. daily vs. monthly use) or strain (e.g. indica vs. sativa). In other words, the true effect of paternal cannabis use may depend on how it was consumed, what exact dosage or type of cannabis was consumed, or exactly when prior to conception exposure occurred – but our study cannot identify effect heterogeneity. Moreover, this study design cannot parse out the effects of chronic cannabis use (e.g. men who used cannabis daily for ten years) versus cannabis use specific during the 3-month preconception period. We lacked data on men's prior cannabis use patterns, so we were unable to know if 'exposed' men in our study were regular cannabis users or if their exposure was limited to the preconception period. Given the animal research on how THC may affect male reproductive organs and sperm quality, it is possible – though not proven – that cannabis has effects that persist after the 3-month spermatogenesis cycle. Additional biologic research is needed to elucidate how long the effects of cannabis on male reproductive health can last. In the meantime, future epidemiologic studies should consider ascertaining information on men's previous cannabis patterns, in addition to their exposure during the preconception period, so analyses can parse out these two distinct exposure profiles.

6.3.3 Future directions

Since this is the first study since the 1980's on paternal preconception cannabis use and risk of birth defects, many future studies are needed to validate and clarify our findings. First, future studies should first investigate this association using biologic measures of exposure. As explained earlier, biologic measure of cannabis use are more valid than self-report (or in this case, maternal report of paternal exposure). While we conducted rigorous bias analysis to assess impacts of exposure misclassification, it is always better to have less measurement error in the first place. Given the case-control nature of the study design, any measurement bias may be

exacerbated if cases and controls have different formats of measurement error, thus highlighting the need for more valid exposure measures in future studies. Future studies should consider measuring THC in urine or hair, though studies would need a prospective design to ascertain these biologic measures of exposure.

Second, future studies should consider using alternative study designs to address the selection bias issue discussed in section 6.3.1 (Strengths & Limitations – Aim 2). Our limitation was conditioning on conception (and survival to a certain point in pregnancy), which is challenging to address. However, a time-to-pregnancy study of couples trying to conceive could theoretically measure paternal cannabis use at multiple time points, and if conception occurs, follow up the infants for adverse birth outcomes. While the paternal cannabis use-birth defects association would still be measured in a population conditioned on conception, this study design would add valuable information on the effects of male cannabis use on likelihood to conceive and time-to-pregnancy, which helps frame and quantify the selection bias. Analytic methods – such as simulations or bias analyses – could then estimate how this selection bias may, or may not, influence effect estimates.

Finally, our study only examined ever/never use of cannabis during the preconception period, but the true effect may be specific to certain dosages, strains, methods of consumption, or timing of use. Future studies should ascertain more specific information on exposure, so they can examine effect-measure modification by these important factors. While dosage has historically been difficult to ascertain since most users are unaware of the exact contents and dosage of the cannabis they use [44], future studies should consider taking advantage of THC and CBD concentration labeling that is now common in states with legal recreational cannabis.

6.4 Conclusions

While both dissertation aims address the broad research area of cannabis use and reproductive health, each aim is quite distinct in its design, analytic methods, and study question it addressed. Aim 1 describes trends in past-month cannabis use in the past decade in our population of interest (men and women of reproductive age) using a technique from demography: the age-period-cohort analysis. This aim is descriptive in nature and helps elucidate shifting trends and life-course patterns of cannabis use. Then, Aim 2 quantifies the effect of preconception paternal cannabis use on 21 types of birth defect phenotypes. This aim is causal in nature and contributes compelling evidence about the potential male-mediated teratogenicity of cannabis use. Notably, the first aim is focused on men and women of reproductive age, regardless of their parity, pregnancy status, or intentions to reproduce, while the second aim is focused on male exposure during the 3-month preconception period among men who were able to conceive. Together, these two aims complement each other by describing trends in exposure broadly among populations of reproductive age, then estimating the causal effect of paternal use on an important adverse birth outcome, both in U.S. populations.

In a nationally-representative study of U.S. men and women of reproductive age, we found that that past-month cannabis use was prevalent and increased from 9.2% in 2002 to 12.3% in 2014. While distinct age, period, and cohort effects were observed, age remains the strongest correlate of past-month use. Despite documented increases in the prevalence of use and rapidly changing state-level policies, little is known about the reproductive health effects of cannabis use. In a rigorous U.S. case-control study of 21 birth defect phenotypes, we found that paternal cannabis use during the 3-months prior to conception was associated with slightly increased risk of diaphragmatic hernia, gastroschisis, cleft lip alone, and hypoplastic left heart syndrome. These associations are novel and inconsistent with previous studies. While future

studies are needed to validate and expand upon our results, these findings indicate that paternal

cannabis use during the preconception period may increase of specific birth defects. As cannabis

policies and social norms continue to change, more research is urgently needed to understand

how cannabis use affects reproductive health and how patterns of cannabis use are changing in

the population.

APPENDIX A: EXPOSURE PREVALENCE BY STUDY YEAR AND SITE IN NBDPS

Table A.1. Prevalence of paternal cannabis use during the 3 months prior to conception in the National Birth Defects Prevention study from 1997 to 2011 (%)

	Arkansas	California	Iowa	Massachusetts	New Jersey	New York	Texas	CDC/Atlanta	North Carolina	Utah
All years	**13.0**	**8.8**	**6.4**	**7.7**	**5.6**	**10.0**	**9.1**	**11.4**	**7.5**	**4.4**
1997	NA	0	0	0	NA	0	6.7	23.1	NA	NA
1998	11.4	5.4	5.6	8.8	0.9	10.8	16.1	10.8	NA	NA
1999	13.3	6.5	6.1	12.0	3.3	10.3	9.7	9.2	NA	NA
2000	7.8	9.2	9.5	10.3	7.4	8.4	9.6	5.7	NA	NA
2001	7.0	11.5	6	4.0	8.9	8.4	7.1	8.4	NA	NA
2002	15.6	12.0	5.1	6.3	9.3	9.7	6.0	11.7	NA	NA
2003	22.6	10.3	3.2	4.6	NA	9.1	12.5	14.0	3.9	2.3
2004	11.9	13.6	10.3	9.0	NA	12.5	6.9	5.3	7.8	4.8
2005	11.4	5.9	6.8	7.7	NA	10.6	7.1	11.2	5.3	4.0
2006	17.3	9.5	4.9	7.7	NA	6.0	7.8	13.6	13.5	2.4
2007	14.0	5.8	7.9	8.1	NA	18	9.3	7.7	9.1	6.7
2008	10.2	5.6	7.1	9.8	NA	11.5	13.2	12.8	50	4.2
2009	10.0	8.8	5.6	9.5	NA	11.1	5.0	17.2	10.9	3.3
2010	17.2	5.6	8.8	5.8	NA	9.1	5.6	16.1	14.0	2.4
2011	15.2	10.9	4.7	5.6	NA	8.3	11.6	13.6	12.5	5.9

*New Jersey did not participate in NBDPS for study years 2003-11; North Carolina and Utah did not participate for study years 1997-2002

*Arkansas and New Jersey did not have any eligible controls in 1997

APPENDIX B: SENSIVITY ANALYSES FOR PATERNAL CIGARETTE SMOKING AND MULTIPLE IMPUTATION

A challenge in our Aim 2 analysis was limited information on paternal cigarette smoking during the 3-month preconception period, a hypothesized confounder. Paternal cigarette smoking was only ascertained during the second half of the study period (2007-2011), and is therefore missing for the first half of the study (~60% of eligible participants had missing paternal smoking). Our original analysis adjustment set did not include paternal smoking so as to maximize our study sample, but here we conduct sensitivity analyses to assess how paternal cigarette smoking may or may not impact our results.

Table B.1 shows adjusted results under three scenarios: [1] fully adjusting (thereby excluding all observations with missing paternal smoking), [2] adjusting for everything except paternal cigarette smoking (thereby including observations with missing paternal smoking), and [3] adjusting for everything except paternal smoking but restricting to observations where paternal smoking is missing. Together, these three scenarios show how selection bias due to measured paternal cigarette smoking and confounding due to paternal cigarette smoking impact the paternal cannabis-birth defect effect estimates.

Table B.2 shows results after multiple imputation of all missing confounder data (including imputation of the >60% paternal cigarette smoking variable). Imputation was conducted using PROC MI and PROC MI ANALYZE where data was imputed n=10 times and effect estimates were combined using Rubin's rule. Results are displayed next to non-imputation adjusted results.

Table B.1. Sensitivity analysis of measurement and adjustment for paternal cigarette smoking, OR (95% CI)

Birth defect phenotype	Crude	Full study period			Restricted to 2007-11		
		Adjusted[1]	Adjusted[2]	Adjusted[3]	Adjusted[1]	Adjusted[2]	Adjusted[3]
Non-heart defects							
Anencephaly and craniorachischisis	**1.43 (1.11, 1.84)**	0.99 (0.59, 1.67)	1.15 (0.80, 1.65)	1.04 (0.62, 1.74)	0.99 (0.59, 1.67)	1.04 (0.62, 1.74)	1.04 (0.62, 1.74)
Spina bifida	0.93 (0.75, 1.15)	1.18 (0.81, 1.72)	1.02 (0.78, 1.33)	1.12 (0.77, 1.63)	1.18 (0.81, 1.72)	1.11 (0.76, 1.61)	1.12 (0.77, 1.63)
Cleft palate alone	1.02 (0.84, 1.23)	0.86 (0.60, 1.25)	0.88 (0.69, 1.12)	0.82 (0.57, 1.18)	0.86 (0.60, 1.25)	0.82 (0.57, 1.17)	0.82 (0.57, 1.18)
Cleft lip with cleft palate	**1.21 (1.04, 1.43)**	1.01 (0.74, 1.39)	1.06 (0.86,1.31)	1.02 (0.75, 1.40)	1.01 (0.74, 1.39)	1.02 (0.75, 1.40)	1.02 (0.75, 1.40)
Cleft lip alone	**1.34 (1.09, 1.63)**	**1.50 (1.02, 2.19)**	1.23 (0.94, 1.60)	**1.48 (1.02, 2.16)**	**1.50 (1.02, 2.19)**	**1.48 (1.01, 2.15)**	**1.48 (1.02, 2.16)**
Esophageal atresia	1.07 (0.83, 1.39)	1.19 (0.74, 1.93)	1.17 (0.84,1.63)	1.22 (0.76, 1.96)	1.19 (0.74, 1.93)	1.21 (0.75, 1.95)	1.22 (0.76, 1.96)
Anorectal atresia/stenosis	1.00 (0.80, 1.25)	1.06 (0.70, 1.61)	1.18 (0.89, 1.56)	1.05 (0.69, 1.59)	1.06 (0.70, 1.61)	1.04 (0.69, 1.58)	1.05 (0.69, 1.59)
Hypospadias second/third degree	0.96 (0.81, 1.13)	1.11 (0.81, 1.51)	1.05 (0.84, 1.32)	1.10 (0.81, 1.50)	1.11 (0.81, 1.51)	1.12 (0.82, 1.52)	1.10 (0.81, 1.50)
Transverse limb deficiency	**1.41 (1.11, 1.79)**	1.52 (0.96, 2.41)	1.20 (0.87, 1.66)	1.41 (0.90, 2.20)	1.52 (0.96, 2.41)	1.47 (0.94, 2.30)	1.41 (0.90, 2.21)
Craniosynostosis	0.77 (0.63, 0.95)	0.96 (0.66, 1.39)	0.92 (0.71, 1.20)	0.95 (0.66, 1.36)	0.96 (0.66, 1.39)	0.95 (0.66, 1.36)	0.95 (0.66, 1.36)
Diaphragmatic hernia	**1.28 (1.02, 1.61)**	1.16 (0.73, 1.84)	1.33 (0.99, 1.79)	1.16 (0.73, 1.83)	1.16 (0.73, 1.84)	1.15 (0.73, 1.82)	1.16 (0.73, 1.83)
Gastroschisis	**3.07 (2.66, 3.54)**	1.11 (0.83, 1.50)	1.25 (0.99, 1.58)	1.14 (0.85, 1.53)	1.11 (0.83, 1.50)	1.15 (0.86, 1.54)	1.14 (0.85, 1.53)
Heart defects							
DTransposition of the great arteries	0.95 (0.72, 1.26)	0.85 (0.50, 1.43)	0.88 (0.62, 1.24)	0.83 (0.50, 1.38)	0.85 (0.50, 1.43)	**	0.83 (0.50, 1.38)
Hypoplastic left heart syndrome	1.24 (0.95, 1.63)	1.53 (0.96, 2.42)	1.38 (0.99, 1.93)	**1.59 (1.01, 2.50)**	1.53 (0.96, 2.42)	**	**1.59 (1.01, 2.50)**
RVOT defects	1.13 (0.96, 1.34)	1.21 (0.89, 1.64)	1.13 (0.91, 1.39)	1.20 (0.89, 1.62)	1.21 (0.89, 1.64)	**	1.20 (0.89, 1.62)

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Birth defect phenotype	Crude	Full study period			Restricted to 2007-11		
		Adjusted[1]	Adjusted[2]	Adjusted[3]	Adjusted[1]	Adjusted[2]	Adjusted[3]
RVOT defects excluding Ebstein cases	1.13 (0.95, 1.34)	1.17 (0.85, 1.61)	1.14 (0.92, 1.41)	1.16 (0.85, 1.59)	1.17 (0.85, 1.61)	**	1.16 (0.85, 1.59)
Pulmonary valve stenosis	1.08 (0.89, 1.31)	1.16 (0.81, 1.65)	1.11 (0.87, 1.41)	1.16 (0.82, 1.64)	1.16 (0.81, 1.65)	**	1.16 (0.82, 1.64)
VSD perimembranous	1.07 (0.88, 1.30)	1.06 (0.59, 1.90)	0.98 (0.77, 1.24)	1.10 (0.62, 1.96)	1.06 (0.59, 1.90)	**	1.10 (0.62, 1.96)
VSD muscular	0.90 (0.65, 1.24)	1.06 (0.57, 1.98)	0.88 (0.59, 1.29)	1.01 (0.55, 1.87)	1.06 (0.57, 1.98)	**	1.01 (0.55, 1.87)
ASD secundum	1.15 (0.98, 1.35)	1.11 (0.81, 1.53)	1.06 (0.87, 1.29)	1.11 (0.82, 1.52)	1.11 (0.81, 1.53)	**	1.11 (0.82, 1.52)
ASD NOS	1.30 (0.97, 1.73)	1.08 (0.47, 2.49)	0.70 (0.47, 1.04)	1.08 (0.47, 2.47)	1.08 (0.47, 2.49)	**	1.08 (0.47, 2.47)

*OR: odds ratio; CI: confidence interval; VSD: ventricular septal defect; ASD: atrial septal defect; NOS: not otherwise specified

[1] Fully adjusted (*paternal* cigarette smoking, other drug use, age, education; *maternal* cannabis use, other drug use, cigarette smoking, age, education, alcohol use, pregnancy intention, household income; study site)

[2] Adjusted for everything except paternal cigarette smoking

[3] Adjusted for everything except paternal cigarette smoking, where paternal smoking is not missing

Table B.2. Sensitivity analysis of multiple imputation of missing confounder data, OR (95% CI)

Birth defect phenotype	Crude	Adjusted‡	Adjusted & Imputed†
Non-heart defects			
Anencephaly and craniorachischisis	**1.43 (1.11, 1.84)**	1.15 (0.80, 1.65)	1.35 (0.97, 1.87)
Spina bifida	0.93 (0.75, 1.15)	1.02 (0.78, 1.33)	0.93 (0.72, 1.20)
Cleft palate alone	1.02 (0.84, 1.23)	0.88 (0.69, 1.12)	0.93 (0.74, 1.17)
Cleft lip with cleft palate	**1.21 (1.04, 1.43)**	1.06 (0.86,1.31)	1.07 (0.88, 1.30)
Cleft lip alone	**1.34 (1.09, 1.63)**	1.23 (0.94, 1.60)	1.27 (0.99, 1.63)
Esophageal atresia	1.07 (0.83, 1.39)	1.17 (0.84,1.63)	1.16 (0.84, 1.59)
Anorectal atresia/stenosis	1.00 (0.80, 1.25)	1.18 (0.89, 1.56)	1.06 (0.81, 1.39)
Hypospadias second/third degree	0.96 (0.81, 1.13)	1.05 (0.84, 1.32)	1.16 (0.94, 1.43)
Transverse limb deficiency	**1.41 (1.11, 1.79)**	1.20 (0.87, 1.66)	1.30 (0.97, 1.75)
Craniosynostosis	0.77 (0.63, 0.95)	0.92 (0.71, 1.20)	0.98 (0.76, 1.27)
Diaphragmatic hernia	**1.28 (1.02, 1.61)**	1.33 (0.99, 1.79)	1.41 (1.07, 1.86)
Gastroschisis	**3.07 (2.66, 3.54)**	1.25 (0.99, 1.58)	1.26 (1.04, 1.53)
Heart defects			
DTransposition of the great arteries	0.95 (0.72, 1.26)	0.88 (0.62, 1.24)	0.95 (0.68, 1.33)
Hypoplastic left heart syndrome	1.24 (0.95, 1.63)	1.38 (0.99, 1.93)	1.27 (0.91, 1.77)
RVOT defects	1.13 (0.96, 1.34)	1.13 (0.91, 1.39)	1.12 (0.92, 1.38)
RVOT defects excluding Ebstein cases	1.13 (0.95, 1.34)	1.14 (0.92, 1.41)	1.13 (0.91, 1.39)
Pulmonary valve stenosis	1.08 (0.89, 1.31)	1.11 (0.87, 1.41)	1.05 (0.83, 1.33)
VSD perimembranous	1.07 (0.88, 1.30)	0.98 (0.77, 1.24)	1.05 (0.83, 1.33)
VSD muscular	0.90 (0.65, 1.24)	0.88 (0.59, 1.29)	0.85 (0.57, 1.27)
ASD secundum	1.15 (0.98, 1.35)	1.06 (0.87, 1.29)	1.06 (0.87, 1.29)
ASD NOS	1.30 (0.97, 1.73)	0.70 (0.47, 1.04)	0.90 (0.62, 1.31)

*OR: odds ratio; CI: confidence interval; VSD: ventricular septal defect; ASD: atrial septal defect; NOS: not otherwise specified

‡ Adjusted for all confounders except paternal smoking; prior to multiple imputation

† Adjusted for all confounders (including paternal cigarette smoking) after multiple imputation of all missing confounder data

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